Intravenous Glargine and Regular Insulin Have Similar Effects on Endogenous Glucose Output and Peripheral Activation/Deactivation Kinetic Profiles

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OBJECTIVE — To compare the effects of intravenously administered long-acting insulin analog glargine and regular human insulin on activation and deactivation of endogenous glucose output (EGO) and peripheral glucose uptake.

RESEARCH DESIGN AND METHODS — In this single-center, randomized, double-blind, crossover euglycemic glucose clamp study, 15 healthy male volunteers (aged 27 ± 4 years, BMI 24.2 ± 0.7 kg/m^2 [mean ± SE]) received a primed continuous intravenous infusion of 40 mU/m^2 of insulin glargine or regular human insulin on 2 different study days in a randomized order. Euglycemia was maintained at 90 mg/dl using a simultaneous variable intravenous infusion of 20% dextrose containing D-[3-^{3}H]glucose. EGO and peripheral glucose disposal kinetics were determined during a 4-h insulin infusion activation period and a 3-h deactivation period.

RESULTS — The results demonstrated no significant difference in activation or deactivation kinetics with respect to EGO and peripheral glucose disposal between insulin glargine and regular human insulin when given intravenously. The mean ± SE time required for 50% suppression of EGO after insulin infusion was 73 ± 23 min for regular insulin and 57 ± 20 min for insulin glargine (NS). The mean maximum rate of glucose disposal was 10.10 ± 0.77 and 9.90 ± 0.85 mg · kg^{-1} · min^{-1} for regular insulin and insulin glargine, respectively (NS). The mean time required for 50% suppression of incremental glucose disposal rate (GDR), defined as the time required for activation from the basal glucose disappearance rate (R_d) to half-maximum insulin-stimulated R_d, was 32 ± 5 and 42 ± 10 min for regular insulin and insulin glargine, respectively (NS). The time required for deactivation from maximum insulin-stimulated GDR to half-maximum GDR after cessation of insulin infusion was 63 ± 5 and 57 ± 4 min for regular insulin and insulin glargine, respectively (NS).

CONCLUSIONS — Activation and deactivation kinetics of EGO and peripheral glucose uptake as well as absolute disposal rate are similar between regular human insulin and insulin glargine when administered intravenously. Thus, the various biological actions of these insulin preparations when given subcutaneously are completely due to their different absorption kinetics.

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Peripheral and endogenous insulin resistance is well documented in obesity and type 2 diabetes (1–3). Abnormalities in the rate of activation of infused insulin on suppression of endogenous glucose output (EGO) and stimulation of peripheral glucose uptake as well as reduced absolute effects of insulin action are important components of this insulin-resistant state (4–8). Glargine insulin (HOE901) is a long-acting insulin when given subcutaneously and has recently been approved in the U.S. for use as a basal insulin in both type 1 and type 2 diabetic patients. Because of a substitution of asparagine with glycine in position A21 of the A-chain of the human insulin molecule and the addition of two arginine molecules on positions B31 and B32 of the B-chain of the human insulin molecule, there is a shift of the isoelectric point from pH 5.4 in native insulin to 6.7 ± 0.2 (9). This makes insulin glargine a soluble insulin preparation at a slightly acidic pH and a less soluble insulin preparation at physiological pH levels. After subcutaneous injection, insulin glargine precipitates in the subcutaneous tissue, which delays its absorption and thereby prolongs its duration of action (10). To date, all the

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Abbreviations: A_{50}EGO, time required for 50% suppression of endogenous glucose output after insulin infusion; A_{50}IGDR, time from basal R_d to half-maximum insulin-stimulated R_d; D_{50}EGO, time required to achieve 50% deactivation from maximum insulin-induced suppression of endogenous glucose output after cessation of insulin infusion; D_{50}IGDR, time required for deactivation from maximum insulin-stimulated glucose disposal rate to half-maximum glucose disposal rate after cessation of insulin infusion; ED_{50}, half maximally effective insulin dose; EGO, endogenous glucose output; FFA, free fatty acid; GDR, glucose disposal rate; hot-GINF, glucose infusion containing D-[3-^{3}H]glucose; IGDR, incremental GDR; R_a, glucose appearance; R_d, glucose disappearance; $R_{d\ max}$, maximum rate of glucose disposal.

