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Pharmacokinetic and glucodynamic variability: assessment of insulin glargine, NPH insulin and insulin ultralente in healthy volunteers using a euglycaemic clamp technique

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Abstract *Aims/hypothesis:* This single-dose, double-blind, randomised, parallel-group study evaluated the reproducibility in systemic exposure and glucodynamic effect of insulin glargine, NPH insulin (NPH) and insulin ultralente (ultralente) using the manually adjusted euglycaemic clamp technique. *Methods:* In total, 36 healthy volunteers received two consecutive s.c. injections (0.4 IU/kg) of glargine, NPH or ultralente with a wash-out period of 7 days between treatments. *Results:* In healthy volunteers, glargine presented well-reproduced flat concentration profiles and no pronounced peaks in activity. NPH, by contrast, showed well-defined peaks in concentration and glucose disposal, while ultralente had highly variable profiles. Within-subject variability (ANOVA) for insulin exposure over 24 h was 15% for glargine and 19% for NPH, compared with 67% for ultralente ($p<0.05$, glargine and NPH vs ultralente). The 49% within-subject variability in total glucose disposal (glucose infusion rate [GIR]-$AUC_{0–24\ h}$) with ultralente was about twice as large as the 22% with NPH ($p<0.05$), but was intermediate with glargine at 31% (p=NS). By contrast, variability in the diurnal time-action profile (SD of diurnal day-to-day differences in GIR) for glargine was 30% ($p<0.05$) and 50% ($p<0.05$) less than with NPH and ultralente, respectively. No serious adverse events were reported. *Conclusions/interpretation:* Although representing insulins of different profiles, glargine and NPH showed a high and similar reproducibility of total absorption and glucodynamic effect, whereas ultralente proved to have poor reproducibility. However, while NPH yields peaks in concentration and activity, glargine shows flat and non-fluctuating profiles resulting in less variation in day-to-day 24-h activity.

Keywords Euglycaemic clamp · Glucodynamic · Insulin glargine · Insulin ultralente · NPH insulin · Pharmacokinetic · Reproducibility

Abbreviations Δ: difference · C_{max}: maximum concentration · CUM: cumulative · GIR: glucose infusion rate · GIR-$AUC_{0–24\ h}$: area under the GIR-time curve up to 24 h · GIR_{max}: maximum GIR · GIR-$t_{50\%}$: time to 50% of GIR-$AUC_{0–24\ h}$ · INS: insulin concentrations · INS-$AUC_{0–24\ h}$: area under the exogenous insulin concentration-time curve up to 24 h · INS-C_{max}: maximum insulin concentrations · INS-T_{max}: times to INS-C_{max} · LLOQ: lower limit of quantification · MSE: mean sum of the error terms · NPH: NPH insulin · T_{max}: time to maximum concentration · ultralente: insulin ultralente

Introduction

Long-acting insulins, which serve to replace endogenous basal insulin release in patients with diabetes, need to provide predictable peakless time-concentration and time-action profiles to mimic the slow, steady rate of insulin secreted in the fasted state. To this day, intermediate- and long-acting insulins, such as NPH insulin (NPH) and insulin ultralente (ultralente), are used as basal insulin therapy. Unfortunately, the pharmacokinetics of these traditional insulin preparations do not match the profiles of endogenous insulin secretion. In particular, intermediate-acting insulin shows a peak-action profile [1] and, as with ultralente, a huge day-to-day variability in absorption after s.c. injection [2]. These undesirable effects result in large fluctuations in systemic exposure, and through this unfavourable insulin action profiles. In particular, large intra-subject variability in onset, extent and duration of action of ultralente causes unpredictable metabolic control, including periods of hypo- and hyperglycaemia. The untimely action of NPH, by contrast, requires extra meals

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or snacks. Consequently, a major barrier to obtaining optimal glycaemic control in patients with diabetes results from their fear of iatrogenic hypoglycaemia caused by volatile fluctuations in the pharmacokinetics of common insulin [3, 4]. Conversely, it is now well established that the hyperglycaemia occurring as a consequence of poor glycaemic control contributes to the development of micro- and possibly macrovascular complications [5, 6].

