

Differential effects of insulin detemir and neutral protamine Hagedorn (NPH) insulin on hepatic glucose production and peripheral glucose uptake during hypoglycaemia in type 1 diabetes

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Abstract

Aims/hypothesis We compared the symptoms of hypoglycaemia induced by insulin detemir (NN304) (B29Lys(ϵ -tetradecanoyl),desB30 human insulin) and equally effective doses of neutral protamine Hagedorn (NPH) insulin in relation to possible differential effects on hepatic glucose production and peripheral glucose uptake.

Methods After overnight intravenous infusion of soluble human insulin 18 participants with type 1 diabetes received subcutaneous injections of NPH insulin or insulin detemir (0.5 U/kg body weight) on separate occasions in random order. During the ensuing gradual development of hypoglycaemia cognitive function and levels of counter-regulatory

hormones were measured and rates of endogenous glucose production and peripheral glucose uptake continuously evaluated using a primed constant infusion of [6,6- $^2\text{H}_2$] glucose. The study was terminated when plasma glucose concentration had fallen to 2.4 mmol/l or had reached a minimum at a higher concentration.

Results During the development of hypoglycaemia no difference between the two insulin preparations was observed in symptoms or hormonal responses. Significant differences were seen in rates of glucose flux. At and below plasma glucose concentrations of 3.5 mmol/l suppression of endogenous glucose production was greater with insulin detemir than with NPH insulin, whereas stimulation of peripheral glucose uptake was greater with NPH insulin than with insulin detemir.

Conclusions/interpretation In participants with type 1 diabetes subcutaneously injected insulin detemir exhibits relative hepatoselectivity compared with NPH insulin, but symptoms of hypoglycaemia and hormonal counter-regulation are similar.

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Abbreviations

C_{\max} Maximum concentration
EGP Endogenous glucose production
HSS Hypoglycaemia symptom score
NPH Neutral protamine Hagedorn
PGU Peripheral glucose uptake

Introduction

In normal physiology insulin is released from the islets within the pancreas into the portal circulation, which flows directly to the liver, a major insulin target organ. The liver extracts up to 60% of the insulin delivered to it with the remainder dispersed into the systemic circulation [1]. In consequence, hepatocytes are exposed to insulin concentrations that are three to four times higher than the other major targets for insulin (adipose tissue and muscle). As a replacement therapy, insulin is delivered as a subcutaneous depot and absorbed into the systemic circulation, through which it is distributed in approximately equal concentrations throughout the body. The normal portal–peripheral insulin gradient is lost, resulting in a relative peripheral hyperinsulinaemia and underinsulinisation of the liver. This metabolic imbalance is inevitable with most existing insulin preparations and may contribute to acute and chronic complications of diabetes.

To reach peripheral target cells (myocytes and adipocytes), insulin must pass through the capillary wall lined by a continuous endothelium, which limits the transfer of large molecules from the circulation into the extravascular space [2]. In contrast, the vascular sinusoids within the liver are lined by highly fenestrated epithelial cells with large pores between them and no basal lamina. Therefore no significant barrier exists between protein molecules within the plasma in the sinusoid and the surface of hepatocytes [3]. Thus an insulin analogue bound to one or more plasma proteins has greater access to hepatocytes than to peripheral target tissues. Insulin detemir (NN304) (B29Lys(ϵ -tetradecanoyl),desB30 human insulin) is a soluble insulin analogue with a C₁₄ fatty acid side-chain at position B29 [4]. In the monomeric state, this side-chain binds to the fatty acid binding sites of albumin in plasma, developing equilibrium between free and albumin bound analogue [5]. Approximately 98% of detemir within the blood stream is bound to albumin [2, 5]. In contrast the prolongation of action of NPH insulin in the subcutaneous depot is dependent on non-covalent associations with zinc atoms and with protamine, such that after absorption into the circulation it exists in the free form identical to native human insulin [6].