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

pharmacokinetic and pharmacodynamic studies comparing glargine with NPH and ultralente insulins (10,11) have been performed after subcutaneous administration. Because there are differences in the amino acid composition of glargine insulin, it is possible that it might have different effects from that of regular human insulin on the rate of suppression of EGO and stimulation of peripheral glucose utilization, independent of its absorption from subcutaneous sites. In contrast to subcutaneous administration, we postulated that glargine insulin would have similar kinetic activity (i.e., rate of activation and deactivation of peripheral insulin action and suppression of EGO) compared with regular human insulin when given intravenously.

In this study, we used the hyperinsulinemic-euglycemic clamp technique in combination with a steady and variable rate of D-[3-^{3}H]glucose tracer infusion to assess differences in activation and deactivation of suppression of EGO and stimulation of peripheral glucose disposal between insulin glargine and regular human insulin.

RESEARCH DESIGN AND METHODS

The study design was a single-dose, double-blind, randomized, crossover trial. The study consisted of four visits: one screening visit (visit 1), two glucose clamp study visits (visits 2 and 3), and one follow-up visit (visit 4). The first glucose clamp study at visit 2 was performed within 14 days of visit 1. The washout period between glucose clamp studies at visits 2 and 3 was at least 7 days. Visit 4 was conducted within 7 days of visit 3.

A total of 15 subjects participated in the study. The first three subjects were treated in a pilot study to refine details of the study methodology. Subsequently, 12 subjects who followed all the study methods described below were included in the study results. Subjects had no family history of diabetes, had no significant medical problems, and maintained regular levels of physical activity. Subjects were not on any medication known to affect glucose metabolism. The mean age of the subjects was 34.8 ± 2.7 years, the mean BMI was 24.2 ± 0.7 kg/m^2, the mean fasting plasma glucose was 89.0 ± 2.2 mg/dl, and the mean fasting insulin was 8.1 ± 2.4 μU/ml. Before each clamp study, all subjects were admitted overnight to the Special Diagnostic and Treatment Unit at the VA San Diego Healthcare System. The study was approved by the University of California San Diego and Veterans Medical Research Foundation Human Subjects Research Review Committee, and all subjects gave written informed consent.

Insulin glargine was supplied by Aventis Pharmaceuticals, and regular human insulin was obtained from Eli Lilly (Indianapolis IN). D-[3-^{3}H] tracer was purchased from New England Nuclear (Boston, MA). Each glucose clamp study consisted of two periods: a basal study period and a glucose clamp study period.

Basal study

Subjects fasted overnight for ~8 h, and then a continuous infusion of D-[3-^{3}H]glucose was started at 0.15 μCi/min at ~3:00 A.M. on each clamp day and was infused for at least 5 h to label the glucose pool and achieve steady state before determination of the basal rate of EGO. Plasma glucose specific activity was measured at −30 min, −20 min, −10 min, and immediately before insulin dosing. Measurement of plasma glucose, immunoreactive insulin, and free fatty acids was also obtained during the basal period.

