A Double-Blind, Randomized, Dose-Response Study Investigating the Pharmacodynamic and Pharmacokinetic Properties of the Long-Acting Insulin Analog Detemir

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OBJECTIVE — To investigate the pharmacodynamic profile and duration of action for five subcutaneous doses of insulin detemir (0.1, 0.2, 0.4, 0.8, and 1.6 units/kg; 1 unit = 24 nmol) and one subcutaneous dose of NPH insulin (0.3 IU/kg; 1 IU = 6 nmol).

RESEARCH DESIGN AND METHODS — This single-center, randomized, double-blind, six-period, crossover study was carried out as a 24-h isoglycemic clamp (7.2 mmol/l) in 12 type 1 diabetic patients.

RESULTS — Duration of action for insulin detemir was dose dependent and varied from 5.7, to 12.1, to 19.9, to 22.7, to 23.2 h for 0.1, 0.2, 0.4, 0.8, and 1.6 units/kg, respectively. Interpolation of the dose-response relationships for AUC_{GIR} (area under the glucose infusion rate curve) revealed that a detemir dose of 0.29 units/kg would provide the same effect as 0.3 IU/kg NPH but has a longer duration of action (16.9 vs. 12.7 h, respectively). Lower between-subject variability was observed for insulin detemir on duration of action (0.4 units/kg insulin detemir vs. 0.3 IU/kg NPH, $P < 0.05$) and GIR_{max} (maximal glucose infusion rate) (0.2 and 0.4 units/kg insulin detemir vs. 0.3 IU/kg NPH, both $P < 0.05$). Assessment of endogenous glucose production (EGP) and peripheral glucose uptake (PGU) resulted in an AOC_{EGP} (area over the EGP curve) of 636 mg/kg (95% CI 279–879) vs. 584 (323–846) and an AUC_{PGU} (area under the PGU curve) of 173 (47–316) vs. 328 (39–617) for 0.29 units/kg detemir vs. 0.3 IU/kg NPH, respectively.

CONCLUSIONS — This study shows that insulin detemir provides a flat and protracted pharmacodynamic profile.

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Traditional basal insulin formulations such as NPH or zinc insulin have limitations such as variability in insulin absorption after subcutaneous injection, resulting in an unpredictable action profile increasing the risk of hypoglycemic episodes, and a pharmacodynamic profile requiring several injections to cover 24 h (1–4).

Recently, long-acting insulin analogs have been biologically engineered to overcome these limitations (5–9). These insulin analogs make use of different principles for achieving a protracted insulin profile, such as changing the isoelectric point (insulin glargine) or acylation of the insulin molecule (insulin detemir). Previous studies have confirmed both a delayed and a sustained blood glucose–lowering effect with insulin detemir compared with NPH insulin in healthy subjects (10,11). However, the results show that a higher molar dose of insulin detemir is needed to achieve comparable glycemic control similar to that observed with NPH insulin (10).

The objective of the present trial was to describe the 24-h pharmacodynamic profile, including duration of action and dose-response relationship, of insulin detemir in subjects with type 1 diabetes and to compare this with NPH insulin.

RESEARCH DESIGN AND METHODS

— In this single-center, double-blind, six-period, randomized, dose-response trial, isoglycemic (7.2 mmol/l) subjects were randomized to a specific treatment sequence encompassing five dose levels of insulin detemir (0.1, 0.2, 0.4, 0.8, and 1.6 units/kg; 1 unit = 24 nmol) and one dose level of NPH insulin (0.3 IU/kg, 1 unit = 6 nmol). The protocol was reviewed and approved by the local ethics committee in Graz, Austria, and the trial was performed in accor-

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Abbreviations: AOC, area over the curve; AUC, area under the curve; EGP, endogenous glucose production; GIR, glucose infusion rate; NEFA, nonesterified fatty acid; PGU, peripheral glucose uptake.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

dance with Good Clinical Practice and the Declaration of Helsinki.

