

Low within- and between-day variability in exposure to new insulin glargine 300 U/ml

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Aims: To characterize the variability in exposure and metabolic effect of insulin glargine 300 U/ml (Gla-300) at steady state in people with type 1 diabetes (T1DM).

Methods: A total of 50 participants with T1DM underwent two 24-h euglycaemic clamps in steady-state conditions after six once-daily administrations of 0.4 U/kg Gla-300 in a double-blind, randomized, two-treatment, two-period, crossover clamp study. Participants were randomized to receive Gla-300 as a standard cartridge formulation in the first treatment period, and as a formulation with enhanced stability through polysorbate-20 addition in the second treatment period, or vice versa. This design allowed the assessment of bioequivalence between formulations and, subsequently, within- and between-day variability.

Results: The cumulative exposure and effect of Gla-300 developed linearly over 24 h, and were evenly distributed across 6- and 12-h intervals. Diurnal fluctuation in exposure (within-day variability) was low; the peak-to-trough ratio of insulin concentration profiles was <2, and both the swing and peak-to-trough fluctuation were <1. Day-to-day reproducibility of exposure was high: the between-day within-subject coefficients of variation for total systemic exposure (area under the serum insulin glargine concentration time curve from time 0 to 24 h after dosing) and maximum insulin concentration were 17.4% [95% confidence interval (CI) 15–21] and 33.4% (95% CI 28–41), respectively. Reproducibility of the metabolic effect was lower than that of exposure.

Conclusions: Gla-300 provides predictable, evenly distributed 24-h coverage as a result of low fluctuation and high reproducibility in insulin exposure, and appears suitable for effective basal insulin use.

Keywords: insulin glargine, pharmacokinetics, type 1 diabetes

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Introduction

Long-acting insulin analogues are designed to establish constant and sustained insulin concentrations over the course of a day, and aim to mimic the endogenous fasting insulin production seen in healthy individuals. In people with type 1 diabetes mellitus (T1DM) they replace endogenous insulin, whereas in people with type 2 diabetes mellitus (T2DM) they complement inadequate insulin secretion. The subcutaneous route of administration and once-daily injections, however, preclude the exact reproduction of physiological oscillating insulin secretion patterns. As a compromise, a long-acting insulin product should present with low diurnal fluctuation in exposure and high between-day reproducibility [1,2]. Such pharmacokinetic (PK) characteristics could help to ensure effective insulin use, allowing accurate dosing and titration and potentially enabling

improved glycaemic control while minimizing the risk of hypoglycaemia [1,2].

Insulin variability can be assessed using PK and pharmacodynamic (PD) variables, but variability attributable directly to insulin exposure is considered the more accurate measure [3], as PD variables are influenced by non-insulin-specific factors [1,3]. Therapeutic doses of basal insulin products are intended to control fasting glucose, whereas the higher basal insulin doses used in experimental settings are similar to lasting prandial insulin effects, overruling the endogenous metabolic equilibrium and requiring high compensatory glucose loads. The strong artificial responses of such high doses are accompanied by high reproducibility according to the sigmoid insulin exposure–effect relationship, while therapeutic exposure results in low glucose demand and less reproducibility. In clinical practice, variability in glycaemic control is subject to lifestyle and treatment regimen, and so is only partly reflective of insulin product characteristics.

Insulin glargine 300 U/ml (Gla-300) has been shown to have more even and prolonged PK and PD profiles compared with glargine 100 U/ml [Gla-100 (Lantus®; Sanofi-Aventis, Frankfurt, Germany)] [4]. Gla-300 also exhibits a glucose-lowering effect similar to that of Gla-100, with a reduced risk of hypoglycaemia [5,6]. For development

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purposes, Gla-300 is being investigated with two formulations; a standard formulation for use with insulin pen cartridges and a formulation for use with vials. Insulin pen cartridges remain airtight during use, whereas insulin contained in vials is exposed to the air, potentially affecting the stability of the insulin molecule [7]. Polysorbate-20 is therefore added to the vial formulation to enhance stability, but should not affect insulin exposure or activity.

The aim of the present replicate-dosing study was to assess exposure to Gla-300 at steady-state conditions in people with T1DM and to demonstrate equivalence between formulations at therapeutic doses of 0.4 U/kg/day. This subsequently also allowed the assessment of within- and between-day variability.

