Pharmacokinetics of ^{125}I-Labeled Insulin Glargine (HOE 901) in Healthy Men

Comparison with NPH insulin and the influence of different subcutaneous injection sites

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OBJECTIVE — To determine the subcutaneous absorption rates and the appearance in plasma of 3 formulations of the long-acting human insulin analog insulin glargine (HOE 901) differing only in zinc content (15, 30, and 80 µg/ml).

RESEARCH DESIGN AND METHODS — We conducted 2 studies. Study 1 compared the subcutaneous abdominal injection of 0.15 U/kg of ^{125}I-labeled insulin glargine[15], insulin glargine[80], NPH insulin, and placebo. In study 2, 0.2 U/kg of insulin glargine[30] was injected into the arm, leg, and abdominal regions. Both studies had a randomized crossover design; each enrolled 12 healthy men, aged 18–50 years.

RESULTS — In study 1, the time in hours for 25% of the administered radioactivity to disappear after bolus subcutaneous injection ($T_{75\%}$) for NPH insulin indicated a significantly faster absorption rate compared with the 2 insulin glargine formulations (3.2 vs. 8.8 and 11.0 h, respectively, $P < 0.0001$). Mean residual radioactivity with NPH insulin was also significantly lower at 24 h (21.9 vs. 43.8 and 52.2%, $P < 0.0001$). The calculated plasma exogenous insulin concentrations after NPH insulin were substantially higher than those with insulin glargine, reaching a peak within the first 6 h after administration before declining. Insulin glargine, however, did not exhibit a distinct peak. Weighted average plasma glucose concentration between 0 and 6 h was significantly lower after NPH compared with insulin glargine ($P < 0.001$).

In study 2, there were no significant differences in the absorption characteristics of insulin glargine between the 3 injection sites ($T_{75\%}$ = 11.9, 15.3, and 13.2 h for arm, leg, and abdomen, respectively) or in residual radioactivity at 24 h.

CONCLUSIONS — Subcutaneous absorption of insulin glargine is delayed compared with NPH insulin. There is little or no difference in the absorption rate of insulin glargine between the main subcutaneous injection sites.

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Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; IRI, immunoreactive insulin; NEFA, nonesterified fatty acid; $T_{75\%}$, time in hours for 25% of the administered radioactivity to disappear after bolus subcutaneous injection.

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

Currently available intermediate- and long-acting insulin preparations do not simulate normal basal insulin secretion and fail to provide optimal glycemic control, with the risk of hypoglycemia and/or hyperglycmia occurring in association with marked insulin peaks and limited duration of effect, respectively (1–3). Recombinant DNA technology, however, has enabled modifications to be made to the human insulin molecule in an attempt to improve the pharmacokinetics of these insulins after subcutaneous administration (4–8).

Insulin glargine (HOE 901) is a di-arginine (30^{B}a-L-Arg-30^{B}b-L-Arg) human insulin analog in which asparagine at position 21^{A} is replaced by glycine. This achieves an increase in the isoelectric point from pH 5.4 (native insulin) to 7.0 and stabilization of the molecule. When injected as a clear acidic solution (pH 4.0), insulin glargine undergoes microprecipitation in the subcutaneous tissue, which retards absorption. Insulin glargine has been formulated in early studies as insulin glargine[15], insulin glargine[30], and insulin glargine[80], which differ only in their zinc content of 15, 30, and 80 µg/ml (2.295, 4.59, and 12.24 µmol/l), respectively.

Studies in animals (9,10) and healthy human subjects (11,12) have shown that insulin glargine exhibits a protracted action profile compared with NPH insulin, which is characterized by a relatively flat plasma insulin concentration profile. In a euglycemic clamp study in healthy volunteers (11), NPH insulin was associated with a rapid onset of effect, peak action occurring at ~6 h, and termination of effect ~16 h after subcutaneous injection. Insulin glargine[15] and insulin glargine[80] exhibited a much slower onset of action and no distinct peak throughout the course of the 24-h study period.