A total of 12 C-peptide–negative (<0.03 nmol/l) subjects with type 1 diabetes entered the trial (seven men and five women, aged 36 ± 12 years, diabetes duration 18 ± 9 years, BMI 23.3 ± 2.3 kg/m^2, HbA_{1c} 7.7 ± 1.0%). Patients with significantly impaired renal or hepatic function or using long-acting insulin formulations were not included. A total of 11 subjects completed the trial. One subject was withdrawn during the third visit because of vein catheterization problems.

The subjects were admitted to the clinic at ~5:00 P.M. on the dosing day. The subjects took their usual basal insulin in the evening before dosing and short-acting insulin only at breakfast and lunch on the dosing day. Between 5:00 and 6:00 P.M., a hand vein was cannulated and kept in a thermoregulated box for sampling of arterialized venous blood. In addition, on the contralateral arm, an antecubital vein was cannulated for infusion of glucose and/or insulin.

At ~6:00 P.M., a variable intravenous infusion of human soluble insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was started to reach isoglycemia at a plasma glucose level of 7.2 ± 0.6 mmol/l (130 ± 10 mg/dl) under constant basal insulin infusion rate as described previously (5,12). A primed-continuous infusion of 10% [6,6-$^2H_2$]glucose was started at ~7:00 P.M., with a priming dose of 4.8 mg/kg infused over 1 min followed by a constant infusion of 0.04 mg · kg^{-1} · min^{-1} (13). At 10:00 P.M. (or later if steady-state conditions were not achieved for at least 1 h), the trial drug was administered subcutaneously in the thigh in accordance with the randomization scheme by a qualified person not otherwise involved in the study to keep the study double blinded. After subcutaneous insulin injection, the rate of the intravenous insulin infusion was decreased gradually in steps of ~25% and ultimately completely withdrawn when plasma glucose consistently decreased to <6.9 mmol/l (<125 mg/dl) as described previously (5). At this time, an intravenous infusion of 10% dextrose was started and continued at a variable rate to maintain plasma glucose level at the target level of 7.2 mmol/l (130 mg/dl) (5). The dextrose infusion was enriched with 2 mg [6,6-$^2H_2$]glucose/ml to prevent a fall in tracer enrichment and consequent errors in glucose turnover determinations, which otherwise occur after insulin administration (14). The exogenous glucose infusion was continued until plasma glucose values exceeded 7.8 mmol/l (140 mg/dl). The trial was stopped 24 h after trial drug administration or earlier if plasma glucose consistently exceeded 11.1 mmol/l (200 mg/dl) in the absence of glucose infusion.

Plasma glucose was measured in duplicates at intervals ≥15 min frequently until trial drug administration, and thereafter with intervals ≥20 min frequently using a glucose oxidase method (Beckman Instruments, Fullerton, CA). Samples for insulin or insulin detemir, nonesterified fatty acids (NEFAs), and [6,6-$^2H_2$]glucose were taken at 30-min intervals from −60 to 360 min and at hourly intervals thereafter.

Glucose and [6,6-$^2H_2$]glucose were simultaneously measured using a modified gas chromatography–mass spectrometry method (13) with di-*O*-isopropylidene acetate derivates. Unlike the method of Hachey et al. (15), two internal standards were used, [$^{13}C_6$]glucose and [$^{13}C_6$-$^2H_7$]glucose. [$^{13}C_6$]glucose and [$^{13}C_6$-$^2H_7$]glucose concentrations were set to approximately the same concentration estimated for glucose and [6,6-$^2H_2$]glucose, respectively. Natural glucose, [6,6-$^2H_2$]glucose, [$^{13}C_6$]glucose, and [$^{13}C_6$-$^2H_7$]glucose were recorded by scanning selected ion monitoring spectra of 287, 289, 293, and 300 m/z, respectively. Tracer enrichment was calculated based on obtained concentrations of glucose and [6,6-$^2H_2$]glucose. NEFAs were analyzed by means of Cobas Mira (Roche Diagnostics, Basel, Switzerland). All methods were validated according to guidelines of the National Committee for Laboratory Standards (NCCLS).

