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Effect of subcutaneous insulin detemir on glucose flux and lipolysis during hyperglycaemia in people with type 1 diabetes

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Aims: To investigate, using a novel non-steady-state protocol, the differential effects of subcutaneous (s.c.) detemir and NPH insulin on glucose flux and lipid metabolism after insulin withdrawal.

Methods: After a period of insulin withdrawal resulting in whole-blood glucose concentration of 7 mmol/l, 11 participants (five men, mean age 41.0 years, mean body mass index 25 kg/m^2) with type 1 diabetes (mean glycated haemoglobin concentration 57 mmol/mol, mean diabetes duration 14 years) received 0.5 units per kg body weight s.c. insulin detemir or NPH insulin in random order. Stable isotopes of glucose and glycerol were infused intravenously throughout the study protocol.

Results: Glucose concentration decreased after insulin treatment as a result of suppression of endogenous glucose production, which occurred to a similar extent with both detemir and NPH insulin. The rate of glucose disappearance (Rd) was not increased significantly with either type of insulin. When the effect of detemir and NPH insulin on glucose flux at glucose concentrations between 9 and 6 mmol/l was examined, glucose rate of appearance (Ra) was similar with the two insulins; however, glucose Rd was greater with NPH insulin than with detemir at glucose concentrations of 8.0, 8.5, 7.0 and 6.0 mmol/l ($p < 0.05$) The percentage change in glycerol Ra, a measure of lipolysis, was greater in the NPH group than in the detemir group ($p = 0.04$).

Conclusions: The results of the study are consistent with the hypothesis that detemir has a lesser effect on the periphery, as evidenced by a lesser effect on peripheral glucose uptake at specific glucose concentrations.

Keywords: insulin analogues, insulin therapy, liver, type 1 diabetes

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Introduction

In normal physiology, insulin is released into the hepatic portal circulation from the pancreatic islets exerting direct effects on the liver. The liver is exposed to a greater concentration of insulin than are other tissues. In this 'first pass', the liver extracts 50–60% of the insulin, the remainder then enters the systemic circulation and reaches the peripheral tissues [1]. Subcutaneous (s.c.) insulin delivery eliminates this gradient, resulting in relative peripheral hyperinsulinaemia and underinsulinization of the liver [2]. This may lead to a variety of metabolic abnormalities, including glycaemic fluctuations, dyslipidaemia and reduction in hepatic insulin-like growth factor-1 production, with a consequent elevation in growth hormone secretion [3]. The metabolic changes are also more pronounced in patients with poor glycaemic control and may be implicated in the long-term micro- and macrovascular complications of diabetes [4].

An insulin analogue exerting greater effects on the liver than the periphery has the potential to partially restore the physiological normal portal to peripheral insulin gradient. A potential mechanism to develop a novel hepato-preferential insulin analogue is by reducing the passage through the endothelial barrier. To reach peripheral target cells, insulin must pass through the capillary wall lined by continuous epithelium [5]. By contrast, highly fenestrated epithelial cells line the vascular sinusoids within the liver, with large pores between them and no basal lamina resulting in no barrier between protein molecules within the plasma, in the sinusoid and on the surface of the hepatocyte. Insulin detemir is a soluble insulin analogue with a C^{14} fatty acid side-chain at position B^{29} [6]. It is 98% bound to albumin in circulation and has difficulty passing through the capillary wall [7]. By contrast, NPH insulin 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 and has no difficulty passing through the capillary wall [8].

The partial hepato-preferential potential of detemir has previously been seen under two experimental conditions: during a euglycaemic-hyperinsulinaemic clamp in healthy individuals [9] and during free fall of glucose concentration to hypoglycaemia in people with type 1 diabetes [10]. The 'gold standard' method for investigating glucose metabolism is the euglycaemic-hyperinsulinaemic clamp, but euglycaemia is the starting condition which falls very near the top of the dose–response curve for insulin action at the liver and near the bottom of that relationship for peripheral tissues. The prevention of hypoglycaemia by clamping serves further to focus attention on peripheral insulin action, as any fall in glucose-dependent glucose uptake resulting from developing

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hypoglycaemia is eliminated by the glucose infusion. Glucose clamp experiments therefore serve to underestimate the vital physiological significance of hepatic insulin action.

Using a novel non-steady-state protocol, the aim of the present study was to delineate the important physiological action of clinically relevant s.c. doses of insulin detemir compared with NPH insulin on the liver versus the periphery at the lower end of the dose–response curve in people with type 1 diabetes. The findings during experimental hyperglycaemia provide unique information on glucose flux and lipolysis with insulin detemir and NPH insulin.