We report here the results of 2 studies in healthy human subjects. In study 1, we compared the absorption rate from the site of bolus subcutaneous injection of ^{125}I-

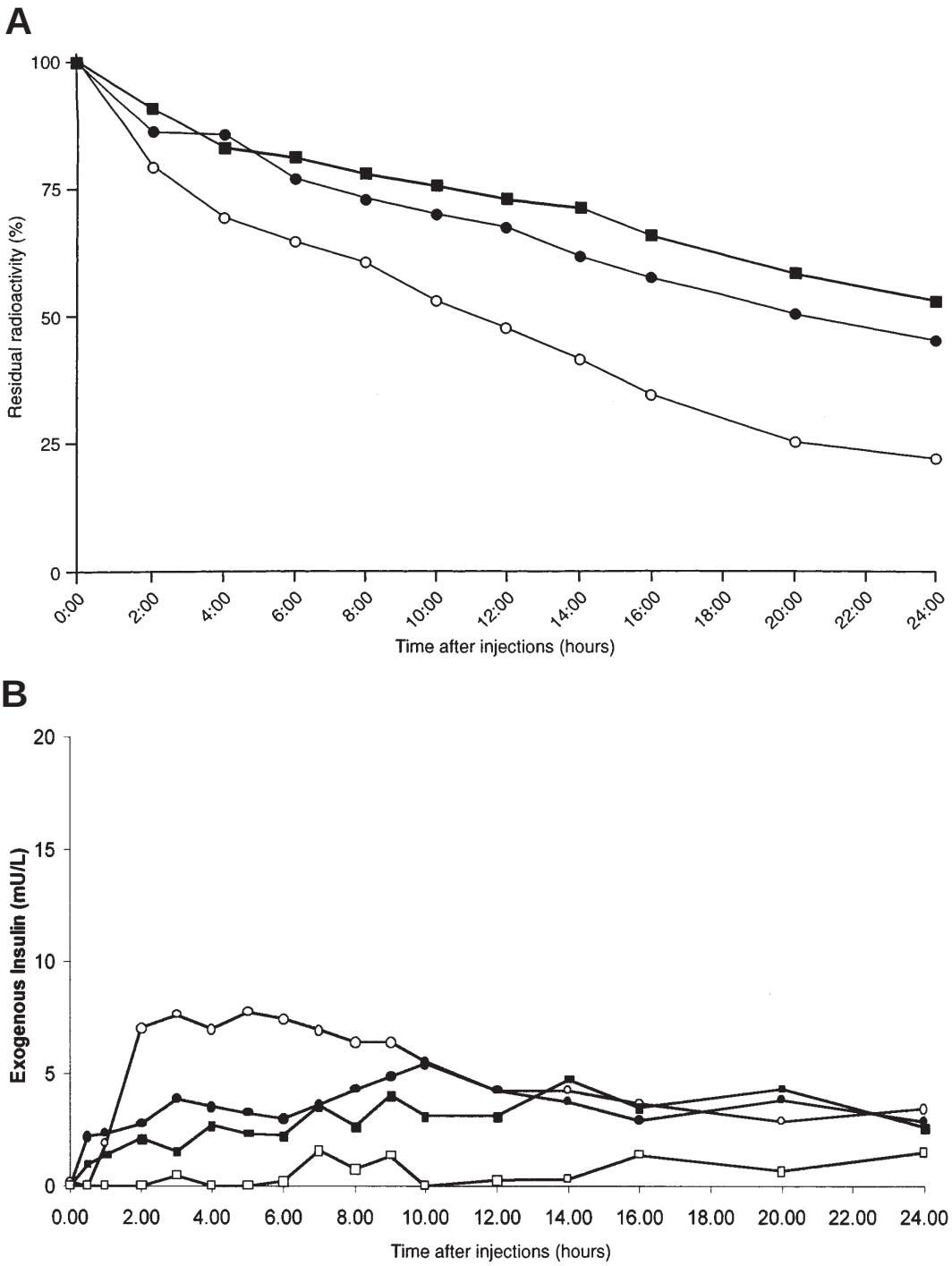

Figure 1—*Comparison of median percentage residual radioactivity* (A) *and exogenous insulin* (B) *at the abdominal injection site for placebo (□), NPH (○), insulin glargine[15] (●), and insulin glargine[80] (■).*

labeled insulin glargine[15] and insulin glargine[80] with that of NPH. In study 2, we compared the absorption rates of ^{125}I-insulin glargine[30] at 3 commonly used subcutaneous injection sites. In both studies, absorption was evaluated by determining rates of disappearance of radioactivity from the subcutaneous injection site and by calculating the exogenous insulin concentration profiles in plasma. Biologic activity was analyzed by comparing changes in blood glucose and nonesterified fatty acid (NEFA) concentrations during the 24-h study periods.

