Pharmacokinetics, Pharmacodynamics, and Modulation of Hepatic Glucose Production With Insulin Glargine U300 and Glargine U100 at Steady State With Individualized Clinical Doses in Type 1 Diabetes

Francesca Porcellati,[1] Paola Lucidi,[1] Paola Candeloro,[1] Patrizia Cioli,[1] Anna Marinelli Andreoli,[1] Gianluca Curti,[2] Geremia B. Bolli,[1] and Carmine G. Fanelli[1]

Diabetes Care 2019;42:85–92 | https://doi.org/10.2337/dc18-0706

OBJECTIVE

This study characterized the pharmacokinetics (PK), pharmacodynamics (PD), and endogenous (hepatic) glucose production (EGP) of clinical doses of glargine U300 (Gla-300) and glargine U100 (Gla-100) under steady-state (SS) conditions in type 1 diabetes mellitus (T1DM).

RESEARCH DESIGN AND METHODS

T1DM subjects (*N* = 18, age 40 ± 12 years, T1DM duration 26 ± 12 years, BMI 23.4 ± 2 kg/m^2, A1C 7.19 ± 0.52% [55 ± 5.7 mmol · mol−1^{-1}]) were studied after 3 months of Gla-300 or Gla-100 (evening dosing) titrated to fasting euglycemia (random, crossover) with the euglycemic clamp using individualized doses (Gla-300 0.35 ± 0.08, Gla-100 0.28 ± 0.07 units · kg^{-1}).

RESULTS

Plasma free insulin concentrations (free immunoreactive insulin area under the curve) were equivalent over 24 h with Gla-300 versus Gla-100 (point estimate 1.11 [90% CI 1.03; 1.20]) but were reduced in the first 6 h (0.91 [90% CI 0.86; 0.97]) and higher in the last 12 h postdosing (1.38 [90% CI 1.21; 1.56]). Gla-300 and Gla-100 both maintained 24 h euglycemia (0.99 [90% CI 0.98; 1.0]). The glucose infusion rate was equivalent over 24 h (1.03 [90% CI 0.88; 1.21]) but was lower in first (0.77 [90% CI 0.62; 0.95]) and higher (1.53 [90% CI 1.23; 1.92]) in the second 12 h with Gla-300 versus Gla-100. EGP was less suppressed during 0–6 h but more during 18–24 h with Gla-300. PK and PD within-day variability (fluctuation) was 50% and 17% lower with Gla-300.

CONCLUSIONS

Individualized, clinical doses of Gla-300 and Gla-100 resulted in a similar euglycemic potential under SS conditions. However, Gla-300 exhibited a more stable profile, with lower variability and more physiological modulation of EGP compared with Gla-100.

[1]*Section of Endocrinology and Metabolism, Department of Medicine, Perugia University School of Medicine, Perugia, Italy*
[2]*Section of Occupational Medicine, Department of Medicine, Perugia University School of Medicine, Perugia, Italy*

Corresponding author: Geremia B. Bolli, geremia.bolli@unipg.it

Received 31 March 2018 and accepted 24 August 2018

Clinical trial reg. no. EudraCT 2015-002135-17, http://eudract.ema.europa.eu/

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc18-0706/-/DC1.

Insulin glargine 300 units · mL^{-1} (Gla-300) is a three-times concentrated glargine 100 units · mL^{-1} (Gla-100); that is, the same units are contained in one-third of the volume (1). Because of its smaller depot and surface area after subcutaneous injection, Gla-300 results into slower and more prolonged absorption, with pharmacokinetics (PK) and pharmacodynamics (PD) different from those of Gla-100 when given at the same fixed dose (0.4 and 0.6 units · kg^{-1}) in subjects with type 1 diabetes mellitus (T1DM) (1). At the same fixed doses, Gla-300 mimics better than Gla-100 the physiology of basal insulin, which in the fasting state has low concentration and is flat, with no definite peak (2). When Gla-300 is compared with Gla-100 in T1DM and type 2 diabetes mellitus in the clinical EDITION program studies, Gla-300 is noninferior in glycemic control and results in a lower risk of hypoglycemia, primarily nocturnal (3–8), as well as in lower day-to-day glucose variability (9).

The slower subcutaneous absorption exposes Gla-300 to greater degradation by proteolytic enzymes (1). In fact, identical doses (0.4 units · kg^{-1} s.c.) of Gla-300 and Gla-100 resulted in lower 24-h plasma insulin exposure (by 17%) and correspondingly lower compensatory exogenous glucose infusion with Gla-300, although with a different distribution profile in T1DM (1). The PK/PD data obtained with the same, fixed insulin doses of Gla-300 and Gla-100 (1) are not necessarily applicable to the clinical situation of T1DM where subjects use individual insulin doses that are generally higher with Gla-300 to compensate for its lower bioavailability when given subcutaneously (6). Individualizing the dose of basal insulin is not only a critical way to ensure that the clamp meets its objective to produce euglycemia but also a meaningful approach to produce PK/PD data of a given basal insulin representative of the needs of the individual subject studied.

The aim of the current study was to characterize PK, PD, and the modulation of endogenous (hepatic) glucose production (EGP) at steady state (SS) with Gla-300 versus Gla-100 using the euglycemic clamp after subcutaneous injection of the individual insulin doses needed in subjects with T1DM to maintain fasting euglycemia.

RESEARCH DESIGN AND METHODS

Subjects

Subjects attending the Diabetes Clinic, Section of Endocrinology and Metabolism, Department of Medicine, University of Perugia Medical School, were enrolled and studied after giving informed written consent, provided they were diagnosed with T1DM, were aged ≥18 but ≤65 years, and with disease duration ≥5 years, A1C ≥6.5% (48 mmol · mol^{-1}) to ≤8.5% (69 mmol/mol), and BMI >20 to ≤27 kg/m^2. Eighteen subjects were studied (Supplementary Table 1). They were all on a basal-bolus insulin regimen and free of detectable micro- and macrovascular complications.

Study Design

This was an investigator-initiated, randomized, single-blind (clamp investigator), two-treatment, two-period, two-sequence crossover study using the euglycemic glucose clamp technique (Supplementary Fig. 1). After 2 weeks of run-in, during which subjects continued the basal-bolus regimen they were on, a computer-generated sequence was used to randomize subjects to treatment with basal insulin Gla-100 (Lantus SoloStar pen; Lantus; Sanofi, Paris, France) or Gla-300 (Toujeo, Toujeo SoloStar pen; Sanofi), both evening dosing, for 3 months. During this period, the subjects titrated basal and prandial insulin continuing the basal-bolus regimen (see below). At the end of the 3 months, subjects underwent the first euglycemic glucose clamp after (evening) a subcutaneous injection of the individual dose of Gla-100 or Gla-300 they were on during the previous 2 weeks. Subjects then underwent a 2-month washout, resuming the basal insulin treatment they were on before randomization. After the subjects were crossed over to the other basal insulin for 3 more months, during which the doses were titrated as in the first period, they were then studied with the second euglycemic glucose clamp following the same procedure as with the first clamp. The study protocol was approved by the Ethical Study Committee of Umbria Region (CEAS) and registered in EudraCT (2015-002135-17). EudraCT, European Union Drug Regulating Authorities Clinical Trials, is the European Clinical Trials database of all clinical trials of investigational medicinal products with at least one site in the EU.

