Insulin detemir is transported from blood to cerebrospinal fluid and has prolonged central anorectic action relative to NPH insulin

Denovan P. Begg¹,², Aaron A. May¹, Joram D. Mul¹,³, Min Liu¹, David A. D’Alessio¹,⁴, Randy J. Seeley¹,⁵, and Stephen C. Woods¹

1 Metabolic Diseases Institute, University of Cincinnati, Cincinnati, OH, USA
2 School of Psychology, UNSW Australia, Sydney, NSW, Australia
3 Joslin Diabetes Center, Harvard Medical School, Boston, MA, USA
4 Department of Medicine, Duke University, Durham, NC, USA
5 Department of Surgery, University of Michigan, Ann Arbor, MI, USA

Running title: Insulin detemir transport to CSF and central action

Key words: Insulin, obesity, transport, blood-brain barrier, central nervous system

Word count: 3993

Number of figures: 6

Corresponding Author

Stephen C. Woods
Metabolic Diseases Institute, University of Cincinnati
2170 East Galbraith Road, Cincinnati, OH 45237
Phone: (+1) 513-558-6799
Fax: (+1) 513-297-0966
Abstract

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, due to increased transport into the central nervous system and/or increased catabolic action within the brain. Transport of DET and NPH insulin (NPH) into the cerebrospinal fluid (CSF) was compared over several hours and following 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 following IP DET than following 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 following DET than following 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.
Introduction

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 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 hours (10). A common problem with insulin treatment is weight gain, patients having insulin doses titrated to achieve glycemic targets often gain from 2-10kg. 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 analogues (15; 16).
It has been proposed that the differential effects on weight loss of DET compared to other insulin formulations is 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 to 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.

**Materials 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 (22-25g) were purchased from Charles River Laboratories (Wilmington, MA). All animals were maintained in AAALAC-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 SMI 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 IACUC.
**CSF sampling**

CSF samples were taken from rats after an overnight fast or mice following a 4-h fast. Animals were placed in the chamber of an anesthetic isoflurane machine (VetEquip, Pleasanton, CA). Following 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 semi-blunted 25-gauge (1½-inch) needle was connected to micro-renathane 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 micro-renathane 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 (~20mm) 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 500µL tube on ice. On average, ~150µL of CSF was collected within 2-min and immediately frozen on dry ice in rats, and ~15µL of CSF was obtained in mice using a glass capillary tube. Animals were immediately sacrificed 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 following peripheral injection

Dose-response experiment 30-min prior to CSF sampling, rats were injected IP with insulin (NPH insulin or DET; 0, 0.1, 0.3, 1, 3, or 10U/kg) and returned to their home-cage. Animals (n=8-10/dose) were anesthetized after 25-min and CSF was sampled precisely at 30-min post-injection.

Time-course experiment Rats (n=6-8/time-point) were administered NPH insulin or DET IP (0.5 U/kg), returned to their home-cage, and had CSF sampled 5, 10, 20, 40, 80, 160 or 320-min post-injection.

Comparison of the appearance of long-acting formulations of insulin, DET and glargine (GLAR) in blood and CSF over time in rats and mice

Rats (n=7-8/group) were injected SC with GLAR (0.5U/kg) or DET (0.5U/kg). The change in dosing route was required as GLAR is only long-acting when injected SC. Following the injection the rats were returned to their home-cage and CSF was sampled at baseline (0), 6, 12, 24, 48 or 72h post-injection. Mice (n=7-8/group) were injected SC with GLAR (1U/kg) or DET (1U/kg). Following the injection mice were returned to their home-cage and CSF was sampled at baseline (0), 6, 12, 24 or 48h post-injection.

Third-ventricular cannulation (3VT)

Each rat was anesthetized with IP ketamine (70mg/kg) and xylazine (6mg/kg), 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 2.2mm 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, 7.5mm ventral to the dura. The cannula placement was fixed with dental acrylic that was anchored to the skull with 3-4 screws. An obturator was inserted that extended 0.5mm below the cannula. At least 5d prior to food intake experiments, cannula placement was confirmed by injecting 10ng of angiotensin II (American Peptides, Sunnyvale, CA) in 1µl saline. Rats that consumed >5ml of water within 30-min were deemed to have a viable cannula. Four rats were excluded based on this criterion.

**Food intake and body weight following 13VT insulin in rats**

Measurements of food intake and body weight were based on an established paradigm (26). Briefly, on each of the 4d prior to an experiment, each animal (n=16) was individually acclimated to handling for 4-5-min/d, and had food removed for the final 4-h of light. All animals underwent this protocol on 3 occasions, 1-week apart, in a randomized crossover manner to receive DET (8mU), NPH insulin (8mU) or 2µL of vehicle (sterile 0.9% saline) 13VT on the experimental days. Injections occurred 1h before dark onset. Food-hoppers were returned at the onset of dark and intake and body weight were assessed after 24 and 48h.

