The Action Profile of Lispro Is Not Blunted by Mixing in the Syringe With NPH Insulin

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OBJECTIVE — To assess the effect of mixing the insulin analog lispro (Humalog) with NPH (Humulin I) before injection on lispro's fast, short action profile.

RESEARCH DESIGN AND METHODS — A total of 12 healthy volunteers received subcutaneous abdominal injections of 0.1 U/kg regular insulin and 0.2 U/kg NPH insulin as follows: lispro and NPH injected separately (treatment group A), lispro and NPH mixed in the syringe up to 2 min before single injection (treatment group B), and human regular insulin and NPH mixed and injected as in group B (treatment group C), on separate occasions, in random order. Plasma glucose was maintained for 12 h by intravenous 20% glucose. Pharmacokinetic and pharmacodynamic parameters were compared by analysis of variance for repeated measures.

RESULTS — Peak plasma insulin levels (2.6 ± 0.8 vs. 2.2 ± 0.6 vs. 1.9 ± 0.6 ng/ml, $P = 0.075$), total glucose infused (121.5 ± 32.8 vs. 135.0 ± 49.0 vs. 117.3 ± 39.9 mg · kg^{-1} · min^{-1}, $P = 0.53$), and maximum glucose infusion rate (GIR_{max}) (8.3 ± 0.9 vs. 8.0 ± 1.7 vs. 7.1 ± 2.4 mg · kg^{-1} · min^{-1}, $P = 0.65$) were not significantly different between treatments. The times until peak insulin concentrations were similar in treatment groups A and B, but significantly shorter than in treatment group C (0.9 ± 0.3 and 1.2 ± 0.2 vs. 2.0 ± 0.4 h, respectively, $P = 0.042$). The times until GIR_{max} were also not different (113.9 ± 41 and 122.0 ± 45 vs. 209.0 ± 51.3 min, , respectively, $P = 0.002$). The glucose infusion rate (GIR) then fell to 50% GIR_{max} more quickly in treatment groups A and B than in treatment group C (239.9 ± 40.5 vs. 292.4 ± 133.3 vs. 399.5 ± 78.3, respectively, $P = 0.005$).

CONCLUSIONS — The action profile of lispro is not attenuated by mixing lispro with NPH in the syringe immediately before injection. The advantages are available to those individuals who need to combine types of insulin before injection to achieve optimal diabetes control.

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The insulin analog lispro [Lys(B28), Pro(B29)] has the same amino acid sequence as endogenous human insulin, except for positions 28 and 29 on the B-chain where lysine and proline are reversed. This results in an insulin molecule with a reduced capacity for self-association, allowing rapid absorption and action after subcutaneous injection (1–3). Insulin-requiring diabetic patients can therefore conveniently inject lispro immediately before meals and also benefit from improved postprandial glycemia (4–7).

Patients commonly mix rapid-acting insulin and NPH in the same syringe and administer the mixture as a single injection. In conventional therapy, this technique reduces the number of injections required per day and it has been suggested as a desirable way to provide background insulin on which to superimpose short-acting, meal-related insulin boluses (8). NPH contains protamine, which binds insulin in subcutaneous tissues and delays its release into the systemic circulation (9). An interaction between lispro and excess protamine in the NPH could result in loss of the favorable action profile of lispro when the two insulin types are mixed in a syringe before injection. Initial investigations, using a formulation of NPH that is now obsolete, did suggest reduction in the favorable action profile of lispro when the two insulin types were mixed before injection (Eli Lilly, Indianapolis, IN; U.S. data sheet 1996). However, the protamine content of NPH, which was not then regulated in the U.S., was unusually high. This has since been standardized and is now in line with European regulations. We aimed to investigate the extent to which mixing lispro in a syringe with the standard formulation of NPH insulin before injection could blunt the favorable glucodynamic profile of lispro in vivo.