Basal Insulin Titration

Basal insulin titration was an open-label process once-twice per week based on frequent face-to-face or telephone (including e-mail, smartphone applications) contacts during both treatment periods. At baseline, all subjects were familiar with frequent self-monitoring of blood glucose (SMBG) (six to eight plasma glucose [PG] measurements daily before and 2 h after each meal, mid-afternoon, and bedtime, and at least twice weekly at 0300 h). After randomization, Gla-100 and Gla-300 (dosing at 2000 h, ±30 min) were both titrated aiming at fasting PG (FPG) between 90 and 110 mg · dL^{-1}. Dose change decision was made no more frequently than 3–4 days based on the difference between bedtime and fasting PG on days in which postdinner PG was 100–130 mg · dL^{-1} (Supplementary Table 2) (10).

Clamp Procedure

The principle of the hyperinsulinemic-euglycemic glucose clamp was used in the current study (Supplementary Fig. 2) (11). The euglycemic feedback (12) and clamp procedure for the purpose of examining PK/PD of long-acting insulin analogs has previously been described in detail (13–15). The study was blind to the clamp investigators who were not aware of the basal insulin treatment the subjects were on. In brief, subjects were admitted to the Clinical Research Center of Department of Medicine, University of Perugia Medical School, between 1430 and 1500 h of the first study day, ~1.5 h after the end of their usual lunch preceded by a subcutaneous injection of rapid-acting insulin dose. They were put at bedrest and maintained supine and fasted until 2000 h the next day. Two superficial venous lines were started. A hand vein of one arm was cannulated retrogradely with a 21-gauge butterfly needle, and the hand was maintained in a hot box or pad (~65°C) for intermittent sampling of arterialized venous blood (16) for PG and hormone measurements. A superficial vein of the contralateral arm was cannulated with an 18-gauge catheter needle for an intravenous (i.v.) infusion of insulin and/or 20% dextrose solution as needed. At 1500 h, a feedback i.v.

infusion of human regular insulin (regular insulin [Eli Lilly Italia SpA], diluted to 1 unit · mL^{-1} in 100 mL saline solution containing 2 mL of the subject's blood), was initiated, whenever needed, using a syringe pump (Injectomat MC Agilia; Fresenius Kabi, Hessen, Germany) to maintain PG at 100 mg · dL^{-1} according to a modified algorithm (13).

At −180 min from time "0 min," (t_0) a primed sterile, pyrogen-free constant infusion (0.222 μmol · kg^{-1} · min^{-1}) of [6,6-$^2H_2$]glucose (Cambridge Isotopes Laboratories, Cambridge, MA) was started and maintained throughout to determine glucose kinetics. During the clamp, a variable rate of infusion of 20% glucose with 2% [6,6-$^2H_2$]glucose was used to avoid non-SS errors (17) in the measurement of glucose turnover, as described previously (18). A peristaltic pump (Volumat MC Agilia; Fresenius Kabi) was used for infusion of glucose, whenever needed, to prevent fall of PG below 95 mg · dL^{-1} during feedback and clamp periods. At 2000 h, the dose of basal insulin, either Gla-100 or Gla-300, the individual subjects were receiving over the last 15 days, was injected subcutaneously 2 cm to the right or left of the umbilicus by a SoloStar pen (4-mm needle, skinfold technique), and subjects underwent the euglycemic clamp for 24 h.

Analytical Methods

Bedside PG was measured in triplicate every 5–15 min using the YSI 2300 STAT glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma C-peptide was measured by radioimmunoassay (Linco Research, St. Charles, MO). The plasma free insulin concentration (free immunoreactive insulin [FIRI]) was measured by a commercial radioimmunoassay kit specific for human insulin (DRG Instruments GmbH, Marburg, Germany) with a range of detection of 0–200 μU · mL^{-1} after polyethylene glycol extraction of antibodies from the plasma (19). A1C was determined by high-performance liquid chromatography using an HI-Auto A1c HA-8121 apparatus (DIC, Daiichi, Kogaku Co., Ltd., Kyoto, Japan) that was Diabetes Control and Complications Trial (DCCT) aligned (upper limit in subjects without diabetes <6.1%). To determine glucose kinetics, arterialized venous blood samples were taken every 30 min for the first 6 h and then every 60–120 min throughout the studies. Glucose enrichment was determined on its penta-acetate (penta-*O*-acetyl-β-D-glucopyranose) derivative by gas chromatography–mass spectrometry (GC HP 6890 II, MS HP 5973/A; Hewlett-Packard Co., Palo Alto, CA) in electron impact ionization mode monitoring the ions 200 and 202 for the unlabeled and D-[6,6-$^2H_2$]glucose, respectively (20).

End Points

The primary study end point was the glucose infusion rate area under the curve (GIR-AUC) versus area under the time curve 0–24 h (GIR-$AUC_{0–24\ h}$). Secondary PD end points included GIR-$AUC_{(0–12\ h)}$, GIR-$AUC_{(12–24\ h)}$, GIR-$AUC_{(0–6\ h)}$, GIR-$AUC_{(6–12\ h)}$, GIR-$AUC_{(12–18\ h)}$, GIR-$AUC_{(18–24\ h)}$, maximum GIR (GIR_{max}), time to 50% of GIR-$AUC_{(0–24\ h)}$, and GIR fluctuations. Other secondary PK end points included FIRI-$AUC_{(0–24\ h)}$ and the various time intervals for FIRI: FIRI-$AUC_{(0–12\ h)}$, FIRI-$AUC_{(12–24\ h)}$, FIRI-$AUC_{(0–6\ h)}$, FIRI-$AUC_{(6–12\ h)}$, FIRI-$AUC_{(12–18\ h)}$, FIRI-$AUC_{(18–24\ h)}$, maximum FIRI concentration (C_{max}), time to 50% of FIRI-$AUC_{(0–24\ h)}$, swing [(FIRI-C_{max} − FIRI-C_{min})/FIRI-C_{min}], fluctuation, and insulin excursions. Other secondary end points were glucose fluxes (EGP, glucose utilization [GU]).

Calculations and Statistical Analyses

The linear trapezoidal rule was used to calculate the AUC for FIRI and GIR (untrasformed data). GIR data were smoothed by taking a three-point moving average to provide data for calculation of GIR_{max}. C_{max} was read directly from the plasma concentration-time data for each subject. To express within-day insulin concentration variability, the following indices were calculated: swing ([C_{max} − C_{min}]/C_{min}), fluctuation over 24 h (F24) in insulin concentration (quantified as cumulated AUCs of an individual's FIRI profile above and below the average FIRI over 24 h), relative degree of fluctuation (100% × [F24/C_{avg}]) and insulin excursion (C_{max} − C_{min}). Within-day GIR fluctuation around the average value was calculated as for FIRI, as previously reported (1). All calculations were made on untransformed data.

