Comparative Pharmacokinetics and Glucodynamics of Two Human Insulin Mixtures

70/30 and 50/50 insulin mixtures

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OBJECTIVE — To compare and contrast the pharmacokinetics and glucodynamics of two insulin mixtures, one of 50% NPH human insulin and 50% Regular human insulin (50/50) and one of 70% NPH human insulin and 30% Regular human insulin (70/30), in healthy male volunteers after subcutaneous administrations of 0.3 U/kg.

RESEARCH DESIGN AND METHODS — We administered single doses of 50/50 and 70/30 insulins to 18 volunteers in a randomized crossover fashion. All subjects received 0.3 U/kg of each mixture separated by at least 7 days. Each dose was given after an overnight fast and during a glucose clamp to maintain a euglycemic state. We measured serum insulin and C-peptide concentrations through frequent blood sampling after each treatment. Pharmacokinetic measurements were calculated from insulin data corrected for C-peptide, including maximum insulin concentration (C_{max}), time to maximum insulin concentration (t_{max}), terminal rate constant (β), area under the curve from 0 to ∞ (AUC_0^∞), and mean residence time (MRT). Pharmacodynamic measurements were summarized from C-peptide concentrations (minimum C-peptide concentration [C_{min}], time to minimum C-peptide concentration [t_{min}], area between the C-peptide baseline and the C-peptide suppression curve [AOC_c], absolute maximal difference from baseline [S_{diff}] and glucose clamp measurements. The glucose clamp measurements included maximum infusion rates (R_{max}) and time to R_{max} (TR_{max}) from glucose infusion rate (GIR) documentation, as well as cumulative glucose infused during the first 4 h ($^4_0G_{tot}$) and total glucose infused (G_{tot}) during the study.

RESULTS — For the pharmacokinetic assessment, statistically greater values of insulin C_{max} and β were found for the 50/50 mixture, whereas the 70/30 mixture had a greater MRT. Statistical differences were also detected in glucodynamics, with greater values of R_{max} and $^4_0G_{tot}$ found with the 50/50 mixture. Notably, differences were not detected for insulin AUC_0^∞ and G_{tot} values.

CONCLUSIONS — Higher insulin concentrations and a greater initial response were present with the 50/50 mixture, but the two mixtures had equivalent bioavailability and cumulative effects. These results support use of the 50/50 mixture in situations where greater initial glucose control is required.

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GIR, glucose infusion rate; R_{max}, maximum rate of infusion; TR_{max}, time to maximum rate of infusion; G_{tot}, total glucose infused; RIA, radioimmunoassay; CV, coefficient of variation; AUC_0^∞, area under the curve from 0 to ∞; V_β/F, apparent volume of distribution; Cl_s/F, apparent total systemic clearance; β, terminal rate constant; $t_{1/2}$, half-life; $^4_0G_{tot}$, cumulative glucose infused during the first 4 h; C_{max}, maximum insulin concentration; C_{min}, minimum C-peptide concentration; t_{max}, time to maximum insulin concentration; t_{min}, time to minimum C-peptide concentration; AOC_c, area between the C-peptide baseline and the C-peptide suppression curve; S_{diff}, absolute maximal difference from baseline; MRT, mean residence time; CI, confidence interval.

Regular insulin and NPH insulin are commonly mixed in clinical practice to take advantage of the features of both formulations to produce extended glucose control with a rapid onset. Mixing these two formulations may allow certain patients with diabetes to control more conveniently glucose excursions from morning and evening meals.

Previous studies have shown that marketed mixtures control glucose as well as or better than the prescribed patient-mixed ratios (1–3). Documented problems with patients mixing their own insulin include the inability of patients to mix insulins in the correct ratios (2) or inaccurate or biased measurements because of dead space in the syringe (4). Premixed insulins minimize the potential for these errors in addition to providing convenience. In some patients with a high frequency of these errors, blood glucose control may improve (2).

Most NPH insulin preparations made today do not contain an excess of protamine. In these modern preparations, the presence of protamine does not appear to retard insulin absorption when Regular insulin is mixed with NPH (5–8). Nonetheless, no direct comparisons have been made between two different premixed NPH and Regular insulin combinations. Therefore, to compare subcutaneous injections of 70/30 and 50/50 mixtures in healthy subjects, we monitored both serum insulin concentrations and induced glucose needs by means of a glucose clamp. In this way, we were able to define differences and similarities between the two insulin mixtures.