RESEARCH DESIGN AND METHODS

Subjects

A total of 12 healthy volunteers (8 men, 4 women) were recruited. They were aged 26.0 ± 4.2 years (mean ± SD) with a BMI of 24 ± 2.4 kg/m^2. Written informed consent was obtained, and all subjects were screened with a medical history, physical examination, electrocardiogram (ECG), toxicology screen, hematology, and renal and liver function tests. Circulating antibodies against human and lispro insulin were also measured. The study protocol was approved by the Research Ethics Com-

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J.R.W. holds stock in Eli Lilly.

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; C_{max}, maximum insulin concentration; early $TR_{[50\%max]}$, time taken to reach 50% maximum GIR; GIR, glucose infusion rate; GIR_{max}, maximum glucose infusion rate; late $TR_{[50\%max]}$, fall back to less 50% maximum GIR; RIA, radioimmunoassay; T_{max}, time of maximum concentration; TR_{max}, time to maximum GIR.

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

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Study protocol and measurements

The following three treatments were administered: treatment A, lispro and NPH insulin administered as separate subcutaneous injections; treatment B, lispro and NPH mixed in a syringe and injected subcutaneously within 2 min; treatment C, human regular insulin and NPH mixed in a syringe and injected subcutaneously within 2 min. All treatments were administered into the subcutaneous tissue of the right and/or left lower abdominal quadrant. The doses of either lispro or human regular insulin were 0.1 U/kg, with 0.2 U/kg NPH. Treatment order was randomized in a three-way crossover design by a volunteer and investigator who were blind to the treatment given using sealed envelopes. A minimum of 3 days and a maximum of 6 weeks separated each treatment.

Subjects fasted from 10:00 P.M. the night before each study. In the morning, a distal vein was cannulated, using 1% lidocaine to anesthetize the skin, and the hand rested in a box warmed to 55°C to arterialize the venous blood (10). Arterialized venous blood samples were taken and plasma glucose concentration was measured at the bedside every 5 min from 30 min before insulin injection to 6 h postinjection and every 15 min thereafter until the end of the study. Glucose (20%) (Clinitec Nutrition, Berkshire, England, U.K.) was infused intravenously to maintain plasma glucose at the fasting level.

Additional blood samples were obtained at baseline (just before insulin administration); every 15 min for 1 h; every 30 min from 1 to 2 h; every hour from 2 to 8 h and at 10 and 12 h for measurement of serum immunoreactive insulin and C-peptide. Samples for serum potassium measurements were collected at 0, 30, 60, 105, 180, 360, 480, 600, and 720 min after insulin dosing.

Plasma glucose was assayed with a YSI 2300 STAT glucose analyzer (Yellow Springs, OH). Serum insulin and insulin lispro concentrations were determined by commercially available radioimmunoassay (RIA) (Coat-A-Count, Diagnostic Products, Los Angeles, CA). Lispro exhibits 100% cross-reactivity with insulin in this RIA method. C-peptide measurements were measured using a commercial RIA kit (DSL, Webster, TX). Potassium was measured as previously described (11).