Glucose fluxes were calculated based on a non-SS assumption, and the total R_a and R_d were calculated by using a modified form of the Steele equation to account for the addition of stable labeled tracer to the exogenous glucose infusate (17). EGP (primarily hepatic) was obtained as the difference between R_a and the exogenous GIR during the clamp. GU was obtained by adding the GIR to the EGP (21). When the EGP yielded a negative number, EGP was assumed to be zero, and the corresponding GIR was assumed as GU.

Point estimates of treatment ratios (Gla-300–to–Gla-100), with 90% CIs, were calculated using ANOVA, which allowed for variation due to sequence, subjects nested within sequence, period, and treatment based on log-transformed data and retransformations. Time to 50% of GIR-$AUC_{(0–24\ h)}$ and time to 50% of FIRI-$AUC_{(0–24\ h)}$ were analyzed nonparametrically using the Wilcoxon rank sum test and Hodges-Lehmann estimates of the treatment differences computed with 90% CIs. Equivalence was to be concluded if the 90% CIs for the PK/PD AUC and C_{max} were completely contained within the interval 0.80–1.25. A total sample size of 18, using two one-sided tests on data from a two-period crossover design, was calculated to provide at least 80% power at a 5% significance level, assuming a true ratio of the means of 1.0 and the coefficient of variation of 0.22, to demonstrate that the 90% CI of the ratios of key PK or PD parameters between treatments would be contained within 0.80–1.25. Data are expressed as arithmetic means (SD), geometric means (95% CI) in tables, and as means and SE in figures. Statistical analysis was usually performed using NCSS 12/PASS 11 software (NCSS, LLC, Kaysville, UT).

RESULTS

Glycemic Control, Body Weight, and Insulin Doses Before Studies

Mean daily blood glucose over the week before studies (SMBG) with Gla-300 and Gla-100 was similar (149 ± 17 and 152 ± 19 mg· dL^{-1}, respectively). No hypoglycemia (PG ≤ 70 mg · dL^{-1}) was reported by SMBG during the 3 days before the studies. A1C similarly decreased from baseline to the end of the 3-month period of treatment with both Gla-300 and Gla-100 (Supplementary Table 1). Body weight did not change significantly from baseline to the end of treatment with Gla-300 and Gla-100 (Supplementary Table 1). The dose of basal insulin at the end of the 3-month period was higher with Gla-300 versus Gla-100,

with a difference of 0.071 units · kg^{-1} (90% CI 0.059; 0.083), whereas the dose of prandial insulin did not change, although the percentage of prandial insulin was lower with Gla-300 versus Gla-100 (Supplementary Table 1).

Intravenous Insulin and Glucose Infusion and PG Concentration Before Clamp Studies

On the first study day, PG at 1500 h (~1.5 h after end of lunch and 5 h before subcutaneous injection of basal insulin and clamp initiation at 2000 h (t_0) was similar with Gla-300 and Gla-100, and decreased slowly to euglycemia by 80 min before t_0, with no difference between the two insulins (Fig. 1). When appropriate, a low dose of i.v. insulin infusion to reach euglycemia was started between 5 and 4.5 h before t_0 and continued until 1.5 h before t_0 when it was virtually zero. The total amount of insulin infused (insulin infusion rate) before t_0 was lower with Gla-300 than with Gla-100 (insulin infusion rate-$AUC_{(-5\ to\ 0\ h)}$, 11.5 ± 7.3 vs. 19.5 ± 9.9 μU·kg^{-1}; 0.57 [95% CI 0.39; 0.81]). When needed, a glucose infusion was started 4.5 h before t_0. GIR increased more with Gla-300 than with Gla-100 until t_0 (GIR-$AUC_{(-5\ to\ 0\ h)}$, 90.1 ± 99.3 vs. 14.5 ± 17.9 mg · kg^{-1}; 7.9 [90% CI 4.17; 15.12]).

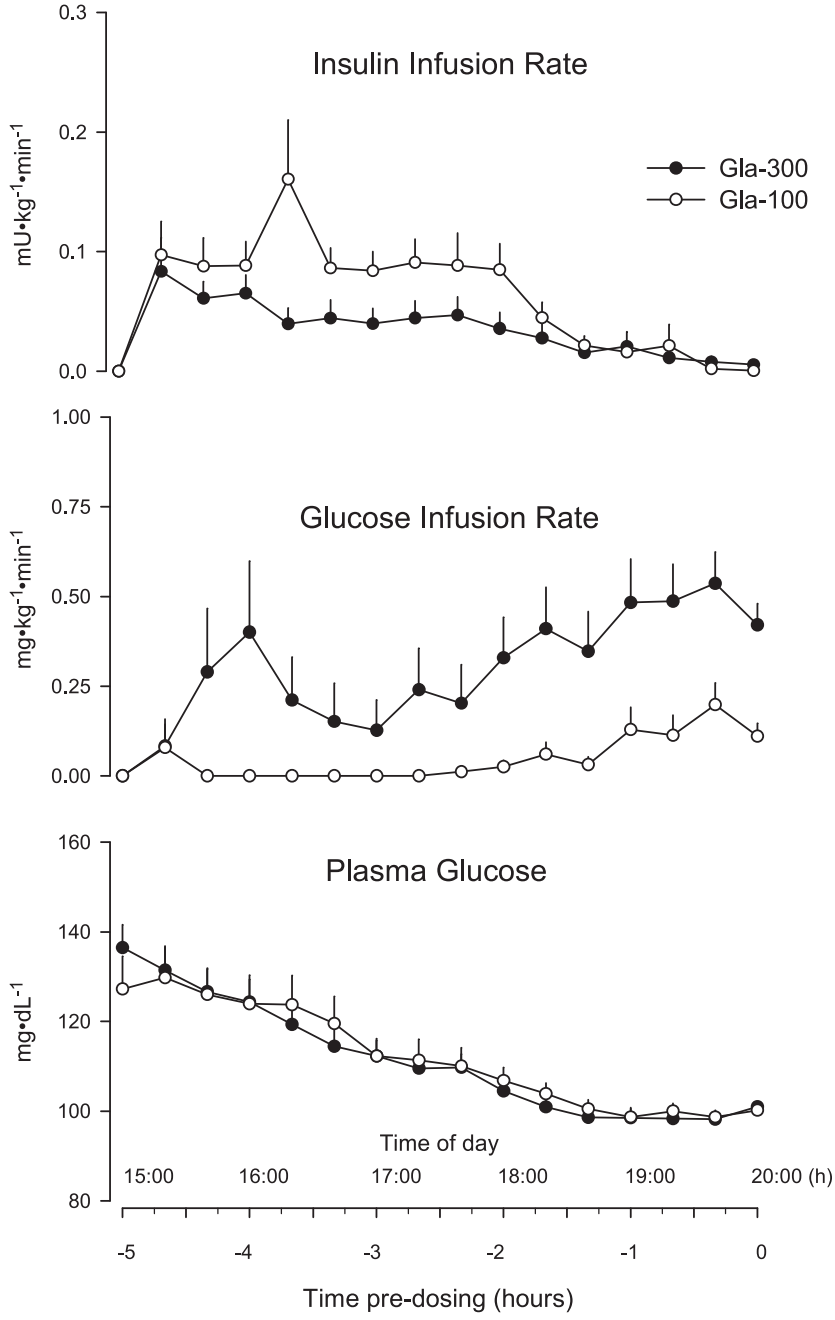

Figure 1—Rates of i.v. insulin and glucose infusions and PG concentration in the 5 h (1500–2000 h) before initiation of the clamp studies at t_0 (2000 h).