Research Design and Methods

This was a single-centre, investigator-led, randomized metabolic study involving people with type 1 diabetes. Ethics approval was granted from the NRES Committee London-Hampstead, REC reference 11/LO.0687. The trial was registered with the European Clinical Trials Database (EudraCT) under the number: 2011-001642-14 and was funded by Novo Nordisk A/S.

Experimental Procedure

Participants were recruited between September 2011 and June 2012 using the diabetes register at the Royal Surrey County Hospital, Guildford, UK. Exclusion criteria included: proliferative retinopathy that had required treatment within the preceding 3 months; impaired hepatic, renal or cardiac function; uncontrolled hypertension, defined as a blood pressure >160/90 mmHg; mental incapacity; pregnancy; and known or suspected allergy to the trial products.

Participants were required to attend three visits. Visit 1 was for screening and informed ethical consent, and visits 2 and 3 were for metabolic studies. There was a minimum interval of 10 days between visits 2 and 3. A randomization block was produced using the randomisation.com website tool. Participants were allocated to receive either 0.5 units per kg body weight s.c.insulin detemir or s.c. NPH insulin, in random order. An independent research nurse administered the insulin. The investigators and participants were blind to the randomization.

A total of 15 participants were randomized and 12 participants completed both metabolic studies. One participant withdrew informed consent before the first metabolic study, 1 was withdrawn after difficulty with venous access and 1 was withdrawn as a result of failure to reach a hyperglycaemic state after 6 h of intravenous (i.v.) insulin withdrawal, thought to be attributable to lipohypertrophy at the participant's s.c. insulin pump site.

Participants were asked not to consume food and to drink only water after 22:00 hours the day before a metabolic study. They were also discouraged from exercising and consuming alcohol the evening before a metabolic study. To minimize any carry-over effect from regular basal insulin, participants using multidose insulin regimes were asked to omit their basal insulin the night before a metabolic study. Participants using continuous s.c. insulin therapy disconnected their insulin pumps at 07:00 hours on the morning of a metabolic study. Metabolic studies commenced at 07:30 hours.

Section A: Isotopic Steady State. All participants were studied in a semi-recumbent position and allowed to drink clear water only. Cannulation was made with an 18-gauge venflon cannula inserted into an antecubital vein in each arm, one for blood sampling and the other for administration of i.v. infusates. After cannulation, baseline blood samples were taken and participants were transferred to an i.v. soluble variable insulin infusion to maintain a whole-blood glucose concentration of 5 mmol/l. A primed (1.7 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.$^2H_2$]glycerol (Cambridge isotopes, CK Gas Products Ltd, Ibstock, UK) were administered from −120 min to the end of the study protocol. Blood samples were taken to measure plasma glucose and glycerol enrichment and concentration between −30 and 0 min.

Section B: Insulin Withdrawal. At isotopic steady state (time 0), insulin was withdrawn and whole-blood glucose allowed to increase to 7 mmol/l.

Section C: Glucose-lowering Effect of Subcutaneous Insulin. Participants were then given 0.5 units per kg body weight of s.c. detemir or NPH depending on randomization order (time 0+). Blood samples were taken to measure plasma glucose and glycerol enrichment and concentration every 20 min until 240 min and then every 30 min until the end of the study protocol. Plasma non-esterified fatty acid (NEFA) concentrations were measured every 60 min. The study was terminated when the whole-blood glucose level fell below 5.0 mmol/l or at 420 min after s.c. insulin injection. Figure S1 shows a flow diagram of the metabolic study methodology.

Analytical Procedures

Throughout the metabolic study, whole-blood glucose concentration was measured using a glucose oxidase technique on a glucose analyser (YSI 2300 Clandon Scientific; Yellow Springs Instruments, Yellow Springs, OH, USA). The whole-blood samples were immediately centrifuged and plasma was later analysed in a laboratory setting.

Total plasma glucose concentrations were measured with a Roche Cobas MIRA analyser using the ABX Pentra glucose kit (Horiba ABX, Northampton, UK) and plasma glycerol concentrations using a Randox glycerol kit (Randox Laboratories, Co. Antrim, UK).The glucose concentration in whole blood has been reported to be 13% lower than the glucose concentration in plasma [11].

The isotopic enrichment of plasma glucose was determined as the trimethylsilyl O methyloxime derivative [12] using gas chromatography-mass spectrometry (model 5975 CMSD inert XL EI/CI MSD; Agilent Technologies, Wokingham, UK). The isotopic enrichment of plasma glycerol was determined as the tert-butyl trimethylsilyl glycerol derivative [13] using a gas chromatography-mass spectrometry model 5973 network MSD (AgilentTechnologies).

