[Lys(B28), Pro(B29)]-Human Insulin

A Rapidly Absorbed Analogue of Human Insulin

DANIEL C. HOWEY, RONALD R. BOWSHER, ROCCO L. BRUNELLE, AND JAMES R. WOODWORTH

[Lys(B28), Pro(B29)]-human insulin (LYSPRO) is an insulin analogue in which the natural amino acid sequence of the B-chain at positions 28 and 29 is inverted. These changes result in an insulin molecule with a greatly reduced capacity for self-association in solution. These clinical studies were designed to compare LYSPRO with human Regular insulin after subcutaneous injection in humans. We wanted to evaluate the effect of adding zinc to LYSPRO on its pharmacokinetics and pharmacodynamics. In addition, we compared the pharmacokinetics and pharmacodynamics of LYSPRO and human Regular insulin after subcutaneous injection to those of human Regular insulin given intravenously. Thus, we compared four treatments: solutions of zinc-free LYSPRO given subcutaneously (*A*), zinc-containing LYSPRO given subcutaneously (*B*), human Regular insulin given subcutaneously (*C*), and human Regular insulin given intravenously (*D*). We gave a 10-U dose of each treatment to 10 healthy (nondiabetic) men during glucose clamps. Serum insulin concentrations peaked more than two times higher (maximum serum insulin level [C_{max}], 698 vs. 308 pM, A vs. C) and in less than half the time (time to C_{max} [T_{max}], 42 vs. 101 min, A vs. C) after subcutaneous injection of zinc-free LYSPRO. At the same time, the glucose infusion rate peaked in about half the time (time to maximum glucose infusion rate [TR_{max}], 99 vs. 179 min, A vs. C) and was slightly but not significantly higher (maximum glucose infusion rate [R_{max}], 3.1 vs. 2.2 mmol/min, A vs. C) than that of human Regular insulin. Although the addition of zinc retarded the absorption of LYSPRO slightly (C_{max}, 550 vs. 698 pM, B vs. A), zinc-containing LYSPRO retained its distinct profile (T_{max}, 53 vs. 42 min, B vs. A). LYSPRO displays faster pharmacodynamic action than human Regular insulin when injected subcutaneously. *Diabetes* 43:396–402, 1994

The purpose of administering Regular insulin with each meal during multiple injection therapy is to maintain postprandial blood glucose excursion as close to normal as possible. In this context, the concentration of Regular insulin may peak too late after injection, and its effects may last too long. This is implied from studies of normal volunteers by Binder et al. (1), Galloway et al. (2,3), and Berger et al. (4). Studies of patients with diabetes conducted by Bhaskar et al. (5) and Gardner et al. (6) indicate that the peak effect of Regular insulin occurs from 2 to 6 h after injection, and its effects may last as long as 16 h (5). The notion that Regular insulin may not provide the desired pharmacodynamic effect can be further illustrated by the studies of Simon et al. (7). In these studies, the glycemic response to a mixed meal in normal volunteers peaked between 26 and 70 min after ingestion. The time required for the glucose levels to return to baseline levels was 6 h on average. Indeed, studies looking for the optimal time to inject Regular insulin before meals suggest that Regular insulin should be given from 30 to 60 min before meals to achieve optimal control of postprandial glucose (8–11). In response to this need, insulin analogues designed for rapid absorption have been developed in an effort to place injection time and peak action of insulin into closer proximity with postprandial glucose excursion (12–14).

[Lys(B28),Pro(B29)]-human insulin (LYSPRO) (Humalog, Lilly, Indianapolis, IN) is an insulin analogue in which

From Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.

Address correspondence and reprint requests to Dr. Daniel C. Howey, Lilly Laboratory for Clinical Research, Eli Lilly and Company, Wishard Memorial Hospital, 1001 West 10th Street, Indianapolis, IN 46202.

Received for publication 16 August 1993 and accepted in revised form 21 October 1993.