Plasma Insulin and Glucose Concentrations and GIR After Subcutaneous Injection of Gla-300 and Gla-100

At t_0, plasma insulin concentration was higher with Gla-300 versus Gla-100 (10.2 ± 1.0 μU · mL^{-1} and 8.2 ± 1.4 μU · mL^{-1}; 1.38 [90% CI 1.15; 1.66]). During the first 6 h after the subcutaneous insulin injection, insulin concentration increased less with Gla-300 than after Gla-100 (FIRI-$AUC_{(0–6\ h)}$, 0.91 [90% CI 0.86; 0.97]) (Table 1). Thereafter, plasma insulin plateaued similarly with Gla-300 and Gla-100 until 12 h, after which time FIRI-$AUC_{(12–24)}$ was 38% greater with Gla-300 until the end of the study (Table 1 and Fig. 2). The maximal serum insulin concentration was lower with Gla-300 versus Gla-100 (0.87 [90% CI 0.81; 0.92]) (Table 1). All indices of the within-day variability of plasma insulin concentration (swing, both absolute and relative fluctuation, and insulin excursion) indicated that this was lower by ~50% with Gla-300 compared with Gla-100 (Table 1).

GIR at t_0 was greater with Gla-300 versus Gla-100 (0.42 ± 0.25 vs. 0.11 ± 0.15 mg · kg^{-1} · min^{-1} (median difference, 0.3 [90% CI 0.17; 0.45]). The overall glucose infused to maintain euglycemia was no different between Gla-300 and Gla-100 over the full 24-h study period (GIR-$AUC_{(0–24\ h)}$, 1.03 [90% CI 0.88; 1.21]) (Table 1 and Fig. 2). However, after the subcutaneous injection, GIR increased less during the first 12 h with Gla-300 compared with Gla-100 (GIR-$AUC_{(0–12\ h)}$, 0.77 [90% CI 0.62; 0.95]) (Table 1), with the greatest difference observed in the first 6 h (GIR-$AUC_{(0–6\ h)}$, 0.71 [90% CI 0.56; 0.91]) (Table 1). Thereafter, GIR was similar up to 18 h, when the trend reversed with higher glucodynamic effect for Gla-300 compared with Gla-100 (GIR-$AUC_{(18–24\ h)}$, 1.91 [90% CI 1.37; 2.68]) (Table 1, Fig. 2, and Supplementary Fig. 3). The maximal GIR was lower by 15% with Gla-300 versus Gla-100 (Table 1 and Fig. 2). Similarly, absolute and relative

Table 1—PK and PD variables after subcutaneous injection of individual doses of insulin Gla-300 and insulin Gla-100 in subjects with T1DM in SS

	0.35 units · kg^{-1}	0.28 units · kg^{-1}	Gla-300–to–Gla-100 ratio
	Gla-300	Gla-100	Point estimate[a] (90% CI)
PK parameters			
FIRI-AUC$_{0–24\ h}$ (μU · h^{-1} · mL^{-1})*^	257 (208; 317)	231 (182; 294)	1.11 (1.03; 1.20)
FIRI-AUC$_{0–12\ h}$ (μU · h^{-1} · mL^{-1})*	139 (115; 168)	145 (116; 181)	0.96 (0.89; 1.04)
FIRI-AUC$_{12–24\ h}$ (μU · h^{-1} · mL^{-1})*	116 (91; 148)	84 (63; 113)	1.38 (1.21; 1.56)
FIRI-AUC$_{0–6\ h}$ (μU · h^{-1} · mL^{-1})*	71 (58; 86)	78 (63; 96)	0.91 (0.86; 0.97)
FIRI-AUC$_{6–12\ h}$ (μU · h^{-1} · mL^{-1})*	68 (57; 82)	67 (52; 86)	1.03 (0.92; 1.14)
FIRI-AUC$_{12–18\ h}$ (μU · h^{-1} · mL^{-1})	59 (47; 75)	48 (36; 63)	1.24 (1.20; 1.38)
FIRI-AUC$_{18–24\ h}$ (μU · h^{-1} · mL^{-1})*	56 (42; 74)	36 (25; 51)	1.58 (1.31; 1.88)
FIRI-C$_{max}$ (μU · mL^{-1})*	14.5 (12.1; 14.4)	16.7 (13.5; 20.8)	0.87 (0.81; 0.92)
$T_{50\%}$–FIRI-AUC$_{0–24}$ (h)§	10 (7; 12)	8.5 (7; 12)	0.5 (0; 1.0)
Swing[b]	1.0 (0.7; 1.4)	3.1 82.3; 4.2)	0.33 (0.26; 0.43)
Fluctuation (μU · mL^{-1})[c]	0.65 (0.5; 0.8)	1.20 (1.1; 1.4)	0.54 (0.45; 0.64)
Fluctuation (%)[d]	6.0 (4.1; 8.6)	12.0 (8.5; 16)	0.51 (0.45; 0.64)
ΔINS (μU · mL^{-1})[e]	7.1 (6; 8.3)	12.3 (10; 15)	0.57 (0.49; 0.67)
PD parameters			
GIR-AUC$_{0–24\ h}$ (mg · kg^{-1})*	981 (803; 1,198)	950 (758; 1,190)	1.03 (0.88; 1.21)
GIR-AUC$_{0–12\ h}$ (mg · kg^{-1})*	464 (345; 613)	604 (476; 766)	0.77 (0.62; 0.95)
GIR-AUC$_{12–24\ h}$ (mg · kg^{-1})*	482 (394; 590)	314 (228; 433)	1.53 (1.23; 1.92)
GIR-AUC$_{0–6\ h}$ (mg · kg^{-1})*	251 (172; 366)	353 (292; 426)	0.71 (0.56; 0.91)
GIR-AUC$_{6–12\ h}$ (mg · kg^{-1})*	165 (98; 277)	229 (153; 344)	0.72 (0.44; 1.18)
GIR-AUC$_{12–18\ h}$ (mg · kg^{-1})	137 (72; 261)	149 (88; 253)	0.92 (0.59; 1.44)
GIR-AUC$_{18–24\ h}$ (mg · kg^{-1})*	280 (230; 341)	144 (101; 205)	1.91 (1.37; 2.68)
GIR-C$_{max}$ (mg · kg^{-1} · min^{-1})*	1.5 (1.3; 1.7)	1.7 (1.5; 2.0)	0.85 (0.79; 0.91)
$T_{50\%}$–GIR-AUC$_{0–24}$ (h)§	11.7 (6.3; 18)	8.3 (4; 13.7)	3.35 (1.85; 5.15)
Fluctuation (mg · kg^{-1} · min^{-1})[c]	0.61 (0.5; 0.8)	0.73 (0.6; 0.9)	0.83 (0.74; 0.93)
Fluctuation (%)[d]	82 (65; 106)	102 (84; 124)	0.81 (0.70; 0.94)