Glucose and Glycerol Calculations

Glucose rate of appearance (Ra), rate of disappearance (Rd) and glycerol production were calculated using Steele's

non-steady-state equations, modified for stable isotope [14]. Before calculation of glucose turnover, time courses were smoothed and interpolated to 5-min intervals using optimal segments technique analysis [15]. The glucose metabolic clearance rate was calculated by dividing glucose Rd by glucose concentration. Because of the variability in endpoint time between the participants, glucose Ra, glucose Rd and metabolic clearance rate were plotted against glucose concentration so a direct comparison of the effect of insulin detemir and NPH insulin on glucose flux could be made at a given plasma glucose level. For each participant the mean glucose Rd, glucose Ra and metabolic clearance rate at glucose concentrations of 9.0, 8.5, 8.0, 7.5, 7.0 and 6.5 mmol/l on the descending slope were calculated. The boundaries for each glucose concentration were ±0.05 mmol/l, so, for example, any plasma glucose concentration between 5.95 and 6.05 mmol/l was considered to be a concentration of 6 mmol/l.

Statistical Analysis

The primary endpoint was the minimum endogenous glucose Ra and secondary endpoints were changes in glucose Rd and glycerol Ra.

All values were expressed as means and standard errors of means (s.e.m.). Glucose Ra, glucose Rd and glycerol Ra at each time interval were compared using a mixed model two-way repeated measures analyis of variance (ANOVA; time × treatment) with *post hoc* Bonferroni correction. A one-way ANOVA was applied for glucose Ra and glucose Rd at a given plasma glucose concentration. All other comparisons were made using paired Student's *t*-tests, and p values <0.05 were taken to indicate statistical significance.

Results

Results for 11 participants were analysed. Cannulation dislodgement resulted in the interruption of metabolic tracer uptake for 1 participant; the results for the participant could not be paired and therefore the participant was excluded. The participant characteristics are shown in Table S1. The male : female ratio was 5 : 6, and the mean ± s.e.m age was 41 ± 4.6 years, weight was 77 ± 4.1 kg, body mass index was 25 ± 0.9 kg/m^2, glycated haemoglobin concentration was 57 ± 1.71 mmol/mol and duration of diabetes was 14 ± 2.6 years. Five participants used continuous s.c. insulin infusion as maintenance therapy and 6 participants used a multidose insulin regime. The mean total daily insulin was 36.1 ± 4.3 international units. Of the 11 participants, 7 received insulin detemir in the first metabolic visit and 4 received NPH insulin.

Plasma Glucose Concentration

The plasma glucose concentration profile for the insulin detemir group and the NPH insulin group were not significantly different (p = 0.37) and are shown in Figure 1. The bimodal distribution in Figure 1 was caused by individual participants' completing the metabolic study before 420 min.

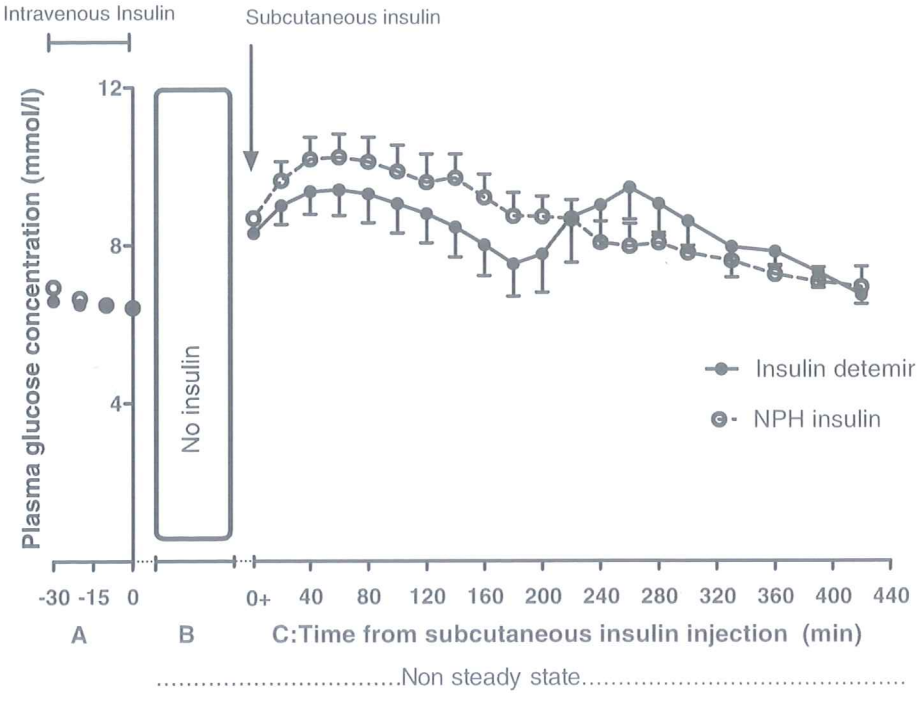

Figure 1. Plasma glucose concentrations plotted against time. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean ± standard error of the mean. At 180 min, n = 11 in both groups; at 260 min, n = 4 in the insulin detemir group and n = 5 in NPH insulin group. At 420 min, n = 2 in the insulin detemir group and n = 4 in the NPH insulin group. A = isotopic steady state; B = period of insulin withdrawal; C = period following subcutaneous insulin injection.