RESEARCH DESIGN AND METHODS

— Premixed vials of 70% NPH and 30% Regular (70/30) and 50% NPH and 50% Regular (50/50) were provided by Lilly (Indianapolis, IN). Both mixtures were human insulin of recombinant origin and contained 100 U/ml, with 1 U equivalent to 38.1 μg (6.56 pmol) of insulin.

This study was performed at the

Lilly Laboratory for Clinical Research (Indianapolis, IN). Study subjects were healthy men between 22 and 45 years of age and within 15% of their ideal body weight for their age, height, and frame size (the Metropolitan Life Health Insurance tables were used as a reference). Before study acceptance, all subjects were given a physical examination, including complete blood and urine chemistry evaluations, a chest X ray, and an electrocardiogram. All subjects had fasting blood glucose concentrations of <6.4 mM. The subjects had normal glucose tolerance tests, with blood glucose concentrations <7.8 mM 2 h after ingestion of 75 g of glucose. All participants gave their written informed consent.

Eighteen volunteers participated in this study. This was a two-way, open-labeled, randomized, crossover study. Each subject was given a single 0.3 U/kg dose of either the 70/30 or 50/50 mixture, and all doses were administered subcutaneously after an overnight fast.

A glucose clamp procedure that used a controlled glucose infusion system (Biostator®, Life Sciences, Miles, Elkhart, IN) was used to maintain a euglycemic state. The glucose clamp was maintained 0.54 mM below the subject's fasting glucose level. The test dose was administered once stabilization was achieved. Blood samples were collected for analysis of insulin and C-peptide at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 18, and 24 h after dose administration. Samples were collected from an antecubital vein contralateral to that used for glucose and saline infusion of the glucose clamp. The infusion rate necessary to maintain the subject's blood glucose was continuously recorded. These glucose infusion rates (GIRs) were then used as a glucodynamic measurement for insulin. From these data, the maximum rate of infusion (R_{max}) and the time when R_{max} occurred relative to the time of injection (TR_{max}) were recorded. In addition, the total glucose infused (G_{tot}) was documented.

The subjects continued to fast and remained at bedrest during the entire data collection period. The subjects received the alternative treatment in the same fashion 7 to 12 days after the first treatment.

Insulin radioimmunoassay (RIA) method

We used a commercially available RIA kit (Insulin Coat-A-Count) from Diagnostic (Los Angeles, CA) to measure the serum concentrations of insulin. We validated the RIA before analysis of study samples, and all analyses were performed in accordance with the kit's instructions. In short, each incubation included buffer, serum, or biosynthetic human insulin standard prepared in kit zero calibrator; ^{125}I-labeled porcine insulin; and a polyclonal guinea pig anti-porcine insulin antiserum. We incubated each binding reaction for 20–22 h at room temperature. Separation of bound and free labeled insulin was achieved by solid-phase antibody methodology using antibody-coated tubes. Assay data were analyzed by VAX computer using a weighted four-parameter logistic model algorithm. The insulin concentration of test samples was estimated from a standard curve of reference insulin that was prepared in the RIA kit's zero calibrator matrix. Each standard curve contained the following concentrations of human insulin: 0, 8.61, 17.2, 43, 86, 170, 430, 860, 1,720, 4,305, 8,610 and 17,220 pM.

The RIA's lower limit of detection was determined to be 8.6 pM. We assessed interassay precision and recovery by adding reference human insulin to serum pooled from fasted nondiabetic adults. Interday precision (percentage coefficient of variation [CV]) (n = 5) was 29.3% at 86 pM, 13.3% at 430 pM, and 13.3% at 4,305 pM. Recoveries for the serum control samples ranged from 92 to 114%. Interassay precision data for standard curve parameters ranged from 3.2% to 19.4%.