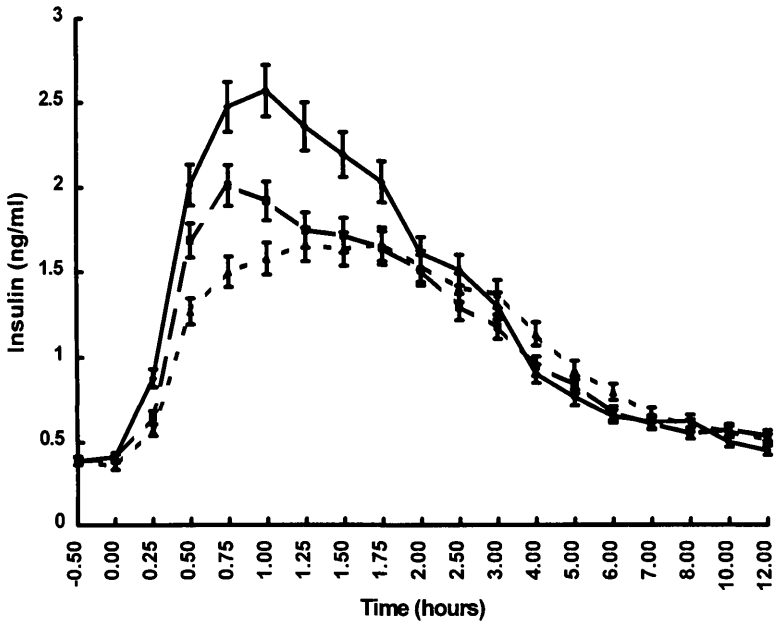

Figure 1—*Mean insulin concentration profiles after subcutaneous injections of 0.1 U/kg of lispro and 0.2 U/kg NPH separately (—◆—), mixed within 2 min before injection (– – ■ – –), and identical doses of regular insulin and NPH mixed before injection (- - -▲- - -).*

Total reactive antibodies (cross-reactive), insulin-specific antibodies (insulin-specific), and lispro-specific antibodies (analog-specific) were measured by RIA at the Corning-Nichols Institute, San Juan Capistrano, California (12).

Analyses. The area under the curve (AUC) for insulin concentration for each subject on each treatment was calculated for the entire study period, AUC 12 h, using the trapezoidal rule. Maximum insulin concentration (C_{max}) and the time the maximum concentration occurred (T_{max}) were determined in each subject, and group data were then compared across treatments.

Glucose infusion rates (GIRs) were calculated individually in 10-min blocks and corrected for deviation from the baseline plasma glucose value (13).

The total amount of glucose infused ($GIR_{12\,h}$) was obtained from the sum of the corrected 10-min GIRs. Maximum glucose infusion rate (GIR_{max}), time to GIR_{max} (TR_{max}), time taken to reach 50% GIR_{max} (early $TR_{[50\%max]}$), and to fall back to less than 50% maximum GIR (late $TR_{[50\%max]}$), and the duration of peak effect were determined individually. The hourly GIR was also calculated for the first 6 h of the study in all treatment groups to assess the action of rapid-acting insulin during that time. To quantify C-peptide suppression, minimum C-peptide concentrations (Cp_{min}) and time to Cp_{min} ($TCpep_{min}$) were documented and compared between treatments. Minimum potassium concentrations (K_{min}) and the time of occurrence (TK_{min}) were also assessed

Comparisons between treatments for pharmacokinetic and glucodynamic values were made by an analysis of variance (ANOVA) model for repeated measures. If a statistically significant treatment effect was observed ($P < 0.05$), pairwise comparisons were made between the treatment means using Student's t test. The study had 80% power to detect a difference of no less than 150 mg/min in GIR_{max} and 1.5 h in TR_{max} between groups with a P value of 0.05 significance level (two-tailed), using a standard deviation for GIR_{max} of 169 mg/min and TR_{max} 1.9 h, as found in the earlier study (Eli Lilly, unpublished data).

RESULTS

Plasma glucose and insulin profiles

Euglycemia was maintained throughout in all studies (5.2 ± 3.3%, 5.1 ± 3.1%, 5.2 ± 3.2% [mean ± coefficient of variation] mmol/l, treatment groups A, B, and C, respectively). Plasma insulin curves are shown in Fig. 1. As shown in Table 1, the AUC 12 h for the insulin curves did not differ between treatment groups (10.8 ± 2.7 vs. 9.9 ± 2.2 vs. 10.1 ± 2.4 ng · h^{-1} · ml^{-1}, ANOVA P = 0.062). Maximum serum insulin concentrations did not differ between groups (ANOVA P = 0.075). The

Table 1—*Pharmacokinetic parameters*

	Treatment group A (lispro and NPH)	Treatment group B (lispro mixed with NPH)	Treatment group C (human regular insulin mixed with NPH)	*P* value
C_{max} (ng/ml)	2.6 ± 0.8	2.2 ± 0.6	1.9 ± 0.6	0.075*
T_{max} (h)	0.9 ± 0.3	1.2 ± 0.2	2.0 ± 0.4	0.042†
AUC 12 h (ng · ml^{-1} · h^{-1})	10.8 ± 2.7	9.9 ± 2.2	10.1 ± 2.4	0.062*

Data are mean ± SD. *No difference across the groups; †treatment groups A and B versus treatment group C.