RESEARCH DESIGN AND METHODS

Study design

Both studies were of a randomized crossover design, with study 1 single-blinded and study 2 unblinded, and carried out at a

Table 1—*Summary of disappearance of radioactivity from the injection site (study 1)*

Variable	Insulin preparation: NPH	Insulin glargine[15]	Insulin glargine[80]	Treatment comparison	Mean treatment difference*	95% CI*	*P**
$T_{75\%}$	3.21 ± 1.37	8.75 ± 3.90	11.01 ± 4.21	NPH/insulin glargine[15]	−5.54	−7.68/−3.41	0.0001
				NPH/insulin glargine[80]	−7.80	−9.93/−5.67	0.0001
				insulin glargine[15]/insulin glargine[80]	−2.26	−4.39/−0.12	0.0311
% Remaining after 24 h	21.90 ± 9.85	43.84 ± 15.04	52.17 ± 15.84	NPH/insulin glargine[15]	−21.94	−30.39/−13.49	0.0001
				NPH/insulin glargine[80]	−30.27	−38.72/−21.82	0.0001
				insulin glargine[15]/insulin glargine[80]	−8.33	−15.85/−0.81	0.0429

Data are means ± SD, means, or CIs. *Based on the ANCOVA model.

single center. Ethical approval was obtained from the local ethics committee, and each subject gave written informed consent to participate. Both studies enrolled 12 healthy men aged 18–44 years with BMI of 18–30 kg/m^2 and a fasting plasma glucose level of <6.0 mmol/l (<108 mg/dl).

On the evening (1800) before each study day, the participants were given a carbohydrate-rich meal and then remained unfed from 2200 onward, except for water. Smoking was not allowed during the study, and the subjects remained rested throughout the study period.

During each study day, frequent blood samples were obtained via a cannula in an antecubital fossa vein, kept patent by a slow-running saline infusion, to facilitate blood sampling for the determination of plasma glucose, immunoreactive insulin (IRI), and C-peptide and NEFA concentrations.

Blood was taken into fluoride oxalate for glucose measurement and into lithium heparin for IRI and C-peptide measurements. Blood samples for NEFA were taken into EDTA. Samples were centrifuged at 4°C, and the supernatant stored at −20°C until assayed.

Blood glucose samples were assayed using a YSI 2300 glucose analyzer (YSI, Hants, U.K.). IRI was measured by a modification of the technique of Heding (13) using antibody M8309 (Novo Nordisk, Copenhagen, Denmark) and insulin glargine as the standard. C-peptide was measured by immunoassay (14) using antibody K6 (Novo Nordisk). Plasma NEFA was assayed using a Wako NEFA-C kit (Alpha, Hants, U.K.).

The protocol for study 1 involved 4 visits (visits 1–4) during which study medication was given, with between-visit washout phases of >7 days. Visits 1–4 were preceded by an overnight fast, and serial pharmacodynamic and pharmacokinetic variables were measured at each visit. The treatment days were randomly allocated and comprised of 3 insulin days and 1 placebo day.

Study 2 involved 3 treatment visits with washout periods of 7–14 days. The order of injection was randomized.

Study medication

All study medications were labeled with radioactive iodine, ^{125}I (50 kBq/dose [1850 µC/dose]), and administered by the same investigator. A single tablet of potassium iodide (120 mg) was given to participants on the evening before each study day to prevent absorption of radioactive iodine by the thyroid gland.