Insulin glargine, a novel, long-acting insulin, represents a true basal insulin with a predictable flat pharmacokinetic profile ensuring real clinical benefit. Several pharmacokinetic and pharmacodynamic studies characterised insulin glargine as an insulin with sustained, prolonged absorption [7], no pronounced peak, a near-24-h duration of action and a lower between-subject variability than NPH or ultralente [8]. A recent study with insulin detemir, another novel, long-acting insulin, compared the between-subject variability of insulin glargine, insulin detemir and NPH in patients with type 1 diabetes [9]. Here, we detail a study that compared the within-subject variability in systemic exposure and glucodynamic effects of insulin glargine with that of NPH and ultralente using the manual euglycaemic clamp technique in healthy volunteers. In addition, diurnal profile reproducibility was assessed [10].

Subjects, materials and methods

Study design

This trial was a single-dose, double-blind, randomised, parallel-group, replicate-design study investigating within-subject variability in systemic exposure and glucodynamics of insulin glargine compared with NPH (Huminsulin basal 100) and ultralente (Ultralong) in healthy volunteers. Thirty-six healthy volunteers were allotted to three groups of 12 subjects per group. Each group received two consecutive injections of one of the study medications. Prior to the second injection, there was a wash-out period of at least 7 days. The study was performed at the Hoechst Marion Roussel Research Centre, Bloemfontein, South Africa, now Farmovs-Parexel.

Study population

Healthy, non-smoking, male volunteers aged 18–33 years (mean 23.1 years), weighing between 66 and 100 kg (mean 79.6 kg), with BMI values of 20.0–26.0 kg/m^2 (mean 23.5 kg/m^2), a normal oral glucose tolerance test and no clinically important abnormalities in their clinical chemistry, ECGs, vital signs and medical history or on physical examination were included in the study. The demographic and baseline characteristics of the study population were similar between groups: 18.3–29.3 years (mean 23.3), 18.9–32.5 years (mean 23.6) and 19.1–27.5 years (mean 22.3) in the insulin glargine (n=12), NPH (n=12) and ultralente (n=12) groups, respectively. Body weight ranges for the insulin glargine, NPH and ultralente groups were 67.1–97.2 kg (mean 77.9), 69.0–91.2 kg (mean 80.5) and 65.6–99.8 kg (mean 80.4), respectively. The BMI ranges were 20.0–25.8 kg/m^2 (mean 22.8), 21.1–26.0 kg/m^2 (mean 23.9) and 22.1–26.0 kg/m^2 (mean 23.7), respectively. All volunteers provided written, informed consent prior to initiation of the investigation; the study was carried out in accordance with the Declaration of Helsinki.

Study medication

Each volunteer received two consecutive (≥7-day wash-out) s.c. injections of insulin glargine (12 subjects), NPH (12 subjects) or ultralente (12 subjects) at a dose of 0.4 IU/kg body weight administered by a physician or nurse otherwise not involved in the study. The peri-umbilical abdominal area was chosen for injection with a 0.13×12 mm single-use syringe with integrated needle (Braun Omnican) for comparison with previous single-dose studies.

Clamp procedure

Subjects remained fasting from 22.00 hours the night before to the end of the entire study procedure. The injection time defined the time zero of the insulin action period, which was monitored for 24 h. Glucose infusion rate (GIR), blood glucose concentration, serum immunoreactive insulin and serum C-peptide concentrations were recorded for pharmacokinetic and pharmacodynamic evaluations. Blood samples were taken from an i.v. cannula, which was inserted into the hand or wrist vein, for measurement of blood glucose, serum insulin and serum C-peptide levels.

Blood glucose concentrations were measured every 10 min after administration of the study medication with a Yellow Springs Instruments 2300S Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA) using the glucose oxidase method. Baseline blood glucose concentration was calculated as the mean value of blood glucose measurements taken at 60, 30, 15 and 5 min prior to study medication. A drop in blood glucose up to a maximum of 10% from baseline signified the initiation of the glucose infusion. A 20% glucose solution was infused at a manually stepwise-adjusted variable rate to restore and maintain the subject's baseline blood glucose concentration.

Pharmacokinetic data collection Serum total immunoreactive insulin was measured at 30, 15 and 5 min prior to and every 60 min up to 24 h after administration of the study medication using an RIA for human insulin with an in vitro cross-reactivity with insulin glargine of about 50%. The lower limit of quantification (LLOQ) was 2.5 μIU/ml. Simultaneous serum C-peptide concentrations were also determined with an RIA with an LLOQ of 0.15 ng/ml (Analytical Services Division, Farmovs Research Centre, Bloemfontein, South Africa). Immunoreactive insu-

lin concentrations (INS) were corrected for endogenous insulin using serum C-peptide levels to yield exogenous insulin concentration profiles and to observe maximum concentrations (INS-C_{max}), times to INS-C_{max} (INS-T_{max}) and to calculate the area under the exogenous insulin concentration-time curve up to 24 h (INS-$AUC_{0–24\ h}$).