Materials and Methods

Study Design

The present study was a double-blind, randomized, two-treatment, two-period, crossover euglycaemic clamp study (Figure S1). The study was performed in compliance with Good Clinical Practice, the Helsinki Declaration and local regulations. The protocol was approved by the ethical review board and all participants provided written informed consent. The study was registered with ClinicalTrials.gov under the number: NCT01838083.

Participants received 0.4 U/kg Gla-300, dispensed from a vial [with polysorbate-20; test (T)] in one treatment period and from a cartridge [reference (R)] in the other treatment period, in a randomized treatment order. Each treatment was administered as a subcutaneous injection, periumbilically using a 1-ml syringe with 1 U per 10 μ l graduation (Beckton, Dickinson and Co., Franklin Lakes, NJ, USA; Product Number 305502). Injections were given by a trained professional once daily, at \sim 20:00 h for 6 days. There was a washout period of 7–21 days between consecutive treatment periods.

The total insulin dose was rounded to the nearest unit. Therefore, if not exactly divisible by 3, the Gla-300 dose needed to be rounded to the nearest graduation mark (1 unit of Gla-300 corresponding to 10/3 μ l).

Participants were to abstain from using other basal or neutral protamine Hagedorn (NPH) insulins before and during the 6 days of treatment and the 24-h clamp. The last dose of usual basal insulin was to be taken \geq 48 h before first study treatment of Gla-300, and the last dose of NPH insulin was to be taken \geq 24 h before the start of Gla-300 treatment. Between discontinuing basal or NPH insulins and the first study dose of Gla-300, only short-acting subcutaneous insulins were to be used. All participants were instructed to adjust their own prandial insulin and caloric load, as needed according to blood glucose measurements and closely supported by the treating physician, on days 1–5 and until 10:00 h on day 6. Participants were instructed to fast from 10:00 h on day 6, \sim 10 h before administration of Gla-300.

Participants

The study enrolled men and women ($n = 50$) aged 18–64 years with T1DM (duration \geq 1 year) as defined by the American

Diabetes Association [8] who were otherwise healthy. Inclusion criteria included total insulin dose <1.2 U/kg/day, basal insulin dose ≥ 0.2 U/kg/day, body weight between 50 and 110 kg, body mass index (BMI) between 18.5 and 30 kg/m², fasting negative serum C-peptide concentration <0.3 nmol/l, glycated haemoglobin (HbA_{1c}) concentration $\leq 9\%$ (≤ 75 mmol/mol) and a stable insulin regimen for at least 2 months before study inclusion. Key exclusion criteria included any history or presence of another clinically relevant disease.

Euglycaemic Clamp Procedure

On day 6 of each treatment period, participants underwent a 24-h euglycaemic clamp using the Biostator™ device (MTB Medizintechnik, Amstetten, Germany). Participants were attached to the Biostator \sim 5 h before receiving medication (\sim 15:00 h). Blood glucose level was adjusted to within a pre-clamp target of 4.4–6.7 mmol/l (80–120 mg/dl) by intravenous infusions of insulin glulisine (Apidra; Sanofi, Paris, France) and glucose. Infusions of glulisine were discontinued at least 30 min before administration of Gla-300. Prior to clamp start, the majority of participants required glucose infusion to reach the clamp target, in line with the mild hyperinsulinaemia caused by the previous fixed daily doses of Gla-300, which were slightly above participants' usual basal insulin dose. Rescue insulin (insulin glulisine) was to be given if blood glucose levels exceeded 13.9 mmol/l (250 mg/l) for >30 min, although this did not occur for any participant.

Serum insulin glargine concentration (INS) was determined using a validated radioimmunoassay with a lower limit of quantification (LLOQ) of 5.02 μ U/ml [30.1 pmol/l (1 μ U/ml = 6 pmol/l)]. Blood samples to assess insulin concentrations were collected 4 h before dosing, immediately pre-dose (time 0) and at 1, 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 h after glargine administration. To exclude falsely high pre-dose values attributable to residual prandial insulin contribution, insulin concentration values at time 0 were replaced by values obtained 24 h after injection of study medication, for statistical inferences and visualization of profiles.

To characterize Gla-300 pharmacologically in this study, an automated clamp was chosen over a manual clamp. Automated clamps establish tight glucose control and provide a bias-free assessment of glucose utilization, but the more frequent measurement of blood glucose levels and adjustment of glucose delivery compared with a manual clamp results in greater minute-by-minute variability of the glucose infusion rate (GIR); this requires mathematical smoothing techniques in order to visualize a glucodynamic profile.