In study 1, the semisynthetic human NPH insulin (HOE 36H, basal-H-insulin 100, Hoechst AG) was administered from 5-ml vials, with each 1-ml suspension containing 100 U human insulin as a crystalline suspension with the excipients NaH_2PO_4, phenol, glycerol, and *m*-cresol at pH 7.3. The recombinant human insulin analog formulations insulin glargine[15] and insulin glargine[80] (Hoechst AG) were also administered from 5-ml vials, with each 1-ml suspension containing 21^A-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin equimolar to 100 U human insulin, together with *m*-cresol and glycerol at pH 4.0, with 15 and 80 µg/ml (2.295 and 12.24 µmol/l) zinc, respectively. All insulin formulations

Table 2—*Summary of plasma exogenous insulin weighted average concentrations for 0–6 and 6–24 h after injection (study 1)*

Variable	Insulin preparation (mU/l): NPH	Insulin glargine[15]	Insulin glargine[80]	Treatment comparison	Mean treatment difference (mU/l)*	95% CI*	*P**
0–6 h	6.69 ± 4.34	3.14 ± 1.91	2.95 ± 2.59	NPH/insulin glargine[15]	3.85	1.40/6.31	0.0017
				NPH/insulin glargine[80]	4.13	1.64/6.62	0.001
				insulin glargine[15]/insulin glargine[80]	0.27	−2.12/2.67	0.8034
6–24 h	4.39 ± 1.78	3.52 ± 1.97	4.03 ± 1.99	NPH/insulin glargine[15]	0.71	−0.81/2.24	0.3121
				NPH/insulin glargine[80]	0.16	−1.39/1.71	0.8208
				insulin glargine[15]/insulin glargine[80]	−0.55	−2.05/0.94	0.4213

Data are means ± SD, means, and CIs. *Based on the ANCOVA model.

Table 3—*Summary of blood glucose weighted average concentrations (study 1)*

Time period	Insulin preparation (mmol/l) Placebo	NPH	Insulin glargine[15]	Insulin glargine[80]	Treatment comparison	Mean treatment difference (mmol/l)*	95% CI*	*P**
0–6 h	5.33 ± 0.33	4.84 ± 0.61	5.17 ± 0.42	5.28 ± 0.34	NPH/placebo	−0.50	−0.71/−0.29	0.0001
					NPH/insulin glargine[15]	−0.35	−0.57/−0.14	0.0012
					NPH/insulin glargine[80]	−0.42	−0.64/−0.21	0.0002
					placebo/insulin glargine[15]	0.15	−0.07/0.36	0.1401
					placebo/insulin glargine[80]	0.08	−0.14/0.29	0.4500
					insulin glargine[15]/insulin glargine[80]	−0.07	−0.30/0.15	0.4790
6–24 h	5.18 ± 0.43	4.69 ± 0.36	4.76 ± 0.46	4.86 ± 0.60	NPH/placebo	−0.50	−0.69/−0.31	0.0001
					NPH/insulin glargine[15]	−0.11	−0.30/0.08	0.2228
					NPH/insulin glargine[80]	−0.14	−0.33/0.04	0.1008
					placebo/insulin glargine[15]	0.39	0.21/0.58	0.0001
					placebo/insulin glargine[80]	0.36	0.17/0.55	0.0003
					insulin glargine[15]/insulin glargine[80]	−0.04	−0.23/0.16	0.6760

Data are means ± SD, means, and CIs. *Based on the ANCOVA model.

were administered as single doses of 0.15 U/kg body wt. The placebo was an insulin diluent consisting of a clear solution of water for injection, NaH_2PO_4, glycerol, and *m*-cresol at pH 7.3, given as a single dose from a 10-ml vial. The study medications were injected subcutaneously into the anterior abdominal wall midway between the umbilicus and the anterior superior iliac spine. The injection technique involved lightly pinching a fold of skin, inserting the needle at a 45° angle to the skin surface, and slowly injecting the test preparations.

In study 2, insulin glargine[30] was administered from 5-ml vials, with each 1-ml solution containing 21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin equimolar to 100 U human insulin, 30 µg/ml (4.59 µmol/l) zinc, *m*-cresol, and glycerol at pH 4.0. Single doses of 0.20 U/kg body wt were injected subcutaneously into *1*) the anterior abdominal wall, midway between the umbilicus and the anterior superior iliac spine; *2*) the arm, midway between the greater tubercle and the lateral epicondyle of the humerus; and *3*) the leg, midway between the trochanter and the lateral epicondyle of the femur. As before, injection technique involved pinching a fold of skin, inserting the needle at a 45° angle to the skin surface, and slowly injecting the test preparations.