LYSPRO, [Lys(B28),Pro(B29)]-human insulin; IGF-I, insulin-like growth factor I; RIA, radioimmunoassay; C_{max}, maximum serum insulin level; T_{max}, time to C_{max}; Cl/F, metabolic clearance; β, terminal rate constant, $t_{1/2}$, terminal elimination half-life; V_β/F, volume of distribution; MRT, mean residence time; AUC_0^∞, area under the serum concentration versus time curve; R_{max}, maximum glucose infusion rate; TR_{max}, time to maximum glucose infusion rate; G_{tot}, total amount of glucose infused up to 12 h; ANOVA, analysis of variance.

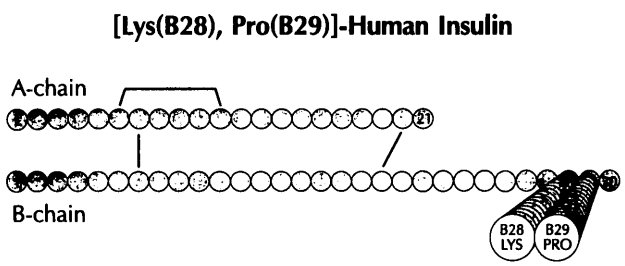

FIG. 1. The amino acid sequence for [Lys(B28),Pro(B29)]-human insulin.

the natural amino acid sequence of the B-chain at positions 28 and 29 is inverted (Fig. 1). These changes result in an insulin molecule with a reduced capacity for self-association (14). Proline at position B28 near the COOH-terminal of the B-chain of human insulin is important for the proper configuration of a β-sheet involving residues B24 through B26 (14). Two insulin molecules align along this surface in an antiparallel orientation to form a nonpolar dimer (15). At this point, the nonpolar dimer interacts with zinc to form a hexamer, the basis of Regular insulin formulations (16). The sequence of lysine at B28 and proline at B29 can be found in insulin-like growth factor I (IGF-I) and is thought to be responsible for its lower degree of self-association in comparison to insulin (14). Accordingly, IGF-I is the model upon which the structure of LYSPRO is based. Despite this minor homology with IGF-I, the affinity of the IGF-I receptor on human placental membranes for LYSPRO is no greater than that for human insulin (17). As a result of these modifications, LYSPRO exhibits monomeric behavior in solution (18), binds zinc less avidly (18), and displays faster pharmacodynamic action than human Regular insulin (Humulin R®, Lilly) in rats and dogs (19) and pigs (20,21). These findings are consistent with the rapid absorption expected from a monomeric insulin injected subcutaneously (22).

As in neutral regular formulations of bovine, porcine, and human insulin (23,24) as well as other analogues of insulin (25), the addition of a small amount of zinc increases the long-term stability of LYSPRO in solution (26). Thus, the clinical studies described were designed to compare LYSPRO with human Regular insulin after subcutaneous injection in humans and to evaluate the effect of adding zinc to LYSPRO on its pharmacokinetics and pharmacodynamics. In addition, we compared the pharmacokinetics and pharmacodynamics of LYSPRO and human Regular insulin after subcutaneous injection to human Regular insulin given intravenously.

RESEARCH DESIGN AND METHODS

The protocol for this study was reviewed by the institutional review board of Indiana University-Purdue University, Indianapolis, IN. Informed consent was obtained from all participants before the study. Before entry into the studies, 10 healthy men were screened using a medical history, physical examination, complete blood count, urinalysis, serum chemistry analysis, electrocardiogram, chest X-ray, and a 2-h glucose tolerance test. Thus, all participants were in good health and met World Health Organization criteria for normal glucose tolerance, i.e., fasting glucose values <6.4 and <7.8 mM 2 h after a 75-g glucose load (27). All of the participants completed their assigned treatments. The participants were similar in regard to age (35.2 ± 5.3 years of age), height (1.77 ± 0.04 m), weight (70.9 ± 5.9 kg), and body mass index (22.6 ± 1.5 kg/m^2). Data are presented as means ± SD. Each participant was expected to maintain his body weight within 2.5 kg of his weight determined on the day before his first treatment throughout the 6-week study.

We compared three drugs: solutions of zinc-free LYSPRO, zinc-containing LYSPRO with ~33 μg/ml of elemental zinc, and human Regular insulin (Humulin R®). All treatments were given subcutaneously into the right or left lower abdominal quadrant. The skin was gently pinched up, and the injection was made into the fold and parallel to the abdominal wall. In addition, human Regular insulin was administered intravenously. A single dose of 10 U was given for each treatment using a randomized, crossover design and without blinding the volunteers, the nurses, or the investigator. A minimum of 5 days separated each treatment. The test materials were administered at a concentration of 100 U/ml.

