CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-017
21-018

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

510/151

Clinical Pharmacology and Biopharmaceutics Review

NDA:	**21-017** **21-018**
Insulin Lispro Protamine Suspension 75%/Insulin Lispro 25% **Insulin Lispro Protamine Suspension 50%/Insulin Lispro 50%** **(Humalog 75/25®)** **(Humalog 50/50®)**	
Sponsor:	**Eli Lilly**
Submission Date:	**21 December 1998**
Type of Submission:	**New Drug Application**
Reviewer:	**Michael J. Fossler**

Synopsis

Eli Lilly has submitted NDAs 21-017 and 21-018 for Humalog 75/25[1] (75% neutral protamine insulin lispro and 25% insulin lispro) and Humalog 50/50 (50% insulin lispro and 50% neutral protamine insulin lispro). These mixtures are intended for use in BID regimens in which both short and long-acting insulins are injected in combination before the morning and evening meals. According to the sponsor, the pre-mixed lispro mixtures are intended to offer convenience as well as the advantages of the faster absorption of insulin lispro.

For these NDAs a total of 8 studies were performed in both healthy volunteers and patients with Type 1 diabetes. Most of these studies utilized the euglycemic clamp methodology.

The Humalog 50/50 formulation required some changes in the concentrations of preservatives in order to conform with European standards. Since these compounds (primarily cresols) are known to promote insulin aggregation, a bioequivalence study was performed to test the performance of the clinical trials formulation relative to the to-be-marketed formulation. The two formulations are bioequivalent with respect to AUC and Cmax. There seems to be a minor increase in tmax for the new formulation, but there is considerable overlap in the two formulations.

During the development of these products, it was found that it was not possible to mix soluble insulin lispro and human NPH insulin and get a stable product. The reason is that exchange of insulin molecules takes place over many months, resulting in a less-than-desirable shelf life. Therefore, a new entity, neutral protamine insulin lispro (NPL) was created for the long-acting portion of the mixes. To compare the new NPL to NPH, a preliminary study in 8 healthy volunteers was performed using the euglycemic clamp. Each volunteer received a 0.4 U/kg injection of either NPL or Humulin N (NPH). The profiles of the two insulins are not identical; NPL is clearly absorbed faster than NPH, demonstrating a higher peak which occurs sooner than with NPH. This difference is also reflected in the pharmacodynamics of the two insulins, with the peak of the glucose infusion rate considerably earlier for NPL than for NPH. Despite its faster absorption (relative to NPH), NPL clearly has the properties of a basal insulin.

The pivotal PK/PD study submitted for the approval of Humalog 75/25 and 50/50 was a five-period, randomized open-label crossover study in 31 healthy volunteers (18 men, 13 women) given the following treatments (0.3 U/kg given in the abdomen) under euglycemic clamp conditions:

- NPL

[1] The naming convention follows the format established when Humulin 70/30 was approved; that is % slow insulin/%fast insulin.

- Humalog 75/25
- Humalog 50/50
- Humalog 25/75
- Humalog R

A linear relationship exists between the percentage soluble insulin lispro and the pharmacodynamic parameters Rmax and $Gtot_{(0\text{-}5\ hrs)}$. The firm used simple linear regression to describe this relationship (figure 2). Although a line does appear to fit the data for Rmax (and, although it is not shown, Gtot(0-5)), the correlation coefficient is low (0.26). This low r^2 is due to significant lack of fit due to auto-correlation, e.g., subjects that have low Rmax values tend to stay relatively low from treatment to treatment. The reviewer performed an exploratory analysis using NONMEM version V. These results showed an improvment in the r^2 value.

Two studies were performed in patients with Type 1 diabetes. Study IOGI was a randomized, incomplete block design in 12 diabetics (10 male, 2 female) who were randomized to receive three of the following four treatments:

- 0.3 U/kg NPL
- 0.3 U/kg NPH
- 0.3 U/kg 75/25
- 0.3 U/kg 50/50

It was found that levels in patients are significantly lower than those found in normal volunteers at equivalent doses. The time to peak values between the two studies are comparable. The firm explains these results by noting that a lispro-specific assay was used in this study, which does not measure endogenous insulin. This assay includes a precipitation step which could result in loss of insulin lispro from the samples. The NPH arm was assayed with a [redacted] method which also includes a [redacted] The values from the NPH arm were not reported because there were so few quantifiable levels. This may be due to loss of insulin during the [redacted] This, however, does not explain the GIR data, which are also lower than what was seen in the normal volunteer study. This suggests that there may be some degree of insulin resistance in these Type 1 patients. Taken together, it appears that although both the PK and PD responses are lower in Type 1 patients than in healthy volunteers, they may not be related. The lower insulin levels are likely an artifact of the assay method, while the PD results may be due to a moderate degree of insulin resistance in the patients.

The second study performed in Type 1 diabetic patients was Study IOFX. This was an open-label, randomized, crossover study in 31 Type 1 diabetics given either Humulin 70/30 or Humalog 75/25 prior to a standard test meal. Humulin was given 30 minutes prior to the meal as is standard practice. The Humalog 75/25 was given immediately before eating. The dose of insulin given was individualized for each patient but kept constant across treatments, based on their responses to previous test meal challenges.. The mean post-prandial blood glucose curves are essentially identical; Humulin shows a larger attentuation of the post-prandial glucose peak which may be due to the greater proportion of soluble insulin contained in this product, Also, the baseline glucose values were slightly but significantly higher in the 75/25 group as compared to the Humulin group (mean of 138.4 vs. 121.2, $p < 0.0001$). The results suggest that Humulin 70/30 and Humalog 75/25 will give clinically equivalent control, but that the Humalog mixture may be given immediately before a meal, which may be more convenient for the patient. A similar study using Humulin 50:50 and Humalog 50/50 (Study IOFY) has been completed and is being analyzed at this time.

Recommendations

The clinical pharmacology and biopharmaceutics portions of NDAs 21-017 and 21-018 are approved. The text under Labeling Comments should be forwarded to the firm.

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Abbreviations used:

Rmax – peak of the glucose infusion rate curve
TRmax – time at which the peak of the glucose infusion rate curve is reached
GIR – glucose infusion rate
IRI – immunoreactive insulin
Gtot, Gtot5 – amount of glucose required in the glucose clamp study. Found by integration of the GIR vs. time curve.

Appendix of Study Summaries (available from DPE-2 upon request)

I. Background

Eli Lilly has submitted NDAs 21-017 and 21-018 for Humalog 75/25 (25% insulin lispro and 75% neutral protamine insulin lispro) and Humalog 50/50 (50% insulin lispro and 50% neutral protamine insulin lispro). These mixtures are intended for use in BID regimens in which both short and long-acting insulins are injected in combination before the morning and evening meals. The pre-mixed lispro mixtures are intended to offer convenience as well as the advantages of the faster absorption of insulin lispro.

Insulin lispro was approved in 1996 as a short-acting soluble insulin (NDA 20-563). It is identical in stucture to human insulin, except the proline and lysine residues at positions 28 and 29 of the B chain are reversed. This reversal results in a reduced tendency for the insulin to form hexamers, which greatly increases its rate of absorption.

For these NDAs a total of 8 studies were performed in both healthy volunteers and patients with Type 1 diabetes. Most of these studies utilized the euglycemic clamp methodology.