C-peptide RIA method

We used a validated competitive RIA method to measure the serum concentrations of C-peptide. This method is similar to other RIA methods for C-peptide (9,10). Briefly, the assay incubation included 100 μL of buffer, serum, or standard biosynthetic human C-peptide; ^{125}I-labeled t-BOC-tyr-human C-peptide (25 pg); and 50 μL of goat anti-human C-peptide antiserum (lot E08–7B2–159–4G, diluted 1:160,000). We incubated each binding reaction for 16–18 h at 4°C. Separation of bound and free labeled C-peptide was achieved by precipitating the bound fraction with a second antibody and 6% polyethylene glycol. After collecting the precipitates by centrifugation, the radioactivity was measured in a γ-counter. We analyzed assay data by VAX computer using a weighted four-parameter logistic model algorithm. The C-peptide concentration of serum test samples was estimated from a standard curve of biosynthetic human C-peptide. Each standard curve contained the following concentrations of C-peptide: 0, 0.008, 0.017, 0.033, 0.083, 0.17, 0.33, 0.83, 1.66, 3.31, 8.28, 16.55 and 33.1 nM.

The RIA's lower limit of detection was determined to be 0.027 nM. We assessed interassay precision and recovery by adding reference human C-peptide to charcoal-stripped serum from fasted nondiabetic adults. Interday precision (percentage CV) (n = 6) was 15.4% at 0.033 nM, 6.0% at 0.33 nM, and 11.0% at 3.31 nM. Recoveries for the serum controls ranged from 109 to 130%. Interassay precision data (percentage CV) for standard curve parameters ranged from 3.5 to 18.5%. Insulin cross-reacted in the RIA <0.001% as well as C-peptide did on a weight basis.

Pharmacokinetic and glucodynamic analyses

Serum concentrations of insulin were used to calculate several pharmacokinetic parameters, including apparent volume of distribution (V_β/F), apparent total systemic clearance (Cl_s/F), the terminal rate constant (β), and half-life ($t_{1/2}$). Calculation of these values was performed using noncompartmental procedures (11).

Table 1—*Summary of pharmacokinetic parameters: insulin corrected for C-peptide*

Parameter	50/50 mixture	70/30 mixture	*P* value
C_{max} (pM)	381 ± 110	231 ± 53.4	<0.001
t_{max} (h)	1.9 ± 0.94	2.2 ± 1.3	NS
AUC_0^∞ (pM/h)	2,342 ± 840	2,238 ± 701	NS*
β (h^{-1})	0.197 ± 0.091	0.129 ± 0.069	0.004
$t_{1/2}$ (h)	3.52†	5.36†	–
MRT (h)	6.43 ± 2.00	10.6 ± 3.50	0.001

Data are means ± SD. *Statistical method: two one-sided Student's *t* tests. † Harmonic means: $t_{1/2}$ not compared statistically; statistical evaluations between treatments dependent on comparisons of β.

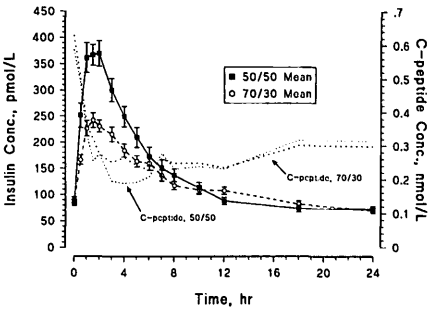

Figure 1—*Mean measured serum insulin and C-peptide concentrations after a single 0.3 U/kg subcutaneous dose of the 50/50 mixture or the 70/30 mixture. Bars represent SE.*

The insulin concentrations were adjusted for endogenous insulin using C-peptide concentrations as an indicator for endogenous insulin production. A method published in other insulin publications (12–14) was used to approximate endogenous insulin concentrations:

$$C'_t = C_t - (C_0)\frac{Cpep_t}{Cpep_0}$$

where C'_t represents the adjusted insulin concentration at time t, C_t the measured insulin concentration at time t, C_0 the insulin concentration at baseline (time 0), $Cpep_t$ the C-peptide concentration at time t, and $Cpep_0$ the C-peptide concentration at baseline.