Pharmacodynamic data collection The time-action profile was characterised by the area under the GIR-time curve up to 24 h (GIR-$AUC_{0–24\ h}$), by the maximum GIR (GIR_{max}), time to 50% of GIR-$AUC_{0–24\ h}$ (GIR-$t_{50\%}$) and the time to GIR_{max} (GIR-T_{max}).

Safety data

Adverse events were reported by the subject or noted by the investigator. Routine laboratory tests included haematology, clinical chemistry and urinalysis. Determination of human insulin antibodies, a physical examination and a 12-lead ECG were also carried out.

Statistical methods

Pharmacokinetics For insulin, area under the concentration-time curve ($AUC_{0–24\ h}$) was calculated according to the linear trapezoidal rule up to 24 h after injection of the study medication. INS-C_{max} was read directly from the derived serum concentrations of exogenous insulin. ANOVA for treatment and subject effect was applied on natural logarithmic (ln)-transformed data. Antilog point estimates with 90% CIs were obtained for the mean ratio 'Clamp 2-Clamp 1' on the ln-scale with period and subject effect per insulin, and for the respective ratios of treatment means (secondary analysis). Non-parametric analysis was performed and 90% CIs calculated for INS-T_{max} according to Steinijans and Diletti.

Pharmacodynamics For glucose, area under the GIR-time curve was calculated as the exact area under the stepwise constant function for the respective time intervals of the 24-h GIR-time profile; the GIR-$t_{50\%}$ was extrapolated from this. The determination of GIR_{max} was based on a 'smoothed three-point running mean' GIR curve for each subject. For each measured value of GIR, a mean GIR value was calculated from the previous, actual and following GIR values (this corresponded to mean values of 20-min intervals from injection of the study medication up to the end of the clamp period). The GIR_{max} was read directly from the smoothed GIR-time curves and the times of GIR_{max} were reported as the blood sampling times corresponding with the GIR_{max}. ANOVA of untransformed data was applied to the area under the smoothed (three-point running means smoother) GIR curve over 24 h (GIR-$AUC_{0–24\ h}$), GIR-$t_{50\%}$ and GIR_{max}, and CIs calculated based on Fieller's theorem. Non-parametric analysis was performed and 90% CIs calculated for GIR-T_{max}. Between-treatment comparisons were conducted as described above.

Variability in pharmacokinetics and pharmacodynamics Within-subject variability was assessed as intra-individual CV values, taken from the mean sum of the error terms (MSE) as calculated by the ANOVA on untransformed values for GIR-$AUC_{0–24\ h}$, GIR_{max}, GIR-$t_{50\%}$ and INS-T_{max}, and on ln-transformed values for INS-AUC and INS-C_{max}. (CV% of untransformed data=SQRT[MSE]/LSM×100; CV% of ln-transformed data=SQRT[(EXP(MSE)–1]×100.) For comparison of variances between treatments, the statistical *F*-distribution was used to compute 90% CIs on the ratio of the two variances.

Profile reproducibility Profile reproducibility was assessed in two steps: by the absolute individual cumulative (CUM) between-day differences in insulin concentration ($\Delta_{absolute}$–INS–CUM) and GIR profiles ($\Delta_{absolute}$–GIR–CUM) and by individual SD values of hourly between-day differences (untransformed, raw data) in insulin concentrations (SD–Δ_{raw}–INS) and GIR (SD–Δ_{raw}–GIR) over 24 h. For comparison between treatments, non-parametric analysis was performed for these metrics. A stem-and-leaf plot for outlier detection was also accomplished. An additional comparison of $\Delta_{absolute}$–INS–CUM was performed with insulin glargine concentrations corrected for the observed underestimation relative to NPH (i.e. equivalence in INS–$AUC_{0–24\ h}$ was assumed).