The following specific questions will be answered about Humalog 75/25 and 50/50:

- How does the PK/PD of neutral protamine insulin lispro (NPL) compare with that of human insulin NPH?
- How do the PK/PD properties of these mixtures compare with each other and with soluble insulin lispro in normal volunteers?
- Are there differences in the PK/PD of these insulin mixtures in Type 1 patients as compared with healthy volunteers?
- What is the optimum time of injection relative to a meal of 75/25?
- How does the PK/PD of Humulin 70/30 compare with Humalog 75/25?

II. Assay Method and Validation

Three assay methods were used in the studies submitted in this NDA. The method used depended on the population studied (e.g., patients vs. normal volunteers) and the type of insulin administered (human insulin vs. insulin lispro). In healthy subjects (in whom circulation insulin antibodies are not of concern) a standard [redacted] insulin assay was used, which can measure both the native and analog molecules. In patients, free insulin (antibodies removed with [redacted]) was measured or, in the case of insulin lispro, a specific assay for the lispro molecule was used. This assay has very little cross-reactivity with human insulin. Relevant assay validation data for the three assays is described below in Table 1.

Table 1: Assay validation summary.

Parameter	Lispro-Specific Assay	Free Human Insulin Assay	Total Human Insulin Assay (used for insulin lispro)
Limit of Quantification			
Dilutional Linearity			
Specificity			
Inter-assay precision			
Inter-assay accuracy			
Stability			

III. Bioavailability and Bioequivalence

Bioequivalence

The Humalog 50/50 formulation required some changes in the concentrations of preservatives in order to conform with European standards. Since these compounds (primarily cresols) are known to promote insulin aggregation, a bioequivalence study was performed to test the performance of the clinical trials formulation relative to the to-be-marketed formulation. Study IOET was a randomized open-label crossover trial in 30 healthy volunteers (20 men, 10 women) and utilized the euglycemic clamp technique. The dose of insulin lispro mix given to each volunteer was 0.2 U/kg.

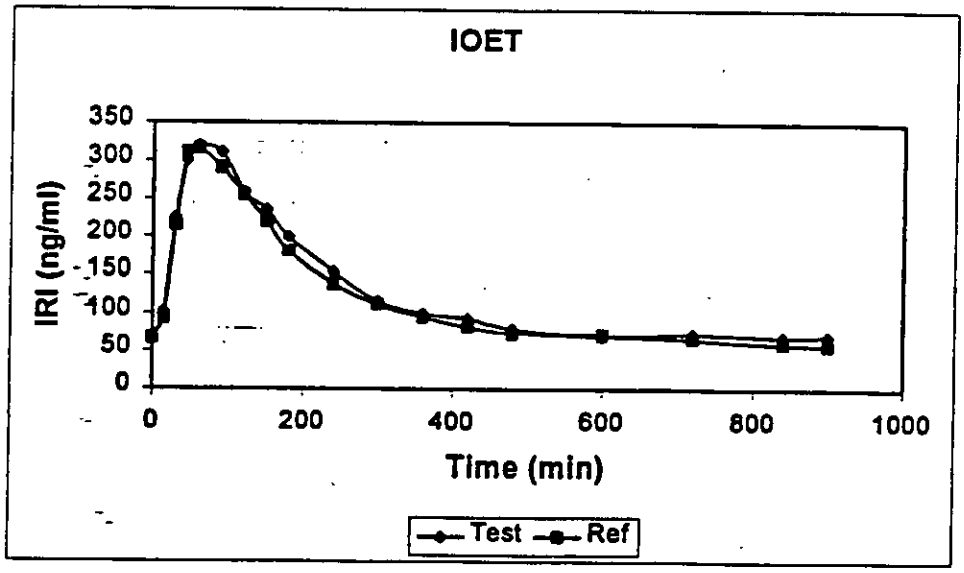
The results of the PK portion of the trial are shown below in Table 2. The two formulations are bioequivalent with respect to AUC and Cmax. There seems to be a minor increase in tmax for the new formulation, but there is considerable overlap in the two formulations, as seen in the inter-quartile ranges for each. The mean serum insulin lispro concentrations are shown in Figure 1.

Table 2: Results summary for Study IOET. Values in the table are mean ± SD, except where otherwise specified.

Parameter	Clinical Trials Formulation (Reference)	To-be-Marketed Formulation (Test)
$AUC_{(0\text{-baseline})}$	7.23 ± 2.05	7.73 ± 2.31
90% CI	*106 (99.9 – 113)*	
C_{max}	2.08 ± 0.61	2.11 ± 2.31
90% CI	*102 (94.2 – 111)*	
$^{1}t_{max}$	60	75

[1]median (inter-quartile range)

Figure 1: Mean serum insulin lispro concentration vs. time curves for the clinical and TBM formulations of Humalog 50/50.



IV. Pharmacokinetics/Pharmacodynamics

Neutral Protamine Insulin Lispro

During the development of these products, it was found that it was not possible to mix soluble insulin lispro and human NPH insulin and get a stable product. The reason is that exchange of insulin molecules takes place over many months, resulting in a less-than–desirable shelf life. Therefore, a new entity, neutral protamine insulin lispro (NPL) was created for the long-acting portion of the mixes. To compare the new NPL to NPH, a preliminary study in 8 healthy volunteers was perfromed using the euglycemic clamp. Each volunteer received a 0.4 U/kg injection of either NPL or Humulin N (NPH). The PK and PD results are shown in Table 3 and in Figures 2 and 3. The profiles of the two insulins are not identical; NPL is clearly absorbed faster than NPH, demonstrating a higher peak which occurs sooner than with NPH. This difference is also reflected in the pharmacodynamics of the two insulins, with the peak of the glucose infusion rate considerably earlier for NPL than for NPH. Despite its faster absorption (relative to NPH), NPL clearly has the properties of a basal insulin.

Table 3: Comparison of the PK/PD properties of NPL and NPH (Study IOBS).

Parameter	NPH	NPL
$AUC_{(0\text{-baseline})}$	907 ± 394	966 ± 447
C_{max} (ng/ml)	1.86 ± 0.47	2.02 ± 0.69
t_{max} (min)	394 ± 166	371 ± 185
R_{max} (mg/min/kg)	3.05 ± 1.34	3.69 ± 1.43
G_{tot} (g/kg)	1.69 ± 0.6	1.90 ± 0.71

Normal Volunteers

The pivotal PK/PD study submitted for the approval of Humalog 75/25 and 50/50 was a five-period, randomized open-label crossover study in 31 healthy volunteers (18 men, 13 women) given the following treatments (0.3 U/kg given in the abdomen) under euglycemic clamp conditions :

- NPL
- Humalog 75/25
- Humalog 50/50
- Humalog 25/75[2]
- Humalog R

A summary of the data is shown in Table 4. There appears to be a linear relationship between the percentage soluble insulin lispro and the pharmacodynamic parameters Rmax and $Gtot_{(0\text{-}5\ hrs)}$. The firm used simple linear regression to describe this relationship (figure 2). Although a line does appear to fit the data for Rmax (and, although it is not shown, Gtot(0-5)), the correlation coefficient is low (0.26). This low r^2 indicates significant lack of fit due to ***auto-correlation***, e.g., subjects that have low Rmax values tend to stay relatively low from treatment to treatment. Simple linear regression is not able to handle this situation in a satisfactory way. Although one could estimate the lack-of-fit component in the linear model and adjust the r^2 value, the same poor fit would result. Another method that might be more satisfactory is to use mixed-effect modeling to estimate individual parameters, and yet estimate inter-subject and residual variabilities at the same time. The reviewer performed an exploratory analysis using NONMEM version V. The results are shown in Table 5 and Figure 3.

