Pharmacokinetic and Pharmacodynamic Head-to-Head Comparison of Clinical, Equivalent Doses of Insulin Glargine 300 units · mL^{-1} and Insulin Degludec 100 units · mL^{-1} in Type 1 Diabetes

Paola Lucidi,[1] Paola Candeloro,[1] Patrizia Cioli,[1] Anna Marinelli Andreoli,[1] Chiara Pascucci,[1] Angela Gambelunghe,[2] Geremia B. Bolli,[1] Carmine G. Fanelli,[1] and Francesca Porcellati[1]

Diabetes Care 2021;44:125–132 | https://doi.org/10.2337/dc20-1033

OBJECTIVE

To prove equivalence of individual, clinically titrated basal insulin doses of glargine 300 units · mL^{-1} (Gla-300) and degludec 100 units · mL^{-1} (Deg-100) under steady state conditions in a single-blind, randomized, crossover study, on the glucose pharmacodynamics (PD) in people with type 1 diabetes (T1D).

RESEARCH DESIGN AND METHODS

Subjects with T1D ($N = 22$, 11 men, age 44.3 ± 12.4 years, disease duration 25.5 ± 11.7 years, A1C 7.07 ± 0.63% [53.7 ± 6.9 mmol · mL^{-1}], BMI 22.5 ± 2.7 kg · m^{-2}), naïve to Gla-300 and Deg-100, underwent 24-h euglycemic clamps with individual clinical doses of Gla-300 (0.34 ± 0.08 units · kg^{-1}) and Deg-100 (0.26 ± 0.06 units · kg^{-1}), dosing at 2000 h, after 3 months of optimal titration of basal (and bolus) insulin.

RESULTS

At the end of 3 months, Gla-300 and Deg-100 reduced slightly and, similarly, A1C versus baseline. Clamp average plasma glucose (0–24 h) was euglycemic with both insulins. The area under curve of glucose infused (AUC-GIR$_{[0–24\ h]}$) was equivalent for the two insulins (ratio 1.04, 90% CI 0.91–1.18). Suppression of endogenous glucose production, free fatty acids, glycerol, and β-hydroxybutyrate was 9%, 14%, 14%, and 18% greater, respectively, with Gla-300 compared with Deg-100 during the first 12 h, while glucagon suppression was no different. Relative within-day PD variability was 23% lower with Gla-300 versus Deg-100 (ratio 0.77, 90% CI 0.63–0.92).

CONCLUSIONS

In T1D, individualized, clinically titrated doses of Gla-300 and Deg-100 at steady state result in similar glycemic control and PD equivalence during euglycemic clamps. Clinical doses of Gla-300 compared with Deg-100 are higher and associated with quite similar even 24-h distribution of PD and antilipolytic effects.

[1]Section of Endocrinology and Metabolism, Department of Medicine, University of Perugia Medical School, Perugia, Italy
[2]Section of Occupational Medicine, Respiratory Diseases and Toxicology, Department of Medicine, University of Perugia Medical School, Perugia, Italy

Corresponding author: Carmine G. Fanelli, carmine.fanelli@unipg.it

Received 3 May 2020 and accepted 12 October 2020

This article contains supplementary material online at https://doi.org/10.2337/figshare.13103027.

The second-generation long-acting basal insulin analogs degludec (both 100 and 200 units · mL^{-1}, Deg-100 and Deg-200) and glargine 300 units · mL^{-1} (Gla-300) show advantages compared with the basal insulin analog of the first generation (glargine 100 units · mL^{-1}, Gla-100) in people with insulin-requiring diabetes, primarily because of the lower risk of hypoglycemia with similar glycemic control (1–6).

An unanswered question is the relative difference(s) between Gla-300 and Deg. The head-to-head studies so far conducted by the respective producing companies have shown controversial results in pharmacokinetics (PK) and pharmacodynamics (PD) (7,8) as well as in clinical outcomes (9,10).

Previous studies have examined PK/PD of the same fixed insulin dose of Gla-300 and Deg-200 (7) and Deg-100 (8) at steady state in people with type 1 diabetes (T1D). However, because Gla-300 has lower bioavailability versus Gla-100 (11,12) and presumably versus Deg as well, the same dose 0.4 units · kg^{-1} of Gla-300 and Deg resulted in lower PD effect of Gla-300 versus Deg-200 (30% in one study) (7), and Deg-100 (14% in the other) (8). In real life, persons with diabetes use higher doses of Gla-300 versus Deg-100 to reach similar glycemic control (9,10). Establishing the PK/PD of individualized clinical doses of Gla-300 and Deg under equivalent glucose-lowering conditions as well as determining possible differences from the fixed doses would therefore be important.

The aim of this study was to establish the PK/PD of Gla-300 and Deg-100 in a head-to-head trial by comparing clinical, individually titrated doses of the two insulins. The primary outcome was to prove equivalence of the glucose-lowering effect (PD) over 24 h of Gla-300 compared with Deg-100, after evening subcutaneous injection of the individually titrated dose of each of the two insulins used by persons with T1D at steady state.

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 T1D, had an age of ≥18 but ≤65 years, a disease duration ≥5 years, an A1C ≥6.5% (48 mmol/mol) ≤8.5% (69 mmol/mol), a BMI >20–≤27 kg · m^{-2}, were naïve to both Gla-300 and Deg, and all were on Gla-100 as basal insulin. Twenty-two 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 independent, investigator-initiated, randomized, single-blind (clamp investigator), two-treatment, two-period, two-sequence crossover study using the euglycemic glucose clamp technique. After 2 weeks of run-in during which subjects continued their basal-bolus regimen, they were randomized, based on a computer-generated sequence, to treatment with basal insulin Gla-300 (Toujeo, SoloStar pen; Sanofi, Paris, France) or Deg-100 (Tresiba, Flex Touchpen; NovoNordisk A/S, Gentofte, Denmark), both given at 2000 h, 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 the evening subcutaneous injection of the individual dose of Gla-300 or Deg-100 they used during the previous 2 weeks. Subjects then underwent 2 months' washout, resuming the basal insulin treatment they were on before randomization. Subsequently, 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, Perugia, Italy) and registered in EudraCT (2016-002725-11) (Supplementary Fig. 1).