Within-day GIR fluctuation around the average value was calculated as for FIRI. [a]Point estimates of treatment ratios with 90% CIs were calculated using a linear mixed-effects model based on log-transformed data and retransformations. *Data are geometric mean (95% CI). §Data are median (min; max) (N = 18). ^FIRI (serum insulin was measured after extraction of antibodies with 30% polyethylene glycol). §Point estimates for treatment effects are based on the Hodges-Lehmann estimate of the median difference with the associated 90% CI. [b]Swing: [(FIRI C$_{max}$ − FIRI C$_{min}$)/FIRI C$_{min}$]. [c]Fluctuation (F24): [(FIRI C$_{max}$ − FIRI C$_{min}$)/C$_{avg}$]. [d]Fluctuation (%): [(100% × F24)/FIRI C$_{avg}$]. [e]ΔINS: (FIRI C$_{max}$ − FIRI C$_{min}$).

fluctuations in GIR around the average were both lower, by 17% and 19%, respectively, with Gla-300 compared with Gla-100 (Table 1).

The PG concentration was no different during the 24-h clamp with Gla-300 (100.5 ± 1.2 mg · dL^{-1}) and Gla-100 (101.4 ± 1.8 mg · dL^{-1}; 0.99 [90% CI 0.98; 1.0]).

Glucose Fluxes (EGP and GU)

Data were available from 14 of the 18 subjects studied (the glucose enrichment was insufficient in 1 subject, the samples were lost in one study in another subject, and the isotope was not infused in 2 subjects). The anthropometric characteristics of the 4 subjects for whom data were missing were not different from those of the rest of the study group. Supplementary Fig. 4 shows the tracer-to-tracee ratio (TTR) in study. The changes of TTR over the 24 h (as well as in the first and second 12-h periods) with Gla-300 and Gla-100 were no different (two-way repeated-measures ANOVA [treatment × time, $F_{15,195}$ = 1.79; P = 0.177, Greenhouse-Geisser correction; power 0.92, with α = 0.05]) At t_0, EGP was lower with Gla-300 (1.85 ± 0.82 vs. 2.07 ± 0.52 mg · kg^{-1} · min^{-1}, respectively; $P < 0.048$). After the subcutaneous injection of basal insulin, EGP decreased both with Gla-300 and Gla-100 as expected. Suppression of EGP was less with Gla-300 compared with Gla-100 in the first 6-h interval (EGP-AUC$_{[0–6\ h]}$, ratio 1.41 [90% CI 1.01; 1.95]), but greater in the 18–24-h interval (EGP-AUC$_{[18–24\ h]}$, ratio 0.57 [90% CI 0.36; 0.92]) (Fig. 3 and Supplementary Fig. 3). However, over the 24-h period, EGP with Gla-300 was similar to that of Gla-100 (ratio 0.98 [90% CI 0.80; 1.21]). GU did not increase after subcutaneous Gla-300 or Gla-100 and was no different with between the two treatments (Fig. 3).

CONCLUSIONS

The current study was undertaken to establish the PK/PD as well as the modulation of EGP using clinical, individual doses of Gla-300 compared with Gla-100 under experimental conditions mimicking as close as possible the clinical situation where subjects with T1DM are usually on different doses of the two insulins (6,7). Such a study design is unprecedented, with one exception (22). Under these conditions, the doses of insulin differ not only between individuals but also in the same individual switching from Gla-100 to Gla-300 and vice versa. In the current study, after a 3-month titration/optimization period, the same glycemic control (A1C) was achieved with 0.07 units · kg^{-1} higher dose of Gla-300 compared with Gla-100, a result consistent with Comparison of a New Formulation of Insulin Glargine With Lantus in Patients With Type 1 Diabetes Mellitus (EDITION 4) (evening dosing group) (6). When these different insulin doses of Gla-300 and Gla-100 were tested in the current study in the same individuals, the two insulins exhibited similar total exposure and required a similar amount of infused glucose over

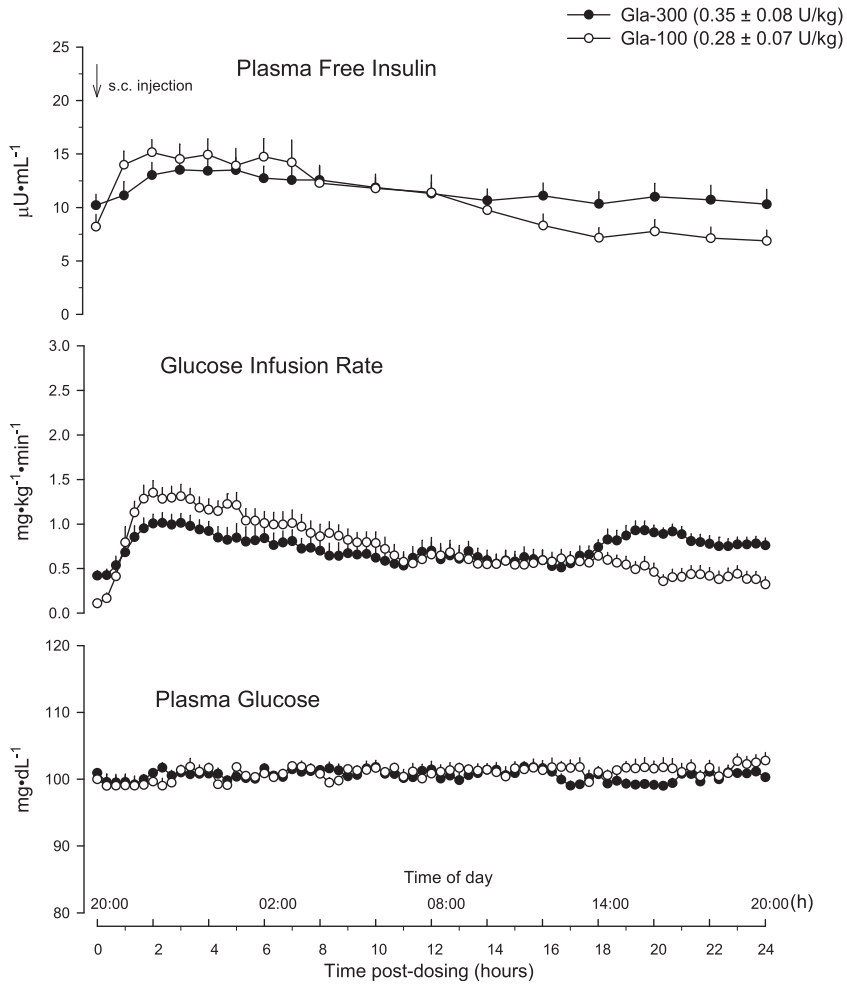

Figure 2—Plasma insulin and glucose concentrations and GIR during the 24-h clamp after subcutaneous injection of clinical doses of Gla-300 and Gla-100 at t_0 (2000 h) under SS conditions.