It has been proposed that because of its protein binding detemir may have a relatively greater effect at the liver than at peripheral target tissues when administered subcutaneously, at least partially restoring the normal liver–peripheral gradient in insulin action [7]. Additional support for this hypothesis comes from studies with a novel insulin analogue that binds to thyroid hormone binding proteins. Thus B1-thyroxylin insulin demonstrated a relatively greater effect on the liver than on peripheral tissues [8]. Glucose clamp studies in participants without diabetes showed that insulin detemir had a greater effect than a preparation of

human insulin on glucose metabolism in the liver and a lesser effect on peripheral tissues [7]; however, clamp studies may not be the best experimental method to investigate this physiological phenomenon.

We designed and performed a novel protocol allowing a gradual fall in plasma glucose concentration to hypoglycaemic levels with continuous assessment of glucose fluxes. We hypothesised that the counter-regulatory response to hypoglycaemia in patients with type 1 diabetes may prove more effective if peripheral tissues are overstimulated to a lesser degree by exogenous insulin. Other studies in participants without diabetes using stepped hypoglycaemic clamps have shown some differences in the perception of hypoglycaemia with insulin detemir, but not in the counter-regulatory response [9]. We report here a study in patients with type 1 diabetes, which compared detemir to neutral protamine Hagedorn (NPH) insulin during a period of gradual free fall of plasma glucose concentration to hypoglycaemic values.

Methods

We conducted a randomised, double-blind, single-centre, two-stage, crossover trial to compare the hypoglycaemic responses to single doses of insulin detemir and NPH insulin in participants with type 1 diabetes. Participants were recruited from the Royal Surrey County Hospital diabetes database. All gave written informed consent and the local Ethics Committee approved the study. Participants were excluded if there were factors that would alter sensitivity to hypoglycaemia, including previous reduced awareness of hypoglycaemia, autonomic neuropathy, treatment with beta-blockers and a total daily insulin dose greater than 100 IU. At initial screening, plasma HbA_{1c} and C-peptide concentrations, full blood count, and renal and liver function were measured.

Study protocol We studied 18 participants, of whom 14 completed both arms of the study satisfactorily; four participants were excluded from the final analysis before it was unblinded, due to failure to achieve hypoglycaemia on both occasions. Height and weight were measured at screening and before each study. The screening weight was used for calculation of the insulin dose administered on each study day.

Participants were asked not to undertake vigorous physical activity and not to drink alcohol, and were excluded if they reported an episode of hypoglycaemia in the 72 h before attendance. Injection of basal insulin was omitted from the evening before participants attended for an overnight variable infusion of soluble (human) insulin to achieve a stable basal plasma glucose concentration of approximately 7 mmol/l by the following morning. This protocol was chosen to ensure that overnight hypoglycaemia was avoided and to enable a washout period of a minimum

34 h prior to subcutaneous injection of the trial insulin. Participants then underwent two studies, one with insulin detemir, the other with NPH insulin, in random order with an interval between studies of 7 to 42 days. Intravenous access was obtained via both ante-cubital fossae, enabling sampling from one and infusion into the other. During the study participants remained fasting, were allowed to ingest water only and were supine throughout. After drawing baseline (unenriched) blood samples, a primed constant infusion of [6, 6-²H₂]glucose (170 mg, 1.7 mg/min) was started. A period of 160 min was allowed for isotopic equilibration, prior to the subcutaneous injection of insulin. The isotope infusion was then continued until the end of the study.

Insulin detemir or NPH 0.5 U/kg was injected subcutaneously into a thigh in a double blind manner by a diabetes specialist nurse not otherwise involved in the trial. This dose was chosen after a pilot study of five participants dosed at 0.4 U/kg failed to achieve adequate hypoglycaemia. An even higher dose might have led to an earlier nadir of blood glucose, but symptoms of hypoglycaemia might have been less tolerable, leading to withdrawal of a larger number of participants. The rate of infusion of intravenous human insulin was gradually reduced to zero over the following 10 min.

The protocol was divided into three sections according to the glucose concentration. Section 1 began with glucose stabilisation prior to injection and lasted until blood glucose had fallen to 4 mmol/l. Section 2 was the period of glucose decline from 4 to 2.4 mmol/l, ending either then or when the symptoms of hypoglycaemia became intolerable or after 8 h from injection. Section 3 was the rescue period, when 20% dextrose primed with [6, 6-²H₂] glucose was infused at 6 mg/kg to restore blood glucose to 6 mmol/l.