Blood samples for pharmacokinetic evaluations were drawn every hour. The measurement of insulin detemir and human insulin was performed using a validated method by Medi-Lab (Copenhagen, Denmark). The insulin detemir assay measures the total concentration (both free and albumin bound) and does not cross-react with human insulin.

Statistical analysis

The primary end point, duration of action, was defined as previously described (5) from the time of 50% reduction in intravenous insulin infusion (onset of action) until plasma glucose consistently exceeded 8.3 mmol/l after the last glucose infusion or after 24 h, whichever came first (end of action). The sample size was determined by specifying a 95% CI with a relative width of 30% for the duration of action.

The secondary pharmacodynamic end points included area under the glucose infusion rate curve (AUC_{GIR}), maximal glucose infusion rate (GIR_{max}), time to GIR_{max}, baseline-adjusted area over the endogenous glucose production curve (AOC_{EGP}), and baseline adjusted area under the peripheral glucose uptake curve (AUC_{PGU}). The EGP and PGU were calculated according to Steele's model for steady and nonsteady state (13,16), as modified by Powrie et al. (17) to account for the addition of stable labeled tracer to the exogenous glucose infusate.

The pharmacokinetic end points included area under the serum insulin curve from zero to infinity (AUC_{ins}), maximal serum insulin concentration (C_{max}), and time to maximal concentration (t_{max}).

The primary objective was assessed in an ANOVA model with duration of action as response variable, period and insulin dose and type of insulin as fixed effects, subject as a random effect, and an error term with a variance depending on insulin dose. From this model, the duration of action was estimated and 95% CIs were constructed for each dose level. In addition, the dose-response relationship was investigated by reducing the statistical model to a model describing the dose dependency of the duration of action through a linear, square root, and a square term.

The end points AUC_{GIR}, GIR_{max}, AOC_{EGP}, and AUC_{PGU} were analyzed using the same ANOVA approach as for the primary end point. For AUC_{GIR}, the dose response could be described by a straight line; however, a square root term was kept in the model to obtain a better fit for the small dose level of insulin detemir. To find the corresponding lower and upper insulin detemir doses, calculation of the range of likely doses by interpolation of the lower and upper confidence limits for NPH was applied.

The dose-response relationships of AUC_{ins} and C_{max} were evaluated using an ANOVA approach with the logarithmical-transformed end points as response variables, dose as fixed effect, and subject as a random effect.