Results

Clamp performance The blood glucose concentration was restored to each individual's fasting blood glucose concentration after study medication and maintained during the entire clamp. The mean within-subject CV of the blood glucose concentration was: (1) after insulin glargine: 4.1% (Visit 2), 4.0% (Visit 3); (2) after NPH: 4.7% (Visit 2), 4.4% (Visit 3); and (3) after ultralente: 4.2% (Visit 2), 4.1% (Visit 3). These CVs are similar to those reported for closed-loop feedback-controlled euglycaemic clamps [11]. There were no relevant differences in mean baseline blood glucose concentrations between visits or treatment groups before administration of the study medication (insulin glargine: 4.5 mmol/l [Visit 2], 4.5 mmol/l [Visit 3]; NPH: 4.5 mmol/l [Visit 2], 4.5 mmol/l [Visit 3]; ultralente: 4.5 mmol/l [Visit 2], 4.5 mmol/l [Visit 3]).

Pharmacokinetic parameters Insulin glargine presented matching serum concentration curves in 10 of 12 subjects. Two subjects were identified with poorly superimposable profiles, but were nevertheless included for analysis. Ultralente presented with distinctly separate serum concentration curves in the majority of subjects, while NPH concentration profiles were fairly reproduced in each individual. As a consequence, insulin glargine and NPH each showed superimposable median profiles, while

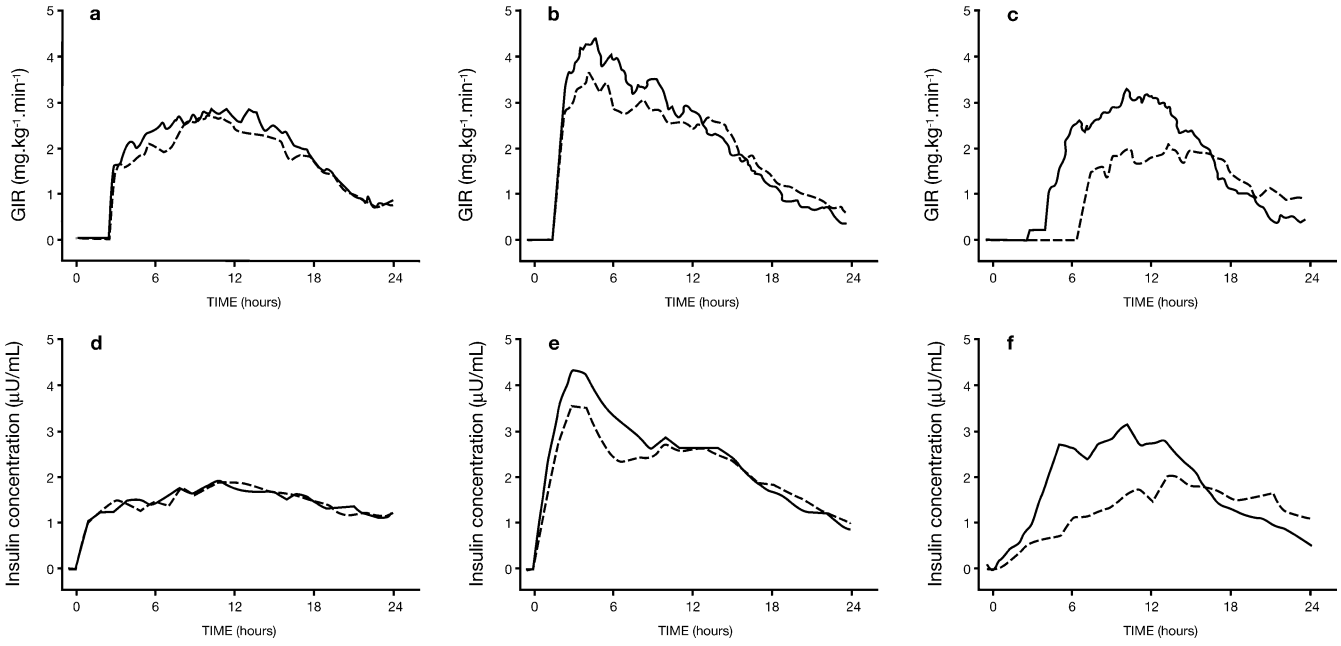

Fig. 1 a–c Pharmacodynamics (average glucose infusion rate [*GIR*]; mg·kg^{-1}·min^{-1}, median) for insulin glargine (**a**), NPH insulin (NPH) (**b**) and insulin ultralente (ultralente) (**c**); median GIR values of Clamp 1 (*solid line*) and (ultralente) Clamp 2 (*dashed line*) are given. **d–f** Pharmacokinetics (exogenous immunoreactive median serum insulin concentrations, estimated from serum immunoreactive insulin and serum C-peptide; µU/ml) for insulin glargine (**d**), NPH (**e**) and ultralente (**f**); median insulin concentrations of Clamp 1 (*solid line*) and Clamp 2 (*dashed line*) are given. The figure shows that generally lower and non-fluctuating serum insulin concentrations, as compared with NPH, were attained for insulin glargine, while ultralente displayed large day-to-day variability

ultralente presented with a day-to-day difference of, on average, 3 h in time (INS-T_{max}) to similar maximum concentrations (INS-C_{max}).