Pharmacokinetics/ pharmacodynamics

The primary pharmacokinetic end point in both studies was the time in hours for 25% of the administered radioactivity to disappear after bolus subcutaneous injection ($T_{75\%}$), with residual radioactivity frequently assessed up to 24 h after administration. Plasma exogenous insulin concentration profiles were estimated, and biological activity profiles, including concentrations of plasma glucose and NEFA, were monitored to evaluate the pharmacodynamic effect of the insulin treatments.

Insulin absorption was estimated using time plots of percentage of residual radioactivity at the injection site. A thallium-activated sodium iodine counter was positioned 50 mm from the site of injection and moved away only at the time of injection. Immediately after injection, the counter was repositioned over the injection site marked on the skin, and the ^{125}I count was read over 5 min. The probe was repositioned using the skin markings for all subsequent ^{125}I measurements every 2 h for 16 h and then at 20 and 24 h after the injection.

The total count rate of radioactivity absorption was calculated by adding counts at the specified times after injection, with the background count rate subtracted from each of these values. The background-corrected count rates were expressed as percentages of the count rate at time of injection.

Plasma insulin concentrations were measured as total IRI. Endogenous insulin concentration was estimated from the IRI:C-peptide ratio during the basal period multiplied by the C-peptide concentration at each time point. Exogenous insulin concentration was then derived by subtracting the calculated endogenous insulin concentration for each sample from the IRI concentration (15). Weighted average concentrations of plasma exogenous insulin were calculated as area under the plasma exogenous insulin concentration versus time curve divided by time for the periods 0–6, 6–24, and 0–24 h after injection. Maximum insulin concentration for the period 0–24 h after injection and time to maximum insulin concentration were also calculated. Similarly, for plasma C-peptide, NEFA, and glucose, the weighted average concentration per period (0–6, 6–24, and 0–24 h), initial and maximal change, and time to minimal and maximal concentrations were also estimated.

Statistical analyses

The Friedman's test was used to analyze minimal and maximal concentrations at time of fasting. The analysis of covariance (ANCOVA) model that we used contained the following effects: treatment (fixed factor), period (fixed factor), treatment sequence (fixed factor), treatment period interaction (fixed factor), and subject (random factor). The levels of fasting plasma glucose, initial exogenous insulin, C-peptide, and NEFA served as covariants for the respective variables. All statistical tests were 2-tailed, with an α level of 0.05. For pairwise comparisons, no α adjustment was performed. Differences between treatments were described using 95% CIs.

RESULTS

— A total of 12 participants completed each study. Subjects in study 1 were aged 18–39 years (median 24.0 years) with BMI between 19.5 and 30.2 kg/m^2 (median 24.1). Study 2 consisted