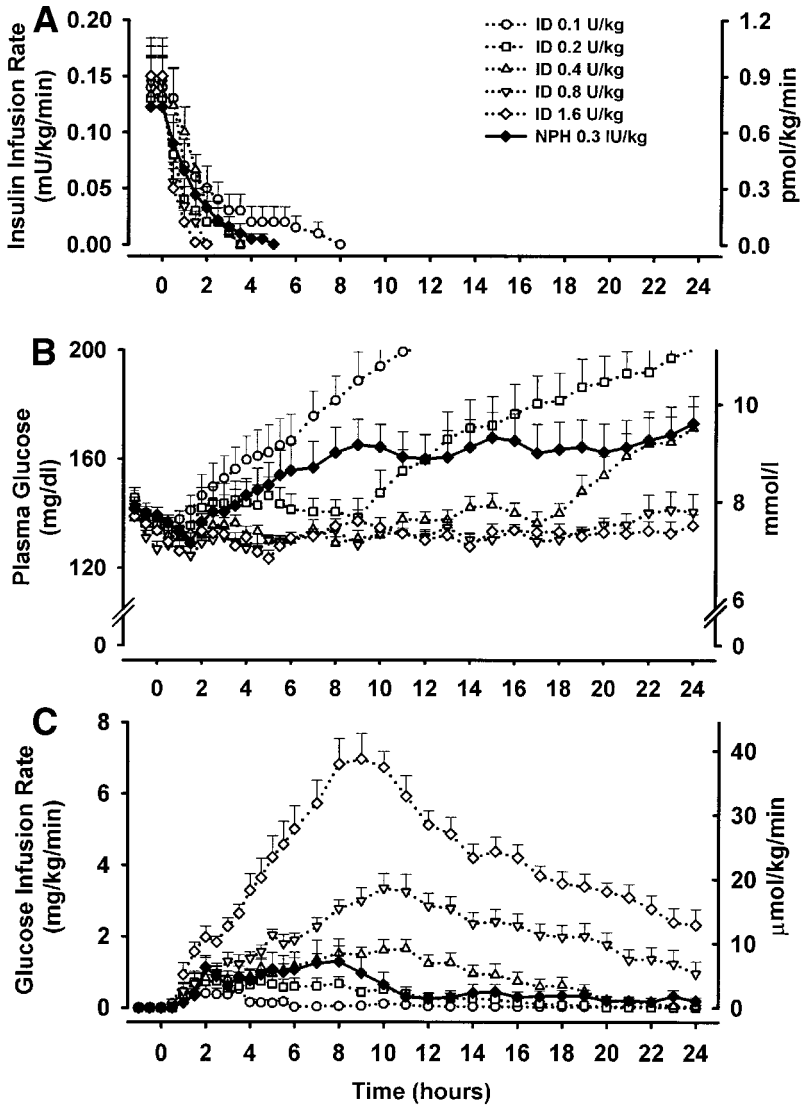

Figure 1—*Time profiles for intravenous insulin infusion rates* (A)*, plasma glucose levels* (B)*, and GIRs* (C) *for after subcutaneous injection of NPH insulin (0.3 IU/kg) and insulin detemir (ID; 0.1, 0.2, 0.4, 0.8, and 1.6 units/kg) in 12 patients with type 1 diabetes. Data are means ± SE.*

RESULTS

Insulin infusion rate

There was a clear dose-dependent effect of insulin detemir, such that higher doses led to a more rapid withdrawal of the intravenous insulin infusion to maintain plasma glucose at 130 mg/dl (7.2 mmol/l) (Fig. 1*A*).

Plasma glucose

Average plasma glucose levels escaping from the clamp target indicate that some, but not necessarily all, subjects are unable to maintain isoglycemia at the given dose level; hence, the steeper the rise in average plasma glucose, the higher the number of subjects who have escaping plasma glucose level at approximately the same time (Fig. 1*B*). As seen from the figure, there was a clear dose-response relationship for insulin detemir, with increasing doses providing less of an increase in average glucose levels. This means that, for the insulin detemir dose levels of 0.8 and 1.6 units/kg, the effect was sufficient to maintain the glucose level for most subjects throughout the 24-h period. NPH insulin (0.3 IU/kg) showed a more gradual increase in average plasma glucose concentrations during the clamp than insulin detemir dose levels of 0.1, 0.2, and 0.4 units/kg, pointing to a more variable time-effect profile and duration of action of NPH compared with insulin detemir. Kaplan-Meier plots confirmed the larger variability for end of action of NPH (not shown).