Glucose clamp study

After basal measurements, the hyperinsulinemic-euglycemic clamp technique was used to measure the activation and deactivation of in vivo metabolic effects of glargine insulin or regular human insulin during a 4-h insulin infusion and 3-h deactivation period. The continuous infusion of D-[3-^{3}H]glucose was maintained throughout the activation and deactivation studies. Additional infusates were administered through an 8-inch intravenous catheter placed into an antecubital vein. Arterialized blood samples were obtained from a retrograde cannulated heated hand vein (12). The clamp was initiated with a 10-min priming dose of insulin infused in a logarithmically decreasing manner to acutely raise the insulin to the desired level (13,14). The priming dose was immediately followed by a constant insulin infusion of 40 mU · m^{-2} · min^{-1} to maintain the desired insulin level. This constant insulin infusion dose approximates the ED_{50} required for both maximal suppression of EGO and stimulation of peripheral glucose uptake in nondiabetic subjects (14). Blood glucose level was determined every 5 min throughout the entire clamp period. A 20% glucose solution containing D-[3-^{3}H]glucose (hot-GINF) was infused at a variable rate to maintain euglycemia at 90 mg/dl. D-[3-^{3}H]glucose was added to the glucose infusate so that the specific activity of the infusate approximated the specific activity that existed in plasma after the 4-h constant tracer infusion. Adding tracer to the glucose infusate allowed the infusion of D-[3-^{3}H]glucose and unlabeled glucose to be varied equally so that the specific activity of the plasma glucose remained approximately constant throughout the study. K_2PO_4/KCl was infused at a rate of 0.16 mmol K^+ per minute to avoid hypokalemia. Plasma glucose specific activity was measured every 10 min for the initial 70 min and every 20 min thereafter. After a constant insulin infusion for 240 min, the insulin infusion was discontinued, but euglycemia was sustained for an additional 180 min to determine the rate and duration of the insulin effect. Serum immunoreactive insulin and C-peptide were measured every 20 min throughout the clamp study period. Measurements of plasma free fatty acid (FFA) levels were obtained twice at baseline (t = −10 min, t = 0), 20 min before stopping the insulin infusion (t = 220 min), immediately before stopping the insulin infusion (t = 240 min), 20 min before stopping the hot-GINF infusion (t = 400 min), and immediately before stopping the hot-GINF infusion (t = 420 min). Subjects were discharged 3–6 h after the clamp study after their safety was ensured.

Measurement of glucose turnover

The glucose turnover rate was assessed during the basal state as well as during the activation and deactivation phases. The subjects received a continuous infusion (0.15 μCi/min) of D-[3-^{3}H]glucose for 4–5 h in the basal state (i.e., before the clamp study) to label the glucose pool. Blood samples for determination of glucose concentration were taken every 5 min, and those for specific activity were taken every 10 min for the first 70 min, then every 20 min thereafter. Glucose turnover was calculated using modified Steele equations for non–steady-state conditions (15).

Measurement of EGO

D-[3-^{3}H]glucose is a suitable tracer substance to measure rates of glucose appearance (R_a) and glucose disappearance (R_d) in vivo under both steady-state and non–steady-state conditions. In the basal state, R_a equals EGO of which the hepatic contribution is ~80% and the renal contribution ~20% (16). During the insulin infusion and subsequent deactivation phase, the rate of EGO is calculated as the difference between the R_a and the infusion rate of exogenous glucose. A_{50}EGO is the time (minutes) required for 50% suppression of EGO after insulin infusion. D_{50}EGO is the time (minutes) required to achieve 50% deactivation from maximum insulin-induced suppression of EGO after cessation of insulin infusion.

Calculation of incremental glucose disposal rate

Incremental glucose disposal rate (IGDR) is defined as the difference between the initial basal GDR and the GDR values during and after cessation of the insulin infusion. The A_{50}IGDR is the time (minutes) from basal R_d to half-maximum insulin-stimulated R_d. D_{50}IGDR is the time (minutes) required for deactivation from maximum insulin-stimulated GDR to half-maximum GDR after cessation of insulin infusion.

Analytical methods

An Eppendorf microfuge (Brinkmann Instruments, Westbury, NY) was used for centrifugation of blood drawn for serum glucose determination. Glucose was measured at the time of blood draw using an automated glucose analyzer (YSI, model 23A; Yellow Springs Instruments, Yellow Springs, OH). Immunoreactive insulin levels were measured in plasma using a human insulin–specific radioimmunoassay kit (Linco Research, St. Charles, MO), according to the manufacturer's instructions. To assess cross-reactivity of the human insulin antibody for glargine insulin, a standard solution of glargine insulin (10 mmol/l) was prepared from powder, and the concentration was confirmed by protein analysis. Various concentrations of this standard solution (0–1,500 pmol/l, or 0–250 mU/ml) were added to fasting serum and plasma samples from several (n = 4) individuals, and the insulin content was measured. This analysis was repeated five separate times. The results were similar for plasma or serum. There was considerable cross-reactivity of the insulin antibody for glargine insulin. Glargine insulin was detected with ~97% efficiency. A correction factor for insulin glargine was determined from the average of the individual correlation curves: measured value (mU/ml) = 0.990 × expected value − 2.16. FFA samples were processed using an in vitro enzymatic colorimetric method (Wako Chemicals, Richmond, VA) (17).