Blood sampling Blood glucose concentration was monitored by frequent blood sampling initially at 20 min intervals, increasing when blood glucose had fallen below 4 mmol/l to 5 to 10 min intervals. Samples were taken for enriched glucose and plasma glucose concentrations every 20 min throughout. Insulin detemir or human insulin concentration was measured every hour throughout the study.

Initially, the counter-regulatory hormones (plasma adrenaline [epinephrine], noradrenaline [norepinephrine], glucagon, serum growth hormone, cortisol and NEFA) were measured every hour. When the blood glucose concentration was at or below 4 mmol/l, these were measured at 20 min intervals until hypoglycaemia nadir, when they were taken just before initiation of the rescue infusion, and at 10 and 30 min thereafter. The target glucose level for termination of the study was 2.4 mmol/l. If this value was not reached within 8 h after subcutaneous injection of the trial insulin, the study was ended. Some participants reached a plateau above 2.4 mmol/l. The study was then stopped at the time limit of 8 h or at a time

when participants could no longer tolerate the symptoms of hypoglycaemia. Once the rescue infusion of 20% dextrose had restored blood glucose to 6 mmol/l, food and drink were given.

Cognitive function tests The cognitive function tests were performed at baseline (−60, −30, −15 min) and then hourly after subcutaneous insulin administration until blood glucose reached 4 mmol/l. Thereafter, the tests were performed every 15 min.

Finger tapping test A computer key was pressed as many times as possible for 10 s. The test was repeated three times at each defined time-point and the mean calculated [10].

Four-choice reaction time test A computer screen randomly displayed a dot in one of four possible fields. The participant had to respond by pressing the correspondingly positioned key on a keypad. Each test was carried out with 100 cycles. Number correct (out of 100), mean correct time (ms) and standard deviation for correct time (ms) were recorded [11].

Hypoglycaemia symptom score A hypoglycaemia symptoms questionnaire based on the Edinburgh Hypoglycaemia Scale [12] with a linear interval scale for each symptom was completed. At each stage the question was asked ‘Do you feel hypo?’ and hypoglycaemia symptom scores (HSS) for autonomic symptoms, neuroglycopenic symptoms and general malaise were graded from 1 (not at all) to 7 (a great deal). This was completed at baseline (−180 min) and hourly after insulin administration until glucose concentration reached 4 mmol/l. After this, the questionnaire was completed every 15 min.

Autonomic function Heart rate and blood pressure were recorded at baseline and every 30 min until blood glucose concentration reached 4 mmol/l. From then they were recorded every 15 min until blood glucose concentration was restored.

Analytical methods The total concentrations of free and bound insulin detemir in serum were measured with an insulin detemir-specific ELISA [13]. Concentrations of serum human insulin and C-peptide were analysed using an insulin ELISA assay (Dako, Glostrup, Denmark).

Plasma concentrations of adrenaline and noradrenaline were quantified by HPLC (Chromosystems, Munich, Germany), glucagon by RIA (Diagnostic Products Corporation, Los Angeles, CA, USA), growth hormone using serum-coated bead immunoradiometric assay (Skybio, Wyboston, UK), NEFA using an enzymatic assay (Randox Laboratories, Crumlin, UK) and cortisol by ELISA (DRG International, Mountainside, NJ, USA).

All isotopic enrichments were measured by gas chromatography–mass spectrometry on a mass selective detector (HP

5971A; Agilent Technologies, Queensferry, UK). The isotopic enrichment of glucose was determined using a penta-*O*-trimethylsilyl-D-glucose-*O*-methoxime derivative analysed by selecting ion monitoring of the ions at charge:mass ratio 319 and 321. Glucose concentrations in blood and plasma were measured by a glucose oxidase method using a glucose analyser (Clandon Scientific; Yellow Springs Instruments, Yellow Springs, OH, USA)

Calculations Endogenous glucose production (EGP) and peripheral glucose uptake (PGU) were calculated using Steele's non-steady state equations [14] adapted by Finegood et al. [15] and modified for stable isotopes. Before calculation of glucose turnover, plasma glucose concentration and glucose enrichment time courses were smoothed and interpolated to 5 min intervals using optical segments technique analysis [16]. For each time-point EGP and PGU were calculated. For each participant in each study the mean EGP and PGU at a glucose concentration of 7.0, 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, 3.5, 3.0 and 2.5 mmol/l were calculated. The boundaries for each concentration were ± 0.05 mmol/l, so for example, any concentration between 5.95 and 6.05 mmol/l was considered to be a concentration of 6 mmol/l. For each concentration there were one, two or three values. The mean EGP and PGU values at each of these glucose concentrations were calculated.