Treatments were administered during glucose clamps conducted using the Biostator (Miles, Mishawaka, IN) and its built-in glucose clamp algorithm (28,29). The participants fasted overnight and continued to fast at bedrest during the glucose clamps, which lasted 12 h. Biostator's glucose analyzer was calibrated to each participant's capillary whole blood glucose level by adjusting the pump ratio to match capillary glucose concentrations. The glucose clamp target for each study was set at 0.28 mM less than the volunteer's fasting blood glucose level determined on the day of each study. Capillary whole blood glucose samples were assayed with a YSI 2300 STAT glucose analyzer (Yellow Springs, OH). Glucose infusion rates and blood glucose values were recorded from the Biostator using a Compaq Deskpro 386/20e (Compaq, Houston, TX) personal computer running Microsoft Windows 3.0 terminal program (Microsoft, Redmond, WA).

We selected a commercially available radioimmunoassay (RIA) kit called Insulin Coat-A-Count (Diagnostic, Los Angeles, CA) for use in these studies. Based on statistical analysis with ALLFIT (30), insulin and LYSPRO produced equivalent displacement curves. We validated the RIA for the analysis of both insulins and performed all RIAs in accordance with the kit's instructions. RIA data were analyzed by a VAX 8800 computer (Digital, Maryland, MA) using a four-parameter logistic model algorithm (31). We estimated the serum concentrations from standard curves of reference insulin or LYSPRO prepared in the RIA kit's insulin-free matrix. For 19 assays, the level of nonspecific binding was 1.92 ± 0.53% (mean ± SD) with a maximum binding of 47.2 ± 2.7%. For serum control samples prepared at 0.5, 2.5, and 25 ng/ml, the mean recoveries ranged from 92 to 126% with interassay coefficients of variation from 8.1 to 21.9%.

We measured serum concentrations of C-peptide by a

competitive RIA. Briefly, each binding reaction contained 250 μl of radioiodinated Tyr-human C-peptide (25 pg), 50 μl of goat anti-human C-peptide antibody (lot E08–7B2–159–4G; diluted 1:20,000), and 100 μl of serum or reference biosynthetic human C-peptide (0.025–100 ng/ml). After mixing, the reaction was incubated for 16–18 h at 4°C. The bound and free forms of C-peptide were then separated by precipitating the bound fraction with a second antibody and 6% polyethylene glycol (32). We counted the radioactivity for the precipitate in a γ-counter and analyzed the data using a four-parameter logistic model algorithm (31).

Venous blood samples were obtained at 0, 15, 30, and 45 min and at 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 h after injection for measurement of serum concentrations of insulin and C-peptide. We used these to calculate several pharmacokinetic parameters for each volunteer for each treatment including maximum serum insulin level (C_{max}) and time to C_{max} (T_{max}). In addition, we used standard noncompartmental methods to calculate metabolic clearance (Cl/F), terminal rate constant (β), terminal elimination half-life ($t_{1/2}$), volume of distribution (V_β/F), and mean residence time (MRT) (33). The values for Cl/F, V_β/F, and MRT depend on estimates of area under the serum concentration versus time curve (AUC_0^∞). Because the calculation of AUC_0^∞ may be much more affected by endogenous insulin secretion than C_{max}, we used C-peptide–corrected serum insulin data to calculate AUC_0^∞. For these calculations, serum insulin concentrations measured after time 0 were corrected by subtracting the fraction of baseline insulin corresponding to the fraction of baseline C-peptide measured in the same sample (34–36), for example,

$$\text{Insulin}_{\text{corrected}} = \text{insulin}_{\text{measured}} - \left(\frac{\text{C-peptide}_{\text{measured}} - \text{C-peptide}_{\text{basal}}}{\text{C-peptide}_{\text{basal}}}\right) \cdot \text{insulin}_{\text{basal}}$$