**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) (Echo NMR, Waco, TX). Mice (n=12/group) then received daily SC injections of either normal saline, DET (1U/kg) or insulin glargine (GLAR, 1U/kg). Animals were weighed and food intake was
monitored weekly for 6-wk, after which time body composition was re-assessed. In a follow-up experiment, mice (n=7-9/group) were injected SC with either normal saline, GLAR (1U/kg) or DET (1U/kg) daily for 6-wk. 24-h 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 (5µL/sample of plasma, 10µL/sample of CSF) was added to an antibody-coated microplate. After incubation for 2h at 4°C, 100µL of anti-insulin enzyme conjugate was dispensed into each well and incubated at room temperature for 30-min. The plate was washed and 100µL of 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 630nm from the absorbance at 450nm. Due 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 (Accuchek; Roche Diagnostics, Indianapolis, IN) (27).

**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 following observations of significant interaction effects (Statistica 7.1; Statsoft). Significance was accepted at p<0.05 with data reported as mean±SEM.
Results

Appearance of DET and NPH insulin in plasma and CSF after its IP administration

IP NPH insulin produced a dose-dependent increase in plasma insulin after 30-min, with a significant increase first occurring at 0.3U/kg (p<0.05; Figure 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 3U/kg dose (Figure 1B). There was a dose-dependent decrease in plasma glucose reflecting the increased plasma insulin (Figure 1C).

Plasma insulin was increased at all doses of DET relative to the 0U/kg group (p<0.05; Figure 1D). The magnitude was dose-dependent, with the 10U/kg group of DET having significantly elevated plasma insulin relative to all other groups (p<0.05). CSF insulin was also elevated following all doses relative to the saline group in a dose-dependent manner (p<0.05). Apparent saturation of CSF insulin occurred by the 3U/kg dose, with no difference between 3 and 10U/kg (Figure 1E). There was a similar dose-dependent decrease in plasma glucose following DET as was observed following NPH insulin (Figure 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 following its IP administration

Injection of 0.5U/kg IP 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 (Figure 2A). CSF insulin
levels also rose rapidly following IP NPH insulin, 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 (Figure 2B). Plasma glucose levels closely tracked plasma insulin levels with an immediate reduction at 5-min and a return to baseline by 80-min (Figure 2C; p<0.05).

Injection of 0.5 U/kg of DET IP 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 following NPH insulin, plasma insulin levels remained elevated 320-min after DET treatment (Figure 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) (Figure 2E). Plasma glucose levels were reduced by DET at a relatively constant level for the duration of the experiment (Figure 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 (ps<0.05) and a more prolonged elevation of insulin in both plasma (160 and 320-min, ps<0.05) and CSF (320-min, p<0.05) following DET. DET also produced a sustained hypoglycemia relative to NPH (80, 160 and 320-min, ps<0.05), see Figure 2.

13VT DET and NPH insulin reduce food intake and body weight
Following 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 (Figure 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 (Figure 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; Figures 3C and 3D).

**Comparison of the appearance of insulin in plasma and CSF following administration to different long-acting formulations of insulin, DET and glargine (GLAR).**

Injection of either DET or GLAR 0.5 U/kg sc 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 (Figure 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 (Figure 4B; ps<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 (Figure 4C; ps<0.05) but, consistent with plasma insulin, remained lower at 24-h only in GLAR-treated animals.
The effect of chronic SC administration of long-acting formulations of insulin on body weight and body composition in mice

Daily SC injection of DET reduced weight gain compared with both saline and GLAR treatment after 3-wk (p<0.05), and this difference was maintained for the remainder of the 6-wk treatment period (p<0.05; Figure 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; Figure 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; Figure 5C). Lean mass did not differ among groups (Figure 5D).

Appearance of insulin in plasma and CSF of mice following SC administration of 1 U/kg of DET and GLAR

In mice, SC DET or GLAR led to an increase in plasma insulin similar what occurred in rats. At the first measured time point after baseline (6-h) plasma insulin was elevated to a greater extent in DET in than GLAR-treated mice (Figure 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 (ps<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 (Figure 6B). Following 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 (Figure 6C; p<0.05). However, the reverse was observed in the CSF, where DET, but not GLAR-treated mice, had increased insulin (Figure 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 following DET and NPH were 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 following 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 following 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 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 blood-brain barrier (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. reported that DET does not enter the brain from the blood in mice (22). However, rather than measuring insulin directly, their group administered labeled DET systemically and measured radioactive counts in TCA-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, when compared to regular insulin and with plasma glucose comparably clamped, DET elicited a significant change in EEG activity in the frontal cortex of men and also caused a significant reduction of food intake. Endogenous insulin is believed to cross the blood-brain-barrier 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.
Following peripheral administration in our rats, insulin in the CSF peaked sooner following IP DET and remained higher longer than treatment with NPH insulin. 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 following 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 to another insulin formulation, GLAR, since it is also used for the same purpose but lacks the attached fatty acid side-chain as 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-wk 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 than women to reduce body fat (36; 40), we utilized male rats in the present experiments. It will be important in future experiments to determine if 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 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 following 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 and colleagues (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 non-homeostatic 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 mellitus (46-48).
Author contributions

D.P.B., R.J.S., D.A.D. and S.C.W. designed the experiments. D.P.B., A.A.M., J.D.M. and M.L. collected and analyzed the data. D.P.B., D.A.D. and S.C.W. wrote the manuscript. R.J.S., A.A.M., J.D.M. and M.L. 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.