T_{max} was similar in treatment groups A and B, but markedly longer in treatment group C (0.9 ± 0.3, 1.2 ± 0.2 vs. 2.0 ± 0.4 h, ANOVA $P = 0.042$).

C-peptide levels were suppressed immediately after the administration of each treatment and remained so with no statistically significant differences between groups.

Glucose infusion rates

The GIRs are shown in Fig. 2. Total glucose infused was not different between studies (121.5 ± 32.8 vs. 135.0 ± 49.0 vs. 117.3 ± 39.9 mg · kg^{-1} · min^{-1}, $P = 0.53$). GIR_{max} was also not significantly different between groups, although the intraindividual variation was large (Table 2). TR_{max} was also not altered by mixing lispro with NPH (113.9 ± 41.0 vs. 122.0 ± 45.0 min, $P = 0.692$, treatment groups A vs. B, respectively) but was significantly longer with regular and NPH in treatment group C (209.0 ± 51.3 min, $P = 0.002$). Analysis of the GIR data in 120-min blocks failed to show any significant difference between treatment groups A and B for the first 6 h. Treatment group C, however, showed a difference at 0–120 min (27.0 ± 8.4 and 25.0 ± 6.4 vs. 13.0 ± 4.9 mg · kg^{-1} · min^{-1}, $P = 0.0015$, treatment groups A, B vs. C, respectively) and 240–360 min (21.6 ± 9.2 and 25.9 ± 11.3 vs. 30.9 ± 11.2, $P = 0.043$, treatment groups A, B vs. C, respectively) as shown in Fig. 3. Treatment groups A and B differed significantly from treatment group C in all the other glucodynamic indexes analyzed (Table 2).

Serum potassium did not show any appreciable differences among all three treatment groups, and no subject showed abnormal insulin antibody titers.

CONCLUSIONS — The rapidly acting analogs of human insulin have been designed to address two problems of conventional regular insulin types: *1*) their slow absorption from the subcutaneous injection site, necessitating administration ideally 1 h and certainly 30 min before meals, and *2*) their prolonged duration of action, increasing risk of delayed hypoglycemia after meals, especially during intensified insulin therapy (4,5). The insulin analog [Lys(B28),Pro(B29)], in which the lysine and proline residues at positions 28 and 29 of the B-chain of the insulin molecule are reversed, is a more rapidly absorbed insulin (1–4) than conventional human regular insulin, offering the advantages of immediate injection before meals and less risk of hypoglycemia later (5–7).

Many patients mix regular-acting with intermediate-acting insulin before injection to minimize the number of injections needed. Concerns were raised that mixing lispro with NPH before an injection might diminish the advantages of the new analog by retarding its absorption from the injection site. Although protamine, the retarding agent of NPH, does not delay the onset of action of conventional regular insulin when the two are given together (6), an early in-house study with lispro and the NPH formulation then available did suggest that the favorable action profile of the analog might be attenuated by NPH. However, the protamine content of the NPH used in the study was unusually high (not regulated in the U.S. at the time of the study), and it was necessary to examine the issue with the now standard NPH formulation. We have shown no difference in the pharmacodynamics of lispro insulin when given with the standardized NPH either as a separate injection or mixed in the syringe before