β was estimated from the terminal log-linear regression of the serum insulin concentration versus time plots. $t_{1/2}$ was calculated from β using the formula $t_{1/2} = \frac{\ln(2)}{\beta}$, apparent V_β/F by $\frac{Vd}{F} = \frac{\text{dose}}{\beta \cdot AUC_0^\infty}$, and apparent systemic clearance by $\frac{Cl}{F} = \frac{\text{dose}}{AUC_0^\infty}$. We estimated MRT ($MRT = \frac{AUMC_0^\infty}{AUC_0^\infty}$, where $AUMC_0^\infty$ is the area under the moment curve) using statistical moment theory (37,38). Unlike half-life, MRT represents a composite of drug distribution and elimination. MRT represents the time needed to eliminate 63.2% of the administered dose and serves as a useful index of the average time a drug remains in the body (39).

The feedback control loop in the glucose clamp algorithm of the Biostator produces frequent fluctuations or cycles in the glucose infusion rate. To reduce the effect of these cycles, we calculated average glucose infusion rates at the same time blood samples were collected for insulin determinations. For the first 6 h of each glucose clamp, 5-min averages were computed from glucose infusion rate observations, e.g., averages taken from 2 min before until 2 min after the blood sample for serum insulin. After 6 h, the interval was increased to 20-min averages, e.g., from 10 min before until 10 min after the blood samples. For the last collection time at 12 h after injection, a 20-min average was taken before the sample collection. From these time-averaged data, we calculated three pharmacodynamic parameters: maximum glucose infusion rate (R_{max}), time to maximum glucose infusion rate (TR_{max}), and total amount of glucose infused up to 12 h (G_{tot}).

Statistical comparisons were performed between the four treatment groups for pharmacokinetic and pharmacodynamic parameters. The comparisons between treatments for pharmacokinetic and pharmacodynamic values were made by an analysis of variance (ANOVA) model for a randomized block design in which the subjects were the blocks. If a statistically significant treatment effect was observed ($P < 0.05$), pair-wise comparisons were performed between the treatment means using Tukey's studentized range test (40) at the $\alpha = 0.05$ level of significance. When the measurements and the variances after intravenous Regular insulin were substantially different from those obtained after the subcutaneous injections, comparisons were performed only between the subcutaneous treatments to satisfy the assumptions of the variance structure. Statistical analysis was performed using the SAS computer program (Cary, NC).

RESULTS

Results of the pharmacokinetic and pharmacodynamic analysis of this study are summarized in Tables 1 and 2 for the four treatments: solutions of zinc-free LYSPRO given subcutaneously (*A*), zinc-containing LYSPRO given subcutaneously (*B*), human Regular insulin given subcutaneously (*C*), and human Regular insulin given intravenously (*D*). Serum insulin and C-peptide data are depicted graphically in Figs. 2 and 3. Serum insulin concentrations peaked highest after intravenous Regular insulin followed by the subcutaneous treatments: LYSPRO, zinc-containing LYSPRO, and Regular insulin. Although mean serum insulin peaked higher after LYSPRO in comparison to zinc-containing LYSPRO (C_{max}, 698 vs. 550 pM, A vs. B), the mean times to peak were not different (T_{max}, 42 vs. 53 min, A vs. B). The AUC_0^∞ for the serum insulin curves did not differ for any of the subcutaneous treatments. β, V_β/F, and $t_{1/2}$ calculated after the administration of both LYSPRO formulations were not different from those obtained for human Regular insulin after intravenous administration. $t_{1/2}$ is similar to that reported previously in the literature for radioiodinated insulin (41–43). The systemic clearance rates (Cl/F) were similar for all subcutaneous treatments. MRT was lowest after intravenous Regular insulin followed by the subcutaneous treatments: LYSPRO, zinc-containing LYSPRO, and Regular insulin. As expected, serum C-peptide paralleled the serum insulin responses by showing sup-