The averaged total systemic insulin exposure (INS-$AUC_{0–24\ h}$) was assayed approximately 40% higher with NPH than with ultralente and insulin glargine, which were equal. INS-C_{max} after NPH was observed on average 4 h (median) after injection, which was significantly earlier than the 12 h after insulin glargine and the 13 h after ultralente (Fig. 1a), despite a wide range of INS-T_{max} occurrences of the latter insulins (Tables 1 and 2).

Pharmacodynamic values The average 24-h glucose disposal (GIR-$AUC_{0–24\ h}$) was similar with insulin glargine and NPH. It was slightly less with ultralente as compared with both insulin glargine and NPH, although the wide margins did not allow for statistical inferences. Insulin glargine and ultralente displayed half of their activity within, on average, 11.5 and 12.5 h, respectively, as compared with 9.5 h with NPH (i.e. within significantly less time; Tables 3 and 4). Very wide ranges of late GIR-T_{max} at low GIR_{max} were observed for insulin glargine and ultralente and distinctly smaller ranges of early GIR-T_{max} at larger GIR_{max} for NPH.

Within-subject variabilities of pharmacokinetics and pharmacodynamics The systemic insulin exposure after ultralente was characterised by fundamentally higher day-to-day (within-subject) variability in core parameters than after insulin glargine or NPH (Fig. 1b). For INS-$AUC_{0–24\ h}$,

Table 1 Replicate pharmacokinetics of insulin glargine, NPH insulin and insulin ultralente parameters

	Study day	Parameter, mean (SD)		
		Insulin glargine[a]	NPH insulin	Insulin ultralente
INS–$AUC_{0–24\ h}$ (µI·U·hml^{-1})	Clamp 1	169 (39)	300 (59)	205 (106)
	Clamp 2	189 (52)	257 (52)	176 (103)
INS–C_{max} (µIU/ml)	Clamp 1	10.0 (2.5)	23.2 (5.0)	16.4 (8.3)
	Clamp 2	12.1 (5.7)	18.4 (2.3)	14.3 (8.4)
INS–T_{max} (h)	Clamp 1	12.4 (4.5)	3.9 (1.4)	10.8 (5.5)
	Clamp 2	11.0 (5.4)	4.7 (3.0)	13.6 (6.0)

INS insulin, C_{max} maximum concentration, T_{max} time to C_{max}
[a]Insulin glargine concentrations are not corrected for differences in cross-reactivity with human insulin (see *Pharmacokinetic data collection*)

Table 2 Comparison of pharmacokinetics between treatments: point estimates (% [90% CI])

	Insulin glargine[a]/NPH insulin	Insulin glargine[a]/insulin ultralente	NPH insulin/insulin ultralente
INS–$AUC_{0–24\ h}$	64 (53, 77)[b]	89 (74, 108)	140 (116, 170)[b]
INS–C_{max}	52 (42, 63)[b]	68 (56, 84)[b]	132 (108, 162)[b]
Median INS–T_{max} (h)	8 (6, 9)[b]	−1 (−4, 2)	−8 (−11, −6)[b]

INS insulin, C_{max} maximum concentration, T_{max} time to C_{max}
[a]Insulin glargine concentrations are not corrected for differences in cross-reactivity with human insulin (see *Pharmacokinetic data collection*)
[b]$p<0.05$

insulin glargine and NPH yielded low within-subject CVs, compared with ultralente. Both insulin glargine and NPH also showed a lower INS-C_{max} within-subject variability compared with ultralente. In line with the flat profile, the CV% in INS-T_{max} was larger after insulin glargine than after either NPH or ultralente (Table 5).

The within-subject GIR variability for GIR-$AUC_{0–24\ h}$ was about twice as large with ultralente as NPH, and intermediate for insulin glargine. The CV% for GIR-$t_{50\%}$ was low and about the same for insulin glargine and NPH, but almost three times as large for ultralente. A similar CV% in GIR_{max} was seen for NPH and insulin glargine, but was larger for ultralente. In any event, there were no statistical differences in variances for any parameter between insulin glargine and NPH, while variances with ultralente were significantly larger for INS–$AUC_{0–24\ h}$, INS–C_{max} and GIR–$t_{50\%}$ compared with insulin glargine and NPH, and for GIR-$AUC_{0–24\ h}$ compared with NPH (Table 5).