[2] This formulation contains 75% soluble insulin lispro and 25% NPL.

Table 4: Summary of PK and PD parameters for Study IODJ. Values in the table are mean ± SD, except where noted. The 90% CI for each parameter were computed using the formulation to the right as the reference (e.g., the 90 % CI's under Insulin lispro were computed using the 25/75 as the reference, etc.).

Parameter	Insulin Lispro	25/75‡	50/50	75/25	NPL
$AUC_{(0\text{-baseline})}$ (ng*hr/ml)	15.9 ± 3.2	15.8 ± 4.4	18.3 ± 5.6	16.1 ± 3.9	15.2 ± 3.6
Mean Ratio (90% CI)	*102 (92.5 – 113)*	*87.8 (79.6 – 96.9)*	*112 (102 – 124)*	*101 (91.8 – 112)*	*na*
$AUC_{(0\text{-5hrs})}$ (ng*hr/ml)	14.7 ± 2.7	10.6 ± 1.8	8.7 ± 1.7	6.21 ± 1.5	3.85 ± 1.03
Mean Ratio (90% CI)	*139 (129 – 149)*	*122 (114 – 131)*	*141 (131 – 151)*	*160 (149 – 172)*	*na*
C_{max} (ng/ml)	6.3 ± 1.4	4.2 ± 0.9	3.2 ± 0.7	2.0 ± 0.7	1.2 ± 0.4
Mean Ratio (90% CI)	*150 (136 – 165)*	*130 (117 – 144)*	*164 (149 – 181)*	*175 (159 – 194)*	*na*
†t_{max} (hrs)	1.0	1.0	1.0	1.0	3.0
Rmax (mg/min/kg)	13.6 ± 3.4	12.2 ± 3.6	11.2 ± 3.0	10.0 ± 3.4	7.9 ± 2.9
Mean Ratio (90% CI)	*113 103 – 125)*	*109 (98.9 – 120)*	*114 (104 – 125)*	*127 (115 – 139)*	*na*
†TRmax (hrs)	2.3	2.5	2.2	2.3	3.3
Gtotal (g/kg)	3.06 ± 0.8	3.8 ± 0.94	3.9 ± 1.1	4.05 ± 1.5	3.9 ± 1.8
Mean Ratio (90% CI)	*83.0 (73.7 – 93.3)*	*97.9 (87.0 – 110)*	*96.7 (85.9 – 109)*	*119 (106 – 134)*	*na*
G(0-5hrs) (g/kg)	2.5 ± 0.56	2.2 ± 0.48	1.9 ± 0.5	1.7 ± 1.1	1.1 ± 0.53
Mean Ratio (90% CI)	*115 (102-129)*	*113 (100-127)*	*123 (109 – 138)*	*174 154 – 196)*	*na*

†median (min – max)
‡not currently seeking approval for this formulation

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Figure 2: Simple linear regression of Rmax as a function of percent soluble lispro. The equation for the line is 0.054x +8.31 (r^2 = 0.26)

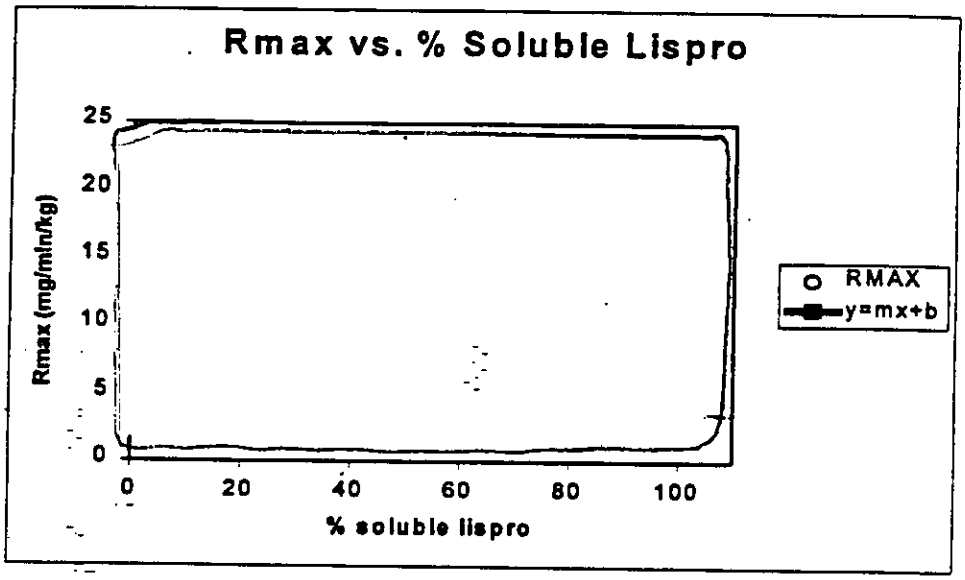
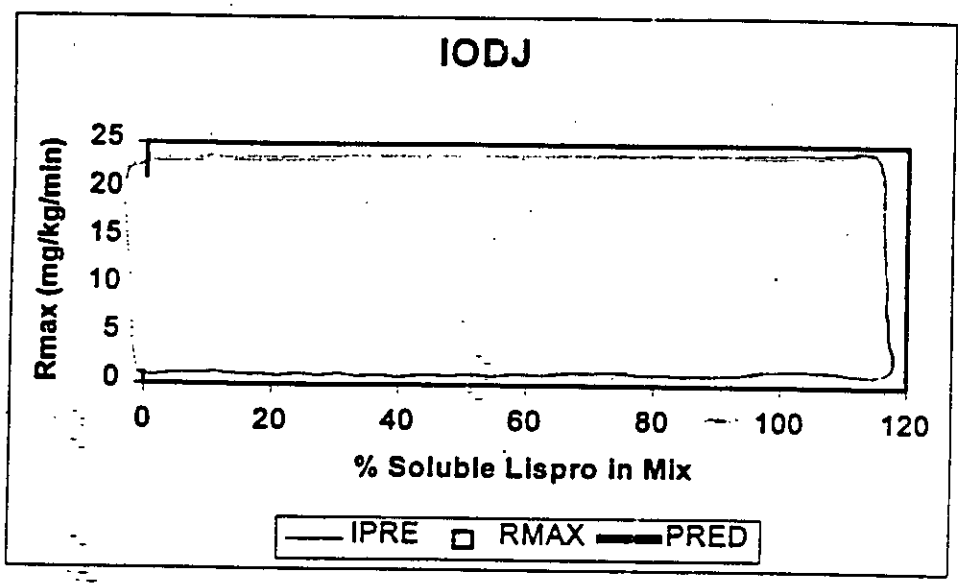


Table 5: Summary of mixed-effect linear regression of IODJ using NONMEM. Computed by reviewer. The r^2 for this model is 0.727, which represents a considerable improvement over the simple linear regression shown above.

Parameter	Mean (range)	SEM of Mean (CV%)
Slope	0.0534	13.7%
Intercept	8.31	6.8%
σ slope (CV%)	36.1%	81%
σ intercept (CV%)	26.6%	28.7%
residual	20.8%	16.5%

Figure 3: Individual observed vs. predicted values for linear regression using NONMEM. IPRE refers to individual prediction, PRED is the population or "typical" prediction.