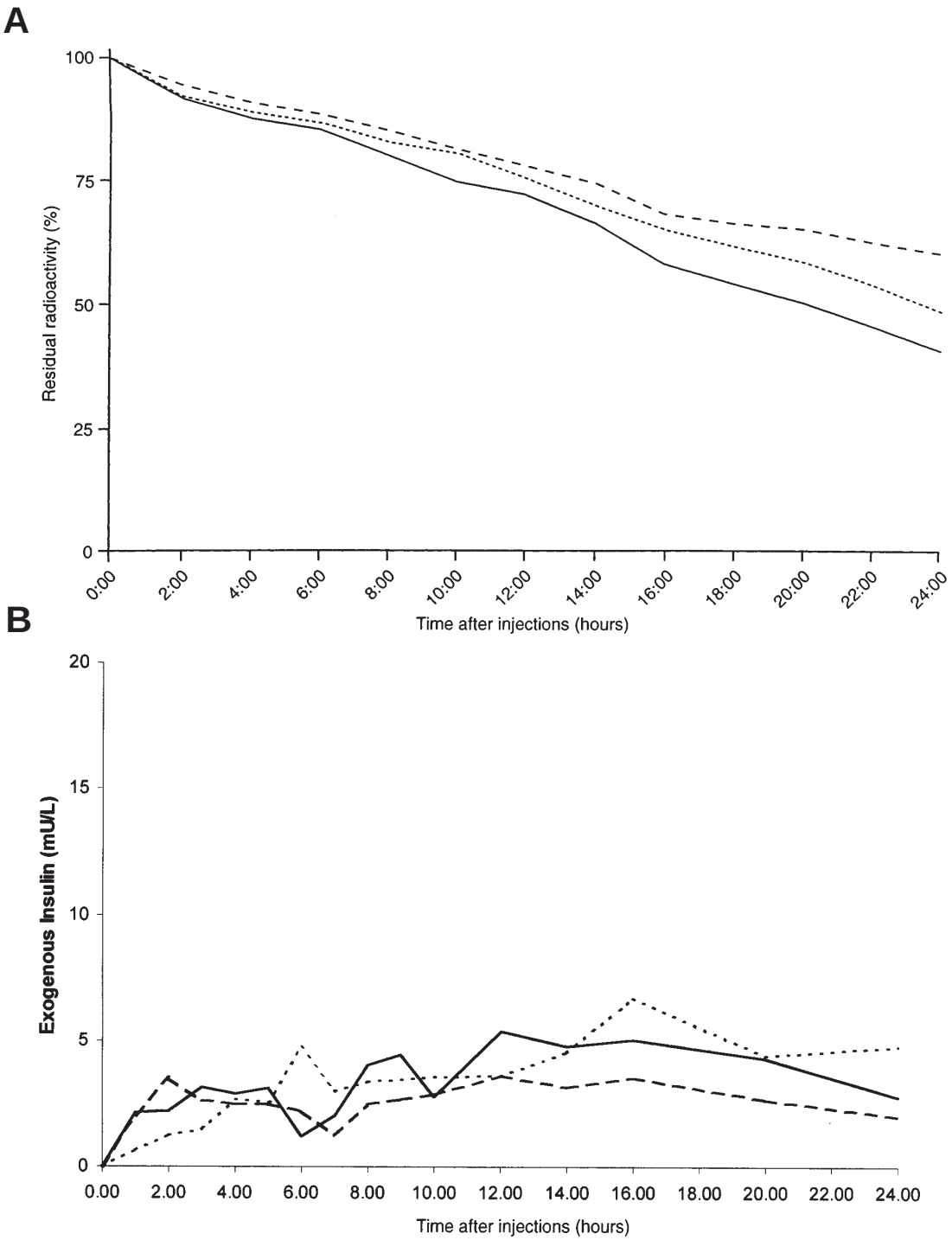

Figure 2—*Comparison of median percentage residual radioactivity (*A*) and exogenous insulin (*B*) for insulin glargine[30] after injection into the arm (—), leg (- - -), and abdomen (– –).*

of healthy men aged 23–44 years (median 32.5) with BMI between 21 and 28 kg/m^2 (median 24.4).

In study 1, absorption was more rapid with NPH insulin than with insulin glargine (Fig. 1). The mean $T_{75\%}$ for NPH insulin differed significantly from those observed for the 2 insulin glargine formulations (i.e., 3.21 vs. 8.75 h for insulin glargine[15] and 11.01 h for insulin glargine[80], $P < 0.0001$) (Table 1). This finding is supported by the mean residual radioactivity of NPH, which was significantly lower at 24 h for the same comparison ($P < 0.0001$). Compared with insulin glargine[80], insulin glargine[15] had a faster absorption rate at $T_{75\%}$ ($P < 0.03$) and yielded less residual radioactivity at 24 h ($P < 0.04$).

These findings for insulin absorption from the subcutaneous tissue were comple-

Table 4—*Summary of disappearance of radioactivity from the injection site for insulin glargine[30] (study 2)*

Variable	Injection site			Treatment comparison	Mean treatment difference ± SD
	Arm	Leg	Abdomen		
$T_{75\%}$	11.9 ± 6.2	15.3 ± 6.2	13.2 ± 4.6	arm/leg	–3.5 ± 6.1
				arm/abdomen	–1.3 ± 7.3
				leg/abdomen	2.1 ± 7.0
% Remaining after 24 h	47.7 ± 17.9	56.3 ± 14.8	57.2 ± 16.0	arm/leg	−8.6 ± 17.2
				arm/abdomen	−9.6 ± 20.4
				leg/abdomen	–1.0 ± 15.3

Data are means ± SD.

mented by analysis of plasma exogenous insulin, glucose, C-peptide, and NEFA concentrations. Plasma exogenous insulin profiles showed clear differences between the pharmacokinetics of NPH and the 2 insulin glargine formulations (Table 2). The mean ± SD plasma exogenous insulin weighted average concentration from 0 to 6 h after injection for NPH was 6.69 ± 4.34 mU/l compared with 3.14 ± 1.91 and 2.95 ± 2.59 mU/l for insulin glargine[15] and insulin glargine[80], respectively. The weighted average concentration from 6 to 24 h for NPH was 4.39 ± 1.78 mU/l compared with 3.52 ± 1.97 and 4.03 ± 1.99 mU/l for insulin glargine[15] and insulin glargine[80], respectively.