TABLE 1
Pharmacokinetic and pharmacodynamic parameters

	Treatments				
	LYSPRO (subcutaneous)	Zinc LYSPRO (subcutaneous)	Regular insulin (subcutaneous)	Regular insulin (intravenous)	ANOVA
Code	A	B	C	D	
Pharmacokinetic parameters					
C_{max} (pM)	698 ± 227	550 ± 229	308 ± 132	9976 ± 4317	A B C D
T_{max} (min)	42 ± 20	53 ± 30	101 ± 40	2 ± 1	AB C D
AUC_0^∞ (nmol · min^{-1} · L^{-1})	71.4 ± 14.6	65.3 ± 9.5	72.7 ± 12.3	103.4 ± 28.0	ABC D
Pharmacodynamic parameters					
R_{max} (mmol/min)	3.10 ± 1.19	3.08 ± 1.14	2.20 ± 1.01	4.02 ± 1.38	CAB D
TR_{max} (min)	99 ± 39	116 ± 43	179 ± 93	23 ± 5	AB C D
G_{tot} (mmol)	450.8 ± 161.8	476.6 ± 157.9	454.7 ± 167.4	280.6 ± 72.2	ABC D

Data are means ± SD; n = 10. For the ANOVA with multiple comparison tests, i.e., Tukey's studentized range test, the designation "AB C D" indicates that treatment parameters for A and B are not different from each other, but A and B are different from C and D. Likewise, C and D are different from one another. "CAB D" indicates that treatments C, A and B and A, B and D are not different, but C, A and B are different from D.

pression of endogenous insulin secretion earlier after injection of the intravenous treatments than the subcutaneous treatments, which did not differ from one another (Fig. 3).

Results of the analysis of the pharmacodynamic parameters are summarized in Table 1. Glucose infusion rates and blood glucose levels are depicted in Figs. 4 and 5. Blood glucose levels were comparable after all subcutaneous insulin treatments. However, blood glucose levels after intravenous Regular insulin dropped below that of subcutaneous Regular insulin and both formulations of LYSPRO at 1 h after injection (Fig. 5). R_{max} peaked highest after intravenous Regular insulin followed by the subcutaneous treatments, which were not different from one another (Table 1). Although C_{max} peaked higher after LYSPRO in comparison to zinc-containing LYSPRO, mean R_{max} values were not different between LYSPRO formulations (3.10 vs. 3.08 mmol/min, A vs. B). In addition, the mean TR_{max} after the LYSPRO formulations was not altered by the addition of zinc (99 vs. 116 min, A vs. B). Both LYSPRO and zinc-containing LYSPRO differ dramatically from subcutaneous Regular insulin in TR_{max} (179 min for C). G_{tot} and AUC_0^∞ were similar for all subcutaneous treatments (Table 1).

DISCUSSION

These studies demonstrate that LYSPRO produces a significantly different pharmacokinetic and pharmacodynamic profile from that offered by human Regular insulin after subcutaneous injection. Serum concentrations of LYSPRO peaked more than two times higher and in less than half the time than human Regular insulin. At the same time, the glucose infusion rate peaked in about half the time and slightly but not significantly higher.

Zinc was added to LYSPRO as a means of increasing its physical stability in solution. Zinc, as an integral part of the insulin hexamer of Regular insulin, has the potential to retard the absorption rate and obliterate the differences between LYSPRO and human Regular insulin. Indeed, the maximum serum insulin concentration after zinc-containing LYSPRO was ~78% of that for LYSPRO without zinc. Yet, the time to peak serum insulin concentration was not significantly different. Both zinc-containing LYSPRO and LYSPRO peaked significantly earlier than human Regular insulin given subcutaneously. Despite this slight decrease in the rate of absorption, no evidence was found that the addition of zinc had an effect on any of the pharmacodynamic parameters.

A combination of results from both in vitro and in vivo

TABLE 2
Pharmacokinetic parameters calculated by noncompartmental methods

	Treatments				
	LYSPRO (subcutaneous)	Zinc LYSPRO (subcutaneous)	Regular insulin (subcutaneous)	Regular insulin (intravenous)	ANOVA
Code	A	B	C	D	
Pharmacokinetic parameters					
β (min^{-1})	0.0158 ± 0.0058	0.0125 ± 0.0043	0.0084 ± 0.0048	0.0243 ± 0.0187	ABD C
$t_{1/2}$ (min)	46	55.4	82.5	46.3	See β
V_β/F (L/kg)	1.03 ± 0.73	1.09 ± 0.41	2.20 ± 1.47	0.72 ± 0.66	ABD C
Cl/F (ml · min^{-1} · kg^{-1})	12.3 ± 1.90	12.3 ± 1.52	12.6 ± 2.20	9.23 ± 2.18	ABC D
MRT (min)	101 ± 43	120 ± 36	235 ± 147	32 ± 46	AB C D

Data are means ± SD; n = 10. $t_{1/2}$ is calculated from the elimination rate constant, β, using the formula $t_{1/2} = \frac{\ln(2)}{\beta}$. $t_{1/2}$ is presented as a harmonic mean. See Table 1 for an explanation of the ANOVA notations.