By allowing for individual variability in slope and intercept values, the fit improves dramatically, with the r^2 going from 0.26 to 0.75. For each 25% increase in soluble lispro (e.g., going from 25% to 50%), the typical Rmax increases by about 1.33 mg/kg/min, which agrees well with the data in Table 4. For a 70 kg subject, this would represent about a 100 mg/min increase in Rmax. The mean GIR vs. time plots as well as mean insulin lispro concentration vs. time are shown in Figures 4 and 5.

Figure 4: Mean Insulin concentration vs. time curves for Study IODJ

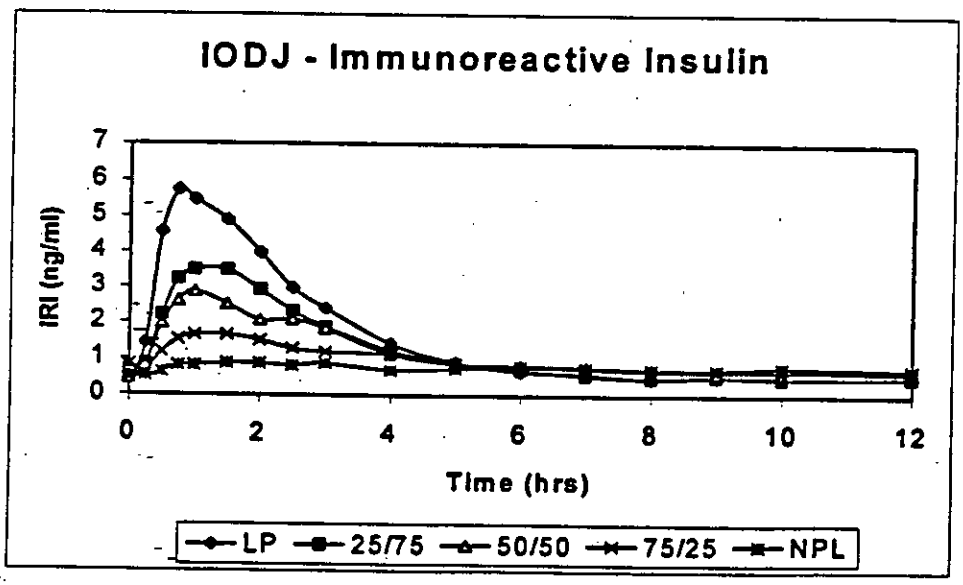
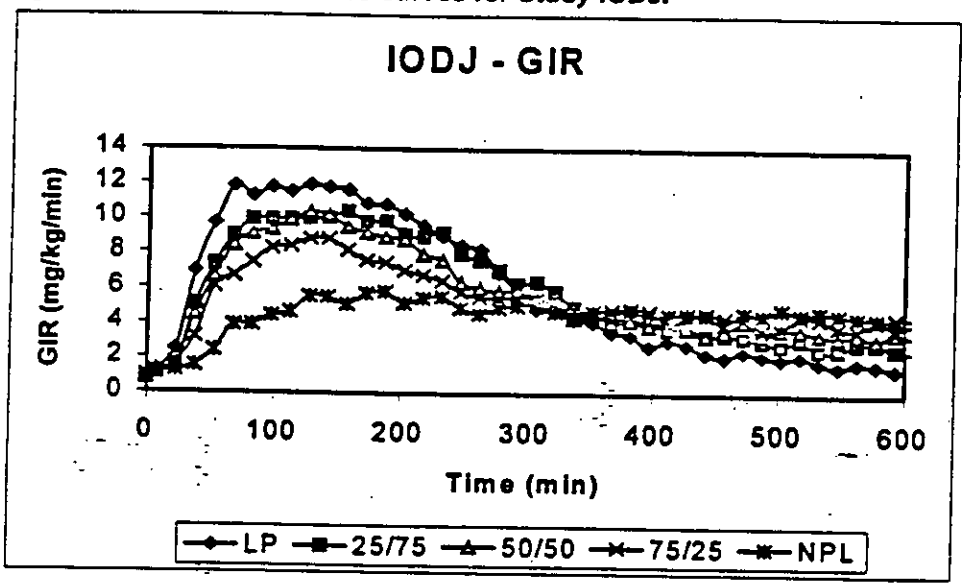


Figure 5: Mean GIR vs. times curves for Study IODJ.



Type 1 Patients

Two studies were performed in patients with Type 1 diabetes. Study IOGI was a randomized, incomplete block design in 12 diabetics (10 male, 2 female) who were randomized to receive three of the following four treatments:

- 0.3 U/kg NPL
- 0.3 U/kg NPH
- 0.3 U/kg 75/25
- 0.3 U/kg 50/50

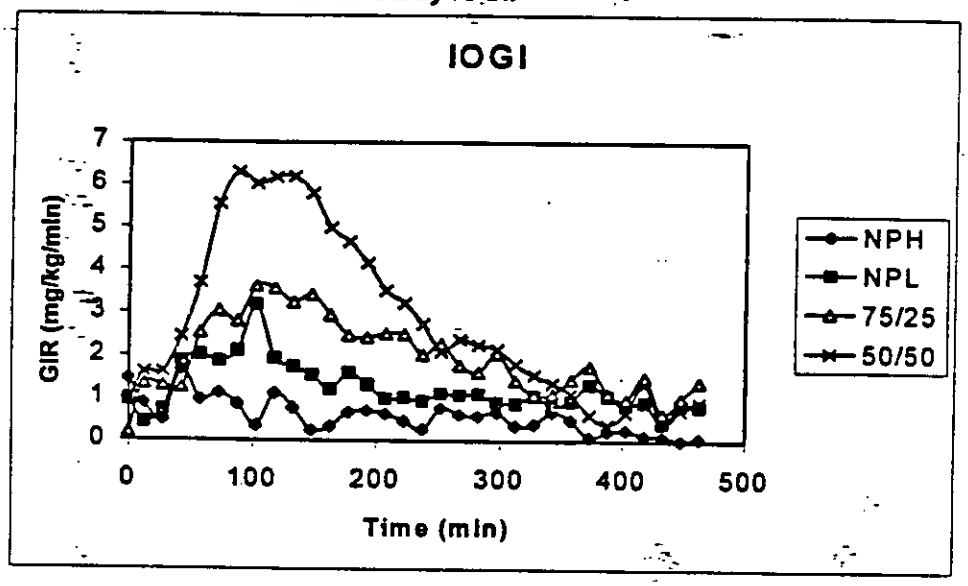
The pharmacokinetics results are shown in Table 5. Comparing these results to those found in Table 4, it is noted that insulin levels in patients are significantly lower than those found in normal volunteers at equivalent doses. The time to peak values between the two studies are comparable. The firm explains these results by noting that a lispro-specific assay was used in this study, which does not measure endogenous insulin. This assay includes a precipitation step which could result in loss of insulin lispro from the samples. Data on the loss of insulin in the assay was not provided in the validation in this submission; however, a similar assay in the original submission showed considerable loss of insulin after the precipitation step. The NPH arm was assayed with a [redacted] method which also includes a [redacted] precipitation step. The values from the NPH arm were not reported because there were so few quantifiable levels. This may be due to loss of insulin during the precipitation step. This, however, does not explain the GIR data. Figure 6 shows the mean GIR values over time; they are also lower than what was seen in the normal volunteer study (compare with Figure 5). This suggests that there may be some degree of insulin resistance in these Type 1 patients. Taken together, it appears that although both the PK and PD responses are lower in Type 1 patients than in healthy volunteers, they may not be related. The lower insulin levels are likely an artifact of the assay method, while the PD results may be due to a moderate degree of insulin resistance in the patients .