Basal Insulin Titration

Basal insulin titration was an open-label process once or 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 plasma glucose (SMPG), 6–8 PG measurements/day before/2-h after each meal, midafternoon, and bedtime and at least 2 times/week at 0300 h). After randomization, both Gla-300 and Deg-100 (dosing at 2000 h ± 30 min) were titrated aiming at fasting PG (FPG) between 90 and 110 mg · dL^{-1}. A dose change decision was made no more frequently than 3–4 days based on the difference between bedtime and fasting PG on days on which the postdinner PG was 100–130 mg · dL^{-1} (13).

Clamp Procedure

The principle of the hyperinsulinemic–euglycemic glucose clamp was used in the current study (14). The manual euglycemic feedback (15) and clamp procedure for the purpose of examining PK/PD of long-acting insulin analogs have previously been described in detail (12,16–18). The study was blind to the clamp investigators who were not aware of the subjects' basal insulin treatment. In brief, subjects were admitted to the Clinical Research Center of the Department of Medicine, University of Perugia Medical School, between 1430 h and 1500 h of the first study day, ~1.5 h after the end of their usual lunch preceded by subcutaneous injection of rapid-acting insulin dose. The subjects were put at bedrest and maintained in the supine position 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 (19) for PG and hormone measurements. A superficial vein of the contralateral arm was cannulated with a 18-gauge catheter-needle for intravenous infusion of human regular insulin and/or 20% dextrose solution, as needed. At 1500 h, a feedback intravenous infusion of human regular insulin (regular insulin [Eli Lilly Italia SpA], diluted to 1 unit/mL in 100 mL of saline solution containing 2 mL of the subject's blood), was initiated, whenever needed, using a syringe pump (Injectomat MC Agilia; Fresenius Kabi, Bad Homburg, Germany) to maintain PG at 100 mg · dL^{-1} according to a modified algorithm (15). At −180 min from time "0 min," 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-steady state errors (20) in the measurement

of glucose turnover, as described previously (21). A peristaltic pump (Volumat MC Agilia; Fresenius Kabi) was used for infusion of glucose (20% solution), whenever needed, to prevent fall of PG $<$95 mg $\cdot$ dL^{-1} during feedback and clamp periods. At 2000 h, the dose of basal insulin, either Gla-300 or Deg-100, the individual subjects had been on for the last 15 days, was injected subcutaneously 2 cm to the right or left of the umbilicus with a SoloStar/FlexTouch pen (4-mm needle, skinfold technique), and then subjects underwent the euglycemic clamp for 24 h (Supplementary Fig. 1).

Analytical Methods

Bedside PG was measured in triplicate every 5–15 min using the YSI 2300 glucose analyzer (YSI 2300 STAT; Yellow Springs Instruments, Yellow Springs, OH). Plasma C-peptide was measured by radioimmunoassay (RIA) (DRG Instruments GmbH, Marburg, Germany). Plasma glucagon was measured by a commercial RIA kit (DRG Instruments GmbH). The insulin concentration was measured by a commercial RIA kit specific for human insulin (DRG Instruments GmbH) with a range of detection of 3.125–200 μU $\cdot$ mL^{-1}, after polyethylene glycol extraction of antibodies from the serum (22) for Gla-300. The insulin degludec assay was performed using guinea pig polyclonal anti-human insulin antibody (Abcam, Cambridge, U.K.). Although the assay is nonspecific, it is likely that total serum degludec was measured (i.e., the sum of free and bound to albumin). HbA_{1c} was determined by high-performance liquid chromatography using an HI-Auto A1C TM HA 8121 apparatus (DIC, Daiichi, Kogaku Co, Ltd, Kyoto, Japan), DCCT aligned (upper limit in subjects without diabetes $<$6.1%). Blood glycerol, β-hydroxybutyrate, alanine, and lactate were measured by previously described fluorimetric methods (23). To determine glucose kinetics, arterialized venous blood samples were taken every 30 min for the first 6 h and then every 60–120 min throughout the studies. Glucose enrichment was determined on its penta-acetate (penta-*O*-acetyl-β-D-glucopyranose) derivative by gas chromatography–mass spectrometry (GC HP 6890 II, MS HP 5973/A; Hewlett-Packard Co, Palo Alto, CA) in electron impact ionization mode monitoring the ions 200 and 202 for the unlabeled and D-[6,6-$^2H_2$]glucose, respectively (24).

End Points

The primary study end point was the area under curve (AUC) of the glucose infusion rate (GIR) versus 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 INS-AUC$_{(0–24\ h)}$ and the various time intervals for INS: INS-AUC$_{(0–12\ h)}$, INS-AUC$_{(12–24\ h)}$, INS-AUC$_{(0–6\ h)}$, INS-AUC$_{(6–12\ h)}$, INS-AUC$_{(12–18\ h)}$, INS-AUC$_{(18–24\ h)}$, and time to 50% of INS-AUC$_{(0–24\ h)}$. Other secondary end points were glucose fluxes (endogenous glucose production [EGP] and glucose utilization), glucagon, free fatty acids (FFA), glycerol, β-hydroxybutyrate, lactate, and alanine.

Calculations and Statistical Analyses

The linear trapezoidal rule was used to calculate the AUC for GIR (untransformed data). GIR data were smoothed by taking a three-point moving average to provide data for calculation of GIR$_{max}$. Within-day GIR fluctuation around the average value was calculated as previously reported (7). All calculations were made on untransformed data. Clamp quality for Gla-300 and Deg-100 was expressed as the coefficient of variation of the PG measurements and as the mean difference between the PG measurements and the target blood glucose level (100 mg/dL).