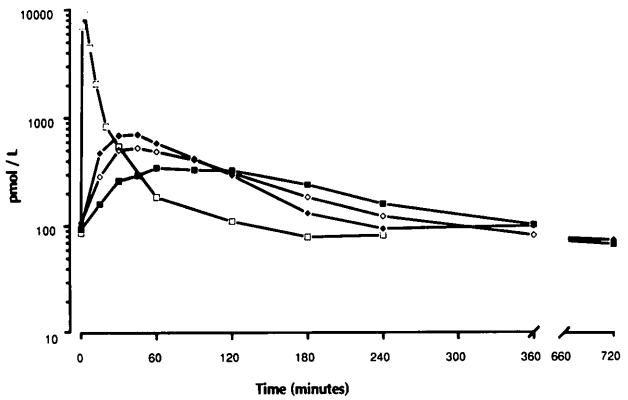

FIG. 2. The mean serum insulin response of 10 normal volunteers to LYSPRO, 10 U subcutaneously (◆); LYSPRO with zinc, 10 U subcutaneously (◇); human Regular insulin, 10 U subcutaneously (■); and human Regular insulin, 10 U intravenously (□).

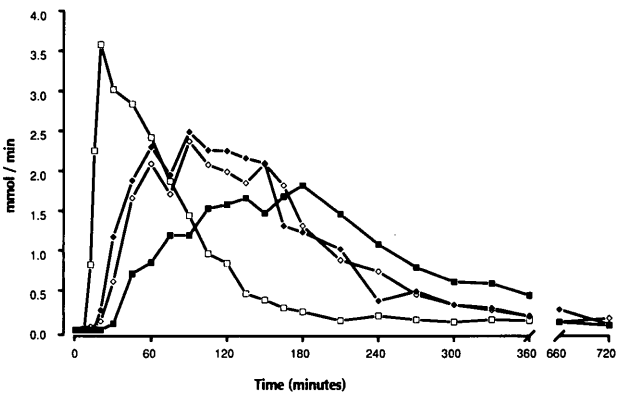

FIG. 4. The mean glucose infusion rate response of 10 normal volunteers to LYSPRO, 10 U subcutaneously (◆); LYSPRO with zinc, 10 U subcutaneously (◇); human Regular insulin, 10 U subcutaneously (■); and human Regular insulin, 10 U intravenously (□).

studies in a variety of species strongly support our contention that LYSPRO and human insulin are equipotent. The insulin receptor affinities on human placental cells and IM-9 lymphocyte for human insulin and LYSPRO are similar (17). Both insulins stimulated [^{14}C]glucose uptake into rat adipocytes to the same degree (17). In vivo studies looking at the effect on lowering blood glucose in rats and dogs (19) and pigs (20,21) implied that these molecules were equipotent. In this study, both total glucose infused and AUC_0^∞ were similar for all of the subcutaneously injected treatments. Taken together, these data support our impression that LYSPRO is fully potent.

A number of findings from this study suggest that the differences we describe between LYSPRO and human Regular insulin are a result of their rates of absorption from the subcutaneous injection site. The serum concentration of insulin after LYSPRO peaks higher and earlier with equivalent values. Equivalent AUC_0^∞ values suggest that equimolar amounts of both drugs were absorbed. The systemic clearance rates (*Cl/F* in Table 2) for LYSPRO and Regular insulin were not different and are similar to rates reported in the literature (35,36,42). The difference in MRT (Table 2) indicates that LYSPRO was retained in the body for a shorter time. The shorter MRT is compatible with faster absorption and more rapid elimination, which produce a shorter duration of action in comparison to human Regular insulin. These findings together support rapid absorption from the injection site as the mechanism of the distinct pharmacodynamic profile offered by LYSPRO.