Pharmacokinetic and Pharmacodynamic Assessments

The INS profiles were characterized according to the area under the INS time curve from time 0 to 24 h post dosing (INS-AUC_{0–24}), the time to 50% of INS-AUC_{0–24}, as well as maximum INS concentration (INS-C_{max}). Body-weight-standardized GIR profiles were characterized by the area under the unsmoothed GIR time curve from time 0 to 24 h post dosing (GIR-AUC_{0–24}), the time to 50% of GIR-AUC_{0–24} and the maximum of the

body-weight-standardized smoothed GIR profiles (GIR_{max}). Further assessments included distribution of exposure and effect over time.

Safety Assessments

Safety assessments, performed in all participants, included adverse events, electrocardiogram variables, vital signs, clinical laboratory measurements, anti-insulin antibodies and local tolerability.

Statistical Analyses

Bioequivalence of Gla-300 Formulations. Point estimates of treatment ratios (T : R), with 90% confidence intervals (CIs), were calculated using a linear mixed effects model (SAS[®] PROC MIXED) based on log-transformed data and re-transformations. Differences between treatments for the time to 50% of INS- and $GIR-AUC_{0-24}$ were analysed non-parametrically based on a Hodges–Lehmann method for paired treatment comparisons; 90% CIs for location shifts between treatments (T – R) were derived.

Within-Day Variability (Fluctuation). Within-day variability in exposure was assessed as the peak-to-trough ratio ($PTR = INS-C_{max}/INS-C_{min}$), the peak-to-trough fluctuation relative to the average INS [$PTF = (INS-C_{max} - INS-C_{min})/INS-C_{avg}$], the bidirectional excursion [$BDE = (INS-C_{max} - INS-C_{min})/2$] and the swing [$(INS-C_{max} - INS-C_{min})/INS-C_{min}$].

Within-day variability in insulin action was calculated as the area between unsmoothed GIR curves and average GIR over the clamp period, per minute.

Between-Day Variability (Reproducibility). To assess within-subject variability, the intra-individual coefficients of variation (CV%; geometric type) of PK and PD variables were calculated from the mean sum of the error terms as calculated by a mixed effects model (using SAS[®] PROC MIXED), evaluating differences in these log-transformed variables between treatment groups. Between-subject CV% was also estimated.

Results

Study Performance

In total, 38 men and 12 women with T1DM, with a mean [standard deviation (s.d.)] age of 42.1 (11.1) years and a mean (s.d.) BMI of 25.4 (3.1) kg/m² [men: age 41.2 (11.1) years, weight 83.2 (11.4) kg, BMI 25.8 (3.1) kg/m²; women: age 45.2 (11.0) years, weight 67.2 (9.0) kg, BMI 24.0 (2.5) kg/m²] on an average basal insulin dose of 0.35 U/kg/day, were randomized, and all participants completed the study. For one participant under treatment R, all serum insulin concentrations were below the LLOQ, therefore, the total number of evaluable profiles for PK analysis was 99 (n = 49 for R and n = 50 for T).

The mean (s.d.) injected doses were 31.62 (5.03) and 31.68 (5.10) U, for treatment R and treatment T, respectively, after accounting for rounding.

Apart from two participants presenting with transitional dawn phenomena in both clamps and a further three in one clamp, blood glucose control aiming for 5.6 mmol/l (100 mg/dl)

was tight, with a median individual average of 5.6 mmol/l (101 mg/dl) at a median individual CV% of 6.4%. On average, participants' blood glucose levels remained ≤ 5.8 mmol/l (105 mg/dl) for the full 24 h (median). The dose of 0.4 U/kg was well adjusted to the participants' needs, as attested by the low glucose demand of only 1.27 mg/kg/min (in total, a mean of 145 g glucose per participant was used during 24 h).

One participant had a very low GIR-AUC at sizeable INS values in one treatment period, with good blood glucose control; these data were included in the analyses as pre-specified. A second participant had no detectable INS at sizeable GIR values in one period, before (4 h pre-dose and time 0) as well as after Gla-300 administration, presumably as a result of technical issues; these INS values were set to missing, while otherwise INS values <LLOQ were set to zero for statistical inferences and visualization of profiles.