Statistical methods The following were compared: baseline adjusted HSS at nadir blood glucose concentration, hypoglycaemic symptoms, hypoglycaemic awareness, blood glucose profile, autonomic response (time taken for heart rate to be $>15\%$ baseline), counter-regulatory hormones, cognitive function, EGP, PGU and the pharmacokinetics of insulin detemir and human insulin.

Data are expressed as mean values with standard deviations or standard errors as appropriate. EGP and PGU were calculated and plotted against blood glucose concentration. Mean profiles for all time-dependent variables were plotted as a function of time using time since trial drug injection and time since blood glucose nadir. A test for no difference in symptoms of hypoglycaemia between insulin detemir and NPH insulin was carried out using ANOVA with insulin preparation, interactions between insulin preparation and dose level and order of visit as fixed effects. A one-way ANOVA was applied to EGP and PGU values for glucose concentrations during hypoglycaemia, (3.5–2.5 mmol/l).

Results

The participants represented a clinically relevant population with type 1 diabetes. Of 18 participants who completed the trial,

four were excluded from the final analysis before the study was un-blinded, due to failure to achieve hypoglycaemia on both visits. The results presented here are for the 14 participants who completed both studies and achieved hypoglycaemia (11 men, three women; age 31 ± 8.2 years; BMI 24.8 ± 1.97 kg/m²; weight 77 ± 9.4 kg; duration of diabetes 13 ± 9.8 years; HbA_{1c} $7.8 \pm 0.71\%$). Of this group, seven achieved target hypoglycaemia and three stopped due to intolerable symptoms of hypoglycaemia after administration of NPH. With insulin detemir, ten of the 14 achieved target hypoglycaemia and four terminated early due to intolerable symptoms.

Hypoglycaemia symptoms and cognitive function A modest increase in HSS was seen during development of hypoglycaemia. The baseline adjusted HSS at nadir blood glucose did not differ significantly between insulin detemir and NPH insulin (estimated difference -1.01 ; 95% CI $-9.55, 7.54$; $p=0.81$). No relevant differences between insulin detemir and NPH insulin were observed in hypoglycaemic awareness endpoints ($p=0.73$). Similarly, no statistical difference was seen in the cognitive function test results between insulin detemir and NPH (Electronic supplementary material [ESM] Table 1) or for 'order of visit' ($p=0.9$)

Glucose metabolism Following administration of insulin detemir or NPH insulin the blood glucose concentration decreased from baseline values of 7.0 ± 1.1 (mean \pm SD) and 7.3 ± 1.2 mmol/l respectively to 2.6 ± 0.5 at 336 ± 77 min and 2.8 ± 0.6 mmol/l at 338 ± 76 min at blood glucose nadir. There were no relevant differences in endpoints related to blood glucose profiles (Fig. 1).

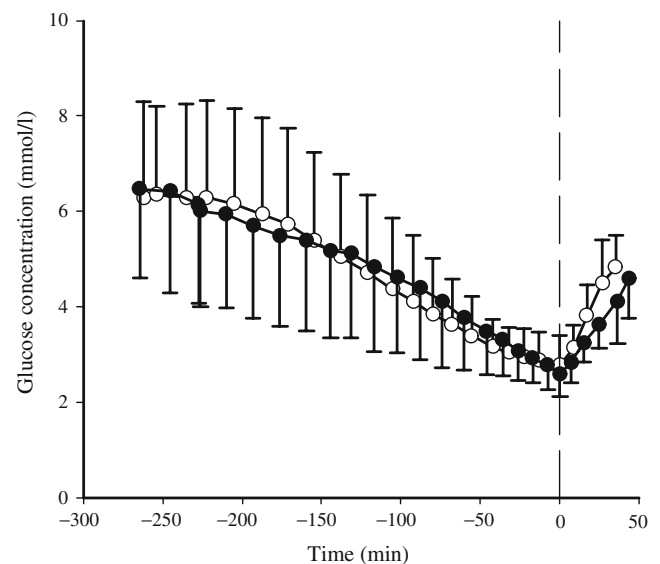


Fig. 1 Glucose concentration plotted against time from glucose nadir. Values are means \pm SEM. Black circles, insulin detemir; white circles, NPH insulin