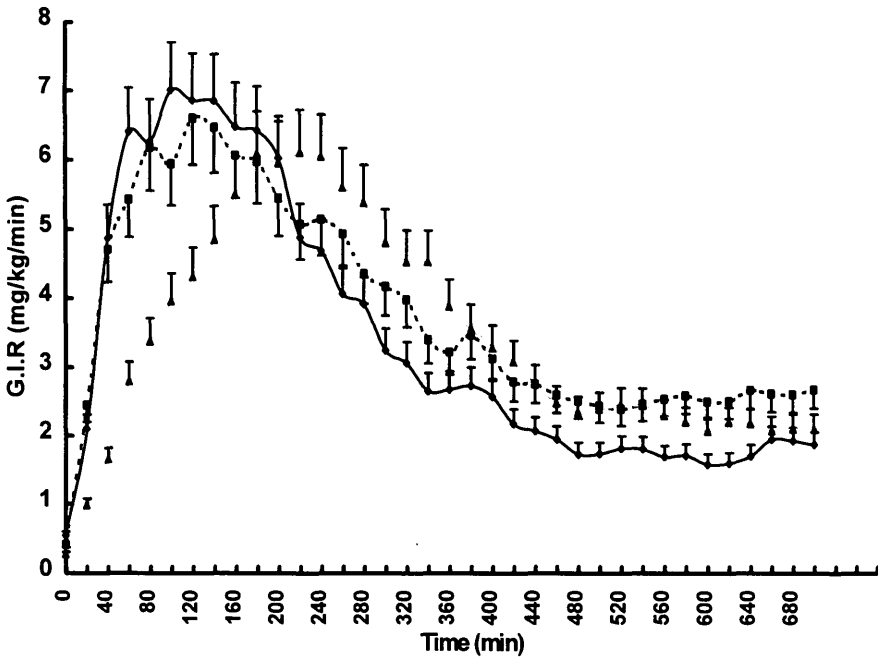

Figure 2—*GIR profiles obtained during the euglycemic clamp after subcutaneous injection of lispro and NPH separately, lispro and NPH administered as a mixture, and regular insulin and NPH administered as a mixture. Subcutaneous injections of 0.1 U/kg of lispro and 0.2 U/kg NPH separately (—◆—), mixed within 2 min before injection (- - -■- - -), and identical doses of regular insulin and NPH mixed before injection (—▲—).*

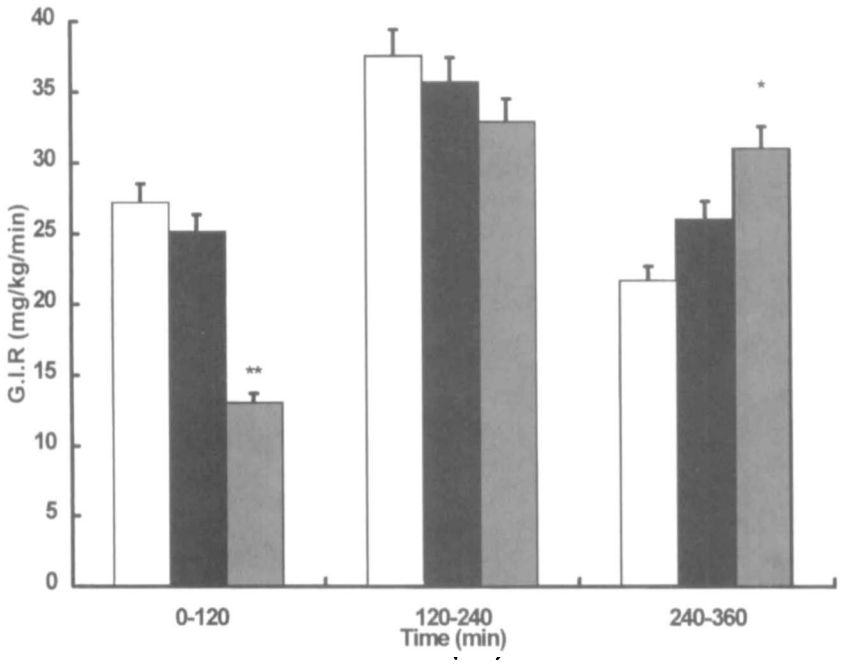

Figure 3—*GIR estimated in 120-min blocks for the first 6 h after subcutaneous injection of insulins: lispro and NPH separately (□), lispro and NPH mixed (■), and regular and NPH mixed (■). **P < 0.002, *P < 0.05.*

administration and injected within 2 min. Both treatments resulted in very similar glucose requirements immediately and for 12 h after the administration to healthy volunteers. Injected separately or after mixing with NPH, lispro showed more rapid absorption than conventional regular insulin, with maximum insulin concentrations attained at an earlier time than after injection of regular human insulin mixed with NPH. Although the maximum serum insulin concentration in the lispro and NPH (mixed) group was only 80% of that seen after separate injections, this difference was not significant and the time to peak serum insulin concentration was not different. A larger sample size may have detected a significant difference in C_{max} between the two lispro groups, as the study only had a power of 57% to detect a difference of 0.5 ng/ml in the means, raising the possibility of a type 2 error. However, the study was powered on the basis of the glucoregulatory effects of the insulin types. We found no evidence that the mixing of lispro and NPH before injection had any effect on insulin action.