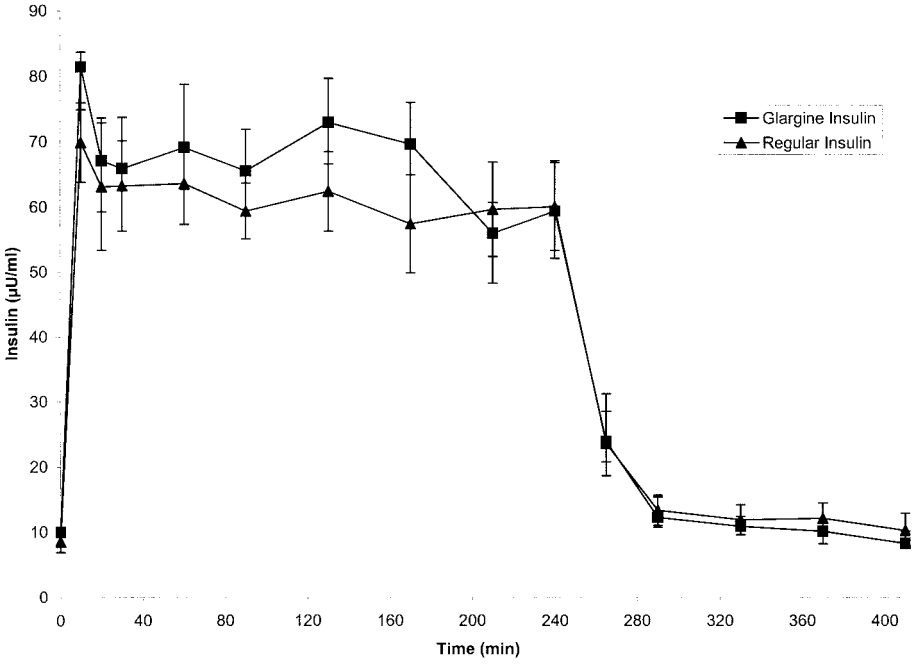

Figure 1—*Mean insulin levels.*

Statistical analysis

The half-times for maximum GDRs for the outcome parameters A_{50}EGO, D_{50}EGO, A_{50}IGDR, D_{50}IGDR, and R_d were compared between the two insulins using ANOVA, with factors for sequence,

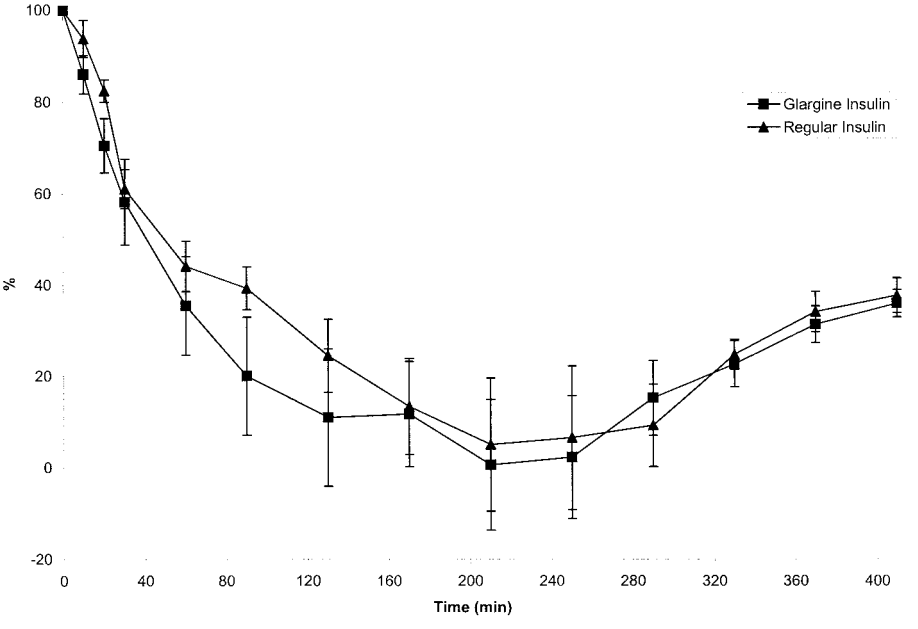

Figure 2—*Mean suppression of hepatic glucose output.*

Table 1—*Half-maximal activation and deactivation of endogenous glucose output, $R_{d\ max}$, and IGDR*