Duration of action

Duration of action for insulin detemir increased dose dependently from 5.7 h at the lowest dose (0.1 units/kg) to 23.2 h at

Table 1—*Pharmacodynamic parameters after subcutaneous injection of NPH insulin and insulin detemir*

	NPH insulin (0.3 IU/kg)	Insulin detemir				
		0.1 units/kg	0.2 units/kg	0.4 units/kg	0.8 units/kg	1.6 units/kg
Onset of action (h)	1.8 ± 1.2	2.0 ± 1.8	2.0 ± 2.5	1.6 ± 1.1	1.1 ± 0.7	0.8 ± 0.3
End of action (h)	15.3 ± 9.0	7.6 ± 6.1	14.0 ± 5.3	21.5 ± 3.3	23.7 ± 0.9	23.9 ± 0.2
Clamps where end of action was truncated at 24 h (%)	33.3	9.1	9.1	41.7	90.9	90.9
Duration of action (h)	12.7 ± 9.9	5.7 ± 6.6	12.1 ± 6.2*	19.9 ± 3.2†	22.7 ± 1.2	23.2 ± 0.3
AUC_{GIR} (mg/kg)	743 (180–1,306)	101 (−204 to 405)†	419 (24–814)	1,184 (667–1,701)	2,879 (2,084–3,675)	5,703 (4,939–6,466)
GIR_{max} ($mg \cdot kg^{-1} \cdot min^{-1}$)	1.6 (0.5–2.6)	0.8 (0.4–1.1)†	1.1 (0.8–1.4)†	1.7 (1.2–2.1)†	3.3 (2.6–4.0)	7.0 (5.8–8.2)
Time to GIR_{max} (h)	6.1 (2.7–9.5)	3.2 (1.5–5.0)	6.2 (4.1–8.3)	8.6 (6.9–10.3)	9.3 (8.6–10.0)	8.7 (7.3–10.2)

Data are means ± SD or estimate (95% CI). Between-subject variability for duration of action, AUC_{GIR}, and GIR_{max} for 0.1, 0.2, and 0.4 units/kg insulin detemir vs. 0.3 IU/kg NPH: *$P < 0.07$; †$P < 0.05$. All other comparisons not significant. Note that a higher percentage of truncated clamps underestimates variability for the respective insulin dose.

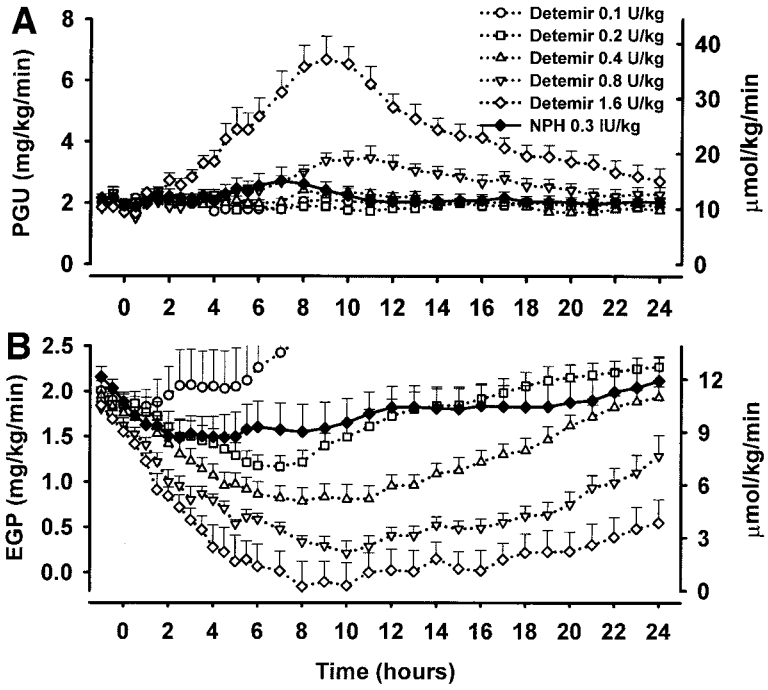

Figure 2—*Time profiles for PGU* (A) *and EGP* (B) *after subcutaneous injection of NPH insulin (0.3 IU/kg) and insulin detemir (0.1, 0.2, 0.4, 0.8, and 1.6 units/kg) in 12 patients with type 1 diabetes. Data are means ± SE.*