Bioequivalence: Reproducibility Between Formulations

For both systemic exposure to Gla-300 and its metabolic effect, bioequivalence between the two treatments was established, as the 90% CIs for the ratio of T : R were within the predefined 0.80–1.25 range (Table 1).

Fluctuation: Within-Day Variability

The PK and PD profiles for each treatment period are shown in Figure 1, and profiles for each participant are shown in Figure S2. Cumulative exposure to Gla-300 ($INS-AUC_{X-Y}/INS-AUC_{0-24}$) developed almost linearly over the 24-h clamp period, with ratios of 0.55 and 0.45 for each 12-h time period (Figure 2A, Table S1). This was reflected in the almost linear cumulative metabolic effect and ratios of 0.53 and 0.47 for each 12-h time period (Figure 2A, Table S1).

Individual absolute INS-AUC and GIR-AUC, as well as the percentage of these parameters observed within each 6-h interval, are shown in Figure 2B. Together with the descriptive statistics of exposure and activity distribution shown in Table S1, Figure 2B shows the rather homogenous within-subject insulin supply and activity over the 24-h interval, despite wide between-subject variability in absolute exposure and activity. The data shown in Figure 2B are also reflective of the generally wider variability in glucose utilization compared with insulin exposure.

Average INS exposure ($INS-C_{avg}$) was 11.3 μ U/ml (68 pmol/l), with a BDE of just 3.3 μ U/ml (20 pmol/l) (Table 2). Diurnal fluctuation in exposure (within-day variability) was low, with a PTR of <2 (Table 2); correspondingly the swing and PTF were <1.

Average GIR (GIR_{avg}) was 1.27 mg/kg/min. Median fluctuation in GIR (within-day PD variability) was 1.0 (interquartile range 0.8–1.1) mg/kg/min. This fluctuation was calculated using unsmoothed GIR data, as the type of smoothing may have a major impact on the calculated variability (Figure S3).

Reproducibility: Between-Day Variability

Reproducibility of total insulin exposure was high, as the within-subject variability (CV%) in insulin exposure was only 17.4% for $INS-AUC_{0-24}$ and 33.4% for $INS-C_{max}$ (Table 3).

Table 1. Equivalence of insulin glargine 300 U/ml formulations in exposure and activity.

	T (Gla-300 + polysorbate-20)	R (Gla-300)	Point estimate (90% CI)
Variable	Geometric mean		Treatment ratio: T : R
INS-AUC ₀₋₂₄ , $\mu\text{U}\cdot\text{h}/\text{ml}$	270	273	1.00 (0.95–1.06)
INS-C _{max} , $\mu\text{U}/\text{ml}$	15.8	15.6	1.02 (0.91–1.14)
GIR-AUC ₀₋₂₄ , mg/kg	1531	1495	1.02 (0.87–1.20)
	Median		Hodges-Lehman shift: T – R
T _{50%} -INS-AUC ₀₋₂₄ , h	10.8	10.7	0.23 (0.01–0.46)
T _{50%} -GIR-AUC ₀₋₂₄ , h	11.4	11.3	–0.33 (–1.04 to 0.38)

Gla-300, insulin glargine 300 U/ml; CI, confidence interval; INS, serum insulin glargine concentration; INS-AUC₀₋₂₄, area under the INS time curve from time 0 to 24 h post dosing; INS-C_{max}, maximum INS; GIR, glucose infusion rate; GIR-AUC₀₋₂₄, area under the GIR time curve from time 0 to 24 h post dosing; GIR_{max}, maximum GIR (based on individually smoothed profiles, LOESS factor 0.06); T, test treatment; R, reference treatment.