The blood glucose weighted average concentrations for all test preparations are shown in Table 3. The levels of fasting blood glucose for all treatments were similar. In the 6 h after injection, blood glucose levels were significantly lower ($P < 0.001$) for NPH than for insulin glargine[15] and insulin glargine[80] and for placebo (Table 3). From 6 to 24 h after injection, however, blood glucose levels were not significantly different for NPH and insulin glargine. Analyses of plasma C-peptide and NEFA for the period 0–24 h after injection indicated patterns consistent with the biological effect of the insulins on blood glucose.

In study 2 (Fig. 2), there were no significant differences in insulin glargine[30] absorption as reflected in $T_{75\%}$ or residual radioactivity 24 h after injection at the 3 injection loci studied by analysis of variance (ANOVA) (Table 4). Neither plasma exogenous insulin concentrations nor blood glucose levels differed according to injection site. In addition, no between-treatment differences were evident for C-peptide or NEFA.

No severe adverse events or injection site reactions were observed in either study.

CONCLUSIONS — Previous studies have shown that insulin glargine is a long-acting human insulin analog that can more closely mimic normal basal insulin secretion than NPH insulin, currently the most widely used insulin preparation for basal insulin replacement (9–12). Early trials in patients with type 1 diabetes have shown insulin glargine to be suitable for use as a basal insulin (16).

In the studies reported here, in study 1, the mean time to disappearance of 25% radioactivity ($T_{75\%}$) from the subcutaneous depot was significantly shorter ($P < 0.0001$) for NPH insulin than for either insulin glargine[15] or insulin glargine[80]. Likewise, mean residual radioactivity after 24 h was lower ($P < 0.0001$) after NPH insulin than after either insulin glargine[15] or insulin glargine[80]. Insulin glargine[15] was absorbed more rapidly than insulin glargine[80] ($P < 0.03$). This difference is consistent with animal studies that show zinc concentration dependency for insulin glargine absorption (9), but the difference in humans is small, with associated plasma glucose profiles suggesting absence of a clinically significant difference.

In study 2, there were little, if any, differences in insulin glargine[30] absorption rates with respect to injection sites. Overall, subcutaneous injections of this formulation into the arm, leg, and abdominal region of healthy volunteers resulted in equal prolongation of absorption of the insulin analog, and all were well tolerated. Statistical comparisons of $T_{75\%}$ and residual radioactivity after 24 h did not indicate a significant difference in absorption between the 3 injection sites. A faster absorption of human ultralente insulin from the abdomen than from the thigh has been demonstrated (17), but this difference was not apparent for bovine ultralente insulin. For NPH (isophane) insulin, no difference was found between subcutaneous injection into the thigh or abdomen (18). The findings in this study suggest that there are only minor, if any, injection site effects with insulin glargine[30].

The conclusions about insulin absorption in studies 1 and 2, which are based on disappearance of radioactivity at the site of injection, are supported by the appearance in plasma of the calculated exogenous insulin and by the glucose profiles. The plasma exogenous insulin profile of NPH in study 1 was consistent with other reports in the literature of NPH absorption, which reaches a distinct peak concentration 4–6 h after subcutaneous injection. In contrast, the plasma exogenous insulin profile of the insulin glargine formulations demonstrated absorption from the subcutaneous injection site at a reduced and relatively constant rate, with no observed prominent peak in plasma insulin concentration. Differences in blood glucose profiles indicated a statistically significant and clinically meaningful difference during the first 6 h after injection, when the absorption of NPH resulted in a relatively more rapid increase in insulin concentration and a greater and earlier hypoglycemic effect.

The results of these 2 studies provide additional evidence to suggest that insulin glargine exhibits the desirable characteristics of a basal insulin intended to provide 24-h glycemic control. Further studies are required to evaluate its full clinical potential as a long-acting insulin analog for basal insulin supply to complement meal-related (bolus) insulin administration.

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