	A_{50}EGO		D_{50}EGO		$R_{d\ max}$		A_{50}IGDR		D_{50}IGDR	
	Regular (min)	Glargine (min)	Regular (min)	Glargine (min)	Regular (mg · kg^{-1} · min^{-1})	Glargine (mg · kg^{-1} · min^{-1})	Regular (min)	Glargine (min)	Regular (min)	Glargine (min)
Subject no.										
1	24	28	NA	NA	10.66	10.66	33	34	113	40
2	34	52	NA	NA	4.11	3.56	5	7	72	90
3	29	32	NA	NA	9.79	9.20	26	43	59	61
4	84	36	5	NA	7.61	7.29	18	13	54	54
5	38	48	NA	NA	13.59	10.43	33	119	53	46
6	100	37	69	NA	8.2	9.42	54	52	49	60
7	315	268	155	61	13.31	12.62	32	94	78	62
8	49	30	NA	NA	8.39	9.55	37	34	54	54
9	42	10	NA	NA	11.9	12.63	26	33	47	48
10	80	43	NA	NA	10.96	8.91	27	24	58	52
11	47	38	NA	NA	11.66	15.45	30	24	66	60
12	34	57	NA	NA	10.45	9.06	68	28	51	60
Mean	73.0	56.6	NA	NA	10.10	9.90	32.4	42.1	62.8	57.3
SE	23.1	19.6	NA	NA	0.77	0.85	4.6	9.5	5.3	3.6
P	—	0.0585	—	NA	—	>0.9	—	>0.3	—	>0.4

NA, not available.

subject within sequence, visit, and insulin. Descriptive results are reported as the mean ± SE. The pair of FFA values for each of the three time periods (i.e., basal, end of insulin infusion, and recovery) was averaged (mean), and each time period was analyzed using the ANOVA described above.

There were no significant sequence or visit effects for any pharmacodynamic parameters ($P > 0.14$). Area under the curve for the moving average smooth R_d was calculated using the means of the moving average smooth R_d values at each time point for each insulin.

RESULTS

Serum insulin levels

The serum insulin levels during and after regular and glargine insulin infusions are shown in Fig. 1. At all time points, the concentrations of both regular and glargine insulin were similar (NS), both during the 240 min of insulin infusion and during the 180 min after cessation of insulin infusion.

Pharmacodynamic data

EGO. The mean basal EGO was 2.13 ± 0.08 mg · kg^{-1} · min^{-1} in the glargine group and 2.06 ± 0.13 mg · kg^{-1} · min^{-1} in the regular insulin group. Both regular and glargine insulin suppressed EGO in a similar manner, as shown in Fig. 2.

At all time points, the suppression of EGO by both regular and glargine insulin was similar (NS), both during the 240 min of insulin infusion and during the 180 min after cessation of insulin infusion. As shown in (Table 1), the time required for 50% suppression of EGO after insulin infusion (A_{50}EGO) for regular insulin was 73.0 ± 23.1 min compared with 56.6 ± 19.6 min for glargine insulin. The effect of insulin glargine on suppression of EGO tended to be slightly faster than that of regular insulin but failed to reach statistical significance.