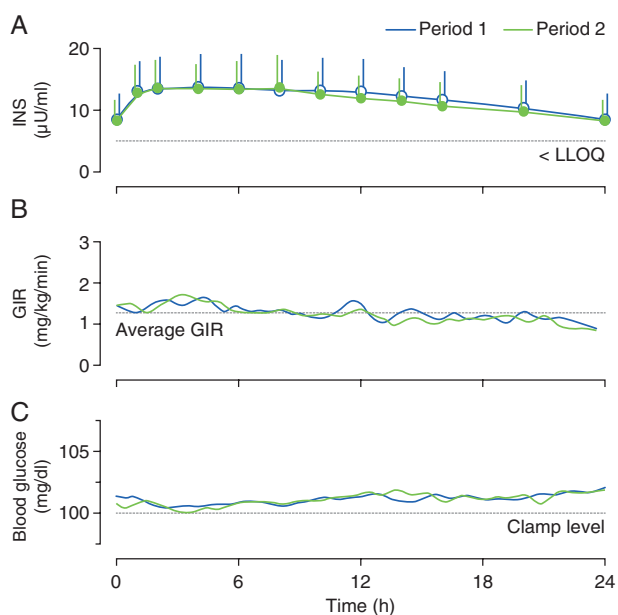


Figure 1. Pharmacokinetic and pharmacodynamic profiles at steady state, by treatment period. Profiles of (A) mean (standard deviation) serum insulin concentration (INS), lower limit of quantification (LLOQ) = 5.02 $\mu\text{U}/\text{ml}$. Two individual far outside values excluded (one in period 1, hour 14 and one in period 2, hour 8). (B) Mean smoothed (LOESS factor 0.06) body-weight-standardized glucose infusion rate (GIR); and (C) mean smoothed (LOESS factor 0.06) blood glucose, with a clamp level of 100 mg/dl.

Excluding outside values [one on treatment R and two on treatment T, defined as having INS values more than three times the interquartile range above the 75th percentile (Q3); >31.4 $\mu\text{U}/\text{ml}$ (188 pmol/l)], the within-subject CV% was reduced to 15.3% for INS-AUC₀₋₂₄ and 19.4% for INS-C_{max}. Reproducibility of the total metabolic effect was lower than that of exposure, reflected in the higher CV% values for GIR-related variables (Table 3). Between-subject variability (CV%) in PK and PD variables is also shown in Table 3.

Safety

The results showed that Gla-300 was generally well tolerated, irrespective of the treatment formulation, and the rates of adverse events were similar between treatments. The frequency

of adverse events was similar for both treatments, occurring in 18 participants while taking the test treatment and in 15 while taking the reference treatment. The most common adverse events were headaches, presumably caused by the clamp procedure, and occurred in 10 participants while taking the test treatment and in 11 while taking the reference treatment. No serious adverse events or deaths were reported during the study. At baseline, 30 participants were negative for anti-insulin antibodies while 20 were positive. In four participants, a conversion from negative to positive anti-insulin antibodies occurred during the treatment period; anti-insulin antibody status remained unchanged in all other participants.

Discussion

The advent of Gla-100 created the concept of a basal-bolus regimen to replace missing or inadequate insulin secretion, or to support failing oral antihyperglycaemic therapy with basal insulin supplementation. Clinical experience with basal insulin supplementation stipulated the design of long-acting insulin products with the most even supply profile possible. Ideally, this low diurnal fluctuation should be accompanied by high day-to-day reproducibility, should allow flexibility in the preferred once-daily dosing interval, and yet should still allow quick dose adjustments. Gla-300 aims to achieve these goals. The more sustained activity beyond 24-h with Gla-300 versus Gla-100, and the short time to steady state, are reported elsewhere [4].

The present study demonstrated bioequivalence of two Gla-300 formulations in terms of PK and PD variables, showing that whether people administer Gla-300 using a vial and syringe, or using an insulin pen, the injected insulin has the same effect. In addition, the crossover design of this equivalence study allowed aspects of variability to be assessed.

Successful treatment with basal insulin products can be adversely affected by variability in glucose response resulting from variability in insulin supply in terms of large diurnal fluctuations and low reproducibility. Although insulin variability can be measured both in terms of exposure (PK) and metabolic effect (PD), only insulin concentrations are a direct measure of variability attributable to the insulin product [3]. The PD response, by contrast, reflects not only the availability of insulin, but largely the within-subject variability in insulin sensitivity and demand, as well as the smoothing algorithm