the highest dose (1.6 units/kg) (Table 1). Duration of action for 0.3 IU/kg NPH insulin was estimated to be 12.7 h. End of action with regard to glucose levels and glucose infusion was not reached within 24 h for all clamps, and for these clamps, the time point for end of action was set to 24 h. The frequency of clamps where end of action was truncated at 24 h, despite further pharmacodynamic effectiveness, is shown in Table 1, indicating that at the higher doses of insulin detemir in particular, the duration of action is underestimated because the clamp was terminated after 24 h. The between-subject variability in duration of action (expressed as SDs) was 6.2 and 3.2 for 0.2 and 0.4 units/kg insulin detemir dose levels, respectively. This between-subject variability was borderline and statistically significantly lower than the SD of 9.9 for 0.3 IU/kg NPH insulin ($P = 0.07$ and $P < 0.001$ for the dose comparisons).

GIR

The mean GIR profiles indicated a maximum effect of NPH insulin around $t = 7$ h (Fig. 1*C*), with a GIR profile in between that observed with 0.2 and 0.4 units/kg insulin detemir. However, the maximum effect for these two insulin detemir doses occurred later ($t = 8$–10 h), was less pronounced, and was followed by a slower decline (Fig. 1*C*). Considerable variability was observed for the individual GIR profiles, in particular for NPH insulin, which is reflected by wide CIs for AUC_{GIR}, GIR_{max}, and time to GIR_{max} as shown in Table 1. The statistical analysis confirmed these observations and showed a significantly lower between-subject variability for 0.1 units/kg insulin detemir vs. 0.3 units/kg NPH for AUC_{GIR} ($P < 0.03$) and a significantly lower between-subject variability on GIR_{max} for insulin detemir doses ≤0.4 units/kg vs. NPH (0.3 units/kg) ($P < 0.05$).

Based on the dose-response model for AUC_{GIR} for insulin detemir, the dose that provided an identical AUC_{GIR} to NPH (0.3 units/kg) was found to be 0.29 units/kg. This corresponded to a molar ratio (insulin detemir to NPH) of 3.9. Duration of action was further estimated at 0.29 units/kg of insulin detemir to be 16.9 h, which was 4.2 h longer than the duration of action estimated for NPH at 0.3 units/kg.

EGP, PGU, and NEFA

The EGP and PGU profiles for the five doses of insulin detemir and the single dose of NPH are shown in Fig. 2*A* and *B*, respectively. Data for AUC_{GIR}, PGU, and EGP are shown in Table 2. When analyzing the dose of insulin detemir (0.29 units/kg) that provided the same AUC_{GIR} as 0.3 IU/kg NPH, AOC_{EGP} resulted in 636 mg/kg (95% CI 279–879) and AUC_{PGU} 173 (47–316). Increasing doses of insulin detemir suppressed NEFA levels; however, there was no tendency toward a preferential effect of any of the tested insulins on NEFA suppression (data not shown).

Pharmacokinetics

Pharmacokinetic parameters are given in Table 2. As for the pharmacodynamic end points, the total and maximal insulin exposure for insulin detemir were clearly dose dependent, whereas the time to maximal insulin exposure did not seem to depend on dose in any obvious way.

CONCLUSIONS

— The aim of the present study was to characterize the pharmacodynamic and pharmacokinetic properties of insulin detemir over a range of clinically relevant doses. The study used a clamp method and defined duration of action as previously described (5). The present study showed a duration of action of 12.7 h with 0.3 IU/kg NPH insulin, which was comparable to that previously reported (13.2 h) by Lepore et al. (5). While a dose of insulin detemir that provided the same pharmacodynamic response as NPH (0.3 IU/kg) was not tested in the present study, the dose that provided a similar response was observed to be between 0.2 and 0.4 units/kg (estimated at 0.29 units/kg).