At baseline (pre-study insulin injection, when all participants were on intravenous soluble human insulin) there were no significant differences in EGP or PGU (Fig. 2a, b).

After injection of insulin, EGP during hypoglycaemia was consistently lower with insulin detemir than with NPH insulin at the same glucose concentration, confirming that insulin detemir exerted a greater suppressive effect on EGP ($p=0.001$). Similarly, after injection of insulin, PGU was lower with insulin detemir than with NPH insulin at the same glucose concentration, showing that insulin detemir also exerted a smaller effect than NPH insulin on PGU ($p=0.005$) (Fig. 2).

Autonomic response At the onset of autonomic response, heart rate was 75 ± 9 and 74 ± 5 beats/min, and systolic and diastolic blood pressure was 123 ± 9 and 128 ± 10 mmHg, and 71 ± 7 and 76 ± 10 mmHg for insulin detemir and NPH insulin respectively. These endpoints did not differ between the two insulins ($p=0.05$).

Counter-regulatory hormones Plasma adrenaline and serum growth hormone concentrations peaked at blood glucose

nadir and then started to decline towards basal levels. Plasma noradrenaline levels peaked at blood glucose nadir then remained stable. There were no significant differences between the effects of insulin detemir and NPH insulin on the counter-regulatory hormones adrenaline (AUC $p=0.30$, maximum concentration [C_{\max}] $p=0.65$), noradrenaline (AUC $p=0.33$, C_{\max} $p=0.81$), glucagon (AUC $p=0.52$, C_{\max} $p=0.34$), growth hormone (AUC $p=0.59$) and cortisol (AUC $p=0.64$, C_{\max} $p=0.25$) at baseline or during hypoglycaemia (Fig. 3) (ESM Table 2). NEFA concentrations when the glucose concentration was below 4 mmol/l were also not significantly different between the two insulins ($p=0.30$).

Insulin concentrations Maximum concentration of insulin detemir occurred at 279 ± 102 min and was 3496 ± 1488 pmol/l; that of NPH insulin occurred at 180 ± 147 min and was 183 ± 144 pmol/l (Fig. 3).

Discussion

In this study we have shown that during hypoglycaemia in type 1 diabetes insulin detemir had a greater effect than NPH insulin on suppression of EGP and a lesser effect than NPH insulin on the stimulation of PGU. No difference was shown between insulin detemir and NPH in awareness of hypoglycaemia, which was measured by the Edinburgh Hypoglycaemia Score and is so crucial to individuals with type 1 diabetes as it allows them to manage control safely and effectively. In addition the counter-regulatory hormone response was not demonstrated to be significantly different between the two insulins. Inclusion of more participants might have revealed subtle differences. However, this seems unlikely, given the high significance shown here for EGP and PGU.

Under normal physiological post-absorptive conditions euglycaemia is maintained by portal insulin acting to modulate hepatic glucose production [17]. In type 1 diabetes, fasting hyperglycaemia is due to increased production of glucose from the liver, i.e. unrestrained glycogenolysis [18].

In the experiments described here, clinically relevant circumstances were reproduced under controlled conditions, allowing us to explore in detail the events that occur when, in type 1 diabetes, an inappropriate excess of subcutaneous insulin results in a drift into hypoglycaemia.