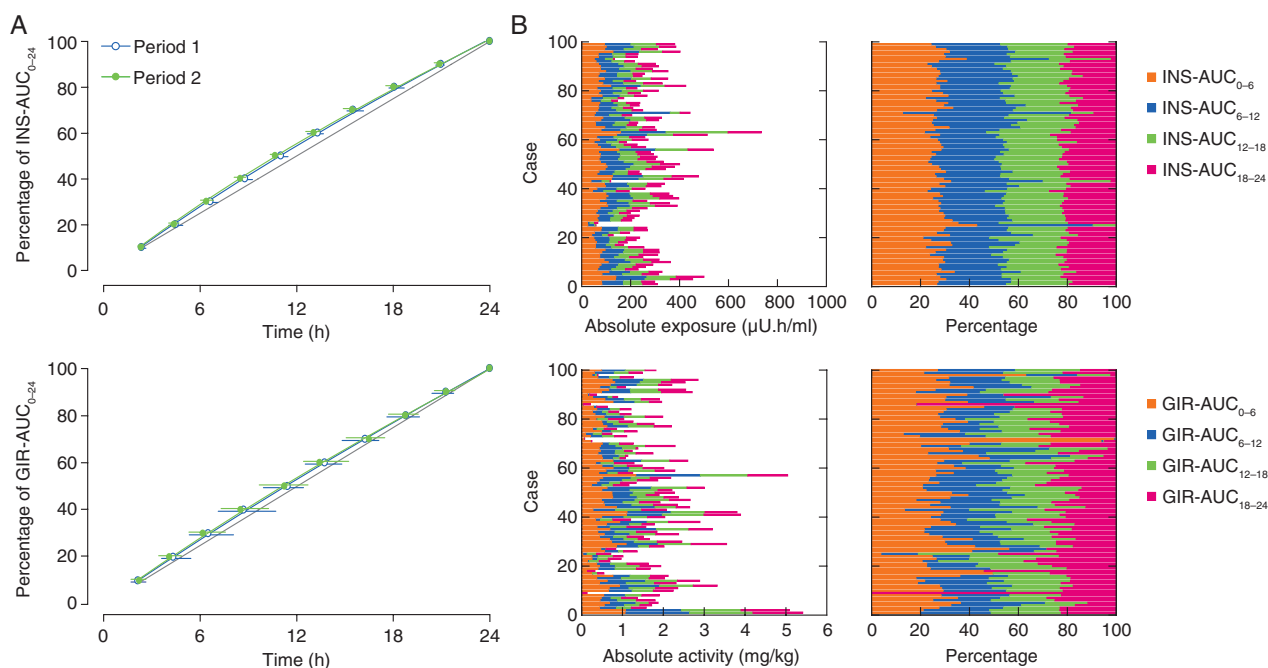


Figure 2. Distribution of insulin glargine 300 U/ml (Gla-300) exposure and activity over 24 h. (A) Mean (interquartile range) time to a given percentage of total exposure and activity (by treatment period). (B) Absolute, and percentage of total, exposure and activity per 6-h time period (by case). Grey lines in (A) represent a perfectly even distribution; cases in (B) are sorted by participant and treatment period; AUC, area under the curve; GIR, body-weight-standardized glucose infusion rate; INS, serum insulin concentration.

Table 2. Within-day variability of insulin glargine.

	INS- C_{\max} , $\mu\text{U/ml}$	INS- C_{avg} , $\mu\text{U/ml}$	INS- C_{\min} , $\mu\text{U/ml}$	BDE, $\mu\text{U/ml}$	PTR	Swing	PTF
n	99	99	99	99	90	90	99
Median	15.0	11.3	8.2	3.3	1.8	0.8	0.6
(interquartile range)	(12.8–17.9)	(9.6–13.8)	(6.5–10.3)	(2.6–4.6)	(1.5–2.1)	(0.5–1.1)	(0.4–0.7)

BDE, bidirectional excursion; INS, serum insulin glargine concentration; INS- C_{avg} , average INS; INS- C_{\max} , maximum INS; INS- C_{\min} , minimum INS; PTF, peak-to-trough fluctuation; PTR, peak-to-trough ratio.

required to visualize the demand profile [1,3]. In addition, PD response is not perfectly synchronized with changes in observed insulin concentrations [9], as displayed by the subtle difference between ratios of INS- and GIR-AUC fractions in this and previous studies [10,11].

Foremost, the results of this study suggest that a therapeutic dose of Gla-300 confers a low level of fluctuation at a high level of reproducibility in exposure, in steady-state conditions in people with T1DM. The bidirectional fluctuation in exposure around the average concentration of 11.3 $\mu\text{U/ml}$ [68 pmol/l; close to a physiological 10 $\mu\text{U/ml}$ (60 pmol/l)] was 3.3 $\mu\text{U/ml}$ (20 pmol/l), which is reflected in a peak-to-trough fluctuation [as defined by the calculation $(\text{INS-}C_{\max} - \text{INS-}C_{\min})/\text{INS-}C_{\text{avg}}$] of 0.6. This is less than the 1.6 observed with Gla-100 (calculated from a previous study [4]) and similar to the values that can be estimated for insulin degludec [12] and PEG-lispro [13], assessed in similar studies.