24 h, but there were differences in the profile. Indeed, the distribution of exposure and effect was more balanced between the first and the second 12-h period of the 24-h study with Gla-300, and thus, the within-day PK/PD variability of Gla-300 was lower than with Gla-100. In addition, Gla-300 appeared to be more consistent in modulating EGP both in the first and the second 12-h period of the 24-h study than Gla-100.

Regarding PK, the current study, with a higher dose of Gla-300 versus Gla-100, originally indicates that the mean plasma insulin concentration over 24 h did not differ (Table 1). This finding is different from that reported with same fixed Gla-300 and Gla-100 dose of 0.4 units · kg-1^{-1}, where plasma insulin over 24 h was lower with the former versus the latter (1). In the current study, there were important differences in plasma insulin profiles between the first and the second 12 h of the 24-h study. Plasma insulin increased less with Gla-300 than with Gla-100 over the first 6 h but remained steady and was consistently greater with Gla-300 than with Gla-100 in the 12- to 24-h interval postdosing (Table 1 and Fig. 2). Overall, the 24-h PK profile of plasma insulin with Gla-300 appeared flatter and more constant over the 24-h observation period than with Gla-100 (Fig. 2). Notably, the 24-h postinjection plasma insulin concentration of Gla-300 was similar to that at predosing at t_0, a value reflecting Gla-300 given the day before day 1 of the study, suggesting consistency (low day-to-day variability) of the same clinical insulin doses of Gla-300, whereas it was lower with Gla-100 (Fig. 2).

The total GIR-AUC over the 24 h of study with Gla-300 and Gla-100 was no different (Table 1). This also is an original finding, which is at variance with the result of less glucose-lowering effect reported with Gla-300 compared with same fixed dose of Gla-100 (1) and establishes equivalence in 24-h PD for individually titrated clinical doses of Gla-300 versus Gla-100. However, there was less variability in glucodynamics (GIR) with Gla-300. In parallel with PK, GIR increased less after the subcutaneous injection of Gla-300 compared with Gla-100 for the first 12 h of the study. Then, GIR was similar between 12 and 18 h and ultimately greater between 18 and 24 h with Gla-300 versus Gla-100.

As expected from the physiology of the insulin dose-response relationship on glucose fluxes (23), the marginal increase in the plasma insulin concentration after the subcutaneous injection of Gla-300 or Gla-100 did not stimulate peripheral GU (primarily insulin-mediated muscle uptake) but had effects especially on suppression of EGP (Fig. 3). The lower suppression of EGP in the initial part of study and its more consistent suppression in the late part, with Gla-300 versus Gla-100, indicates a more physiological modulation of EGP with the former compared with the latter basal insulin. The absolute values of EGP observed in this study should be interpreted with caution due to the unprecedented infusion of the tracer for 24 h (24). Previous observational studies in normal (25) and T1DM subjects (26) have reported an increase in TTR and underestimation by ~30% of EGP after long- versus short-term infusion of the tracer. This has been interpreted as the result of glucose recycling (in the current study possibly from glycogen via incorporation and release of [6,6-$^2H_2$]glucose) (26) and/or failure to achieve complete equilibrium of the tracer with the tracee during the short infusion (25). In the present studies, the TTR increased slowly for the initial 8 h; then, interestingly, it decreased in the early morning hours and, ultimately, increased again in the last part of the study (Supplementary Fig. 4). However, the changes of TTR were superimposable with the two insulins, indicating that even if there were some tracer recycling, this was most likely similar with Gla-300 and Gla-100, and the relative differences of EGP observed with Gla-300 and Gla-100 are real. This interpretation is independently supported by the GIR changes, which, in the present experimental model of hyperinsulinemic-euglycemic clamp with low plasma insulin concentrations and

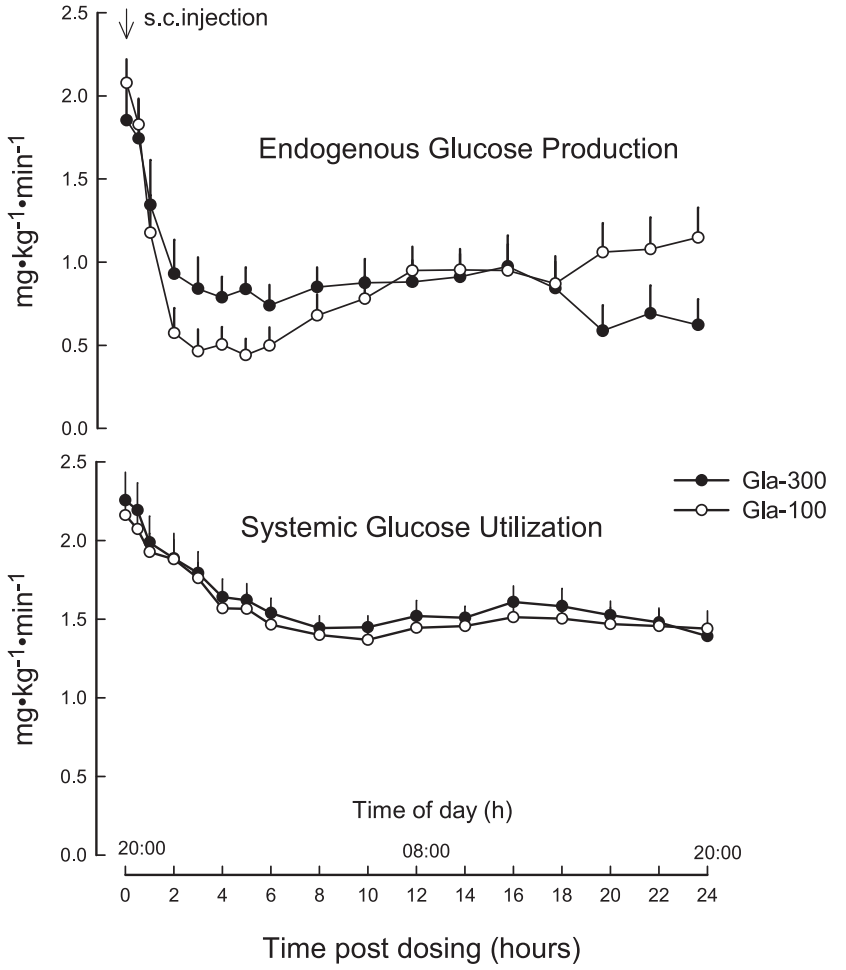

Figure 3—Rates of EGP and systemic GU during the 24 h after subcutaneous injection of clinical doses of Gla-300 and Gla-100 at t_0 (2000 h) under SS conditions.

no increase in muscle glucose uptake, reflect primarily changes in EGP (23).