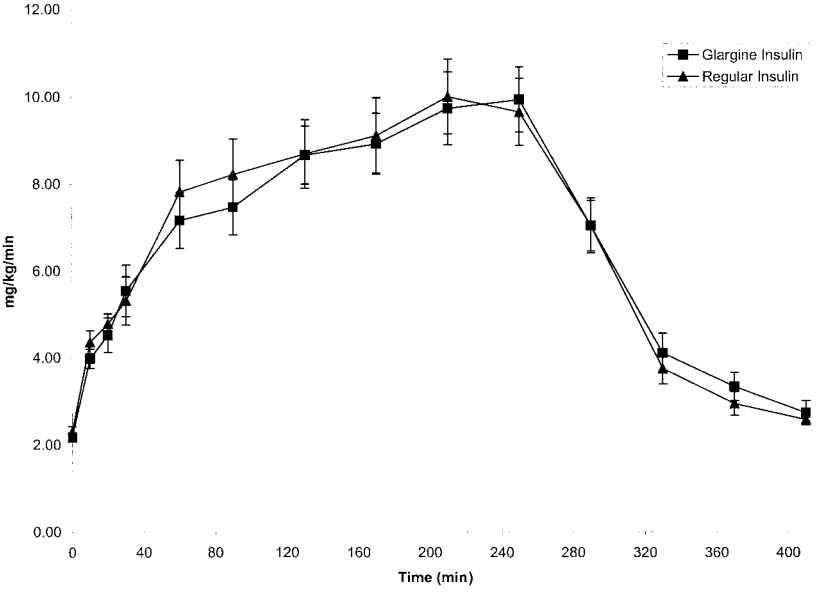

Figure 3—*Mean glucose disposal rate* ($R_{d\ max}$).

The time for D_{50}EGO could not be determined in most subjects after both insulin infusions because deactivation of EGO suppression did not reach the 50% level during the 180-min deactivation period. In other words, after 180 min of deactivation, EGO was still >50% of the absolute maximum insulin-induced EGO suppression for both regular and glargine insulin. However, the D_{50}EGO (Table 1) was achieved for three subjects (subjects 4, 6, and 7) for regular insulin and one subject (subject 7) for glargine insulin. In the other subjects, the deactivation of EGO suppression presumably reached the D_{50}EGO level sometime beyond the designated 180-min deactivation time period in most subjects.

Maximum rate of glucose disposal

The maximum rate of glucose disposal ($R_{d\ max}$) is shown in Table 1 and Fig. 3. At all time points, the suppression of EGO by both regular and glargine insulin were similar (NS), both during the 240 min of insulin infusion and during the 180 min after cessation of insulin infusion.

The $R_{d\ max}$ for regular insulin was 10.10 ± 0.77 mg · kg^{-1} · min^{-1}, and it was 9.90 ± 0.85 mg · kg^{-1} · min^{-1} for glargine insulin. There was no significant difference (NS) in the $R_{d\ max}$ between regular and glargine insulin. No significant effects of sequence or period were found ($P > 0.2$).

IGDR. There was no difference in either the activation or deactivation kinetics of IGDR (defined as the difference between the initial basal R_d and the R_d values during and after cessation of the insulin infusion) between regular and glargine insulin (Table 1). The A_{50}IGDR was 32 ± 5 min for regular insulin and 42 ± 10 min for glargine insulin (NS). Similarly, there was no significant difference in deactivation between regular and glargine insulin; D_{50}IGDR was 63 ± 5 min for regular insulin and 57 ± 4 min for glargine insulin.

FFAs. FFA levels were obtained at the basal period, at the end of the clamp, and during the recovery period (Table 1). FFA levels (mmol/l, mean ± SE) for regular insulin were 0.45 ± 0.06, 0.09 ± 0.004, and 0.33 ± 0.04 for the basal, end, and recovery periods, respectively. FFA levels for glargine insulin were 0.43 ± 0.05, 0.09 ± 0.01, and 0.37 ± 0.03 for the basal, end, and recovery periods, respectively. There were no significant differences in FFA levels between regular and glargine insulin at any of the three time periods (all $P > 0.2$).

CONCLUSIONS — This study has clearly demonstrated that after intravenous administration, there are no significant differences between glargine and regular insulin to suppress EGO or to promote peripheral glucose disposal. Further, the activation and deactivation kinetics of insulin action on glucose disposal and endogenous glucose suppression were similar for both glargine and regular human insulin. After intravenous administration, both glargine and regular insulin resulted in nearly similar plasma insulin concentrations, which persisted for 3 h after the two insulin infusions were discontinued. Thus, although subcutaneous administration of glargine insulin leads to a significant delay in its absorption, when administered intravenously, this pharmacokinetic difference is eliminated. Further, in keeping with similar pharmacokinetic profiles, both glargine and regular insulin also had similar pharmacodynamic characteristics after intravenous administration, as discussed below.