Table 5: Mean pharmacokinetic parameters in Type 1 patients (Study IOGI).

Parameter	NPL	75/25	50/50
AUC(0-baseline) (ng*hr/ml)	1.97 ± 1.92	4.22 ± 2.22	4.34 ± 1.57
AUC(0-5 hrs) (ng*hr/ml)	0.831 ± 0.538	1.24 ± 0.63	3.44 ± 0.876
Cmax (ng/ml)	0.27 ± 0.15	1.06 ± 0.37	1.46 ± 0.57
[1]Tmax (hrs)	2.0	1.25	1.0

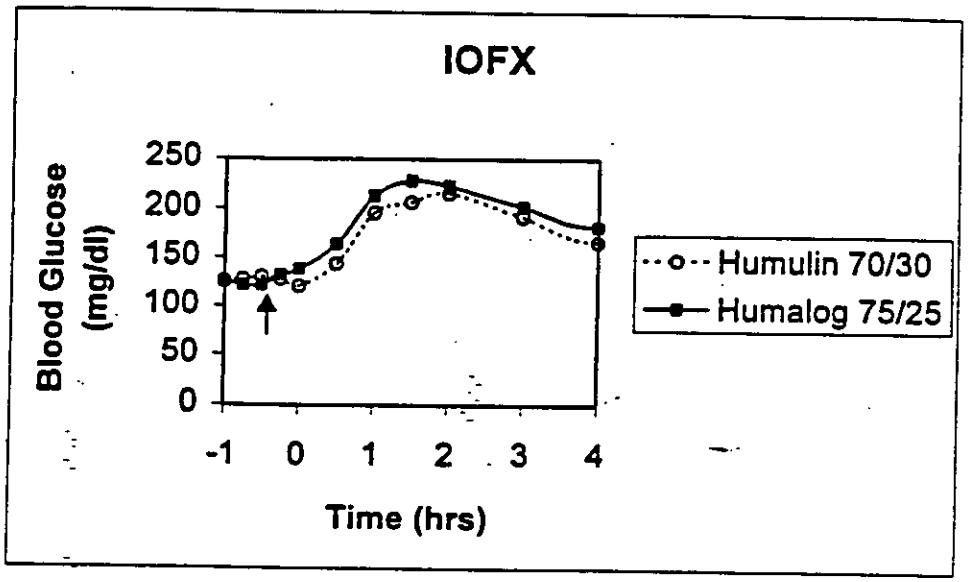
[1]median (min – max)

Figure 6: Mean GIR vs. time curves for Study IOGI.



The second study performed in Type 1 diabetic patients was Study IOFX. This was an open-label, randomized, crossover study in 31 Type 1 diabetics given either Humulin 70/30 or Humalog 75/25 prior to a standard test meal. Humulin was given 30 minutes prior to the meal as is standard practice. The Humalog 75/25 was given immediately before eating. The dose of insulin given was individualized for each patient, based on their responses to previous test meal challenges. Figure 7 shows the mean blood glucose profile in the post-prandial period. The curves are essentially identical; Humulin shows a larger attentuation of the post-prandial glucose peak which may be due to the greater proportion of soluble insulin contained in this product. Also, the baseline glucose values were slightly but significantly higher in the 75/25 group as compared to the Humulin group (mean of 138.4 vs. 121.2, $p < 0.0001$). The results suggest that Humulin 70/30 and Humalog 75/25 will give clinically equivalent control, but that the Humalog mixture may be given immediately before a meal, which may be more convenient for the patient. A similar study using Humulin 50:50 and Humalog 50/50 (Study IOFY) has been completed and is being analyzed at this time.

Figure 6: Mean post-prandial blood glucose values as a function of time. (Study IOFX). Solid arrow indicates time of Humulin dosing; time of Humalog dosing is time zero. A snack was given 4 hours post for both treatments.



V. Dosage and Administration

As with all insulin products, the dosing is highly individualized for each patient.

VI. Formulation

The formulations for each to-be-marketed product is listed in Table 6 below.

Table 6: Formulation of Humalog mixture products. Amounts in the table are per mL.

Ingredient	75/25	50/50
Insulin Lispro		
Dibasic Sod. Phosphate	3.78 mg	3.78 mg
Glycerin	16 mg	16 mg
Phenol	mg	mg
m-cresol	1.76 mg	2.2 mg
Protamine SO_4	0.28 mg	0.19 mg
Zinc Oxide	mg Zn^{++}	qs to 0.0305 mg Zn^{++}
HCL/NaOH	qs to adjust pH	qs to adjust pH

VII. Reviewer Conclusions

- The PK/PD properties of the basal insulin used in the Humalog mixtures, NPL, are similar but not identical to human NPH. NPL is absorbed slightly faster than NPH (as estimated by the peak) but still has the characteristics of a basal insulin.
- In healthy volunteers, the peak of the glucose infusion rate (Rmax) is linearly related to the amount of soluble insulin lispro in each mixture, as might be expected.

- Although the general pattern of the PK/PD properties seen in healthy volunteers is preserved in Type 1 diabetics, lower levels of insulin lispro were seen in patients. This is likely a result of the antibody precipitation step used in the assay. The glucose infusion rates seen in patients are also significantly lower, suggesting that these Type 1 patients had a moderate degree of insulin resistance.

- 75/25 injected just before a standard meal gave a similar post-prandial glucose profile as compared with Humulin 70/30 given 30 minutes before eating. The conclusion is that Humalog 75/25, like Humalog R, may be given just prior to a meal.

VIII. Comments to firm

None at this time.

IX. Labeling Comments

The following comments apply to the labeling for both 75/25 and 50/50:

1) In Figures [redacted] the x-axes should be truncated at 12 hours post-dose in order to emphasize the relevant portions of the curves.

2) Figures [redacted] should be combined into one graph, with all but the most relevant comparisons deleted. For 75/25, these would be 75/25 and Humulin 70/30. Similarly, for 50/50, these would be 50/50 and Humulin 50/50. It should be made clear that these are cross-study comparisons.

X. Signatures

/S/ 12/2/99

Michael J. Fossler, Pharm.D., Ph.D.

Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

FT initialed by Hae-Young Ahn, Ph.D., Team Leader /S/ 12/2/99

version: Final
Recommendation: AP

Briefing held 12/2/99. Present: Chen, Hunt, Ahn, Johnson, Fossler

CC: NDA 21-017(orig., 1 copy), NDA 21-018 HFD-510(Koller, Rhee), HFD-850(Lesko), HFD-870(M. Chen, Fossler, Ahn), HFD-340(Vish), Central Document Room (Barbara Murphy)

Appendix: Study Summaries

Clinical Study Synopsis: Study F3Z-MC-IOBS

Title:	Pharmacokinetics of Intermediate-Acting Formulation of Insulin Lispro
Investigators:	[redacted]
Study Centers:	There was one study center.
Dates of Study:	September 1994 through December 1994
Clinical Phase:	Phase 1
Objectives:	To compare the pharmacokinetics and glucodynamics of insulin lispro protamine suspension (NPL) with Humulin® N (NPH).
Methodology:	Nonrandomized, open-label, crossover study.
Number of Subjects:	Male: 3, Female: 5, Total: 8.
Diagnosis and Inclusion Criteria:	Men and women 18 years and older, determined to be healthy by a physical examination, laboratory tests, ECG, and chest x-ray. Participants exhibited normal glucose tolerance (fasting and 2-hour levels following ingestion of 75 g of glucose). None of the subjects had a history of diabetes mellitus, gastrointestinal or hepatic disorders, atopy or allergy to drugs, or cardiovascular disease. Subjects were currently free of hepatic or renal function impairment, as assessed by clinical laboratory tests and physical examination.
Dosage and Administration:	Test Product: Insulin lispro protamine suspension (100 U/mL) 0.4 U/kg; CT03474 Reference Therapy: Humulin N (human insulin [recombinant DNA origin] isophane suspension) (100 U/mL) 0.4 U/kg; Lot: 8MG42 Each product was given as a single subcutaneous dose to all subjects. For each subject, treatments were separated by at least 7 days and given during a manual glucose clamp.
Duration of Treatment:	Insulin lispro protamine suspension: 1 day (1 dose) Humulin N: 1 day (1 dose).

Criteria for Evaluation:

Pharmacokinetics and Glucodynamics: Standard pharmacokinetic and glucodynamic parameters were used as comparative indices between the treatments. Maximum insulin (IRI) concentrations (C_{max}), time to C_{max} (t_{max}), and area under the serum concentration-versus-time curve (AUC) were the pharmacokinetic measures; the maximum glucose infusion rate (R_{max}), time to R_{max} (TR_{max}), and the total glucose infused (G_{tot}) were the glucodynamic measures.

Safety: All adverse events were reported to the sponsor regardless of their causality.

Statistical Methods:

An analysis of variance was applied to compare the NPH and NPL pharmacokinetic and glucodynamic parameters. This method was suited to a two-way crossover design, and considered treatment and subject effects.

Summary and Conclusions:

No deaths and no serious adverse events were reported during this study.

- Subcutaneous administration of NPL results in delayed and prolonged absorption of insulin lispro in healthy subjects.
- The peak activity (R_{max}) of NPL is similar to that of NPH, although the time of peak activity (TR_{max}) may occur earlier.
- The overall hypoglycemic activity of NPL is similar to that of NPH as determined by the total glucose requirement during the glucose clamp (G_{tot}).
- Maximum IRI concentrations (C_{max}) and time to C_{max} (t_{max}) are similar between NPL and NPH. Overall exposure, as assessed by area under the serum concentration-versus-time curves, was similar.
- The activity profile of NPL is consistent with an intermediate-acting insulin.

Clinical Study Synopsis: Study F3Z-MC-IOCM

Title:	Pharmacokinetics of Free Mixtures of Insulin Lispro and Insulin Lispro Protamine Suspension
Investigators:	[redacted]
Study Centers:	There was one study center.
Dates of Study:	May 1995 through August 1995
Clinical Phase:	Phase 1
Objectives:	To compare the pharmacokinetics of insulin lispro, insulin lispro protamine suspension (NPL), and extemporaneously prepared insulin lispro/NPL mixtures. Secondarily, to obtain preliminary indications of the immunogenic response to NPL by measuring insulin antibodies.
Methodology:	Randomized, balanced incomplete block, open-label, crossover study.
Number of Subjects:	Male: 3, Female: 7, Total: 10; Age: 21-30, inclusive.
Diagnosis and Inclusion Criteria:	Healthy men and women 18 years and older. Participants met the National Diabetes Data Group criteria for normal glucose tolerance. Subjects had no history of diabetes mellitus, gastrointestinal or hepatic disorders, atopy or allergy to drugs, or cardiovascular disease. Subjects were free of hepatic or renal function impairment, as assessed by clinical laboratory tests and physical examination.
Dosage and Administration:	Test Product: Insulin lispro protamine suspension (100 U/mL; NPL); CT-04255; insulin lispro (100 U/mL) CT-03509. These two products were mixed extemporaneously just prior to dosing in 3 ratios: 25, 50, and 75% insulin lispro with NPL. Reference Therapy: 0.3 U/kg doses of 100% NPL and 100% insulin lispro Three of the five preparations (the 3 extemporaneous mixtures, NPL, and insulin lispro) were given as a single subcutaneous dose in each subject, separated by at least seven days. All administrations were given during a manual glucose clamp.

Duration of Treatment:	All treatments were 1 day in duration (1 dose)
Criteria for Evaluation:	Pharmacokinetics and Glucodynamics: Maximum insulin (IRI) concentrations (C_{max}), time to C_{max} (t_{max}), and area under the serum concentration-versus-time curve (AUC) were pharmacokinetic measures used. The maximum glucose infusion rate (R_{max}), time to R_{max} (TR_{max}), and the total glucose infused (G_{tot}) were the glucodynamic measures used. Additional parameters used to define the rapid-acting portion of the treatments were 0-5 hour AUC and G_{tot} values (AUC_0^5, ${}_0^5G_{tot}$). AUC values from 5 hours until return to baseline and G_{tot} values from 5 hours until the end of the clamp procedure (AUC_5^r, ${}_5^{end}G_{tot}$) were used as indices of the prolonged action of all treatments. Safety--All adverse events were reported to the sponsor regardless of their causality.
Statistical Methods:	Simple regression analyses were performed between several pharmacokinetic and glucodynamic parameters (C_{max}, AUC_0^r, AUC_0^5, AUC_5^r, R_{max}, G_{tot}, ${}_0^5G_{tot}$, ${}_5^{end}G_{tot}$) and the percent of soluble insulin lispro contained in the extemporaneous mixtures. An analysis of variance was applied to compare the pharmacokinetic and glucodynamic parameters among treatments, with selected pairwise contrasts. In addition to the omnibus test of equality among the five formulations, a single-degree of freedom contrast for linear trend was tested. Specific comparisons between adjacent formulations were conducted via t-tests based on the ANOVA error variance.
Summary and Conclusions:	There were no deaths and no serious adverse events reported during this study. With the exception of one moderate event, all events were mild and resulted in no residual effects. Post-baseline antibody measurements were in the normal reference range. Evaluations of the pharmacokinetic and glucodynamic data showed the t_{max} and TR_{max} values were different only for the NPL treatment. A positive linear relationship was present between the percent of soluble insulin lispro and C_{max}, AUC_0^5, R_{max}, and ${}_0^5G_{tot}$ values. A negative linear relationship was found between the percent of soluble insulin lispro and AUC_5^r and ${}_5^{end}G_{tot}$ values. G_{tot} values were not statistically different between treatments, although comparisons between treatments suggest a negative curvilinear relationship may exist.

The rapid onset of activity characteristic of insulin lispro was maintained in all insulin lispro/NPL mixtures. Extemporaneous mixing of insulin lispro and NPL does not alter the expected absorption time-activity profile of soluble insulin lispro.