Profile reproducibility The individual absolute cumulative between-day differences in insulin concentration ($\Delta_{absolute}$–INS–CUM) were similar with insulin glargine and NPH, with and without adjustment for the imbalance in INS-$AUC_{0–24\ h}$. Absolute individual between-day differences were significantly less for insulin glargine than after ultralente. The variation in the hourly between-day differences, expressed as individual SD in insulin concentrations (SD–Δ_{raw}–INS), was less for insulin glargine profiles than for NPH (similar when adjusted for the imbalance), and significantly less than for ultralente (Fig. 2). Accordingly, the absolute between-day differences in the time-action profiles ($\Delta_{absolute}$–GIR–CUM) were similar with insulin glargine and NPH, but less compared with ultralente. The variation in the between-day differences (SD–Δ_{raw}–GIR) with insulin glargine, however, was 30% less compared with NPH and 50% less compared with ultralente (Fig. 2). Moreover, both results were statistically significant when two extreme values in the insulin glargine group were excluded, as indicated by a stem-and-leaf plot analysis (Table 6).

C-peptide Suppression of endogenous insulin secretion assessed by a reduction in C-peptide concentration was slightly more rapid with NPH than with insulin glargine or ultralente, but was uniformly the same at >50% after the

Table 3 Replicate glucodynamics of insulin glargine, NPH insulin and insulin ultralente

	Study day	Parameter, mean (SD or range)		
		Insulin glargine	NPH insulin	Insulin ultralente
GIR–$AUC_{0–24\ h}$ (mg/kg)	Clamp 1	2,558 (1,400)	2,993 (1,091)	2,593 (1,313)
	Clamp 2	2,987 (1,820)	2,847 (1,133)	2,025 (1,360)
GIR–$t_{50\%}$ (h)	Clamp 1	12 (2)	9 (1)	11 (2)
	Clamp 2	11 (2)	10 (1)	15 (3)
GIR_{max} ($mg \cdot kg^{-1} \cdot min^{-1}$)	Clamp 1	3.2 (1.3)	5.0 (1.7)	4.1 (2.0)
	Clamp 2	3.7 (2.7)	4.4 (1.8)	3.3 (2.0)
Median GIR–T_{max}, h (range)	Clamp 1	8.6 (4.4–11.7)	4.6 (2.0–10.0)	8.1 (4.5–15.4)
	Clamp 2	10.1 (4.0–22.2)	4.9 (3.5–13.7)	13.1 (5.5–23.5)

GIR glucose infusion rate, T_{max}, time to C_{max}, *GIR–$t_{50\%}$* time to 50% of GIR–$AUC_{0–24\ h}$

Table 4 Comparison of glucodynamics between treatments: point estimates (% [90% CI])

	Insulin glargine/NPH insulin	Insulin glargine/insulin ultralente	NPH insulin/insulin ultralente
GIR–$AUC_{0–24\ h}$	93 (67, 128)	120 (83, 179)	129 (97, 176)
GIR–$t_{50\%}$	125 (118, 132)[a]	92 (83, 100)	73 (65, 82)[a]
GIR_{max}	72 (52, 97)[a]	93 (64, 133)	129 (97, 174)
Median GIR–T_{max} (h)	3.6 (2.1, 5.3)[a]	−1.9 (−4.1, 0.8)	−5.7 (−7.8, −3.0)[a]

GIR glucose infusion rate, GIR_{max} maximum GIR, T_{max} time to GIR_{max}, *GIR-$t_{50\%}$* time to 50% of GIR-$AUC_{0–24\ h}$
[a]$p<0.05$

Table 5 Within-subject variability: CV% (95% CI)

	Insulin glargine	NPH insulin	Insulin ultralente
INS–$AUC_{0–24\ h}$	15 (8, 22)[a]	19 (11, 28)[b]	67 (35, 99)
INS–C_{max}	29 (16, 41)[a]	21 (12, 31)[b]	64 (34, 95)
INS–T_{max}	48 (26, 69)	36 (20, 52)	37 (20, 55)
GIR–$AUC_{0–24\ h}$	31 (17, 45)	22 (12, 32)[b]	49 (26, 72)
GIR_{max}	27 (15, 38)	23 (13, 33)	38 (20, 57)
GIR–$t_{50\%}$	13 (7, 19)[a]	11 (6, 17)[b]	32 (17, 47)

