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Effect of subcutaneous insulin detemir on glucose flux, lipolysis and electroencephalography in type 1 diabetes

The aim of the present study was to investigate the effects of subcutaneous detemir on glucose flux, lipid metabolism and brain function. Twelve people with type 1 diabetes received, in random order, 0.5 units/kg body weight detemir or NPH insulin. Glucose concentration was clamped at 5 mmol/l then increased to 10 mmol/l. Glucose production rate (glucose Ra), glucose uptake (glucose Rd) and glycerol production (glycerol Ra) were measured with a constant intravenous infusion of [6,6-$^2H_2$]glucose and [$^2H_5$]glycerol. Electroencephalography direct current (DC) and alternating current (AC) potentials were measured. While detemir induced similar effects on glucose Ra, glucose Rd and glycerol Ra during euglycaemia compared with NPH, it triggered a distinct negative shift in DC potentials, with a significant treatment effect in frontal cerebrocortical channels ($p < 0.001$). AC spectral power showed significant differences in theta and alpha frequencies during euglycaemia ($p = 0.03$). Subcutaneous detemir exerts different effects on brain function when compared with NPH in people with type 1 diabetes. This may be an important mechanism behind the limitation of weight gain with detemir.
Keywords: glucose metabolism, insulin detemir, lipid metabolism, type 1 diabetes, weight

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RESEARCH LETTER

Introduction

Exogenous insulin, as administered to people with type 1 diabetes, is frequently associated with weight gain or weight stability rather than weight loss. Detemir is associated with less weight gain compared with NPH insulin [1,2]. Detemir may influence appetite and reduce energy intake through greater direct effects on the brain [1]. Alternatively, it may have a higher action in the liver than peripheral tissues and therefore a less anabolic effect on peripheral tissues [3], or may result in a reduction in defensive snacking [4].

The aim of the present study was to delineate the important physiological action of clinically relevant subcutaneous doses of detemir compared with NPH insulin on glucose flux and lipolysis, measured using stable isotope techniques, and brain function, measured by electroencephalography (EEG) direct current (DC) potentials and alternating current (AC) potentials, in people with type 1 diabetes.

Methods

This was an investigator-led, double-blind crossover metabolic study for people with type 1 diabetes. Participant inclusion is shown in Table S1.

Ethics approval was granted by the NRES Committee London - Hampstead. REC reference 11/LO.0687. The trial was registered in the European Clinical Trials Database (EudraCT) number: 2011-001642-14 and funded by Novo Nordisk A/S.

Participants omitted their basal insulin the night before a metabolic study. At study commencement they were transferred to a soluble variable rate insulin infusion to maintain a blood glucose concentration of 5 mmol/l. Primed (170 mg) continuous infusion (1.7 mg/min) of [6,6-$^2H_2$]glucose and 0.4 mg/kg body weight/min of [1,1,2,3,3-$^2H_5$]glycerol (Cambridge isotopes, CK Gas Products Ltd, Ibstock, UK) were administered from −120 min. At isotopic steady state, participants were given 0.5 units/kg body weight of subcutaneous detemir or NPH insulin, depending on randomization order. The variable rate insulin infusion was tailed off over 90 min and blood samples taken to measure glucose and glycerol concentration and enrichment and non-esterified fatty acids (NEFA) concentration at predetermined time points. A variable infusion rate of dextrose, spiked with [6,6-$^2H_2$]glucose, maintained blood glucose at 5 mmol/l until 210 min and then 10 mmol/l until 300 min. To prevent rapid changes in tracer to tracee ratio of glucose, 20% dextrose was spiked with 4 mg/g of [6,6-$^2H_2$]glucose tracer at the start of the euglycaemic and hyperglycaemic period.

The EEG recordings were taken using a portable recorder (SKU:M97130 Vitaport; Temec Instruments B.V., Kerkrade, Netherlands). DC potential recordings were obtained from frontal (F3,F4), frontocentral (FC3,FC4) and central (C3,C4) electrodes. AC potentials were recorded from F3,F4,FC3,FC4 and occipital (01,02) electrodes. Each electrode was referenced to contralateral mastoid electrodes. The Karolinska Drowsiness test was undertaken at predetermined time points [5]. Participants were required to stare at a dot with their eyes open (3 min) and stay immobile with their eyes closed (3 min).

Analytical Procedures

Blood glucose concentrations were measured using a glucose analyser (YSI 2300; Clandon Scientific, Yellow Springs

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Instruments, Yellow Springs, OH, USA). Plasma glucose concentrations were measured on a Cobas MIRA using ABX Pentra glucose kit (Horiba ABX, Northampton, UK), plasma glycerol concentrations using a Randox glycerol kit and plasma NEFA concentrations using a Randox Calorimetric kit (Randox Laboratories, Co., Crumlin, UK).