The definition of onset of action (50% decrease of intravenous infusion) in our trial is a conservative estimate and likely to be influenced by several factors such as insulin sensitivity and clamp quality. Most likely, onset of action of subcutaneous insulin occurs earlier, and therefore, duration of action is underestimated. However, conclusions regarding duration of action remain unaltered when an alternative onset definition (injection time until glucose >8.3 mmol/l; Table 1) was used.

The interpolation in the present study used a saturated model, allowing the description of a multitude of dose-response relationships, but was simplified by removing insignificant terms. Based on the reduced dose-response model for AUC_{GIR}

for insulin detemir, the dose that provided an identical AUC_{GIR} to 0.3 units/kg NPH (743 mg/kg) was estimated to be 0.29 units/kg. While the limitations of interpolation are acknowledged, it served to improve the validity of the comparison between NPH and insulin detemir, given the limitations of the studied doses, i.e., a comparison of 0.3 IU/kg NPH insulin to 0.2 and 0.4 units/kg insulin detemir would either be under- or overestimating the difference between the two insulin preparations, respectively. In addition, linear interpolation would have provided an almost identical result (0.285 units/kg).

Based on interpolation, an insulin detemir dose of 0.29 units/kg would provide a 4.2-h longer duration of action than 0.3 IU/kg NPH (16.9 vs.12.7 h). In a previous study, this prolongation of action enabled type 1 diabetic subjects to inject insulin detemir before dinner without deterioration of glycemic control during nighttime (18). Fasting blood glucose levels were still significantly lower with this insulin detemir administration regimen in comparison with NPH administered at bedtime, without increasing the risk for nocturnal hypoglycemia.

This study was not designed to investigate differences in variability between the treatments. However, individual pharmacodynamic responses to NPH insulin were characterized by unpredictability of time and extent of peak action and a relatively small average metabolic effect. Neither the average BMI of 23 kg/m^2 (range 19–27) nor the average basal insulin infusion rates indicate a particular prevalence of insulin resistance in our population or lower insulin sensitivity on NPH study days. Furthermore, resuspension of NPH insulin was performed by standardized procedures before injection, and the injection site was not changed between visits. In contrast, pharmacodynamic parameters obtained with insulin detemir showed less between-subject variability. The present study does not allow conclusions to be drawn on within-subject variability. However, lower within-subject variability was observed in a recently published clamp trial (19), and treatment with insulin detemir has been reported to result in lower and less variable glucose levels in clinical trials (18,20–22). The reduction of nocturnal hypoglycemic episodes found in the clinical studies could be clearly attributed to the reduced action peak observed with insulin detemir (20,21).

In conclusion, insulin detemir showed a linear dose response over a range of clinically relevant doses. Futhermore, insulin detemir provides a flat and protracted pharmacodynamic profile.

Table 2—*Pharmacodynamic and pharmacokinetic parameters of subcutaneous injection of NPH insulin and insulin detemir*

	NPH insulin	Insulin detemir				
	(0.3 IU/kg)	0.1 units/kg	0.2 units/kg	0.4 units/kg	0.8 units/kg	1.6 units/kg
AOC_{EGP} (mg/kg)	568 ± 482	129 ± 150	447 ± 291	988 ± 536	1,454 ± 435	1,874 ± 345
AUC_{PGU} (mg/kg)	324 ± 490	34 ± 28	77 ± 84	387 ± 509	1,084 ± 958	3,448 ± 990
$AUC_{0-\infty}$ [(pmol/l) · min]	—	1,232,081 ± 1,118,694	1,680,803 ± 924,942	3,709,330 ± 1,765,748	6,715,456 ± 2,664,617	14,235,324 ± 6,181,450
C_{max} (pmol/l)	216 ± 240	1,434 ± 920	2,896 ± 1,910	4,422 ± 1,774	7,278 ± 2,809	16,535 ± 9,344
t_{max} (min)	327 ± 285	305 ± 124	365 ± 153	410 ± 80	415 ± 109	371 ± 110

Data are means ± SD.

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