Glucose fluxes were calculated based on a nonsteady state 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 (20). EGP (primarily hepatic) was obtained as the difference between R_a and the exogenous GIR during the clamp. Glucose utilization was obtained adding the GIR to the EGP (12). When the EGP yielded a negative number, EGP was assumed to be zero, and the corresponding GIR was assumed as glucose utilization.

Point estimates of treatment ratios (Gla-300–to–Deg-100), with 90% CIs (Table 1 and Supplementary Table 3), 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 INS-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 PD AUC (i.e., AUC of GIR) and maximum concentration were completely contained within the interval 0.80–1.25. A total sample size of 22, in an equivalence test of means 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, when the true ratio of the means is 1.0 and the coefficient of variation is 0.24, to demonstrate that the 90% CI of the ratios of key PD parameters between treatments would be contained within the equivalence limits of the mean ratio 0.80–1.25. Data are expressed as arithmetic means (SD) and median (interquartile range) in text, geometric means (90% CI) in tables, and as means and SE in figures. Statistical analysis was usually performed using NCSS19/PASS11 (NCSS, LLC, Kaysville, UT).

RESULTS

Glycemic Control, Body Weight and Insulin Doses Before Studies

At the end of the 3-month treatment period, A1C and body weight were similar with Gla-300 and Deg-100 (Supplementary Table 2). The mean dose of basal insulin at the end of the 3-month period was higher with Gla-300 (0.34 $\pm$ 0.08 units $\cdot$ kg^{-1}) versus Deg-100 (0.26 $\pm$ 0.06 units $\cdot$ kg^{-1}), a difference of 0.084 units $\cdot$ kg^{-1} (95% CI 0.071–0.097). This dose was kept stable over the last 2 weeks and injected in the clamp studies. Similarly, the mean dose of prandial insulin did not change in the same period of time, although the amount of prandial insulin was higher with Gla-300 versus Deg-100, a mean difference of 0.038 units $\cdot$ kg^{-1} (95% CI 0.015–0.06). No hypoglycemia (PG $\leq$70 mg $\cdot$ dL^{-1}) was reported (SMBG) during the 3 days before the studies.

Intravenous Insulin and Glucose Infusion and PG Concentration Before Clamp Studies

On the first study day, at 1500 h ($\sim$1.5 h after the end of lunch and 5 h before

Table 1—Pharmacokinetic and pharmacodynamic variables

	Gla 300 (0.34 units · kg^{-1})	Deg 100 (0.26 units · kg^{-1})	Gla-300-to-Deg-100 ratio point estimate[a] (90% CI)
PK parameters			
INS-$AUC_{0–24\ h}$ ($\mu U \cdot h^{-1} \cdot mL^{-1}$)*	394 (335–462)	11,072 (9,763–12,557)	—
INS-$AUC_{0–12\ h}$ ($\mu U \cdot h^{-1} \cdot mL^{-1}$)*	208 (179–243)	5,868 (5,189–6,636)	—
INS-$AUC_{12–24\ h}$ ($\mu U \cdot h^{-1} \cdot mL^{-1}$)*	184 (155–220)	5,203 (4,572–5,921)	—
INS $AUC_{0–6\ h}$ ($\mu U \cdot h^{-1} \cdot mL^{-1}$)*	105 (90–122)	2,769 (2,456–2,123)	—
INS $AUC_{6–12\ h}$ ($\mu U \cdot h^{-1} \cdot mL^{-1}$)*	103 (88–121)	3,098 (2,732–3,514)	—
INS $AUC_{12–18\ h}$ ($\mu U \cdot h^{-1} \cdot mL^{-1}$)*	94 (79–111)	2,790 (2,457–3,169)	—
INS $AUC_{18–24\ h}$ ($\mu U \cdot h^{-1} \cdot mL^{-1}$)*	90 (75–109)	2,415 (2,113–2,753)	—
$T_{50\%}$ INS-$AUC_{0–24}$ (h)§	11 (9; 13)	11.5 (9; 13)	−0.5 (−0.5 to 0)
PD parameters			
GIR-$AUC_{0–24\ h}$ (mg · kg^{-1})*	1,121 (951–1,322)	1,087 (883–1,372)	1.04 (0.91–1.18)
GIR-$AUC_{0–12\ h}$ (mg · kg^{-1})*	486 (341–639)	489 (374–641)	0.99 (0.7–1.4)
GIR-$AUC_{12–24\ h}$ (mg · kg^{-1})*	573 (482–681)	514 (350–755)	1.11 (0.84–1.47)
GIR $AUC_{0–6\ h}$ (mg · kg^{-1})*	236 (170–330)	206 (154–275)	1.15 (0.75–1.75)
GIR $AUC_{6–12\ h}$ (mg · kg^{-1})*	245 (168–356)	268 (196–366)	0.91 (0.59–1.42)
GIR $AUC_{12–18\ h}$ (mg · kg^{-1})*	266 (206–314)	276 (195–391)	0.96 (0.75–1.93)
GIR $AUC_{18–24\ h}$ (mg · kg^{-1})*	291 (251–339)	261 (221–308)	1.12 (0.97–1.29)
GIR C_{max} (mg · kg^{-1} · kg^{-1})*	1.42 (1.25–1.61)	1.61 (1.36–1.79)	0.88 (0.79–0.98)
$T_{50\%}$ -GIR-$AUC_{0–24}$ (h)§	12.2 (8.5; 24)	12.3 (8.3; 16)	0 (−1 to 1)
Fluctuation[b] (mg · kg^{-1} · min^{-1})	0.46 (0.34–0.56)	0.57 (0.48–0.67)	0.80 (0.68–0.94)
Fluctuation[c] (%)	59 (44–78)	76 (58–97)	0.77 (0.63–0.92)