After s.c. insulin injection, each individual was observed to have a rise in plasma glucose concentration and then a fall. Individual participants only crossed a plasma glucose concentration once on the descending slope: 11 participants in both study groups at 180 min; 4 participants in the insulin detemir group and 5 participants in the NPH insulin group at 260 min; and 2 participants in the insulin detemir group and 4 participants in the NPH insulin group at 420 min.

Plasma glucose concentration at isotopic equilibrium (time 0) was 6.4 ± 0.4 for the insulin detemir study and 6.4 ± 0.3 the NPH study. The period of insulin withdrawal (Section B) varied for each metabolic study (Table S2). The time to s.c. injection was significantly quicker for participants who used continuous s.c. insulin infusion ($p = 0.006$; 36.0 ± 8.9 min for the detemir group and 58.0 ± 12.9 min in the NPH group) than for participants who used a multidose insulin regime (115 ± 22.3 min for the detemir group and 95.0 ± 20.8 min for the NPH group). The glucose-lowering effects of s.c. insulin given during the metabolic study, however, were not significantly different between participants who were on multidose insulin regimes and those who used a continuous s.c. insulin infusion regime ($p = 0.46$).

Because of the time variability during the insulin withdrawal period, the glucose-lowering effect of s.c. insulin (Section C) was plotted as real time from injection of s.c. insulin. At the point of s.c. insulin injection (time 0+), the mean ± s.e.m. plasma glucose concentrations were 8.3 ± 0.3 and 8.7 ± 0.4 mmol/l, respectively. After administration of s.c. insulin, the plasma glucose concentration continued to rise and achieved peak values of 9.4 ± 0.6 mmol/l at 51 ± 7.0 min in the detemir study and 10.3 ± 0.6 mmol/l at 62.2 ± 7.8 min in the NPH study.

Glucose Metabolism

At isotopic steady state, there was no significant difference in glucose flux in the insulin detemir study and the NPH study.

Glucose Rate of Appearance and Disappearance Plotted Against Time. Mean ± s.e.m. glucose Ra fell rapidly in the first 180 min (11 participants) from 15.01 ± 0.60 to 6.81 ± 0.78 μmol/kg/min in the detemir study and from 16.58 ± 0.69 to 7.59 ± 0.71 μmol/kg/min in the NPH insulin study (ANOVA $p = 0.47$). Glucose Rd remained unchanged in both study groups (ANOVA $p = 0.32$; Figure 2).

Glucose Rate of Appearance and Disappearance Plotted Against Glucose Concentration. Because of the variability in metabolic endpoint time between the participants, glucose Ra and glucose Rd were plotted against glucose concentration so a direct comparison of insulin action could be made at a given plasma glucose concentration. At glucose concentrations of 8.5 to 6 mmol/l, glucose Ra decreased from 8.2 ± 0.9 to 5.8 ± 0.5 μmol/kg/min in the insulin detemir study and from 8.2 ± 0.5 to 6.3 ± 0.6 μmol/kg/min in the NPH study, with no significant difference in glucose Ra between the insulin treatments (Figure 3). At glucose concentrations of 8.5 to 6 mmol/l, glucose Rd decreased from 11.0 ± 0.6 to 9.5 ± 0.7 μmol/kg/min with insulin detemir and was maintained between 11.5 ± 0.6 and 11.4 ± 0.4 μmol/kg/min with NPH insulin (Figure 3). Glucose Rd was greater with NPH insulin than with detemir at glucose concentrations of 8.0, 8.5, 7.0 and 6.0 mmol/l ($p < 0.05$).

Glucose metabolic clearance rate increased with decreasing glucose concentrations in both studies but was higher in the NPH insulin study at glucose concentrations between 8 and 6.0 mmol/l (Figure 3). At 8 mmol/l, the metabolic clearance rate was 1.3 ± 0.07 ml/kg/min in the insulin detemir study and 1.5 ± 0.04 ml/kg/min in the NPH study ($p = 0.04$) and at 6 mmol/l, the metabolic clearance rate was 1.59 ± 0.09 ml/kg/min in the insulin detemir study and 1.91 ± 0.08 ml/kg/min in the NPH study ($p = 0.03$).

