Comparison of Insulin Aspart and Lispro
Pharmacokinetic and metabolic effects

**OBJECTIVE** — To compare insulin levels and actions in patients with type 1 diabetes after subcutaneous injection of the rapid-acting insulin analogs aspart and lispro.

**RESEARCH DESIGN AND METHODS** — Seven C-peptide–negative patients with type 1 diabetes (two men and five women) were studied at the General Clinical Research Center at Temple University Hospital two times, 1 month apart. Their plasma glucose was normalized overnight by intravenous infusion of insulin. The next morning, they received subcutaneous injections of either aspart or lispro (9.4 ± 1.9 U) in random order. For the next 4–5 h, their plasma glucose was clamped at ~5.5 mmol/l with a variable infusion of 20% glucose. The study was terminated after 8 h.

**RESULTS** — Both insulin analogs produced similar serum insulin levels (250–300 pmol/l) at ~30 min and disappeared from serum after ~4 h. Insulin aspart and lispro had similar effects on glucose and fat metabolism. Effects on carbohydrate metabolism (glucose uptake, glucose oxidation, and endogenous glucose production) peaked after ~2–3 h and disappeared after ~5–6 h. Effects on lipid metabolism (plasma free fatty acid, ketone body levels, and free fatty acid oxidation) appeared to peak earlier (at ~2 h) and disappeared earlier (after ~4 h) than the effects on carbohydrate metabolism.

**CONCLUSIONS** — We conclude that both insulin aspart and lispro are indistinguishable from each other with respect to blood levels and that they are equally effective in correcting abnormalities in carbohydrate and fat metabolism in patients with type 1 diabetes.

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Abbreviations: EGP, endogenous glucose production; FFA, free fatty acid, GIR, glucose infusion rate; GRa, rate of glucose appearance; GRd, rate of glucose disappearance.

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

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Informed written consent was obtained from all subjects after explanation of the nature, purpose, and potential risks of the study. The study protocol was approved by the Institutional Review Board of Temple University Hospital.

Study design
All subjects were admitted to the General Clinical Research Center at Temple University Hospital the day before the studies. Their evening dose of insulin was withheld. At 6:00 p.m. they were fed a standard meal consisting of 53% carbohydrate, 15% protein, and 32% fat. After that, they fasted for the duration of the study but were allowed water ad libitum.

During the night, their blood glucose concentration was maintained between 5.5 and 6.7 mmol/l with an intravenous infusion of regular insulin. The insulin infusion was discontinued at ~7:00 a.m. the following day. At ~6:00 a.m. an infusion of [6,6-2H2]glucose was started and continued until the end of the study. At ~8:00 a.m. they received a subcutaneous injection into the abdominal wall of a rapidly acting insulin analog (either aspart or lispro) at a dose equal to one-half of their normal daytime insulin dose. All subjects received both insulins, 1 month apart, in random order. The patients, the nurses who collected the blood, and the technician who analyzed the blood were all unaware as to which analog was given.

After injection of the insulin analogs, plasma glucose levels were kept at ~5.5 mmol/l with a variable infusion of 20% dextrose in water (5). Eight hours after insulin injection, the study was terminated; the patients restarted on their normal insulin regimen, were fed a meal, and were discharged from the hospital.

Glucose turnover
Glucose turnover was determined using the stable isotope [6,6-2H2]glucose as described (6). To assure isotope equilibration, the tracer infusion was started 120 min before initiation of the clamp starting with a bolus of 30 µmol, followed by a continuous infusion of 0.3 µmol·kg−1·min−1. Blood was collected at 30- to 60-min intervals (~120, ~30, 0, 30, 60, 120, 180, 240, 300, 360, 420, and 480 min) for determination of isotope enrichment. Rates of glucose appearance (GRa) and disappearance (GRd) were calculated from the isotope enrichments before (~30 to 0 min) and during the 8-h study using Steele’s equation for nonsteady state (7). Underestimation of GRa during hyperinsulinemia was avoided by adding [6,6-2H2]glucose (6.9 mmol/100 ml) to the unlabeled glucose infused to maintain euglycemia.