C_{max}, maximum concentration. *Data are geometric mean (95% CI). §Data are median (interquartile range) ($N = 22$). [a]Point estimates of treatment ratios with 90% CIs were calculated using a linear mixed-effects model based on log-transformed data and retransformations. [b]Absolute fluctuation (F24): (GIR AUC above and below the mean). [c]Relative fluctuation (%): (100% × [F24/GIR mean]).

subcutaneous injection of basal insulin and clamp initiation at 2000 h [time 0, T0]) PG was similar with Gla-300 and Deg-100, then decreased slowly and reached euglycemia between 60 and 40 min before T0, with no difference between the two insulins (Fig. 1). When appropriate, a low-dose intravenous infusion of human regular insulin to reach euglycemia was started between 5 and 4.5 h before T0 and gradually decreased until 20 min before T0, when it was totally withdrawn. The insulin infusion rate (IIR), as median IIR-$AUC_{(-5\ to\ 0\ h)}$, before T0 was 23.8 (28) and 16 (14.5) units · kg^{-1} (estimated difference, 7.5 $\mu U \cdot kg^{-1}$ [95% CI −3.45 to 21.3, $P = 0.291$]) with Gla-300 and Deg-100, respectively. When needed, a glucose infusion was started 4.5 h before T0. The GIR, as median GIR-$AUC_{(-5\ to\ 0\ h)}$ was 38.7 (88) and 57.9 (165) mg · kg^{-1} (estimated difference 1.35 mg · kg^{-1} [95% CI −122 to 57, $P = 0.961$]) with Gla-300 and Deg-100, respectively.

Insulin, PG Concentrations, and GIRs

At T0, mean serum free insulin concentration with Gla-300 was 15.5 ± 5.2 $\mu U \cdot mL^{-1}$ and increased to a peak of 22.2 ± 8.7 $\mu U \cdot mL^{-1}$ at a median time of 5 h (7.5 h). Thereafter, serum free insulin returned to baseline values by the end of the studies. Owing to the measurement of total plasma insulin concentration (i.e., the albumin-bound + free fractions) with Deg-100, the numbers were more elevated versus those of Gla-300. The mean insulin concentration was 393 ± 109 $\mu U \cdot mL^{-1}$ at T0 and increased to a peak of 567 ± 149 $\mu U \cdot mL^{-1}$ at 7 h (1 h) after dosing. Thereafter, the total plasma Deg-100 concentration decreased slowly and was similar to baseline by the end of studies (Fig. 2).

The mean GIR at T0 was similar with Gla-300 (0.54 ± 0.31 mg · kg^{-1} · min^{-1}) and Deg-100 (0.68 ± 0.58 mg · kg^{-1} · min^{-1}; mean difference −0.14, 95% CI −0.46 to 0.18), as expected with long-acting insulin at steady-state. The overall glucose infused to maintain euglycemia was equivalent between Gla-300 and Deg-100 over the full 24-h study period (GIR-$AUC_{[0–24\ h]}$, 1.04 [90% CI 0.91–1.18]) (Fig. 2, Table 1). However, after the subcutaneous injection, 6-h fractions of the GIR-$AUC_{(0–24\ h)}$ with Gla-300 were 15% greater compared with Deg-100 during the first 6 h, then stabilized to a level lower by 9% compared with Deg-100 and, finally, was 12% greater than Deg-100 in the last 6 h of the studies (Supplementary Fig. 2). The GIRmax was lower by 12% with Gla-300 versus Deg-100 (Table 1). At 24 h, the values of GIR were similar to those of T0 both for Gla-300 (0.54 ± 0.30 and 0.79 ± 0.37) and Deg-100 (0.69 ± 0.58 and 0.64 ± 0.37) at T0 and 24 h, respectively, both mg · kg^{-1} · min^{-1}. Both absolute and relative fluctuation in GIR around the average were lower with Gla-300 compared with Deg-100 by 20% and 23%, respectively (Table 1). GIR-AUC every 6 h was generally evenly distributed with both Gla-300 and Deg-100 (Supplementary Fig. 2).

The mean PG concentration was no different during the 24 h clamp with Gla-300 (99.3 ± 3.7 mg · dL^{-1}) and Deg-100 (99.0 ± 4.3 mg · dL^{-1}; mean difference 0.36, 95% CI −1.5 to 2.6) (Fig. 2). The quality of the performance of clamp studies was good, as indicated by the coefficient of variation of plasma glucose measurements and the mean difference between the PG measurements and the target blood glucose level for Gla-300 (3.6 ± 1.7 vs. 3.6 ± 1.8%, $P = 0.849$) and Deg-100 (2.98 ± 1.81 vs. 2.86 ± 1.66 mg · dL^{-1}, $P = 0.752$). Individual GIR (top panel) and PG (bottom panel) profiles during the euglycemic clamp studies are given in Supplementary Fig. 3.

Glucose Fluxes (EGP and Glucose Utilization)

At T0, EGP was similar with Gla-300 and Deg-100 (mean values 1.70 ± 0.68 vs. 1.54 ± 0.88 mg · kg^{-1} · min^{-1}, respectively; mean difference 0.16 [95% CI, −0.24 to 0.57], $P = 0.411$). After subcutaneous injection of basal insulin, EGP

