1 **Research Letter**

2 **Effect of Subcutaneous Insulin Detemir on Glucose Flux, Lipolysis and**
3 **Electroencephalography in Type 1 Diabetes.**

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Abstract

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 0.5Units/kgBW detemir or NPH insulin. Glucose concentration was clamped at 5mmol/L then increased to 10mmol/L. Glucose production rate (glucose Ra), glucose uptake (glucose Rd) and glycerol production (glycerol Ra) were measured with a constant iv infusion of [6,6²H₂]glucose and [2H₅]glycerol. Electroencephalography direct (DC) and alternating (AC) current potentials were measured. While detemir induced comparable effects on glucose Ra, glucose Rd and glycerol Ra during euglycaemia, compared with NPH, it triggered a distinct negative shift in DC-potentials, with 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.

Key terms: lipid metabolism. Insulin detemir, glucose metabolism, type 1 diabetes, weight

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 liver than peripheral tissues and therefore a less anabolic effect on peripheral tissues (3) or result in a reduction in defensive snacking (4).

This study aimed 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 EEG DC-potentials and 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 supplement table1.

Participants omitted their basal insulin the night before a metabolic study. At commencement they were transferred to a soluble variable rate insulin infusion (VRII) to maintain a blood glucose concentration of 5mmol/l. Primed (170mg) continuous infusion (1.7mg/min) of [6,6-²H₂]glucose and 0.4mg/kg BW/min of [1,1,2,3,3-²H₅]glycerol (Cambridge isotopes, CK Gas Products Ltd, UK) were administered from -120 min. At isotopic steady state, participants were given 0.5Units/kgBW of subcutaneous detemir or NPH insulin depending on randomisation order. The VRII was tailed off over 90 minutes and blood samples taken to measure glucose and glycerol concentration and enrichment and NEFA concentration at predetermined time points. A variable infusion rate of dextrose spiked with [6,6-²H₂]glucose maintained blood glucose at 5mmol/l until 210minutes and then 10mmol/l until 300minutes. To prevent rapid changes in tracer to tracee ratio of glucose, 20% dextrose was spiked with 4mg/g of [6,6-²H₂]glucose tracer at the start of the euglycaemic and hyperglycaemic period.

EEG recordings were taken using a portable recorder (SKU:M97130 Vitaport; Temec Instruments B.V. 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 (O1,O2) 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 (3minutes) and stay immobile with their eyes closed (3minutes).

Analytical Procedures

Blood glucose concentrations were measured using a glucose analyser (YSI 2300 Clandon Scientific, Yellow Springs Instruments, Ohio, USA). Plasma glucose concentrations were measured on a Cobas MIRA using ABX Pentra glucose kit (Horiba ABX, Northampton, UK), plasma glycerol concentrations using Randox glycerol kit and plasma NEFA concentrations using a Randox Calorimetric kit (Randox Laboratories, Co.Antrim, UK)

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

Glucose Ra and Rd and glycerol Ra were calculated using Steele's non-steady state equations modified for stable isotopes (8). EEG data was exported as European Data Format (EDF) file and imported into ProFusion PSG3 (Compumedics Ltd, Abbotsford 3067, Australia).

Median values for consecutive 5minute periods were referenced to 0V using the average voltage from insulin dosing.

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. Spekhofstraat2, Netherlands). Using a 2second window and applying Fast Fourier Transform up to a frequency of 32Hz, giving a resolution of 0.5Hz spectral power (mV) was calculated for 8 frequency bands.

Statistical Analysis

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

Results

Twelve participants completed the metabolic study (8 females, 4 males). Mean age 33.5 ± 4.7 years, weight 70.0 ± 2.5 kg, BMI 24.5 ± 0.8 kg/m², HbA1c $6.9 \pm 0.7\%$, diabetes duration 16.1 ± 2.4 years). Five 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 (supplement fig1).

Glucose Ra and glucose Rd were similar during the euglycemic period. During the hyperglycemic period glucose Rd was higher with NPH insulin than detemir ($p=0.003$) (supplement fig2). Glycerol Ra and NEFA concentrations were similar ($P=0.09$).

DC-potentials

DC-potentials showed a greater negative shift in the detemir group than the NPH group ($p=0.002$) (Fig1). The data was 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 (supplement table2).

AC- 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 presented in supplemental table3. Supplemental table4 demonstrates 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 comparable 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 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 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 due detemir's lipophilic property (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.

This study does not identify what the changes are driving biologically. Hallschmid 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 (11). 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 type1 diabetes, clinically relevant doses of subcutaneous detemir exerts stronger effects on brain function and seems 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

David Russell-Jones 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. Derk Jan Dijk has received advisory board honoraria from Novo Nordisk.