The fairly even distribution of Gla-300 exposure over 24-h sees the weakly expressed maxima within the first 6 h, in line with a shift from ~ 12 h for the first injection due to accumulation towards steady-state supply within 3–4 days. Using

a different retarding principle (formation of multi-hexamer complexes upon subcutaneous injection and subsequent binding to plasma albumin), insulin degludec is reported to show a similar even distribution of exposure and activity at steady state in people with T2DM [14] and T1DM [10]. In a study of people with T2DM, Heise et al. [11] reported an almost equal distribution (12-h fractions) of insulin degludec activity over 24 h at steady state, irrespective of dose (GIR-AUC₀₋₁₂/GIR-AUC₀₋₂₄ of 0.49, 0.53 and 0.50 at 0.4, 0.6 and 0.8 U/kg), at exposure ratios (INS-AUC₀₋₁₂/INS-AUC₀₋₂₄) of 0.53 for all doses. In contrast, the 6-h fractions of total activity showed considerable variability (0.27, 0.22, 0.20 and 0.31) for a therapeutic dose of 0.4 U/kg, while the two higher doses present fractions that are more strongly aligned. Corresponding 6-h fractions of insulin degludec activity in people with T1DM maintained a fairly constant distribution across doses, but with the majority of activity apparently occurring between 6 and 18 h [12].

Recently, a study comparing Gla-100 with a long-acting insulin product using hydrodynamic size as the absorption retarding principle, with strong albumin binding (PEG-lispro; LY2605541), presented Gla-100 activity with 0.25 for the first

Table 3. Between-day variability in pharmacokinetic and pharmacodynamic variables.

	Within-subject CV% (90% CI)	Between-subject CV% (90% CI)
INS-AUC ₀₋₂₄ *	17.4 (15–21)	34.8 (29–43)
	15.3 (13–19)†	34.1 (28–43)†
INS-C _{max} *	33.4 (28–41)	25.6 (16–35)
	19.4 (17–24)†	23.0 (18–30)†
GIR-AUC ₀₋₂₄ ‡	34.8 (30–42)	43.2 (34–55)
GIR _{max} ‡	27.9 (24–34)	23.9 (16–32)

AUC, area under the curve; CI, confidence interval; CV%, coefficient of variation; INS, serum insulin concentration; GIR, body weight standardized glucose infusion rate.

*CV% is of geometric type.

†Excluding outliers. Three participants excluded from this analysis owing to presenting INS-C_{max} outlier values (>31.4 µU/ml), one participant on the reference treatment and two on the test treatment.

‡CV% is based on untransformed data.

6 h, declining to 0.17 for the 18–24-h fraction, while PEG-lispro activity increased from 0.18 to 0.24 over the same time periods [15]. Similarly, in Trial 1993 comparing insulin degludec with insulin glargine, most of the insulin glargine activity was observed in the first 12–18 h after dosing (31, 29 and 23% for the first three 6-h periods, respectively, then declining to 17% from 18 to 24 h) [12]. By comparison, Gla-300 presents with sustained, and thus more evenly distributed, activity over 24 h compared with Gla-100. This is in line with a previous study showing that Gla-300 has more even PK and PD profiles than Gla-100, with a reduced swing in insulin concentrations [4]. In the present study, within-day variability (fluctuation) in insulin exposure with Gla-300 was low over the entire 24-h period, and the swing was similar to that previously reported [4]. This low diurnal fluctuation in insulin exposure is expected to be of clinical relevance by reducing an individual's risk of hyperglycaemia and hypoglycaemia. It is possible that a reduced hypoglycaemic risk may alleviate the fear of such episodes, helping to remove one of the main barriers to glycaemic management [16].

It should be noted that, although an even distribution of glucose utilization also suggests low fluctuation in activity, assessing this latter variable requires prior smoothing of raw data profiles by predefined algorithms, which can render the results arbitrary. Applying a conservative LOESS smoothing procedure (such as a factor of 0.06) to visualize GIR profiles therefore results in greater fluctuation compared with stronger smoothing.