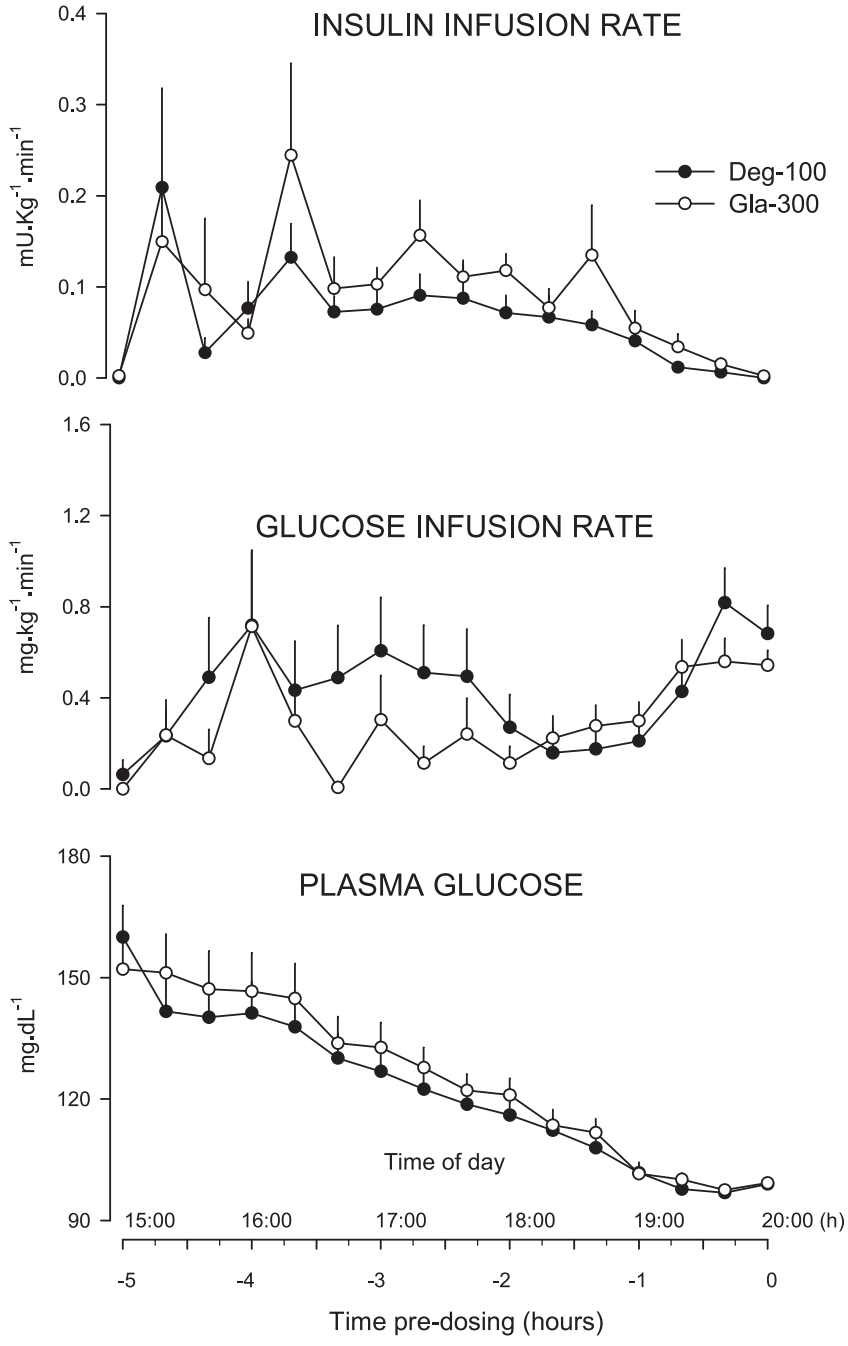

Figure 1—Feedback intravenous insulin and/or glucose infusion and PG concentration in the 5 h before subcutaneous injection of Gla-300 and Deg-100 at 2000 h (T0).

decreased both with Gla-300 and Deg-100, as expected. Overall, suppression of EGP with Gla-300 was similar to that of Deg-100 over 24 h (Fig. 3). However, there were differences in the profiles. In fact, Gla-300 suppressed more EGP compared with Deg-100 in the first 6 h and last 6 h of the clamp study, by 24% and 20%, respectively, whereas Gla-300 suppressed EGP less during 12–18 h versus Deg-100, by 27% (Fig. 3 and Supplementary Fig. 2). At the end of the study EGP was lower with Gla-300 ($0.54 \pm 0.0.48$ mg · kg^{-1} · min^{-1}) versus Deg-100 (0.79 ± 0.46 mg · kg^{-1} · min^{-1}; −0.25, 95% CI −0.5 to −0.07). Rates of glucose utilization at T0 were similar with Gla-300 and Deg-100 and did not increase after administration of either basal insulin. Rates of glucose utilization decreased slightly and similarly with the two insulins (1.42 ± 0.34 mg · kg^{-1} · min^{-1} and 1.44 ± 0.41 mg · kg^{-1} · min^{-1} at 24 h postdosing, Gla-300 and Deg-100, respectively) (Fig. 3).

Plasma Glucagon, Plasma FFAs, Blood Glycerol, and β-Hydroxybutyrate

Plasma glucagon was not different with Gla-300 and Deg-100 both at T0 and after subcutaneous insulin dosing (Supplementary Fig. 4).

The mean plasma FFA concentrations were similar at T0 with Gla-300 (274 ± 138 μmol · L^{-1}) and Deg-100 (284 ± 153 μmol · L^{-1}). After subcutaneous injection, FFA-AUC$_{(0–24)}$ was also similar to Gla-300 versus Deg-100. However, FFA-AUC$_{(0–12)}$ was 14% lower, indicating initial greater FFA suppression with Gla-300 compared with Deg-100 (Supplementary Fig. 4 and Supplementary Table 3).

Blood glycerol and β-hydroxybutyrate exhibited a pattern similar to that of FFA, with greater suppression of these metabolites, by 14% and 18%, respectively, with Gla-300 versus Deg-100 during the first 12 h of the study (Supplementary Fig. 4 and Supplementary Table 3).

Blood Alanine and Lactate

Blood alanine and lactate were similar at baseline and did not change over the 24 h with either insulin, indirectly suggesting no insulin-stimulated muscle glucose metabolism, as independently indicated by the lack of increase in glucose utilization (Supplementary Fig. 5 and Supplementary Table 3).