APPEARS THIS WAY
ON ORIGINAL

Clinical Study Synopsis: Study F3Z-MC-IODJ(b)

Title:	Pharmacokinetics of Insulin Lispro Premixtures: A Comparison of Insulin Lispro, Low Mixture, Mid Mixture, High Mixture, and Insulin Lispro Protamine Suspension
Investigators:	[redacted]
Study Centers:	There was one study center.
Dates of Study:	November 1995 through May 1996
Clinical Phase:	Phase 1
Objectives:	The objectives of this study were to determine the safety of NPL and manufactured insulin lispro/NPL mixtures and to determine and demonstrate the pharmacokinetic and pharmacodynamic differences between the formulations.
Methodology:	Randomized, open-label, 5-way crossover study.
Number of Subjects:	Male: 18, Female: 13, Total: 31; Age: 22-33, inclusive.
Diagnosis and Inclusion Criteria:	Men and women 18 years and older, determined to be healthy by a physical examination, laboratory tests, ECG, and chest x-ray. Participants met the National Diabetes Data Group criteria for normal glucose tolerance. No subject had a history of diabetes mellitus, gastrointestinal or hepatic disorders, atopy or allergy to drugs, or cardiovascular disease. Subjects were currently free of hepatic or renal function impairment.
Dosage and Administration:	Test Products: High Mixture (HM; 75% lispro, 25%NPL; CT04735), Mid Mixture (MM; 50% lispro, 50% NPL; CT04737), and Low Mixture (LM; 25% lispro, 75% NPL; CT05093). Reference Therapies: Insulin lispro protamine suspension (NPL), CT04722, and insulin lispro (Humalog) CT04345. Both test products and reference therapies were supplied as 100 U/mL injectable solutions.

	All 5 preparations were given as a single abdominal subcutaneous dose in each subject, separated by at least 5 days. All administrations were given during a glucose clamp.
Duration of Treatment:	All treatments were 1 day in duration (1 dose).
Criteria for Evaluation:	Pharmacokinetics and Glucodynamics–Standard pharmacokinetic and glucodynamic parameters were used as comparative indices between the treatments. Maximum insulin (IRI) concentrations (C_{max}), time to C_{max} (t_{max}), and area under the serum concentration-versus-time curve (AUC) were the pharmacokinetic measures; the maximum glucose infusion rate (R_{max}), time to R_{max} (TR_{max}), and the total glucose infused (G_{tot}) were the glucodynamic measures. Additional parameters were used as indices of the rapid-action portion of the treatments: 0-5 hour AUC and G_{tot} values (AUC_0^5, ${}_0^5G_{tot}$). AUC values from 5 hours until return to baseline and G_{tot} values from 5 hours until the end of the clamp procedure (AUC_5^r, ${}_5^{end}G_{tot}$) were used as indices of the prolonged action of all treatments. Safety–All adverse events were reported to the sponsor regardless of their causality.
Statistical Methods:	An analysis of variance was applied to compare the pharmacokinetic and glucodynamic parameters among all treatments, with pairwise comparisons between the adjacent formulations (for example, low mix and mid mix), defined by insulin lispro content. This method was suited to a multiple crossover design and accounted for sequence and period effects and their interactions. In addition, regressions were performed between the various pharmacokinetic and glucodynamic parameters and the percent soluble insulin lispro in each treatment.
Summary and Conclusions:	There were no deaths and no serious adverse events reported during this study. All events were mild and resulted in no residual effects. With the exception of decreases in hemoglobin and erythrocyte count, no clinically significant laboratory abnormalities were observed. The decreases in hemoglobin and erythrocyte count are felt to be due to the blood sampling that occurred during the study treatments. Insulin antibody measurements showed no increase over a minimum of 6 weeks. Evaluations of the pharmacokinetic and glucodynamic data showed the t_{max} and TR_{max} values were statistically greater only for the NPL treatment. A positive linear relationship

was present between the percent soluble insulin lispro and C_{max}, AUC_0^5, R_{max}, and ${}_0^5G_{tot}$ values. A negative linear relationship was found between the percent of soluble insulin lispro with AUC_5^t and ${}_5^{end}G_{tot}$ values. This study showed that the rapid onset of activity characteristic of insulin lispro was maintained in all manufactured insulin lispro/NPL mixtures. Each manufactured insulin lispro/NPL mixture has a distinct glucodynamic and pharmacokinetic profile.

APPEARS THIS WAY
ON ORIGINAL

Clinical Study Synopsis: Study F3Z-MC-IOET

Title:	Bioequivalence of CT07806 Versus CT07807
Investigators:	This study included 1principal investigator.
Study Centers:	This was a single center study.
Dates of Study:	July 1997 through October 1997.
Clinical Phase:	Phase I.
Objectives:	To assess bioequivalence of a new formulation of insulin lispro mid mixture (CT07807) relative to the extant formulation (CT07806) following subcutaneous administration.
Methodology:	Open-label, randomized, crossover design in healthy subjects. Pharmacokinetic parameters from IRI serum concentration-versus-time profiles were used for assessment of bioequivalence. Glucodynamic parameters from glucose clamp methodology were used for comparing glucodynamic response between formulations.
Number of Subjects:	Male 20, Female 10, Total 30.
Diagnosis and Inclusion Criteria:	Healthy human subjects of either gender who: signed informed consent; were 18 years or older; had serum creatinine < 1.5 mg/dL; had BMI ≤ 27 kg/m^2; and had a normal oral glucose tolerance test within 6 months.
Dosage and Administration:	<u>Test Product:</u>

	Insulin Lispro Mid Mixture (MM), new formulation (CT07807), 0.2 U/kg given as a single subcutaneous dose,100 U/mL (3.5 mg/mL). Reference Therapy Insulin Lispro MM, extant formulation (CT07806), 0.2 U/kg given as a single subcutaneous dose, 100 U/mL (3.5 mg/mL).
Duration of Treatment:	Single dose of each formulation

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ON ORIGINAL

Criteria for Evaluation:

Pharmacokinetics

Bioequivalence between formulations was assessed based on C_{max} and AUC_{0-tb}. The 90% confidence intervals for the ratio of means was assessed against conventional bioequivalence limits (80% to 125%).

Glucodynamics

Glucodynamic parameters, R_{max}, TR_{max}, and G_{tot} were calculated from glucose clamp data. If the 95% confidence interval for a given glucodynamic parameter did not include zero, the glucodynamic parameter was considered to be significantly different between formulations.

Safety

Adverse events and vital signs.

Statistical Methods:

Pharmacokinetics: An analysis of variance suited to crossover design was applied in comparing the formulations. The 90% confidence intervals for the ratio of means were tested against standard bioequivalence limits.

Glucodynamics: An analysis of variance suited to crossover design was applied in comparing the formulations. The 95% confidence intervals for the difference in means were constructed.

Summary and Conclusions:

Study results show that the extant and new MM formulations are both biopharmaceutically and pharmacodynamically equivalent. The new formulation was bioequivalent to the extant formulation for AUC_{0-tb} (AUC, from time = 0 to return to baseline) and C_{max} as assessed by serum IRI concentrations. No glucodynamic differences were observed between the two formulations based upon the comparison of R_{max}, TR_{max}, and G_{tot} values. There was no difference in the safety profile between the extant and new MM formulations in subjects.