Non-esterified Fatty Acid and Glycerol Metabolism

After s.c. insulin, suppression of NEFA concentration was delayed in the detemir study compared with NPH study (Figure 4). The mean plasma glycerol concentration achieved peak values of 100.6 ± 19.8 in the detemir study and 148.8 ± 35.1 μmol/l in the NPH study before the glycerol concentration began to decrease at 50.9 ± 7.0 and 62.3 ± 7.8 min (ANOVA $p = 0.86$; Figure 4).

The mean glycerol Ra was higher in the NPH study (4.0 ± 0.8 μmol/l/kg/min) compared with the glycerol Ra achieved in the insulin detemir study (2.8 ± 0.4 μmol/l/kg/min), although did not achieve statistical significance (mean difference −1.20 μmol/l/kg/min, confidence interval −2.45 to 0.096 *t*-test $p = 0.07$). To account for this difference, glycerol Ra is presented as change from the point of s.c. insulin injection. The percentage change in glycerol Ra was consistently greater in the NPH study group than in the detemir group (ANOVA $p = 0.04$; Figure 5).

Discussion

After a period of insulin withdrawal in participants with type 1 diabetes, insulin detemir had a lesser effect on glucose uptake into peripheral tissues but a similar effect on hepatic glucose production when compared with NPH insulin at the same glucose concentration. In addition, there was less suppression of the glycerol production rate with insulin detemir. Insulin detemir therefore has the potential to partially restore the physiological portal-peripheral insulin gradient.

In type 1 diabetes with severe hyperglycaemia, glucose production and glucose uptake is increased to above-normal levels [16,17]. Without adequate direct insulin action in the liver [18,19], the excess glucose production is so great that even an increase in glucose-dependent peripheral glucose uptake and the associated glycosuria fail to limit progressive hyperglycaemia. A possible explanation for the excess peripheral glucose uptake is that hyperglycaemia to which people with uncontrolled type 1 diabetes are chronically exposed could induce slow adaptive changes by changing the efficiency of the glucose transport system or activating some enzymatic systems capable of being affected by glucose concentration [20,21].