The low variability in exposure with Gla-300 is related to the mechanism of protraction. The PK results indicate that, compared with Gla-100, Gla-300 has a more gradual and prolonged release of insulin glargine from the subcutaneous depot at the injection site [4]. The resulting apparent extended terminal half-life (INS-t_{1/2}) of 17–19 h confers accumulation of steady-state concentrations within 3–4 days with Gla-300, compared with 2 days for Gla-100 with an INS-t_{1/2} of 12 h, flattening the exposure profile. In addition to demonstrating the low within-day fluctuation of Gla-300, the present study also shows that the between-day (within-subject) variability in total exposure to Gla-300 (CV% of INS-AUC₀₋₂₄) was numerically

lower, at 17.4%, than previously demonstrated for Gla-100 in a similarly designed study [1]. In a clinical setting, high reproducibility would be a major advantage when titrating an individual's insulin dose, owing to a more predictable insulin exposure.

As expected, the reproducibility of PD variability was lower than that of PK variability, demonstrated by the between-day within-subject CV% values. It has previously been shown [1] that the glucodynamic response variability of clinically relevant doses in modestly hyperinsulinaemic euglycaemic clamp experiments is approximately twice that of the PK variability. This observation is confirmed by replicate studies in T1DM with rapid-acting insulin analogue products at 0.3 U/kg, presenting a PK variability of 7% at a PD variability of 17% (data not shown).

The latter observation also indicates that shifting the activity towards the linear portion of the insulin exposure–effect relationship, as demonstrated with rapid-acting analogue products, reduces the impact of individual between-day differences in the metabolic state and lowers variability *per se*. Measuring and comparing PD variability is therefore prone to confounding from factors not related to insulin release, and so these results must be interpreted carefully.

For example, a recent study compared PD variability between insulin degludec and insulin glargine in people with T1DM [17]. The results of that study suggested that insulin degludec was associated with a reduction in PD variability compared with Gla-100, using parameters derived from GIR profiles. Notably, the average glucose utilization was higher with insulin degludec. A previous study of Gla-100 in healthy individuals reported CV% values for GIR-AUC₀₋₂₄ and GIR_{max} of 34 and 36%, similar to the 35 and 28% seen in the present study with Gla-300 in T1DM, rather than the 82 and 60% reported by Heise et al. [17]. In addition to differing study populations, limitations of such comparisons lie in the different study designs used; parallel rather than crossover in the degludec trial, and quadruplicate [1] versus triplicate [17] or duplicate readings (in the present study).

The strengths of the present study include: the fact that the euglycaemic clamp was performed under steady-state conditions, reflecting real life more closely than a single-dose scenario; the inclusion of only individuals with T1DM who had negligible endogenous insulin production, which might otherwise have interfered with PK measurements; and the calculation of variability derived directly from insulin exposure. The main limitation of the study relates to the experimental clamp setting, as it is difficult to extrapolate such results directly to blood glucose variation experienced by people with diabetes in clinical practice; however, the results reported are consistent with previous studies of Gla-300 showing less glycaemic excursion and lower within-subject variability [18], as well as equivalent glycaemic control with less hypoglycaemia [5,6], compared with Gla-100.

In summary, once-daily administration of Gla-300 at a therapeutic dose of 0.4 U/kg provides predictable and evenly distributed 24-h coverage, owing to low diurnal fluctuation in insulin glargine exposure at a high level of between-day reproducibility.

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Conflict of Interest

R. H. A. B, I. N, L. T and K. B are employees of Sanofi. C. K is an employee and co-owner of Profil. C. K has received research funds from Boehringer Ingelheim, Dance Pharmaceuticals, Hoffmann LaRoche, Johnson&Johnson, Eli Lilly, Novo Nordisk, Novartis, Noxxon, Sanofi and Servier. C. K has received speaker and travel grants from Sanofi.

R. H. A. B contributed to the study conception and design, data analysis and interpretation, and was responsible for the development of the manuscript. I. N, L. T, K. B and C. K contributed to the study conception, design, data analysis and discussion, and reviewed/edited the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Study design.

Figure S2. Serum insulin concentration, body-weight-standardized glucose infusion rate and blood glucose profiles after multiple doses in steady state, by treatment period and participant.

Figure S3. The effect of smoothing algorithms on body-weight-standardized glucose infusion rate profiles.

Table S1. Within-day distribution of area under the serum insulin concentration time curve and the area under the unsmoothed glucose infusion rate time curve as 6- and 12-h fractions of total area under the curve.

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