The differential modulation of EGP, the primary determinant of PG concentration with Gla-300 compared with Gla-100, might translate into practical implications for subjects with T1DM. On one hand, the lower and smoother suppression of EGP in the initial part of the current study with evening dosing of Gla-300 versus Gla-100 possibly reduces the risk for nocturnal hypoglycemia at this vulnerable time of day (27) with former compared with latter basal insulin, a result reported by the EDITION studies with evening dosing (3–8). On the other hand, the more consistent suppression of EGP with Gla-300 versus Gla-100 18–24 h postdosing predicts less hyperglycemia in the afternoon with the former than with the latter. This effect may be beneficial to those T1DM subjects who present hyperglycemia in the late afternoon and are sometimes given basal insulin twice daily (28). In the EDITION JP 1, Gla-300 resulted in lower predinner PG versus Gla-100 (29).

In the current study, the within-day variability of Gla-300 was lower compared with Gla-100 both for PK and PD (Table 1). Swing and fluctuation of PK were lower with Gla-300, and fluctuation of PD was also lower with Gla-300 compared with Gla-100. Similar results have been previously reported, however, in healthy subjects (30) and with the same fixed doses of insulin (30–32).

The PK and PD data of the current study are qualitatively consistent with those of Becker et al. (1). However, the current study design makes the results more directly applicable to the real life of T1DM subjects than those of the fixed doses by Becker et al. (1). In addition, the population in the current study was more homogeneous, with all subjects at run-in on the same basal insulin (Gla-100). In contrast to previous studies, the current study included a relevant (predominant) number of female subjects who were studied in the follicular phase of their menstrual cycle on both occasions.

In the current study, great care was given to the preparation of subjects immediately before the clamp experiments to achieve similar metabolic conditions before the Gla-300 and Gla-100 study, as shown in Fig. 1. Ensuring that subjects are in reproducible metabolic conditions at t_0 of the clamp studies with a crossover study design is, of course, important for reliable interpretation of data generated.

The results of the current study were obtained with evening dosing. Additional studies are needed to examine PK/PD of Gla-300 and Gla-100 given in the morning (a more and more attractive time of injection for patients) (6,9,30) to establish whether differences in circadian rhythm (18,33–35) may affect the PK/PD observed in the current study with evening dosing.

The current study has limitations such as the number of subjects studied and the single-blind not double-blind study design. Strengths are the PK/PD data generated in a homogeneous group of subjects, including women, in fair glycemic control, all treated with the same basal insulin Gla-100 at baseline, the reproducible metabolic conditions before paired clamp studies, and the novel approach to study PK/PD of Gla-300 and Gla-100 with individually titrated clinical doses of each insulin resulting in matched glycemic control.

In conclusion, the study at SS indicates that PK/PD of both Gla-300 and Gla-100, titrated to fasting euglycemia in subjects with T1DM, have similar glucose-lowering effects over 24 h. However, evening dosing of Gla-300 exhibits potentially favorable differences in PK/PD profiles and in modulating EGP that result in lower insulin activity at night, greater insulin activity in the afternoon, and lower within-day PK/PD variability. Thus, evening dosing of Gla-300 has the potential of reducing the risk of nocturnal hypoglycemia, improving predinner hyperglycemia, and reducing within-day glucose variability in subjects with T1DM.

Acknowledgments. The authors are grateful to Dr. Elena Volpi, Sealy Center on Aging, University of Texas Medical Branch, Galveston, TX, for her comments on the interpretation of glucose fluxes.
Duality of Interest. The study is an investigator-initiated trial, partially funded by Sanofi. F.P. has received honoraria for lectures and consultations

from Sanofi, Eli Lilly, Menarini, and Medtronic. P.L. has received travel grants for scientific meetings from Sanofi and Menarini. G.B.B. has received honoraria for lectures and consultations from Sanofi and Menarini. C.G.F. has received honoraria for lecturing and consultations from Sanofi and travel grants from Menarini. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. F.P. enrolled patients, performed clamps, analyzed data, wrote the clinical protocol, and reviewed and edited the manuscript. P.L. enrolled patients, performed clamps, analyzed data, and reviewed and edited the manuscript. P.Ca. performed clamps, laboratory assays, glucose turnover measurements, and reviewed and edited the manuscript. P.Ci. and A.M.A. performed clamps and reviewed and edited the manuscript. G.C. performed laboratory assays, glucose turnover measurements, and reviewed and edited the manuscript. G.B.B. provided the study concept and design, supervised the protocol development and the research, enrolled patients, and wrote the manuscript. C.G.F. enrolled patients, performed clamps, analyzed data, performed statistical analysis, and wrote the manuscript. C.G.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the 77th Scientific Sessions of the American Diabetes Association, San Diego, CA, 9–13 June 2017.