CV (ANOVA) taken from the mean sum of the error terms, *INS* insulin, C_{max} maximum concentration, T_{max} time to C_{max}, GIR_{max} maximum glucose infusion rate, $GIR\text{-}t_{50\%}$ time to 50% of GIR-$AUC_{0–24\ h}$
[a]$p<0.05$, insulin glargine vs insulin ultralente
[b]$p<0.05$, NPH insulin vs insulin ultralente

Table 6 Profile reproducibility: ratio of medians

	Insulin glargine/NPH insulin	Insulin glargine/insulin ultralente
$\Delta_{absolute}$INS–CUM	0.83 (0.73[c])/1.29[a] (1.14[a,c])	0.41[b] (0.37[b,c])
$\Delta_{absolute}$GIR–CUM	0.95 (0.84[c])	0.60[b] (0.54[b,c])
SD–Δ_{raw}–INS	0.67[b] (0.58[b,c])/1.04[a] (0.90[a,c])	0.49[b] (0.43[b,c])
SD–Δ_{raw}–GIR	0.70[b] (0.66[b, c])	0.48[b] (0.46[b,c])

Δ difference, *INS* insulin, *CUM* cumulative, *GIR* glucose infusion rate
[a]Normalised for $INS–AUC_{0–24\ h}$
[b]$p<0.05$
[c]Excluding outliers

6th hour with each of the insulins (mean change from baseline: insulin glargine, −67% vs −62%; NPH, −66% vs −64%; ultralente, −65% vs −66%; all Clamp 1 vs Clamp 2).

Safety No serious adverse events were reported. Adverse events, reported in 19 subjects, were most frequently mild-to-moderate headaches, which occurred in similar numbers between the groups, and iron-deficiency anaemia (six subjects). Both events were related to the clamp procedure.

Discussion

This study assessed the day-to-day variability in the time-concentration and time-action profiles of insulin glargine compared with NPH and ultralente, employing the manual euglycaemic clamp technique in healthy volunteers. Euglycaemia was reliably established with blood glucose concentrations varying by <5% within each subject's clamp period, with C-peptide suppression as a surrogate for the reduction of endogenous insulin release, which was uniformly the same in all clamps from the 6th hour onwards. In the present study, insulin was injected into the abdomen rather than the thigh. Although the abdomen is no longer commonly used as an injection site we do not believe that this procedure influenced the results, as supported by others [7].

There were clear differences in the time-concentration profiles of the three insulins. Although both insulin glargine and NPH displayed low variability in systemic exposure, injection of NPH produced defined early peak exposures. Ultralente, which in general affords a flat profile, was associated with an ill-defined onset in absorption and, consequently, large variability in systemic exposure.

These insulin concentration profiles are fully corroborated by the glucodynamic profiles. Insulin glargine demonstrated a very reproducible activity profile with no pronounced peak and an early, modest onset in glucose lowering, requiring a soft onset in equalising glucose infusion to commence within 2–3 h and be sustained for 24 h. NPH, by contrast, required a more robust glucose infusion to commence prior to the second hour after injection and thus generated a defined peak in glucose supply at the fifth hour, after which it declined. Ultralente actually presented two markedly different profiles with a highly variable onset in both glucose lowering and corresponding glucose infusion, although it eventually displayed a profile with no pronounced peak.

Concerning assessment of within-subject variability in glucodynamics, there are limitations on euglycaemic clamps of basal insulins, and on manual clamps in non-diabetic subjects in particular [12]. The euglycaemic-hyper-