CONCLUSIONS

The current study used clinical doses of basal insulin analogs Gla-300 and Deg-100 used by individual subjects with T1D in their everyday life to optimize fasting glycemic control. In a crossover design, after 3 months of optimal titration of these basal insulins, glycemic efficacy was similar between the two insulins, although the dose of Gla-300 was higher than that of Deg-100. However, when such clinically titrated, individualized doses of both insulins were tested during euglycemic clamps, performed at the end of each 3-month period of treatment, similar 24-h postdosing GIRs were achieved, resulting in establishing PD equivalence between Gla-300 and Deg-100 similar to the glycemic efficacy achieved during clinical treatment.

Under these novel, unprecedented experimental conditions of PD equivalence, the action profile of both insulins remained stable overall with a quite similarly even distribution over 24 h and similar suppression of EGP, glucagon, and

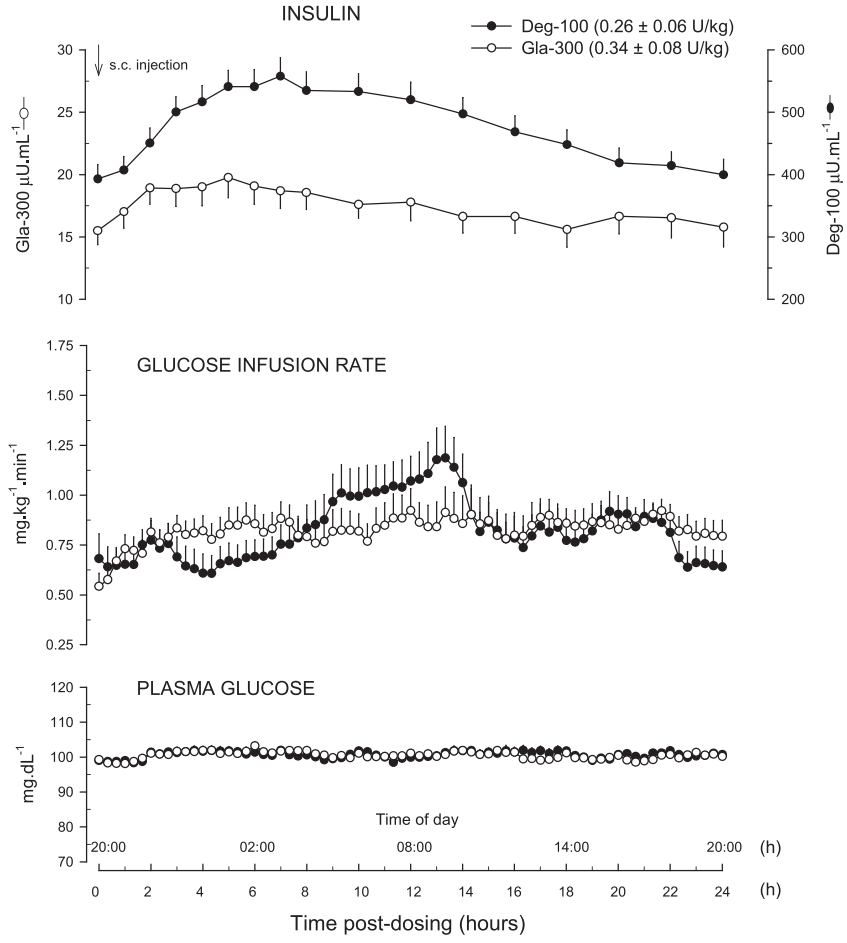

Figure 2—Insulin (serum free insulin Gla-300, plasma total [free + albumin-bound] insulin Deg-100), rates of glucose infusion, and PG concentration in the Gla-300 and Deg-100 studies. s.c., subcutaneous.

lipid metabolism. However, both absolute and relative fluctuation of GIR (within-day variability) was lower with Gla-300 compared with Deg-100 by 20% and 24%, respectively (Table 1).

At different, although similar glucose-lowering doses used by individual subjects with T1D in their everyday life and examined in the current study, Gla-300 and Deg-100 both maintain euglycemia for 24 h postdosing with only a modest glucose-lowering effect, as indicated by the relatively low GIR in the euglycemic clamps (Fig. 2). In fact, there was adequate suppression of EGP without stimulation of glucose utilization (Fig. 3). Taken together, these results suggest that the clinical doses of Gla-300 and Deg-100 used in the present studies were in the therapeutic range, mimicking the physiology of endogenous basal insulin secretion in the fasting state with smooth modulation of EGP without stimulating peripheral glucose utilization (25,26). This is different from the fixed-dose studies where GIR was consistently more elevated throughout the 24-h clamp (7,8). Although glucose turnover was not determined in those studies (7,8) it is likely that based on GIR values achieved, $\geq$1.8–2.0 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}, EGP was fully suppressed and peripheral glucose utilization stimulated to some extent (7,8). Also in those studies (7,8), because of its lower bioavailability, the same fixed dose (0.4 units $\cdot$ kg^{-1}) of Gla-300 showed quite a highly variable distribution of GIR throughout the 24 h, with a large plateau-nadir extent between 6 and 18 h. During this time interval, hyperglycemia developed in some individuals for whom the clamp was no longer euglycemic (7,8). This is in contrast with the 24-h even distribution of GIR with clinical doses of Gla-300, equivalent to those of Deg-100, in the current study (Fig. 2, Table 1, and Supplementary Fig. 2).

Taken together, the above considerations suggest that the PK/PD of clinical, equivalent doses of Gla-300 and Deg-100 in the current study mirror the action profile of these two insulins in persons with T1D more closely than those shown by the fixed-dose studies (7,8).