Clinical Study Synopsis: Study F3Z-MC-IOFX

Title:	Insulin Lispro Low Mixture (LM) vs. Human Insulin 30/70 Following a Standard Test Meal in Patients with Type I Diabetes
Investigators:	This study included 1 principal investigator.
Study Centers:	This was a single center study.
Dates of Study:	11 July 1997 - 18 June 1998.
Clinical Phase:	Phase II.
Objectives:	The primary objective of this study was to compare the postprandial glucodynamic response after administration of insulin lispro LM (LM) immediately prior to a standard test meal with human insulin 30/70 administered 30 minutes prior to an identical test meal in patients with type I diabetes.
Methodology:	Open-label, randomized, 2-way crossover study. Glucose stabilization was achieved prior to administration of the standard dinner test meal.
Number of Subjects:	39 patients enrolled, 14 male, 25 female 31 patients included in the analysis
Diagnosis and Inclusion Criteria:	Enrolled patients had type I diabetes verified by a negative fasting serum C-peptide measurement and had received insulin therapy for at least 30 days prior to entering the study. Thirty-nine patients were enrolled in the study. Data from 31 of the 39 patients were included in the final analysis.
Dosage and Administration:	<u>Test Product</u> Insulin lispro low mixture (25% insulin lispro/75% insulin lispro protamine suspension) Supplied in 3 mL cartridges - 100 units/mL. <u>Reference Therapy</u> Human insulin 30/70 (clinical trial material) Supplied in 3 mL cartridges - 100 units/mL.
Duration of Treatment:	Single test meal exposures to each insulin.
Criteria for Evaluation:	<u>Efficacy</u> The pharmacokinetic parameters determined included the area under the serum insulin concentration versus time

curve (AUC), the maximum insulin concentration attained (C_{max}), and the time to the maximum concentration (t_{max}). Blood glucose measurements collected after the standardized meal were used to evaluate glucodynamic response. From these measurements, the maximum glucose concentration, time to the maximum glucose concentration, the maximum glucose excursion from baseline, and time to the maximum glucose excursion were calculated.

Safety

Safety assessments included the reporting of adverse events.

Statistical Methods:

An analysis of variance was applied to compare the pharmacokinetic and glucodynamic parameters between treatments. Based on an unbalanced two-period crossover design, the statistical model included sequence, period, and treatment as fixed factors and patient nested within sequence as a random effect. In addition, baseline blood glucose was added as a covariate for some pharmacodynamic parameters. Single degree of freedom contrasts were constructed to obtain estimates of treatment effects. Least-square means were used in all analyses.

Summary and Conclusions:

The following statements summarize the study results:

No serious adverse events were reported during the study period. No clinically significant increases in insulin antibody levels were observed during the study period. Postprandial glucodynamic profiles observed with insulin lispro LM administered immediately prior to the meal and human insulin 30/70 administered 30 minutes prior to the meal were similar in patients with type I diabetes. Comparisons of insulin lispro LM and human insulin 30/70 pharmacokinetic parameters showed insulin lispro LM was more rapidly absorbed and maximum concentrations were similar between the two treatments. No difference in the incidence of hypoglycemia was observed between treatment groups. The dose of insulin lispro LM and human insulin 30/70 may have been too low to adequately control blood glucose following the standard test meal.

This study supports the following conclusions:

Insulin lispro LM has a similar safety profile to that of human insulin 30/70. Insulin lispro LM is more rapidly absorbed when compared to human insulin 30/70. The glucodynamic control provided by insulin lispro LM administered immediately prior to the meal is comparable to that of human insulin 30/70 administered 30 minutes prior to the meal when identical doses of each treatment are administered.

Clinical Study Synopsis: Study F3Z-MC-IOGI

Title:	Administration of Insulin Lispro Protamine Suspension (NPL), Low Mixture, and Mid Mixture: A Comparison with Administration of Human NPH in Patients with Type I Diabetes
Investigators:	This study included 1 principal investigator.
Study Centers:	This was a single center study.
Dates of Study:	June 1997 through October 1997.
Clinical Phase:	Phase II.
Objectives:	To investigate the pharmacokinetics and pharmacodynamics of insulin lispro protamine suspension (NPL), insulin lispro low mixture (LM), insulin lispro mid mixture (MM), and Humulin N (NPH) in patients with type I diabetes mellitus.
Methodology:	This was an open-label, randomized, 3-way balanced incomplete block study involving 12 patients with type I diabetes mellitus.
Number of Subjects:	10 Male, 2 Female; 12 Total.
Diagnosis and Inclusion Criteria:	Patients with type I diabetes and negative (< 0.3 nM) fasting serum C-peptide concentrations were enrolled in the study. All patients were using intensive insulin therapy. Enrolled patients monitored their blood glucose routinely for at least 60 days prior to entry into the study. The total daily dose of insulin did not exceed 2.0 U/kg.
Dosage and Administration:	<u>Test Product:</u> NPL 0.3 U/kg; 100 U/mL; LM 0.3 U/kg; 100 U/mL; MM 0.3 U/kg; 100 U/mL <u>Reference Therapy</u> NPH 0.3 U/kg; 100 U/mL
Duration of Treatment:	Single dose of 3 of the 4 possible formulations.
Criteria for Evaluation:	<u>Pharmacokinetics</u> Maximum drug concentrations (C_{max}) produced and the times those concentrations occurred after administration (t_{max}), and the area under the serum concentration versus time curve (AUC_0^t) from the time of dosing (time=0) until the return to baseline (t') were the primary parameters. In addition to AUC_0^t calculations, partial AUCs from 0 to 5

hours after dosing (AUC_0^5) and from 5 hours until the return to baseline (AUC_5^t) were calculated.

Glucodynamics
Mean blood glucose measurements were calculated from 0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 15, and 15 to 19 hours after dosing, with the last measurement reflecting a 4-hour mean. Additionally, an 8-hour mean blood glucose was calculated (0 to 8 hours after dosing). From the glucose infusion rates required to maintain euglycemia, the maximum infusion rates (R_{max}, mg/min) and the times to maximum infusion rate (TR_{max}, hr) were documented. The cumulative amount of glucose infused during data collection (G_{tot}, gm, until clamp cessation) was recorded.

Safety
Adverse events and vital signs.

Statistical Methods:

An analysis of variance was applied to compare the pharmacokinetic and glucodynamic parameters between treatments. Based on a balanced incomplete block design, the statistical model included treatment as a fixed factor and patient as a random effect. In addition, baseline blood glucose was added as a covariate for the glucodynamic parameters. Single degree of freedom contrasts were constructed to test *a priori* comparisons. The analysis was conducted for all patients who enrolled and completed the protocol. Least-square means were used in all analyses.

Summary and Conclusions:

The following statements summarize the study results: No serious adverse events were reported during the study period. No clinically significant increases in insulin antibody measurements were observed during the study period. A linear relationship was observed between two glucodynamic parameters (R_{max}, ${}_0^5G_{tot}$) and the percentage of soluble insulin lispro present in all lispro-containing formulations. TR_{max} was not significantly different between LM and MM and between LM and NPL, but was notably shorter for LM and MM compared to NPL. A positive linear relationship was observed between two pharmacokinetic parameters (C_{max}, AUC_0^5) and the percentage of soluble insulin lispro present in all insulin lispro-containing formulations. The relationships between corresponding pharmacokinetic and glucodynamic parameters (C_{max} and R_{max}, AUC_0^5 and ${}_0^5G_{tot}$) modeled in healthy subjects were predictive of the results in patients with type I diabetes.

The following statements summarize the conclusions of this study: Pharmacokinetic and glucodynamic profiles observed with NPL, LM, and MM in patients with type I diabetes are consistent with those observed in healthy subjects. The observed pharmacokinetic and glucodynamic differences between NPL and LM, and LM and MM, satisfy the requirements for insulin mixtures in the draft guidance for diabetes

compounds. Glucose clamp studies performed in healthy subjects are predictive of insulin activity in patients with type I diabetes.