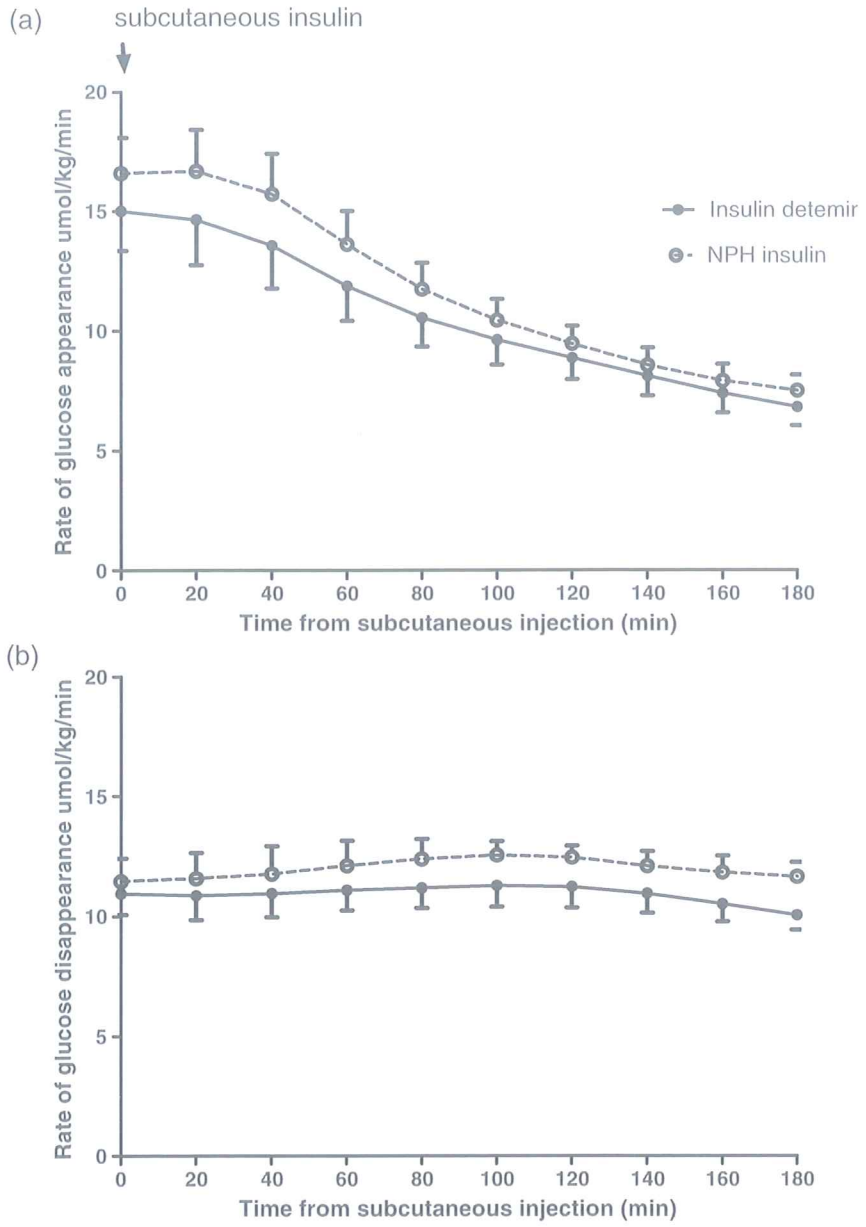

Figure 2. (a) Glucose rate of appearance (b) glucose rate of disappearance plotted against time from subcutaneous injection. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean ± standard error of the mean. Time plotted until 180 min, where n = 11 in both groups.

As the ethics committee was concerned that the study design would increase the risk of diabetic ketoacidosis, s.c. insulin was administered at a whole-blood glucose concentration of 7 mmol/l, a lower concentration than originally intended. This resulted in mean peak plasma glucose concentrations of only 9.4 ± 0.6 mmol/l in the detemir group and 10.3 ± 0.6 mmol/l in the NPH group. Many of the participants' peak glucose concentrations were below the renal glucose threshold and the majority of participants reached the metabolic endpoint before 420 min. Withdrawal of soluble i.v. insulin was also quicker for participants using the continuous s.c. insulin infusion regime than for those using multidose insulin regimes. The difference may be attributable to a carry-over effect of basal s.c. insulin. Insulin concentration data are not reported as detemir is protein-bound and it is difficult to measure free insulin concentrations accurately; however, there was no significant difference in the glucose-lowering effect seen during the metabolic study between the continuous s.c. infusion of insulin group and the multidose insulin group.

The study was designed to compare clinically and physiologically relevant low doses of insulin similar to those that might be used as basal insulin replacement. Previous studies have reported that at low physiological insulin concentrations, the direct glucose-lowering effect occurs mainly by reduction