We included an intravenous treatment of human Regular insulin as a comparator that provided pharmacokinetic data for human Regular insulin without the effects of absorption. LYSPRO had a shorter half-life in comparison to subcutaneously injected human Regular insulin but similar to that of intravenously administered Regular insulin. The half-life calculated for human Regular insulin after subcutaneous injection clearly does not represent the actual disposition of human Regular insulin. The longer half-life after subcutaneous injection reflects the slower absorption rate of Regular insulin from the subcutaneous injection site. After subcutaneous injection, the elimination rate of Regular insulin is limited by its absorption rate. This condition is called the flip-flop phenomenon (44). Thus, the calculated β for subcutaneously injected Regular insulin is altered by persistent absorption of insulin from the subcutaneous injection site. This phenomenon commonly occurs when drugs with fast elimination rates, such as oral penicillin G (45), intramus-

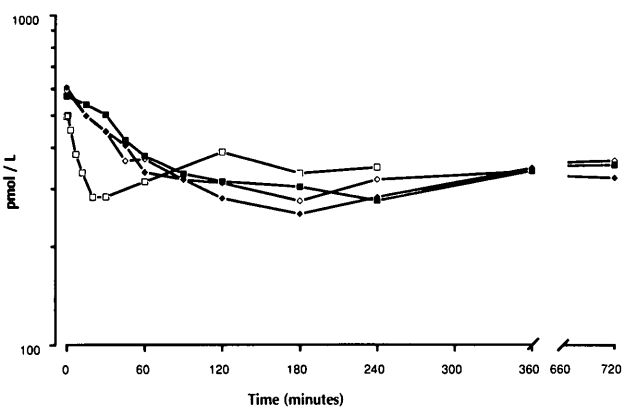

FIG. 3. The mean serum C-peptide response of 10 normal volunteers to LYSPRO, 10 U subcutaneously (◆); LYSPRO with zinc, 10 U subcutaneously (◇); human Regular insulin, 10 U subcutaneously (■); and human Regular insulin, 10 U intravenously (□).

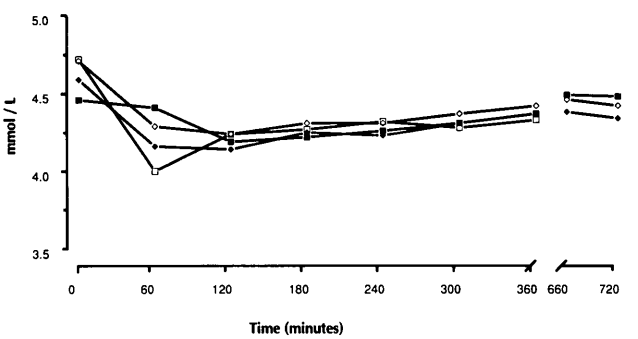

FIG. 5. The mean whole blood glucose response of 10 normal volunteers to LYSPRO, 10 U subcutaneously (◆); LYSPRO with zinc, 10 U subcutaneously (◇); human Regular insulin, 10 U subcutaneously (■); and human Regular insulin, 10 U intravenously (□).

cular ampicillin and dicloxacillin (46), and oral zidovudine (47), are administered in situations in which they have slow absorption rates. Under these circumstances, parameters that are dependent upon an accurate estimation of β, such as V_β/F, may be unreliable. Indeed, we would expect V_β/F for both insulins to be similar, because they are large, polar molecules, the structures of which differ only slightly. The calculated mean V_β/F for LYSPRO from this study is 40% larger than that resulting from intravenous human Regular insulin and 50% of the value determined for subcutaneously injected human Regular insulin. These differences might suggest that the subcutaneous bioavailability of LYSPRO is greater than that for human Regular insulin. However, they are more likely to be a result of the effect of the absorption rates on the calculation of the β. The addition of intravenous LYSPRO to future human pharmacology studies will clarify this problem.

In conclusion, the absorption rate of LYSPRO with or without additional zinc is significantly more rapid than that of human Regular insulin after subcutaneous injection. With its more rapid absorption and shorter duration of action, LYSPRO may offer an advantage over human Regular insulin in the control of blood glucose after meals.

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