References

1. Becker RH, Dahmen R, Bergmann K, Lehmann A, Jax T, Heise T. New insulin glargine 300 units·mL^{-1} provides a more even activity profile and prolonged glycemic control at steady state compared with insulin glargine 100 units·mL^{-1}. Diabetes Care 2015;38:637–643
2. Bolli GB, Gottesman IS, Cryer PE, Gerich JE. Glucose counterregulation during prolonged hypoglycemia in normal humans. Am J Physiol 1984;247:E206–E214
3. Riddle MC, Bolli GB, Ziemen M, Muehlen-Bartmer I, Bizet F, Home PD; EDITION 1 Study Investigators. New insulin glargine 300 units/mL versus glargine 100 units/mL in people with type 2 diabetes using basal and mealtime insulin: glucose control and hypoglycemia in a 6-month randomized controlled trial (EDITION 1). Diabetes Care 2014;37:2755–2762
4. Yki-Järvinen H, Bergenstal R, Ziemen M, et al.; EDITION 2 Study Investigators. New insulin glargine 300 units/mL versus glargine 100 units/mL in people with type 2 diabetes using oral agents and basal insulin: glucose control and hypoglycemia in a 6-month randomized controlled trial (EDITION 2). Diabetes Care 2014;37:3235–3243
5. Bolli GB, Riddle MC, Bergenstal RM, et al.; EDITION 3 Study Investigators. New insulin glargine 300 U/ml compared with glargine 100 U/ml in insulin-naïve people with type 2 diabetes on oral glucose-lowering drugs: a randomized controlled trial (EDITION 3). Diabetes Obes Metab 2015;17:386–394
6. Home PD, Bergenstal RM, Bolli GB, et al. Glycaemic control and hypoglycaemia during 12 months of randomized treatment with insulin glargine 300 U/mL versus glargine 100 U/mL in people with type 1 diabetes (EDITION 4). Diabetes Obes Metab 2018;20:121–128
7. Matsuhisa M, Koyama M, Cheng X, et al.; EDITION JP 1 Study Group. Sustained glycaemic control and less nocturnal hypoglycaemia with insulin glargine 300U/mL compared with glargine 100U/mL in Japanese adults with type 1 diabetes (EDITION JP 1 randomised 12-month trial including 6-month extension). Diabetes Res Clin Pract 2016;122:133–140
8. Terauchi Y, Koyama M, Cheng X, et al. New insulin glargine 300 U/ml versus glargine 100 U/ml in Japanese people with type 2 diabetes using basal insulin and oral antihyperglycaemic drugs: glucose control and hypoglycaemia in a randomized controlled trial (EDITION JP 2). Diabetes Obes Metab 2016;18:366–374
9. Bergenstal RM, Bailey TS, Rodbard D, et al. Comparison of insulin glargine 300 units/mL and 100 units/mL in adults with type 1 diabetes: continuous glucose monitoring profiles and variability using morning or evening injections. Diabetes Care 2017;40:554–560
10. Porcellati F, Lucidi P, Bolli GB, Fanelli CG. Nocturnal glycemic control with glargine titration based on bedtime in addition to fasting plasma glucose in type 1 diabetes (Abstract). Diabetes 2016;65(Suppl. 1):A78
11. Andres R, Swerdoff T, Pozefsky T, Coleman D. Manual feedback technique for the control of blood glucose concentration. In *Automation in Analytical Chemistry*. Skeggs LT Jr., Ed. New York, Mediad, 1966, p. 486–491
12. De Feo P, Perriello G, Ventura MM, et al. Studies on overnight insulin requirements and metabolic clearance rate of insulin in normal and diabetic man: relevance to the pathogenesis of the dawn phenomenon. Diabetologia 1986;29:475–480
13. Lepore M, Pampanelli S, Fanelli C, et al. Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro. Diabetes 2000;49:2142–2148
14. Porcellati F, Rossetti P, Busciantella NR, et al. Comparison of pharmacokinetics and dynamics of the long-acting insulin analogs glargine and detemir at steady state in type 1 diabetes: a double-blind, randomized, crossover study. Diabetes Care 2007;30:2447–2452
15. Porcellati F, Lucidi P, Bolli GB, Fanelli CG. How to accurately establish pharmacokinetics/pharmacodynamics of long-acting insulins in humans: relevance to biosimilar insulins. Diabetes Care 2015;38:2237–2240
16. McGuire EA, Helderman JH, Tobin JD, Andres R, Berman M. Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J Appl Physiol 1976;41:565–573
17. Gastaldelli A, Coggan AR, Wolfe RR. Assessment of methods for improving tracer estimation of non-steady-state rate of appearance. J Appl Physiol (1985) 1999;87:1813–1822
18. Lucidi P, Porcellati F, Rossetti P, et al. Pharmacokinetics and pharmacodynamics of therapeutic doses of basal insulins NPH, glargine, and detemir after 1 week of daily administration at bedtime in type 2 diabetic subjects: a randomized cross-over study. Diabetes Care 2011;34:1312–1314
19. Kuzuya H, Blix PM, Horwitz DL, Steiner DF, Rubenstein AH. Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes 1977;26:22–29
20. Wolfe RR. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York, Wiley-Liss, 1992, p. 425–426
21. Lucidi P, Rossetti P, Porcellati F, et al. Mechanisms of insulin resistance after insulin-induced hypoglycemia in humans: the role of lipolysis. Diabetes 2010;59:1349–1357
22. Morrow LA, Hompesch M, Jacober SJ, Leng Choi S, Qu Y, Sinha VP. Glucodynamics of long-acting basal insulin peglispro compared with insulin glargine at steady state in patients with type 1 diabetes: substudy of a randomized crossover trial. Diabetes Obes Metab 2016;18:1065–1071
23. Rizza RA, Mandarino LJ, Gerich JE. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am J Physiol 1981;240:E630–E639
24. Vella A, Rizza RA. Application of isotopic techniques using constant specific activity or enrichment to the study of carbohydrate metabolism. Diabetes 2009;58:2168–2174
25. Tigas SK, Sunehag AL, Haymond MW. Impact of duration of infusion and choice of isotope label on isotope recycling in glucose homeostasis. Diabetes 2002;51:3170–3175
26. Mallad A, Hinshaw L, Dalla Man C, et al. Nocturnal glucose metabolism in type 1 diabetes: a study comparing single versus dual tracer approaches. Diabetes Technol Ther 2015;17:587–595
27. Graveling AJ, Frier BM. The risks of nocturnal hypoglycaemia in insulin-treated diabetes. Diabetes Res Clin Pract 2017;133:30–39
28. Ashwell SG, Gebbie J, Home PD. Twice-daily compared with once-daily insulin glargine in people with Type 1 diabetes using meal-time insulin aspart. Diabet Med 2006;23:879–886
29. Matsuhisa M, Koyama M, Cheng X, et al.; EDITION JP 1 Study Group. New insulin glargine 300 U/ml versus glargine 100 U/ml in Japanese adults with type 1 diabetes using basal and mealtime insulin: glucose control and hypoglycaemia in a randomized controlled trial (EDITION JP 1). Diabetes Obes Metab 2016;18:375–383
30. Becker RH, Frick AD, Teichert L, et al. Fluctuation and reproducibility of exposure and effect of insulin glargine in healthy subjects. Diabetes Obes Metab 2008;10:1105–1113
31. Gerich J, Becker RH, Zhu R, Bolli GB. Fluctuation of serum basal insulin levels following single and multiple dosing of insulin glargine. Diabetes Technol Ther 2006;8:237–243
32. Becker RH, Nowotny I, Teichert L, Bergmann K, Kapitza C. Low within- and between-day variability in exposure to new insulin glargine 300 U/ml. Diabetes Obes Metab 2015;17:261–267
33. Bailey TS, Pettus J, Roussel R, et al. Morning administration of 0.4 U/kg/day insulin glargine 300 U/mL provides less fluctuating 24-hour pharmacodynamics and more even pharmacokinetic profiles compared with insulin degludec 100 U/mL in type 1 diabetes. Diabetes Metab 2018;44:15–21
34. Radziuk J, Pye S. Diurnal rhythm in endogenous glucose production is a major contributor to fasting hyperglycaemia in type 2 diabetes. Suprachiasmatic deficit or limit cycle behaviour? Diabetologia 2006;49:1619–1628
35. Porcellati F, Lucidi P, Cioli P, et al. Pharmacokinetics and pharmacodynamics of insulin glargine given in the evening as compared with in the morning in type 2 diabetes. Diabetes Care 2015;38:503–512