A glucose infusion apparatus (Biostator®) was used to perform the glucose clamp procedure. The Biostator® uses an intravenous glucose sensor with glucose measured on a continuous basis. Appropriate glucose infusions were made automatically after insulin administration to maintain blood glucose at the preset levels. The GIR, G_{tot}, and blood glucose values were recorded and input into an IBM-compatible computer. Average GIR measurements were calculated every 30 min for the first 12 h then hourly until the final blood collection (24 h after dosing). Each subject's data were consolidated, allowing for times when the glucose clamp needed to be stopped and restarted (i.e., if catheters needed changing because of clogging). During early blood collections, a 5-min infusion average was computed from GIR observations beginning 2 min before until 2 min after the blood sample was collected. At late collection times (after 12 h), this calculation was extended to an 11-min infusion average, from 5 min before until 5 min after the blood samples were collected, except for the last collection time, which was a 10-min average just before the sample collection (at 24 h). R_{max} (mg/min) and TR_{max} (h) were derived from these time-averaged data. Two cumulative G_{tot} values were collected: the total glucose infused during the first 4 h ($^4_0G_{tot}$) and the total glucose infused for the duration of the study (G_{tot}).

The C-peptide data were considered a response measurement to exogenous insulin administration. We attempted to quantify this response by measuring the minimum C-peptide concentrations observed (C_{min}, nM), the time to C_{min} (t_{min}, h), the area between the C-peptide baseline ($Cpep_0$) and the C-peptide suppression curve (AOC_c, nM/h), and the absolute maximal difference from baseline (S_{diff}, nM). Calculation of AOC_c used $Cpep_0$ as a standard reference value and was performed using the trapezoidal rule. The baseline C-peptide was assumed to remain constant during the entire collection period.

In addition to these measurements, a relationship between the insulin serum concentrations and the GIR values at each time was constructed using an effect compartment model (21). From this relationship, estimates of onset, maximum response, and duration of action were produced for both 70/30 and 50/50 insulins.

Statistical analysis

The comparisons between treatments for pharmacokinetic and glucodynamic values were made by an analysis of variance accounting for subject and period effects. In addition, insulin AUC was compared between the treatments by using two one-sided Student's *t* tests as an assessment of bioavailability equivalence (15). Treatments were considered statistically different when $P < 0.05$. The statistical program SAS was used for all statistical comparisons.

RESULTS— All 18 subjects completed the study, and no serious complaints or adverse events were encountered.

Table 1 summarizes the pharmacokinetic measurements of insulin with statistical inferences. The mean serum insulin and C-peptide concentrations from both treatments are given in Fig. 1. The C_{max} values were statistically different between treatments, confirming the apparent differences shown in Fig. 1. In addition, the β and mean residence time values were also significantly different. No carryover effects were detected for any parameter.

Figure 1 suggests that the higher insulin concentrations from the 50/50 mixture appeared to suppress endoge-

Table 2—*Summary of C-peptide parameters*

Parameter	50/50 mixture	70/30 mixture	*P* value
C_{pep0} (nM)	0.59 ± 0.20	0.63 ± 0.24	—
C_{min} (nM)	0.13 ± 0.086	0.16 ± 0.13	NS
t_{min} (h)	5.0 ± 2.7	8.6 ± 5.1	0.031
AOC_c (nM/h)	7.51 ± 2.69	8.47 ± 3.67	NS
S_{diff} (nM)	0.46 ± 0.14	0.46 ± 0.17	NS

Data are means ± SD. AOC_c calculated by the trapezoidal rule up to 24 h, with the C-peptide baseline as a reference.

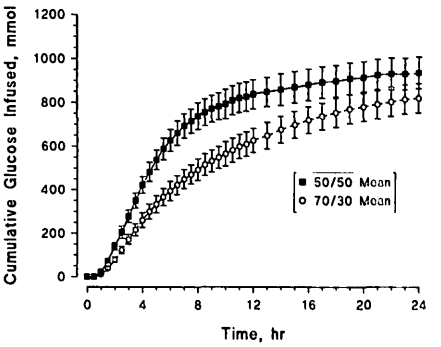

Figure 3—*Mean cumulative glucose infused to maintain euglycemia after administration of the 50/50 mixture or the 70/30 mixture. Bars represent SE.*

nous insulin production to a greater extent as measured by the mean C-peptide data. However, endogenous insulin suppression does not appear to be significant between treatments. Table 2 shows the comparison of C-peptide parameters between treatments, with only the t_{min} values showing statistical significance.