The current study also examined PK of clinical doses of Gla-300 and IDeg-100. Based on the reliable measurement of free insulin, the PK of clinical doses of Gla-300 are rather stable and evenly distributed over the 24 h, as previously reported (4,11,12). Deg-100 exhibited a stable profile and was evenly distributed over the 24 h as well, although with a 22% greater peak activity. However, it is difficult to compare the serum free insulin concentrations of Gla-300 with those of plasma Deg-100, because the former is the biologically active fraction of circulating insulin, whereas the latter is the total insulin circulating in blood (i.e., the sum of the albumin-bound fraction [major and inactive fraction] and the free fraction [minor fraction]. Thus, the interpretation of PK of degludec is more qualitative than quantitative, and the possibility that relative changes of the total concentration over time reflect the dynamics of free degludec insulin remains an unproven assumption. However, at 24 h postdosing, the free insulin of Gla-300 and total insulin of Deg-100 both approach the T0 values, suggesting consistent insulin exposure day to day.

The current study has examined also the effects of Gla-300 and Deg-100 on suppression of glucagon and lipolysis. In this regard, differences between Gla-300 and Gla-100 have been recently reported (27). In the present studies, there were no differences between clinical doses of Gla-300 and Deg-100 on suppression of glucagon and lipolysis over 24 h. There was, however, an initial delay in suppression of lipolysis observed with the latter (Supplementary Fig. 3), which is consistent with initial PD (Fig. 2 and Supplementary Fig. 2). This finding is similar to that observed with insulin detemir versus Gla-100 with subcutaneous dosing (17) and versus human regular insulin versus intravenous dosing (28), suggesting a class effect of delayed onset of action on glucose and lipid metabolism after administration of acylated insulins versus comparators. However, the absolute differences of FFA, glycerol, and β-hydroxybutyrate observed in the current study are transient and appear modest, remaining in the range of normal concentrations.

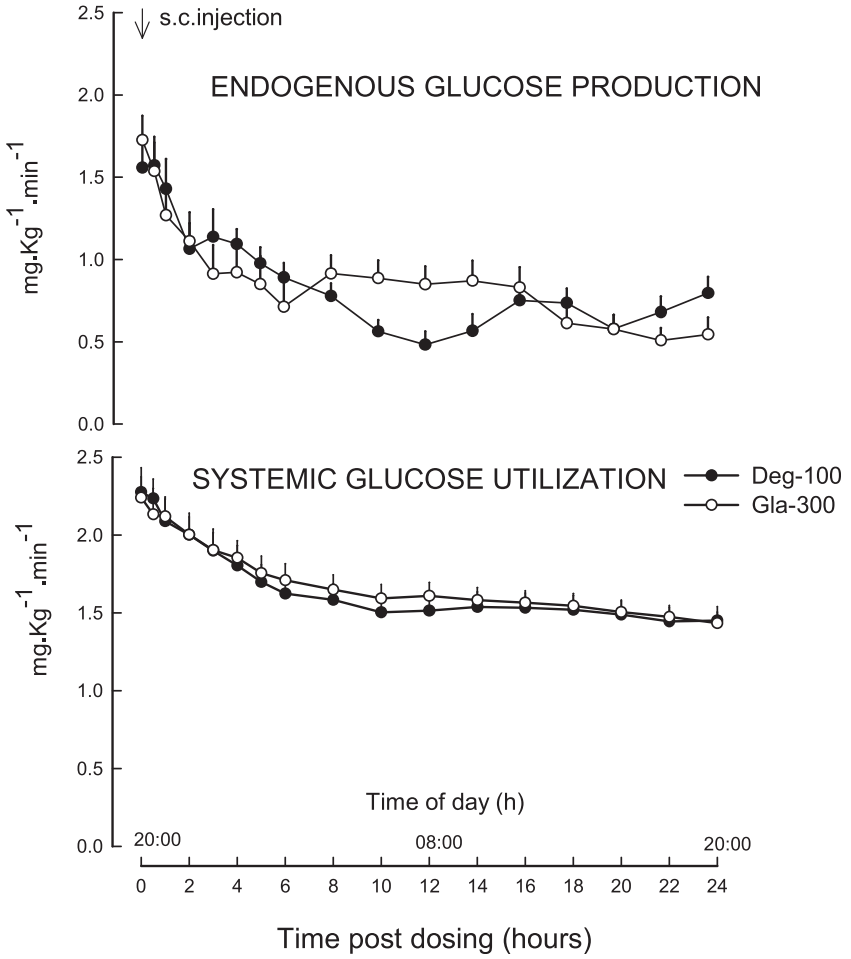

Figure 3—Rates of EGP and glucose utilization in the Gla-300 and Deg-100 studies. s.c., subcutaneous.

The clinical doses of Gla-300 in the current study are higher than those of Deg-100 by ~32%. This was in fact predicted by the lower PD of Gla-300 versus Deg-100 and Deg-200 in previous studies at fixed doses in T1D (7,8), and demonstrated in type 2 diabetes clinical trials (9,10). The higher clinical Gla-300 doses versus comparator basal insulins (12,29) are not the result of the lower potency of Gla-300 but of its lower bioavailability after subcutaneous dosing (11,12). In fact, when Gla-300 is given intravenously in dogs, PK and PD of the same dose Gla-300 and Gla-100 are similar (30).

The current study has some limitations. Neither the end nor the duration of action of Gla-300 and Deg-100 could be established because the clamp study lasted only 24 h. Because the current study has examined PK/PD of the two insulins given in the evening, the results cannot be immediately applied to morning dosing, and ad hoc studies are required in this respect. Finally, PK/PD day-to-day variability of the two insulins was not examined.