It is well known that insulin suppresses EGO (8), and in the present study, the time required for 50% suppression of EGO after insulin infusion A_{50}EGO was 57 ± 20 min for glargine insulin and 73 ± 23 min for regular insulin. In a previous study by Prager et al. (7), the A_{50}EGO in lean nondiabetic subjects after a similar 40 mU · m^{-2} · min^{-1} glucose clamp with regular insulin infusion was 18 ± 6 min. However, in that study, the steady-state plasma insulin levels attained during the clamp were higher at ~100 mU/ml, whereas in our study, the steady-state insulin levels obtained during the clamp were ~75 mU/ml. In addition, in the study by Prager et al., subjects tended to be leaner, with a BMI of 22 ± 1 kg/m^2, whereas in our study, the BMI was 24 ± 1 kg/m^2. It must also be mentioned that in our study, one subject (subject 7, who had a BMI of 22 kg/m^2 and a fasting insulin level of 3 mU/ml) had extremely high A_{50}EGO values of 315 and 268 min during regular and glargine insulin infusions, respectively. This subject thus suppressed his EGO to 50% of his basal rate—not during the 240 min of insulin infusion, but well after the insulin infusion had been stopped. However, he demonstrated the same behavior after exposure to both glargine and regular insulin.

It was also observed in the present study that glargine insulin acted more rapidly to suppress EGO than regular insulin, with the A_{50}EGO for insulin glargine being 57 ± 20 vs. 73 ± 23 min for regular insulin. However, this was only a trend and was not statistically significant ($P = 0.0585$) and probably has little, if any, clinical significance. Another feature of this study was that the time for D_{50}EGO could not be determined in most subjects after both insulin infusions, because deactivation of EGO suppression did not reach the 50% level after 180 min of deactivation. In other words, after 180 min of deactivation, suppression of EGO was still >50% of the absolute maximum insulin-induced EGO suppression for both regular and glargine insulin. In the study by Prager et al. (7), the lean nondiabetic control subjects had D_{50}EGO values of 59 ± 8 min. However, in that study, the duration of insulin infusion was only 180 min, whereas in the present study, the insulin infusion was continued for 240 min. It is possible that the longer infusion period of insulin in our study may have led to prolonged deactivation. It is presumed that D_{50}EGO would have occurred at some point beyond the 180 min deactivation period during which the subjects were studied. This delayed deactivation of EGO is interesting but probably not clinically significant, because neither glargine nor regular insulin reached D_{50}EGO consistently within 180 min.

Similar to the effects on EGO suppression, there were no differences in the absolute GDRs achieved after either regular or glargine insulin infusion. The mean $R_{d\ max}$ with insulin glargine was 9.90 ± 0.85 vs. 10.10 ± 0.77 mg · kg^{-1} · min^{-1} for regular insulin. However, it is possible that despite having similar $R_{d\ max}$ values, there are kinetic differences in the onset (activation) and offset (deactivation) of insulin glargine's biologic effects compared with regular insulin. To calculate these kinetic differences, we calculated the IGDR, which was defined as the difference between the basal GDR and the measured R_d values during the insulin infusion and subsequent deactivation phase and also the half-times for activation and deactivation (A_{50}IGDR and D_{50}IGDR) (8). The time taken to achieve 50% $R_{d\ max}$ (A_{50}IGDR) in the case of glargine insulin

was 42 ± 10 vs. 32 ± 5 min for regular insulin, and the D_{50}IGDR was 57 ± 4 and 63 ± 5 min, respectively. Thus, there did not appear to be any kinetic differences between glargine and regular insulin during the activation and deactivation phase of insulin's biological action.

These similar effects on both EGO and peripheral glucose disposal support the conclusion that both glargine and regular insulin have similar end-organ metabolic effects when administered intravenously. This conclusion is further supported by the fact that there was no difference in the suppression of FFA levels both during steady-state insulin infusion and after stopping the insulin infusion.

In conclusion, despite differences in the amino acid composition of glargine insulin, which leads to unique absorption kinetics when given subcutaneously, there were no significant differences in absolute glucose disposal rates or activation/deactivation kinetics on EGO and $R_{d\ max}$ between regular human insulin and glargine insulin when administered intravenously. The delayed biological action of insulin glargine when given subcutaneously appears to be due to its different absorption kinetics.

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