Plots of mean GIR versus time for both treatments are provided in Fig. 2, with a summary of the glucodynamic parameters given in Table 3. These glucodynamic data agree with the plots of serum concentration versus time (Fig. 1), which indicate that the 50/50 mixture induced a significantly greater maximum glucose demand (R_{max}) than did the 70/30 mixture. These data also suggest the effects from the 70/30 mixture may be more prolonged. Figure 3 compares the cumulative total glucose infused for both treatments during the course of the study. As expected, the 50/50 mixture appeared to induce a greater glucose need more rapidly than the 70/30 mixture. This initial greater glucose demand was statistically significant ($^{4}_{0}G_{tot}$, Table 3). However, both mixtures achieve asymptotes that are not significantly different (G_{tot}, Table 3). Figure 4 shows the blood glucose values for both treatments during the study and verifies that the clamp procedure was successful in maintaining equivalent and constant blood glucose levels for both treatments.

Figure 5 shows the predicted glucose infusion rates based on the effect compartment model, with 95% confidence intervals also indicated. Using the 10% E_{max} value as an indicator of a clinically observable response, estimates of onset, peak, and duration were obtained as defined in a previous publication (21). These estimates are provided in Table 4.

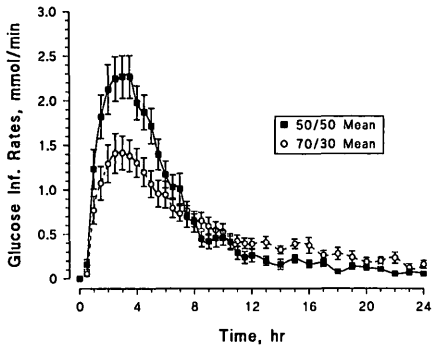

Figure 2—*Mean glucose infusion rates needed to maintain euglycemia after administration of the 50/50 mixture or the 70/30 mixture. Bars represent SE.*

CONCLUSIONS— This study compared the pharmacokinetics and glucodynamics of two different mixtures of an intermediate-acting insulin (NPH) and a rapid-onset insulin (Regular). Differences between NPH and Regular insulin pharmacokinetics and pharmacodynamics are well-known (14,16–18). Mixtures of these two insulins have also been studied, but such studies have generally been limited to 70/30 or mixtures with greater percentages of NPH insulin (18,19).

Distinct differences in the serum concentration versus time curves were found between the two mixtures (Fig. 1). The greater C_{max} of the 50/50 mixture is expected because of the greater percentage of the rapid-onset insulin (Regular). Additionally, the 70/30 mixture returns to baseline concentrations at a later time. This, again, is a factor of the greater amount of Regular versus NPH insulin in the 50/50 mixture when compared with the 70/30 mixture. The β values were also statistically different between mixtures, with the 70/30 mixture showing a longer half-life. Subcutaneously administered

Table 3—*Summary of glucodynamic parameters*

Parameter	50/50 mixture	70/30 mixture	*P* value
R_{max} (mg/min)	512 ± 165	335 ± 142	<0.001
TR_{max} (h)	3.3 ± 1.0	3.5 ± 1.3	NS
G_{tot} (g)	169 ± 53.8	148 ± 51.0	0.119
$^{4}_{0}G_{tot}$ (g)	75.6 ± 30.6	46.7 ± 25.2	<0.001

Data are means ± SD.

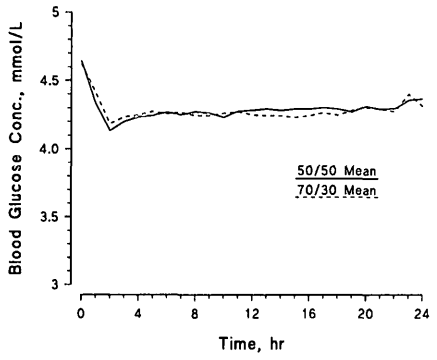

Figure 4—*Mean blood glucose concentrations that verify euglycemia after administration of the 50/50 mixture or the 70/30 mixture. Error bars have not been included for reasons of clarity.*

insulin (both Regular and NPH) is subject to absorption-rate-limited elimination, where β actually represents the absorption rate constant (11,20). Therefore,