Acknowledgements

This work was supported by NIH grants to S.C.W. DK017844, DK095440, S.C.W. and M.L. DK092779, D.A.D. DK57900, and R.J.S. DK54080. D.P.B. was supported by an NHMRC of Australia Early Career Fellowship 1013264.

Disclosure statement

D.A.D. consults for Interarcia, Lilly, and Novo Nordisk; receives research support from Johnson and Johnson and Mannkind, and participates in clinical trials sponsored by Sanofi-Aventis. R.J.S. has consultancies, research support or is a paid speaker with the following companies: Ethicon Endo-Surgery/Johnson & Johnson, Novo Nordisk, Merck, Novartis, Angiochem, Zafgen, Takeda, Ablaris, Pfizer, Eli Lilly, Zealand Pharma.
References

14. Dornhorst A, Luddeke HJ, Koenen C, Merilainen M, King A, Robinson A, Sreenan S, Group PS: Transferring to insulin detemir from NPH insulin or insulin glargine in type 2 diabetes patients on basal-only therapy with oral antidiabetic drugs improves glycemic control and reduces weight gain and risk of hypoglycaemia: 14-week follow-up data from PREDICTIVE. Diabetes Obes Metab 2008;10:75-81
Figure 1. Mean (±SEM) concentration of insulin in plasma and CSF, and glucose in plasma, 30-min following the IP administration of insulin detemir (DET) or NPH insulin at different doses in rats. IP NPH insulin and DET produced similar levels as a function of dose in plasma (A, 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 U/kg dose (B, E). Plasma glucose decreased in a dose-dependent manner following IP NPH insulin or DET (C, F). Significant differences are described in the text.

Figure 2. Mean (±SEM) concentration of insulin in plasma and CSF, and glucose in plasma, at different time points following the IP administration of 0.5 U/kg of insulin detemir (DET) or NPH insulin in rats. Plasma insulin rose rapidly following the IP injection of NPH insulin, peaking at 5-min and then decreasing (A). CSF insulin levels also rose rapidly following IP NPH insulin, the peak occurring at around 40-min, and levels returning to baseline by 320-min (B). Plasma glucose dropped sharply following IP NPH insulin, returning to baseline by 80-min (C). IP 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 following IP 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 following DET to a constant level for the duration of the experiment (F). Significant differences are described in text.

Figure 3. 13VT DET and NPH insulin reduce food intake and body weight in rats. 13VT 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 post infusion
(C); however, only the DET group maintained the weight loss after 48 h (D). * p<0.05 vs. CON, + p<0.05 vs. NPH insulin. Data are mean ±SEM.

**Figure 4. Comparison of the appearance of insulin in the plasma and CSF following the SC administration of two long-acting formulations of insulin, DET and glargine (GLAR) in rats.**

Injection of either DET or GLAR at 0.5 U/kg sc 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 mean ±SEM.

**Figure 5. Chronic sc administration of DET reduces food intake and body weight relative to SC 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 CON at Week 6 (A). Total food intake was reduced in the DET group compared with that in both GLAR and CON (B). Fat mass was lower in DET, and higher in GLAR, compared with CON (C). Lean mass was not altered by any treatment (D). * p<0.05 vs. CON, + p<0.05 vs. GLAR. Data are mean ±SEM.

**Figure 6. Appearance of insulin in plasma and CSF at different times following the administration of SC DET and GLAR in mice, and the effect of chronic DET and GLAR treatment on CSF insulin.**

Injection of either DET or GLAR (1 U/kg, sc) 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). 24 h 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, but not GLAR-treated mice, had elevated insulin. * p<0.05 vs. CON, + p<0.05 vs. GLAR. Data are mean ±SEM.
Figure 1

A

B

C

D

E

F

241x127mm (300 x 300 DPI)
Figure 3

(A) 24-h food intake (g)

(B) 48-h food intake (g)

(C) Δ 24-h body weight (g)

(D) Δ 48-h body weight (g)

CON, NPH, DET
Figure 4

- A: Plasma insulin (ng/mL) over time (h).
- B: CSF insulin (ng/mL) over time (h).
- C: Plasma glucose (mg/dL) over time (h).

Different symbols represent different groups: DET and GLAR.
Figure 6

A

Plasma insulin (ng/mL)

Time (h)

0 6 12 24 48

B

CSF insulin (ng/mL)

Time (h)

0 6 12 24 48

C

Plasma insulin (ng/mL)

CON GLAR DET

D

CSF insulin (ng/mL)

CON GLAR DET

187x140mm (300 x 300 DPI)