Isotopic enrichment of plasma glucose was determined as the trimethylsilyl-O-methyloxime derivative [6], using a gas chromatography-mass spectrometry model 597S CMSD inertXL EI/CI MSD (Agilent Technologies, Wokingham, UK). The isotopic enrichment of plasma glycerol was determined as the tert-butyltrimethylsilyl glycerol derivative [7] using a gas chromatography-mass spectrometry model 5973 network (Agilent Technologies).

Glucose production rate (glucose Ra), glucose uptake (glucose Rd) and glycerol production (glycerol Ra) were calculated using Steele's non-steady state equations modified for stable isotopes [8]. EEG data were exported as a European Data Format (EDF) file and imported into ProFusion PSG3 (Compumedics Ltd, Abbotsford, VIC, Australia). Median values for consecutive 5-min periods were referenced to 0 V using the average voltage from insulin dosing.

The AC channels were re-exported from ProFusion as an EDF file with filtering applied. The EDF was then imported into Vitascore (Temec Instruments B.V.). Using a 2-s window and applying Fast Fourier Transform up to a frequency of 32 Hz, giving a resolution of 0.5 Hz spectral power (mV) was calculated for eight frequency bands.

Statistical Analysis

Glucose Ra, glycerol Ra and glucose Rd data were subjected to two-way analysis of variance (ANOVA) including treatment (detemir vs NPH) and time as a repeated measure. The Bonferroni method was used to correct for multiple comparisons. DC and AC potential data were subjected to two- and three-way ANOVA. Data are expressed as mean and standard error of the mean.

Results

Twelve participants completed the metabolic study (8 women, 4 men). Their mean age was 33.5 ± 4.7 years, weight 70.0 ± 2.5 kg, BMI 24.5 ± 0.8 kg/m^2, glycated haemoglobin 6.9 ± 0.7% and diabetes duration 16.1 ± 2.4 years. Five participants used continuous subcutaneous insulin infusions.

Glucose and Glycerol Metabolism

The plasma glucose concentration profile for detemir and NPH insulin were similar (euglycaemia, $p = 0.30$; hyperglycaemia, $p = 0.61$). Plasma glucose at isotopic equilibrium was 6.9 ± 0.4 mmol/l in the detemir group and 6.0 ± 0.3 mmol/l in the NPH group (Figure S1).

Glucose Ra and glucose Rd were similar during the euglycaemic period. During the hyperglycaemic period glucose Rd was higher with NPH insulin than detemir ($p = 0.003$; Figure S2). Glycerol Ra and NEFA concentrations were similar ($p = 0.09$).

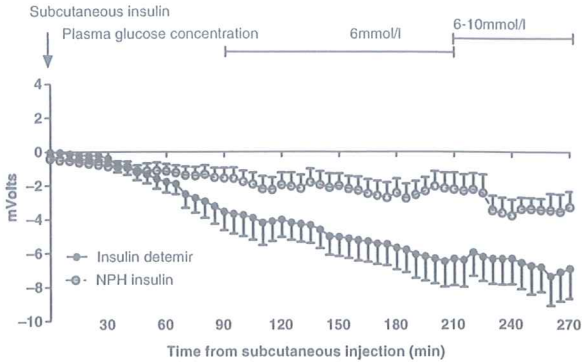

Figure 1. Mean direct current potential averaged across all subjects and all electrodes and plotted against time relative to the subcutaneous injection. Data are expressed as means and standard error of the mean.

Direct Current Potentials

The DC potentials showed a greater negative shift in the detemir group than in the NPH group ($p = 0.002$; Figure 1). The data were then modelled to account for missing data, participant effects and periodicity. There was a significant treatment by channel interaction ($p < 0.001$). Table 1 shows the effect of treatment for each individual channel. There were significant treatment and treatment by time interactions for channels F3 and F4, during euglycaemia (Table S2).

Alternating Current Spectral Power

During 'eyes-open', combined channels showed treatment effects in the theta frequency band during euglycaemia ($F(1,39) = 4.87$, $p = 0.03$). The treatment interactions and treatment by channel interactions are shown in Table S3. Table S4 shows the treatment by time interaction for each channel in the theta and alpha band. No significant treatment effects were obtained during 'eyes-closed'.

Discussion

This is the first study to provide evidence that when compared with NPH insulin, clinically relevant doses of subcutaneous detemir may act differently in the brain. While eliciting similar effects on glucose flux and lipolysis during euglycaemia, detemir triggered a greater negative shift in DC potentials. The negative shift was of a global nature, with the greatest effect in the frontal cerebrocortical regions.

Interpretation during hyperglycaemia is difficult as the differences may result from increased rate of peripheral glucose uptake in the NPH group rather than a direct difference of insulin action in the brain. There were also differences in AC potentials, with activation of theta and alpha activity in the frontocentral cerebrocortical regions. The exact significance remains unclear.