In conclusion, new findings of the current study in T1D are the equivalence of PD, and similar suppression of EGP, glucagon, and lipolysis with clinical doses of Gla-300 versus Deg-100 for the same glycemic control. There was a similarly even distribution of insulin activity of Gla-300 and Deg-100 over the 24 h post-dosing, however with lower within-day variability for Gla-300. Additional head-to-head studies are needed in T1D to establish whether the differences in within-day variability of Gla-300 and Deg-100 of the current study translate into meaningful clinical outcomes.

Acknowledgments. The authors are deeply grateful to the subjects who volunteered for the study. The authors also thank Dr. Gabriele Perriello, Section of Endocrinology and Metabolism, Department of Medicine, University of Perugia, for his invaluable help in organizing and ensuring the clamp studies in the hospital research center.

Funding. This was an independent study, not supported by companies, but supported only by the University of Perugia.

Duality of Interest. The study is an investigator-initiated trial. P.L. has received travel grants for scientific meetings from Sanofi, MSD, Novo, Abbott, 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. F.P. has received honoraria for lectures and consultations from AstraZeneca, Sanofi, Eli Lilly, Mundipharma, and Medtronic. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. 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., A.M.A., and C.P. performed clamps and reviewed and edited the manuscript. A.G. 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. F.P. enrolled patients, performed clamps, analyzed data, wrote the clinical protocol, and reviewed and edited 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 at the 79th Scientific Sessions of the American Diabetes Association, San Francisco, CA, 7–11 June 2019.

References

1. Ratner RE, Gough SC, Mathieu C, et al. Hypoglycaemia risk with insulin degludec compared with insulin glargine in type 2 and type 1 diabetes: a pre-planned meta-analysis of phase 3 trials. Diabetes Obes Metab 2013;15:175–184
2. Ritzel R, Roussel R, Bolli GB, et al. Patient-level meta-analysis of the EDITION 1, 2 and 3 studies: glycaemic control and hypoglycaemia with new insulin glargine 300 U/ml versus glargine 100 U/ml in people with type 2 diabetes. Diabetes Obes Metab 2015;17:859–867
3. Heise T, Hermanski L, Nosek L, Feldman A, Rasmussen S, Haahr H. Insulin degludec: four times lower pharmacodynamic variability than insulin glargine under steady-state conditions in type 1 diabetes. Diabetes Obes Metab 2012;14:859–864
4. 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
5. Mathieu C, Hollander P, Miranda-Palma B, et al.; NN1250-3770 (BEGIN: Flex T1) Trial Investigators. Efficacy and safety of insulin degludec in a flexible dosing regimen vs insulin glargine in patients with type 1 diabetes (BEGIN: Flex T1): a 26-week randomized, treat-to-target trial with a 26-week extension. J Clin Endocrinol Metab 2013;98:1154–1162

6. Riddle MC, Bolli GB, Home PD, et al. Efficacy and safety of flexible versus fixed dosing intervals of insulin glargine 300 U/mL in people with type 2 diabetes. Diabetes Technol Ther 2016;18:252–257
7. Heise T, Nørskov M, Nosek L, Kaplan K, Famulla S, Haahr HL. Insulin degludec: lower day-to-day and within-day variability in pharmacodynamic response compared with insulin glargine 300 U/mL in type 1 diabetes. Diabetes Obes Metab 2017;19:1032–1039
8. Bailey TS, Pettus J, Roussel R, et al. Morning administration of 0.4U/kg/day insulin glargine 300U/mL provides less fluctuating 24-hour pharmacodynamics and more even pharmacokinetic profiles compared with insulin degludec 100U/mL in type 1 diabetes. Diabetes Metab 2018;44:15–21
9. Rosenstock J, Cheng A, Ritzel R, et al. More similarities than differences testing insulin glargine 300 units/mL versus insulin degludec 100 units/mL in insulin-naive type 2 diabetes: the Randomized Head-to-Head BRIGHT Trial. Diabetes Care 2018;41:2147–2154
10. Philis-Tsimikas A, Klonoff DC, Khunti K, et al.; CONCLUDE Study Group. Risk of hypoglycaemia with insulin degludec versus insulin glargine U300 in insulin-treated patients with type 2 diabetes: the randomised, head-to-head CONCLUDE trial. Diabetologia 2020;63:698–710
11. 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
12. Porcellati F, Lucidi P, Candeloro P, et al. 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. Diabetes Care 2019;42:85–92
13. 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
14. 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, pp. 486–491
15. 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
16. 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
17. 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
18. 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
19. 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
20. 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
21. 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
22. 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
23. Fanelli CG, De Feo P, Porcellati F, et al. Adrenergic mechanisms contribute to the late phase of hypoglycemic glucose counterregulation in humans by stimulating lipolysis. J Clin Invest 1992;89:2005–2013
24. Wolfe RR. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York, Wiley-Liss, 1992, pp. 425–426
25. 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
26. Bolli GB, Gottesman IS, Cryer PE, Gerich JE. Glucose counterregulation during prolonged hypoglycemia in normal humans. Am J Physiol 1984;247:E206–E214
27. Lucidi P, Porcellati F, Cioli P, et al. Greater suppression of glucagon, lipolysis, and ketogenesis with insulin glargine U300 as compared with glargine U100 in type 1 diabetes mellitus. Diabetes Technol Ther 2020;22:57–61
28. Rossetti P, Porcellati F, Ricci NB, et al. Different brain responses to hypoglycemia induced by equipotent doses of the long-acting insulin analog detemir and human regular insulin in humans. Diabetes 2008;57:746–756
29. Home PD, Bergenstal RM, Bolli GB, et al. New insulin glargine 300 units/mL versus glargine 100 Units/mL in people with type 1 diabetes: a randomized, phase 3a, open-label clinical trial (EDITION 4). Diabetes Care 2015;38:2217–2225
30. Werner U, Korn M, Tennagels N. Insulin glargine 300 U/mL and insulin glargine 100 U/mL show equipotent in vivo blood glucose lowering when administered intravenously in dogs. Diabetes Technol Ther 2016;18(S1):A119