Roselle Herring carried out the metabolic studies, interpreted data and drafted the manuscript. Roselle Herring 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, Fariba Shojaee-Moradie carried out the metabolic studies, sample analysis and interpreted data. Nicola Jackson assisted with sample analysis. Sig Johnsen provided statistical support. Richard Knight conducted the EEG analyses. Richard Jones, A.Margot Umpleby, Derk-Jan and David L Russell-Jones participated in the design of the study, interpreted data, and reviewed and edited the manuscript.

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Figure 1 legend. The mean DC-potential averaged across all subjects and all electrodes and plotted against time relative to the subcutaneous injection. Data were expressed as means and standard errors of means (SEM).

Table 1. DC-Potentials (mV). The 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: left frontal	F4: right frontal	C3: left central	C4: right central	FC3: left frontocentral	FC4: right frontocentral
Euglycaemic clamp (90-210 minutes)						
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 minutes)						
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
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Supplemental Figure 1 legend. Plasma glucose concentration plotted against time in the clamp protocol. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean±SEM, n=12

Supplemental Figure 2 legend. Glucose rate of appearance (Ra) and glucose rate of disappearance (Rd) plotted against time. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean±SEM, n=12

Supplemental Figure 3 legend. Glycerol concentration and glycerol rate of production (Ra) plotted against time. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean $\pm$ SEM, n=12

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Inclusion Criteria	Exclusion Criteria
Type 1 diabetes greater than 12 months	Proliferative retinopathy that had required treatment within the preceding 3 months
BMI less than 35	Impaired hepatic function
Over 18 years of age	Impaired renal function
HbA1c of greater or equal to 37mmol/mol (5.5%) and less than 75mmol/mol (9%)	Impaired cardiac function

Insulin administered via multiple daily dosing regime or continuous subcutaneous insulin infusion.	People with uncontrolled hypertension defined as a blood pressure greater than 160/90mmHg
	Mental incapacity
	Pregnancy
	suspected allergy to the trial products.

Supplement table 1. Inclusion and exclusion criteria

Supplement Table 2. DC potentials (mV): Treatment by time interaction for channel F3 and F4 during the euglycaemic clamp (120 – 210 mins) and hyperglycaemia period (210 – 270 mins).

Time (minutes)	Difference in LS means (mV)	Std Error	Confidence Intervals
Euglycaemic clamp period: F3 (Left frontal)			
120	-8.143	2.95	(-14.414, -1.872)
150	-7.523	3.82	(-15.613, 0.567)
180	-7.646	4.33	(-16.794, 1.501)
210	-7.504	4.96	(-17.967, 2.958)
Hyperglycaemia period: F3 (Left frontal)			
240	4.295	5.15	(-10.177, 18.766)
270	4.426	5.53	(-9.725, 18.577)
Euglycaemic clamp period: F4 (Right frontal)			
120	-3.278	4.16	(-12.311, 5.755)
150	-2.613	4.68	(-12.704, 7.477)
180	-1.565	5.147	(-12.624, 9.494)
210	-1.059	5.58	(-13.020, 10.902)
Hyperglycaemia period: F4 (Right frontal)			
240	0.797	5.03	(-9.975, 11.570)
270	1.566	5.31	(-9.806, 12.939)

Supplement Table 3. AC-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.

	Low Delta 0-0.5Hz	Delta 0.5-4.5Hz	Theta 5-7.5Hz	Alpha 8-11.5 Hz	Sigma1 12-14.5Hz	Beta-1 15-19.5Hz	Beta-2 20-24.5Hz	Beta-3 25-31.5.Hz
'Eyes open' PERIOD								

Treatment effect	F(1,41) = 0.11, p=0.74	F(1,42) = 0.03, p=0.86	F(1,39) =4.87, P=0.03	F(1,38) =3.10, P=0.08	F(1,36) =0.26, P=0.61	F(1,36) =0.39, P=0.53	F(1,39) =1.61, P=0.21	F(1,34) =0.76, P=0.39
Treatment by channel interaction	F(10,38) = 1.85, p=0.08	F(10,38) =0.86, P=0.57	F(10,41) =1.19, P=0.33	F(10,40) =0.65, P=0.76	F(10,38) =0.30, P=0.98	F(10,41) =0.43, P=0.93	F(10,42) =0.67, P=0.75	F(1,34) = 0.63, P=0.43
'Eyes closed' PERIOD								
Treatment effect	F(1,36) = 0.57, P=0.46	F(1,32) = 0.49, P=0.47	F(1,35) =2.48, P=0.12	F(1,45) =0.59, P=0.44	F(1,36) =0.75, P=0.39	F(1,35) =0.25, P=0.61	F(1,36) =0.26, P=0.61	F(1,36) =0.55, P=0.93
Treatment by channel interaction	F(10,41) = 0.62, P=0.79	F(10,41) =0.27, P=0.98	F(10,41) =0.71, P=0.71	F(10,39) =1.04, P=0.43	F(10,40) =0.61, P=0.80	F(10,42) =1.37, P=0.22	F(10,41) =1.80, P=0.09	F(1,41) = 1.55, P=0.16

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