Endogenous glucose production
Endogenous glucose production (EGP) was calculated as the difference between the isotopically determined GRa and the glucose infusion rates (GIR) needed to maintain stable blood glucose levels during insulin infusion (EGP = GRa – GIR).

Indirect calorimetry
Respiratory gas exchange rates were determined at 30- to 60-min intervals with a metabolic measurement cart as previously described (8). Rates of protein oxidation were estimated from the urinary nitrogen excretion after correction for changes in urea nitrogen pool size (9). Rates of protein oxidation were used to determine the nonprotein respiratory quotient. Rates of fat oxidation were determined with the tables of Lusk, which are based on an nonprotein respiratory quotient of 0.707 for 100% oxidation and 1.00 for 100% carbohydrate oxidation.

Body composition
Body composition was determined by bioimpedance analysis (10).

Analytical procedures
Plasma glucose was measured with a glucose analyzer with the glucose oxidase method every 15–20 min. Free insulin levels were determined in deproteinized serum by radioimmunoassay with a specific antibody that cross-reacts only minimally (<0.2%) with proinsulin (Linco, St. Charles, MO). This antiserum recognizes equally human insulin, insulin lispro, and aspart (Fig. 1). Plasma fatty acids were determined enzymatically in chilled plasma with a kit (Wako, Richmond, VA). C-peptide was determined by radioimmunoassay (Linco). Plasma β-hydroxybutyrate and acetoacetate were determined enzymatically.

Statistical analysis
All data are expressed as means ± SEM. Statistical significance was assessed using two-way repeated measures ANOVA and
two-tailed Student’s t test. A P < 0.05 was considered significant.

RESULTS

Insulin levels
Insulin concentrations rose from 39 ± 6 to 256 ± 63 pmol/l 30 min after injection of insulin aspart and from 43 ± 10 to 286 ± 99 pmol/l 30 min after injection of insulin lispro (P = 0.24). After that, insulin levels declined at similar rates in both groups reaching basal levels ~4 h after injection (Fig. 2).

GIR
GIR reached 17.3 ± 5.2 and 15.3 ± 4.3 μmol·kg⁻¹·min⁻¹ 120 min after injection of insulin aspart and lispro, respectively (P = 0.61) (Fig. 2). After that, GIR decreased in both groups, reaching levels not different from 0 at ~300 min.

Plasma glucose
Preinjection glucose concentrations were 7.3 ± 0.6 and 7.2 ± 0.4 mmol/l (P = 0.68), respectively, in the insulin aspart and insulin lispro groups. After injection of the insulin analogs, blood glucose was prevented from falling for ~300 min by infusion of exogenous glucose. After that, glucose levels rose, reaching 12.1 ± 2.8 and 10.7 ± 1.6 mmol/l (P = 0.67), respectively, in the insulin aspart and lispro groups at the end of the study (Fig. 2).

EGR
EGR rose from 16.6 ± 1.4 to 26.8 ± 6.6 and from 14.5 ± 1.1 to 27.6 ± 8.6 μmol·kg⁻¹·min⁻¹, respectively, at ~120 min in the insulin aspart and lispro groups. EGR then declined in both groups, reaching basal levels after ~240 min. There were no statistically significant differences between the two groups at any time (P = 0.61) (Fig. 3).

Carbohydrate and free fatty acid oxidation
Basal carbohydrate oxidation rates were similar in both groups. They increased comparably after analog injection until ~3 h and then declined. Carbohydrate oxidation rates in both groups were not statistically significant from each other at any time during the studies (P = 0.69). Similarly, free fatty acid (FFA) oxidation rates first decreased and then increased. There were no statistically significant differences between the two groups (P = 0.22) (Fig. 4).