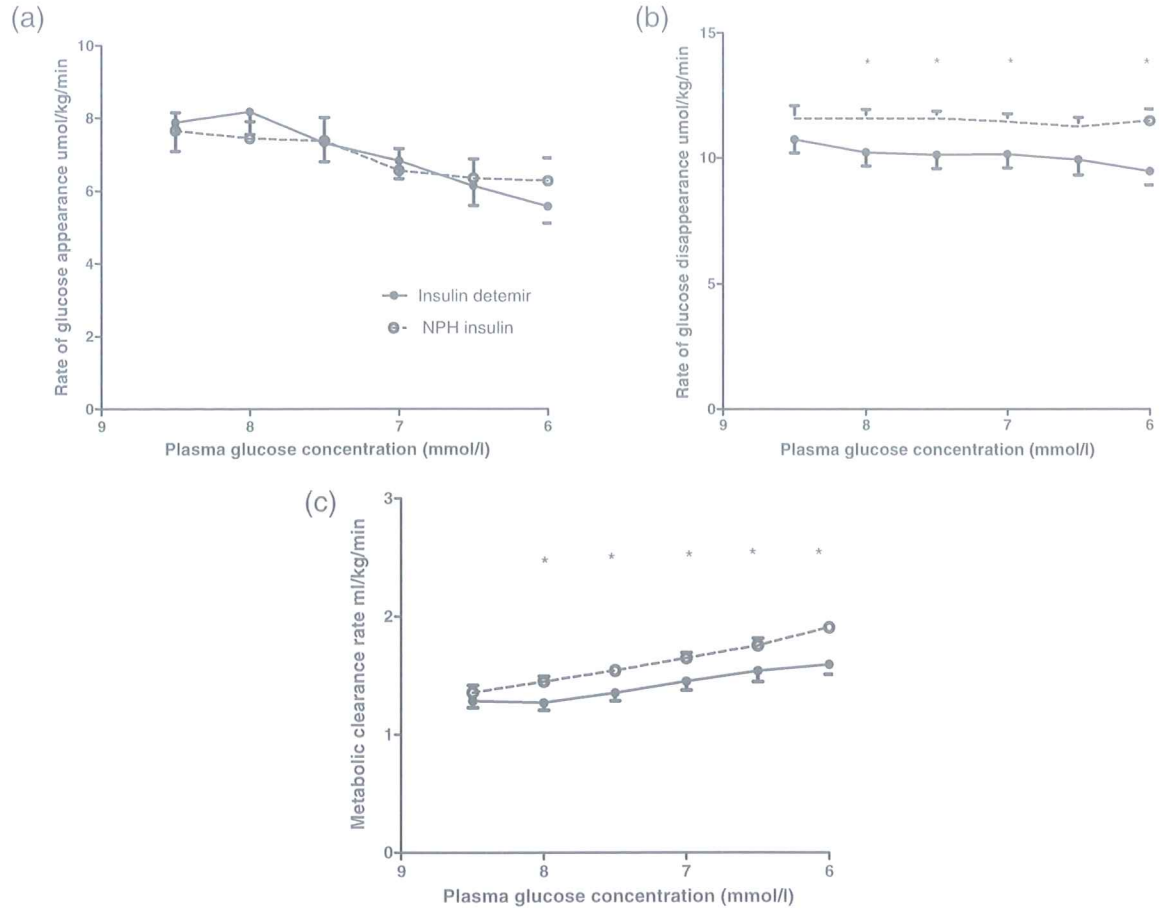

Figure 3. (a) Glucose rate of appearance at each glucose concentration. (b) Glucose rate of disappearance at each glucose concentration. (c) Metabolic clearance rate at each glucose concentration. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean ± standard error of the mean. *$p < 0.05$.

in hepatic glucose production [22]; this formed the basis of the low-dose insulin i.v. infusions to treat diabetic ketoacidosis [23]. Over time, however, the role of insulin on peripheral glucose uptake has been favoured as the dominant action of insulin in lowering glucose concentration [24]. This is in part attributable to the euglycaemic-hyperinsulinaemic clamp being recognized as the gold standard method for investigating the action of insulin preparations on glucose metabolism [25]. The present study confirmed that basal s.c. insulin acts primarily to suppress hepatic glucose production. The effect of insulin on peripheral glucose uptake remained unchanged, suggesting that, at elevated glucose concentrations, the majority of peripheral glucose uptake is insulin-independent. Approximately 60–80% of glucose uptake may be non-insulin-mediated [26]. It is also unlikely that the high glucose concentration was responsible for the reduction in hepatic glucose production, as suppression by hyperglycaemia has been reported to be deficient in diabetes [27].

The action of insulin on adipose tissue was measured by the rate of glycerol appearance, a measure of lipolysis, and the suppression of NEFA concentration. After s.c. insulin administration there was a rapid suppression of glycerol production. The suppression of glycerol production was seen before complete suppression of hepatic glucose production, supporting the evidence that lipolysis is extremely sensitive to the action of insulin [28].

With regard to the differential effects of NPH insulin and insulin detemir, there was no difference in hepatic glucose production between NPH insulin and insulin detemir at the low end of the insulin dose–response curve; however, s.c. insulin detemir has a lesser effect on peripheral glucose uptake at specific glucose concentrations. There was also less suppression of glycerol production and NEFA concentration with detemir when compared with NPH insulin over time, and a greater percentage change in glycerol production in the NPH group than in the detemir group. This provides further evidence to support the hypothesis that insulin detemir has a lesser effect on peripheral tissues and thus has the potential to partially restore the physiological portal-peripheral insulin gradient. Detemir's albumin-binding property is an important potential mechanism as detemir bound to albumin may be prevented by the endothelial barrier from reaching receptor sites on cells of peripheral tissues; however, the other unique property of insulin detemir is the partial non-receptor-mediated clearance