We tested the hypothesis that at any given value of hypoglycaemia glucose flux would be greater with NPH insulin than with insulin detemir. The results support this suggestion. As insulin detemir-induced hypoglycaemia developed in these patients, both glucose uptake and

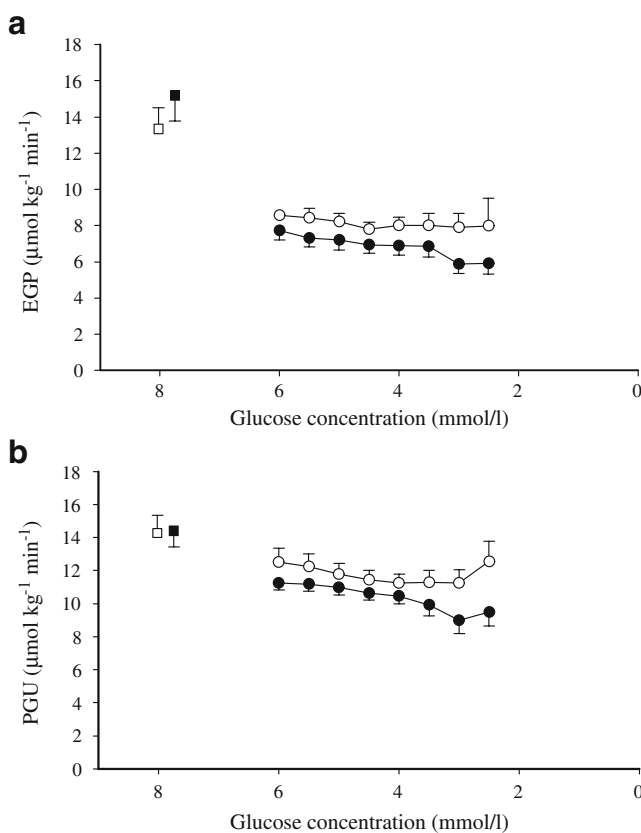
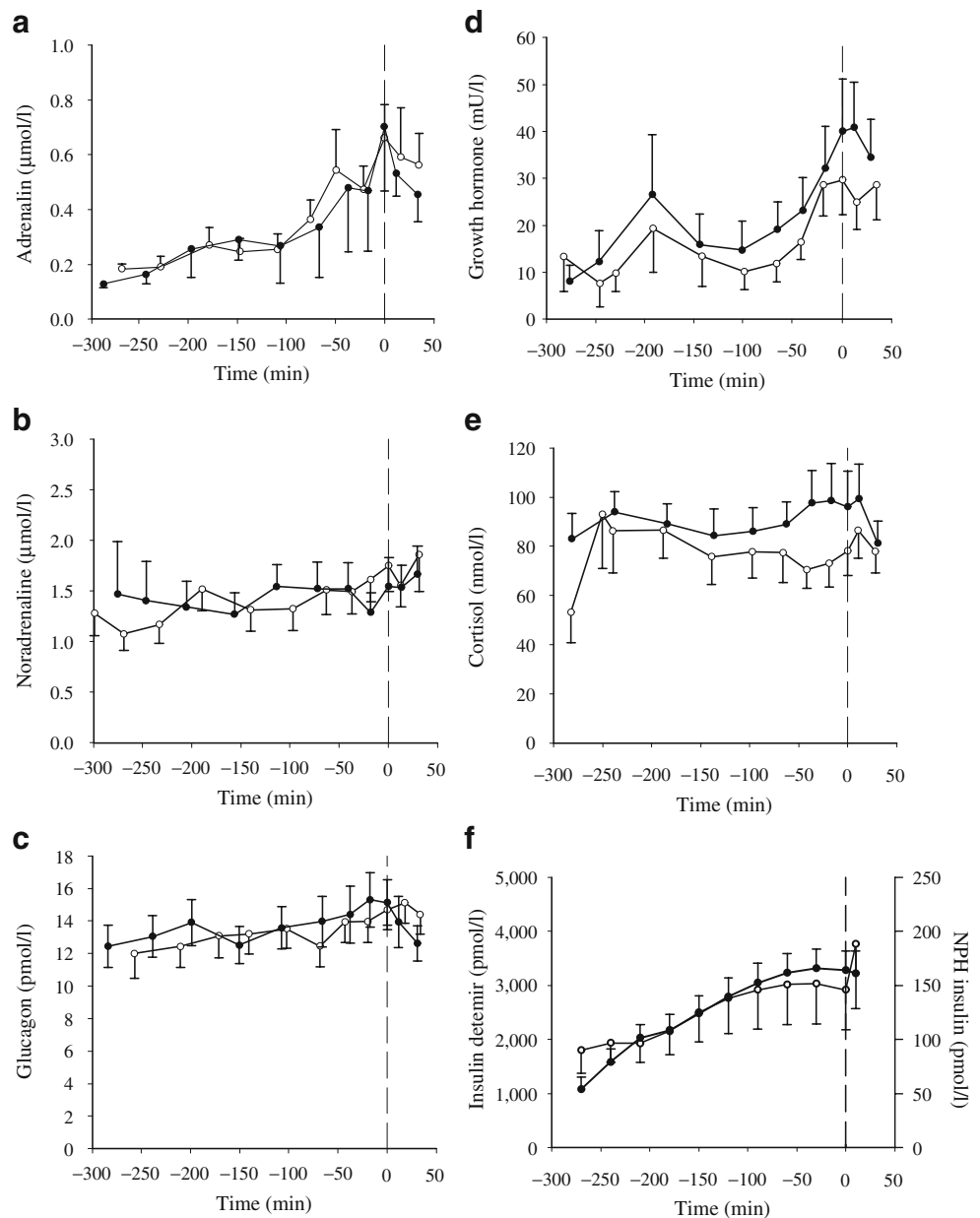