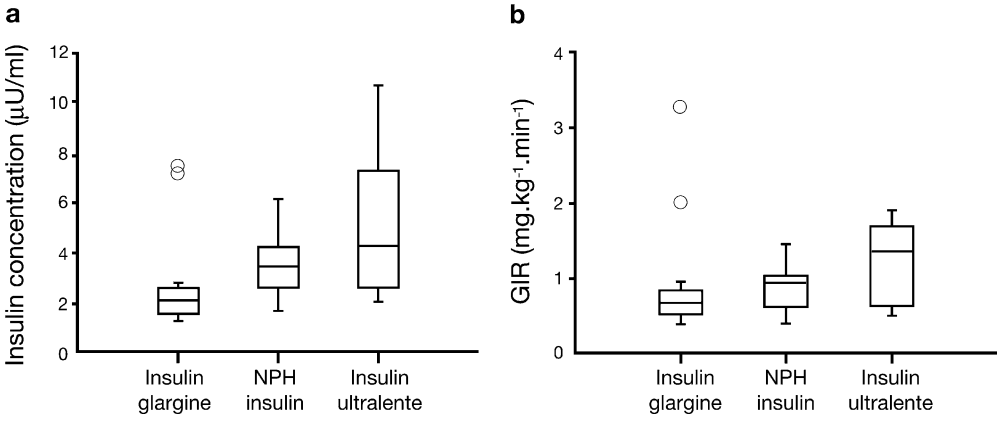

Fig. 2 Distribution of SD of diurnal individual day-to-day differences in insulin concentration (**a**) and glucose infusion rate (*GIR*; **b**)

insulinaemic clamp technique was developed to examine insulin sensitivity, which requires elevated steady-state insulin concentrations [11, 13]. However, glucose disposal in lasting euglycaemic–hyperinsulinaemic clamps becomes subject to increasing insulin sensitivity and non-oxidative glucose uptake [14]. Therefore, in line with the complex experimental interplay between a required reduction in blood glucose concentration of up to 10% that triggers the counter-glucose infusion and that, thus, follows the genuine onset of glucose-lowering activity, it is not so much the inherent pharmacokinetics of a basal insulin, but rather the sluggish onset of the manual adjustment of this infusion that determines the early variability in GIR. As a result, over the complete 24-h period after injection, the within-subject variability of the GIR parameters was lowest when the insulin effect was strongest, which was seen with NPH. The large variability of ultralente action, by contrast, is predominantly due to the erratic onset in absorption and, in turn, action.

The more recent investigation of insulin detemir, NPH and insulin glargine, all intended for basal insulin replacement, using a Biostator-based euglycaemic clamp in patients with type 1 diabetes, is in line with this notion [9]. Tight blood glucose control is at the expense of larger fluctuation in Biostator-adjusted glucose infusion, which in turn requires vigorous smoothing of GIR readings to generate similar smooth biological response profiles as compared with manually adjusted clamps [15]. The more robust onset of action after insulin detemir resulting in less fluctuation in glucose infusion may explain, at least in part, the perceived less variable profile as compared with insulin glargine.

Overall, the common summary parameters do not appropriately reflect the actual day-to-day variation in fluctuation in concentration or in activity over 24 h, which are of interest to a patient using a basal insulin. To this end, the profile reproducibility was assessed. Although the direct comparison of insulin glargine and NPH for variations in concentration was limited by the presumed bioanalytical underestimation of insulin glargine, insulin glargine presented at least equal profile reproducibility to NPH, even when adjusted to equivalence in systemic exposure to NPH, and superiority to ultralente. More importantly, superiority in reproducibility was visible in the glucodynamic profile, where insulin glargine presented 30–50% less variation over 24 h compared with NPH and ultralente, respectively.

The flat time-concentration profile seen in healthy volunteers under controlled conditions was confirmed to be well reproducible in patients with type 1 diabetes at steady-state [16]. Therefore, from a clinical viewpoint, since hypoglycaemia is one of the most common and feared side-effects of insulin therapy [4], the use of preparations such as insulin glargine, which have a smooth 24-h profile and low within-patient variability, is favourable and may encourage greater adherence to insulin therapy. Insulin glargine is associated with a lower incidence of nocturnal hypoglycaemia compared with NPH [17, 18], and this could be due to the relatively flat action profile of insulin glargine compared with NPH. Further, the more opportune activity profile of insulin glargine facilitates treating to target HbA_1c levels with a lower incidence of hypoglycaemia. Indeed, it has already been demonstrated that insulin glargine enables treating to a target HbA_1c of <7% [19]. In addition, when treated to a comparable HbA_1c target, patients receiving insulin glargine experience a significantly lower incidence of severe hypoglycaemia compared with patients receiving NPH [20].

In summary, although representing insulins of different action profiles, under experimental conditions in manual euglycaemic clamps in healthy volunteers, the reproducibility of time-concentration and glucodynamic effects was high and favourable for insulin glargine and NPH, but low for ultralente. Insulin glargine generally shows low day-to-day, within-subject variations in serum insulin concentration and the corresponding glucodynamic effect, which implies improved reproducibility of basal glucose control with insulin glargine.

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Duality of interest

R.H.A. Becker is an employee of Aventis Pharma, a company of the sanofi-aventis group.

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