These are important data from a clinical point of view. A large number of patients with diabetes choose to minimize their number of injections by mixing rapid- and intermediate-acting insulin. Equally importantly, there are now suggestions that the best way to use the new rapid-acting insulin analogs in multiple daily injection regimens may be to mix them with a small amount of intermediate-acting insulin (8). The combination of the two insulin types may be particularly beneficial if injected immediately before meals, with a dose of lispro to achieve rapid short duration hyperinsulinemia to control the prandial glycemic response and a small dose of NPH to sustain insulin levels until the next meal (17). Such a balanced combination may overcome the recognized problem with multiple daily injections of lispro, where preprandial hyperglycemia has been observed, secondary to the short action profile of the analog (18). Our data suggest that such a regimen will not result in a loss of speed of onset of action of the analog. The major drawback of this is the need to mix insulin types before every meal because the long-term stability of the lispro-NPH mix has not been demonstrated. A very recent study of premixed formulations of lispro and a new insulin analog preparation, NPL, has recently been reported (19), but our present data only allow us to support the use of an NPH-lispro mix if the mixture is administered within 2 min of being made.

Our data, which show a similar need in total glucose required to maintain euglycemia among three regimens, supports the contention that lispro and regular human insulin are equipotent and that the addition of NPH did not result in a reduced potency of lispro. However, the more rapid rise in the GIRs necessary to maintain euglycemia was noticed in both the lispro treatment groups when compared with regular human insulin and NPH (mixed) treatment group. This was due to a slower rise in glucose requirement (a proxy for insulin action) and to a slower fall and suggests that the mixing of regular human insulin with NPH gives the least chance of controlling postprandial glycemia without delayed hypoglycemia.

We can conclude that the rapid glucose-lowering effect of lispro is not impaired

Table 2—*Glucodynamic parameters*

	Treatment group A (lispro and NPH)	Treatment group B (lispro mixed with NPH)	Treatment group C (regular insulin mixed with NPH)	*P* value
GIR_{max} (mg · kg^{-1} · min^{-1})	8.3 ± 0.9	8.0 ± 1.7	7.1 ± 2.4	0.65*
TR_{max} (min)	113.9 ± 41.0	122.0 ± 45.0	209.0 ± 51.3	0.002†
Early $TR_{[50\%max]}$ (min)	44.5 ± 7.6	47.1 ± 5.1	99.6 ± 29.8	0.004†
Late $TR_{[50\%max]}$ (min)	239.9 ± 40.5	292.4 ± 133.3	399.5 ± 78.3	0.005†
Duration of peak activity (min)	122.0 ± 52.6	134.1 ± 58.0	164.3 ± 46.2	0.369*
Total glucose infused (mg · kg^{-1} · min^{-1})	121.5 ± 32.8	135.0 ± 49.0	117.3 ± 39.9	0.53*

Data are means ± SD. *No differences across the groups; †treatment groups A and B versus treatment group C.

by mixing with NPH before injection, at least when the injection is administered within 2 min of mixing. This has important clinical implications. The potential advantages for lispro include the recommendation that it be administered immediately before eating (often the patients' preference) and that it may diminish late hypoglycemia, especially with exercise (20) or at night (21) in well-controlled diabetes. However, there is a risk of it running out between meals, especially when the time of the meals is widely spread apart, which may be ameliorated by mixing lispro with a little NPH. Our data suggest that this mixing is feasible, without significant loss of either the fast onset or the short duration of the insulin effect. The convenience and postprandial glucose-lowering properties of lispro are also available to those who like to mix insulin types to simplify their treatment regimens.

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