Although we cannot determine the underlying molecular mechanism, the greater action of detemir in the brain may be related to its albumin-binding or the novel method of protraction. The capillary endothelial barrier in peripheral tissue

Table 1. Direct current potentials (mV). Effect of treatment and treatment by time interaction (time as a repeated measure) for each channel during the euglycaemic clamp period and hyperglycaemia period.

	F3: lef t frontal	F4: right frontal	C3: left central	C4: right central	FC3: left frontocentral	FC4: right frontocentral
Euglycaemic clamp (90–210 min)						
Treatment interaction	F(1,20) = 25.6, p < 0.0001*	F(1,31) = 23.8, p < 0.0001*	F(1,28) = 1.02, p = 0.32	F(1,23) = 0.49, p = 0.49	F(1,17) = 0.66, p = 0.43	F(1,23) = 7.40, p = 0.01*
Treatment by time interaction	F(7,24) = 3.67, p < 0.008*	F(7,25) = 5.26, p < 0.008*	F(7,27) = 1.84, p = 0.12	F(7,25) = 2.77, p = 0.03*	F(7,22) = 2.84, p = 0.03*	F(7,27) = 2.67, p = 0.03*
Hyperglycaemic period (210–300 min)						
Treatment interaction	F(1,1) = 0.56, p = 0.60	F(1,14) = 0.16, p = 0.70	F(1,5) = 0.59, p = 0.48	F(1,12) = 0.40, p = 0.54	F(1,11) = 1.51, p = 0.25	F(1,11) = 1.15, p = 0.31
Treatment by time interaction	F(3,1) = 0.38, p = 0.80	F(3,16) = 1.60, p = 0.23	F(3,10) = 3.69, p = 0.05*	F(3,14) = 5.95, p = 0.008*	F(3,13) = 1.14, p = 0.37	F(3,13) = 1.46, p = 0.27

* < 0.05.

may limit the transfer of detemir from the circulation into the extravascular space. Detemir may also cross the blood–brain barrier (BBB) more easily than human insulin [9], perhaps as a result of detemir's lipophilic properties [10]. Alternatively, there may be more unbound active detemir available to bind to the insulin receptor in the brain as albumin is very low in the cerebrospinal fluid. Finally, detemir could cross the BBB equally but have different binding affinities to the insulin receptors located within the brain. What is evident is that further work in the field is still required.

The present study does not identify what the changes are driving biologically. Hallschmid et al. [11] associated the shift in DC potential with reduced food intake in healthy subjects, suggesting intravenous detemir had an enhanced anorexigenic impact on the central nervous system that controls nutrient uptake. A lower intake of energy with detemir than with NPH has also been reported, further supporting reduced food intake as a potential mechanism for the weight-sparing effect [1]. Finally, the vagus nerve is the most important link between the gut, pancreas and liver to the brain and appears to be involved in the control of food intake [12]. The indirect action of insulin on the hypothalamus and interaction with hepatic glucose production is of interest.

In conclusion, in people with type 1 diabetes, clinically relevant doses of subcutaneous detemir exert stronger effects on brain function and seem to have a tissue-selective action with preference for brain tissue compared with peripheral tissues. This may be an important mechanism behind the limitation of weight gain.

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Conflict of Interest

D. R.-J. has received research funding or advisory board or lecture fee honoraria from Novo Nordisk. The remaining authors declare no duality of interest associated with this manuscript. D. J. Dijk has received advisory board honoraria from Novo Nordisk.

R. H. carried out the metabolic studies, interpreted the data and drafted the manuscript. R. H. takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript, F. S.-M. carried out the metabolic studies, sample analysis and interpreted data. N. J. assisted with sample analysis. S. J. provided statistical support. R. K. conducted the EEG analyses. R. J., A. M. U., D.-J. D. and D. L R.-J. participated in the design of the study, interpreted data and reviewed and edited the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Plasma glucose concentration plotted against time in the clamp protocol. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean ± standard error of the mean, n = 12.

Figure S2. Glucose rate of appearance and glucose rate of disappearance plotted against time. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean ± standard error of the mean, n = 12.

Table S1. Inclusion and exclusion criteria.

Table S2. Direct current potentials (mV). Treatment by time interaction for channel F3 and F4 during the euglycaemic clamp (120–210 min) and hyperglycaemia period (210–270 min).

Table S3. Alternating current spectral power (mV). The treatment and treatment by channel interaction (channel and time were repeated measures) for combined channels at each band frequency during the euglycaemic clamp period.

Table S4. Treatment by time interaction for alternating current spectral power (mV) in band 3 (theta) and band 4 (alpha) for each individual channel during 'eyes open'.

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