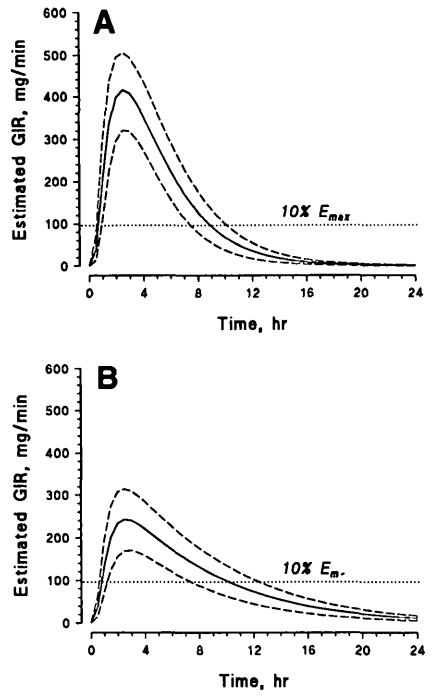

Figure 5—*Predicted glucose infusion rates from the effect compartment model for both the 50/50 mixture (A) and the 70/30 mixture (B). ____, Mean values; – – – –, 95% CIs about the means. The 10% E_{max} level also is indicated, above which a clinically observable effect is expected.*

Table 4—*Predicted onset, peak, and duration of activity*

Mixture	Onset (h)	Peak (h)	Duration (h)
50/50	0.5–1.0	1.5–4.5	7.5–10
70/30	0.75–1.5	1.5–6	7–12.5

comparisons of the calculated β values actually represent comparisons of the absorption rate constant. Because Regular insulin is absorbed more quickly than NPH (16), one would expect the 50/50 insulin to have a greater value for β (and thus, a shorter half-life of absorption) because more Regular insulin is present in this mixture. Despite these differences, the AUC_0^∞ values are not significantly different between the two mixtures, which indicates that the two mixtures have equivalent bioavailability. These data suggest that the greater amount of protamine present in the 70/30 mixture does not present a problem with insulin bioavailability and, therefore, confirms previous observations (6–8). Additionally, the t_{max} values were not significantly different, which suggests that one could expect maximum effects to occur at the same time for both mixtures.

The C-peptide curves show a greater reduction at early times with the 50/50 mixture, as expected. However, the AOC_c values are equivalent between treatments, again supporting equivalent bioavailability between the two formulations. The only C-peptide parameter that was significantly different between treatments was t_{min}, which suggests that the maximum reduction of C-peptide occurred at a later time with the 70/30 mixture. However, this comparison must be interpreted with caution because the variability of this parameter was large with both treatments.

The GIR time-action profile (Fig. 2) reflects the serum insulin concentration versus the time curve and shows that R_{max} for the 50/50 mixture is significantly greater but occurs at the same time as that for the 70/30 mixture. As expected from the results of the higher initial insulin concentrations, these data show a greater amount of glucose control at earlier times with 50/50. These data are supported by the significantly greater amount of glucose infused during the first 4 h ($^4_0G_{tot}$) using the 50/50 mixture. However, Fig. 2 shows that the effects of the 70/30 mixture appear to last longer. This longer duration of action was confirmed by the effect compartment modeling, which suggests that the duration of action for 50/50 is 7.5–10 h and that of 70/30 is 7–12 h for a 0.3 U/kg dose. Again, the differences in serum insulin concentrations at later times between the two formulations (Fig. 1) support this finding. In addition, the equivalency of net glucose infused (G_{tot}) between treatments supports the greater glucose control at later times with the 70/30 mixture. The equivalence of bioavailability and net glucodynamics between mixtures suggests that the two mixtures could be interchanged reliably when differences in glucose control arise.

To summarize, the 50/50 mixture produces greater initial serum concentrations and induces a greater initial glucose demand during a euglycemic glucose clamp compared with the 70/30 mixture. However, the 70/30 mixture has a longer duration of action than does the 50/50 mixture. The two mixtures exhibit equivalent bioavailability and net glucose demands. Thus, the 50/50 mixture can be used interchangeably with the 70/30 mixture when more immediate glucose control is required. Such a requirement typically may occur in the morning, when insulin resistance is at its greatest.

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