Fig. 2 (a) EGP and (b) PGU at glucose concentrations between 3.5 and 2.5 mmol/l ($p=0.001$ and $p=0.005$ respectively). Values are means \pm SEM. Black squares, insulin detemir at baseline; white squares, NPH insulin at baseline; black circles, insulin detemir post-baseline; white circles, NPH insulin post-baseline

Fig. 3 Counter-regulatory hormones plotted against time from glucose nadir. **(a)** adrenaline, **(b)** noradrenaline, **(c)** glucagon, **(d)** growth hormone and **(e)** cortisol. **f** NPH insulin (white circles) and insulin detemir (black circles) levels. Values are mean \pm SEM



glucose production were significantly lower than with identical low glucose concentrations induced by NPH insulin. The results were striking. At baseline on human soluble insulin there were no differences in EGP and PGU between studies. After dosing with either insulin detemir or NPH, differences appeared, with detemir having a greater effect on suppression of EGP and less effect on stimulation of PGU. In contrast, with NPH insulin, equivalent levels of hypoglycaemia were partly dependent on a relatively greater stimulation of glucose disposal.

In previous studies, participants have undergone a stepwise hypoglycaemic clamp [9], with insulin detemir being administered via the intravenous route and compared with human insulin in participants without diabetes. Results

were similar to this study, with counter-regulatory hormone response not shown to be significantly different. However, a difference in maximal responses of some symptoms to hypoglycaemia were higher with insulin detemir. In our study, the important methodological difference was that insulin detemir was administered subcutaneously in a more physiological manner to participants with type 1 diabetes, mimicking the clinical setting. In this context it should be noted that the intravenous route of a protein-bound insulin whose free component cannot be measured is always difficult to interpret and compare with the licensed subcutaneous route [19].

Although not tested by the experiments described here, it is possible that counter-regulatory mechanisms would more

adequately correct hypoglycaemia occurring with less inappropriate insulin stimulation of PGU. The counter-regulatory hormone response to insulin acts predominantly on stimulation of EGP. The differential and more physiological effect of insulin detemir suggested by this study could explain the reduced episodes of hypoglycaemia and lower weight gain observed in clinical studies comparing detemir with NPH [20–23]. Alternatively, it is possible that differences in the effects of detemir and native insulin in the brain [9] could exert influence in these respects, perhaps via an autonomic mechanism.

Results presented here are consistent with the notion that detemir has a relatively hepatoselective effect, adding weight to the suggestion that the capillary endothelial barrier in peripheral tissue limits the transfer of detemir insulin from the circulation into the extravascular space. Further potential clinical advantages of this phenomenon deserve exploration.

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Duality of interest D. L. Russell-Jones has been paid honoraria for lectures by Novo Nordisk. L. Westergaard and H. Haahr are employed by Novo Nordisk. The remaining authors declare that there is no duality of interest associated with this manuscript.

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