Plasma FFA and ketone bodies
Basal plasma FFA levels decreased from 413 ± 65 to 130 ± 19 and from 620 ± 109 to 207 ± 40 μmol/l, 180 and 120 min, respectively, after injection of insulin aspart and insulin lispro. After that, FFA levels rose in both groups reaching 1,071 ± 112 and 1,195 ± 185 μmol/l after 480 min. The differences between the groups were not statistically significant (P = 0.40). Ketone body concentrations changed in parallel with FFA concentrations. Again, there were no significant differences between the two groups at any time (P = 0.70) (Fig. 5).
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C-peptide and glucagon
Preinjection levels of C-peptide (0.07±0.04 and 0.08±0.04 nmol/l, P=0.71) and glucagon (49±5 and 46±6 pg/ml, P=0.82) for the aspart and lispro groups, respectively, did not change significantly throughout the studies.

CONCLUSIONS

Comparison of insulin levels
After subcutaneous injection of an equal amount of either insulin lispro or aspart into the same patients, free insulin reached similar concentrations (~250–300 pmol/l) at approximately the same time (30–60 min postinjection), and after that free insulin disappeared from serum at comparable rates, reaching preinjection levels at ~240 min.

Insulin analog concentrations were measured with an anti-insulin antibody that bound insulin, insulin lispro, and aspart with equal affinity over the range of insulin levels observed in this study. Therefore, serum levels of these three insulins could be directly compared with each other.

A limitation of our study was that blood was sampled only every 30–60 min. This precluded precise definition of peak insulin levels, the time to reach peak levels, and the time of disappearance of injected insulin from the blood. Nevertheless, these limitations do not detract from the observation that both insulins produced virtually superimposable insulin concentration curves.

The results of the current study are similar to those of two other studies in which the pharmacokinetics of the two insulin analogs were compared (11,12). One study used a single blind, random crossover design in seven patients with type 1 diabetes and 5- to 10-min blood sampling during the first hour after injection. They reported that after subcutaneous injection of 10 units of either analog, plasma profiles resembled each other but lispro showed a slightly more rapid uptake and peaked and declined marginally faster. A second study, which appeared while our study was under review, showed complete equivalence of lispro and aspart with respect to pharmacokinetics profiles and effectiveness controlling postprandial glucose excursions (12).

Comparison of insulin actions
To our knowledge, this is the first study comparing action of lispro and aspart on carbohydrate and fat metabolism in the same patients. We found no significant differences comparing the actions of both analogs on GRd (>80% of which occurs in skeletal muscle [13]), on EGP (>80% of which occurs in the liver [14]), on GIR (reflecting a combination of insulin action on muscle and liver), and on carbohydrate oxidation. All of these effects reached ~2–3 h and had disappeared after ~5–6 h. As shown in Fig. 3, the nonsignificant differences in GRd and EGP were in opposite directions (i.e., insulin aspart was more active promoting glucose uptake but less active suppressing EGP).

This supports the notion that the bioactivity profiles of the two insulins are interchangeable.

Similarly, there were no significant differences comparing actions of the two analogs on plasma FFA and ketone body levels nor on FFA oxidation. (Changes in plasma FFA levels closely reflect insulin action on lipolysis and is a very sensitive indicator of insulin action [15].) Insulin effects peaked at ~2 h and had disappeared after ~4 h. These results are in accord with previously published data on effects of insulin lispro (16,17) and insulin aspart (18–20).

Thus, the actions of both insulin analogs on carbohydrate and on lipid metabolism appeared to be indistinguishable from each other. This was not surprising in view of the similarity of their blood levels. Because of the small number of patients studied, we cannot completely rule out the possibly of small differences in some of the action of these two insulin analogs. Nevertheless, our data suggested that these two rapidly acting insulin analogs should be equally effective in treating the metabolic abnormalities of patients with type 1 diabetes.

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