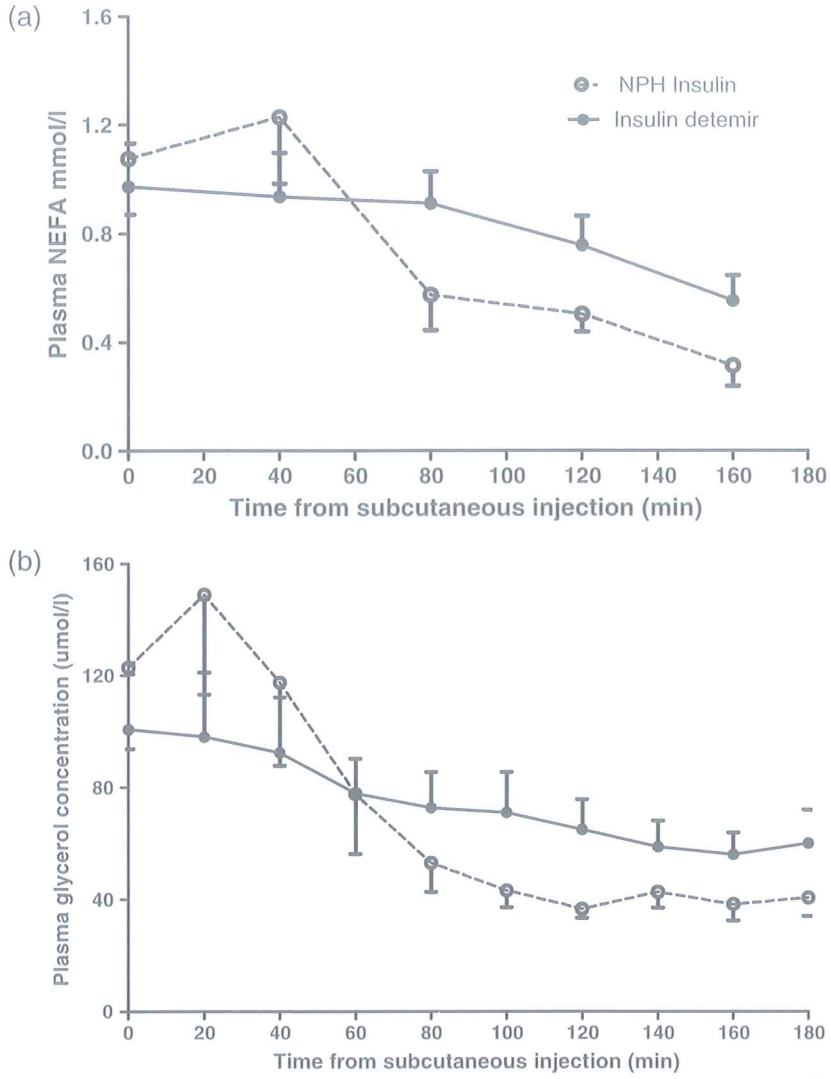

Figure 4. (a) Non-esterified fatty acid (NEFA) concentration and (b) glycerol concentration plotted against time from subcutaneous injection. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean ± standard error of the mean, n = 11.

and it is possible that the low receptor-binding affinity and altered pharmacokinetic properties of detemir could contribute to the reduced biological activity in the periphery [29].

A number of insulin analogues, which may offer a more physiological insulin supplementation are currently in development. Because of their molecular size they have the potential of hepatoselectivity combined with a longer duration of action [30]. Similar to insulin detemir, insulin degludec has the structural addition of glutamic acid and may be hepato-preferential; however, there are no clamp data available [31]. In most clinical trials weight gain tended to be greater with degludec than with glargine of detemir, suggesting that degludec may in fact not be hepato-preferential [30,32]. LY2605541 is a new insulin analogue currently in phase III clinical trials. This analogue is insulin lispro covalently bonded to 20 000 Dalton monomethoxypoly(ethylene glycerol) moiety at lysine B28, increasing the hydrodynamic diameter, which results in a prolonged duration of action [33]. Interestingly, clamp data have shown LY2605541 to have a preferential hepatic effect with modest weight loss and lower nocturnal hypoglycaemia [32,34]. Information is also beginning to emerge about another insulin analogue known only as 'insulin 327', which is acylated with 22-carbon length fatty diacid to promote strong but reversible binding to plasma albumin [35]. Initial data suggest that insulin 327 may have hepato-preferential effects [35].

In conclusion, the results of the present study suggest that this novel protocol may give a more physiological assessment of s.c. basal insulin action than a simple clamp protocol. The results are also consistent with the hypothesis that detemir has a relatively hepato-preferential effect, adding weight to the suggestion that the capillary endothelial barrier in adipose tissue and muscle limits the transfer of free insulin detemir from the circulation into the extravascular extracellular space. The differential and more physiological effect of

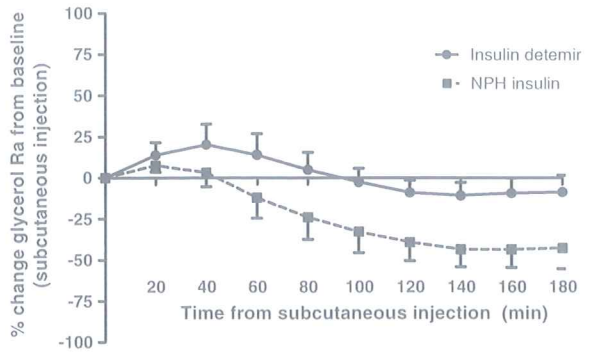

Figure 5. Percentage change in glycerol rate of appearance (Ra) after subcutaneous insulin injection. Closed circles represent insulin detemir and open circles represent NPH insulin. Values are mean ± standard error of the mean, n = 11.

insulin detemir suggested by the present study may explain the reduced episodes of hypoglycaemia, reduced episodes of nocturnal hypoglycaemia and lower weight gain observed in clinical studies when comparing detemir with conventional insulin preparations of NPH [36–38] and the reduced weight gain [39] with comparable glycaemic control [40] when compared with insulin glargine.

Acknowledgement

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Conflict of Interest

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

R. H. carried out the metabolic studies, interpreted data, drafted the manuscript and 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. R. J., A. M. U. 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. Flow diagram of the metabolic study methodology.

Table S1. Participant characteristics.

Table S2. Time (min) to reach blood glucose concentration of >7 mmol/l after intravenous insulin withdrawal.

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