

The Alteration of Aspart Insulin Pharmacodynamics When Mixed With Detemir Insulin

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OBJECTIVE—Mixing rapid acting insulin analogs with detemir insulin to minimize daily injections has been adopted as a common regimen, especially for some children with type 1 diabetes, despite the manufacturing company's caution against mixing these analogs in the same syringe. The effect of this practice on the pharmacodynamics (PD) of rapid-acting insulin has not been widely studied. This crossover, randomized study was undertaken to determine whether mixing aspart with detemir insulin has an adverse effect on the early glucodynamic action of rapid-acting insulin analog in humans.

RESEARCH DESIGN AND METHODS—Eight adolescents with type 1 diabetes (age 17.3 ± 0.6 years and A1C $7.3 \pm 0.3\%$) had two euglycemic glucose clamps during which 0.2 units/kg aspart and 0.4 units/kg detemir insulin were injected either as a separate or single mixed injection in random order.

RESULTS—Mixing the two insulins diminished the peak and overall early aspart insulin action with significantly lower maximum glucose infusion rate (GIR_{max} separate 6.1 ± 0.7 mg/kg/min vs. mix 4.5 ± 0.5 mg/kg/min; $P = 0.03$) values and the area under curve for GIR during the first 3 h of the insulin action study (separate 757 ± 105 mg/kg vs. mix 491 ± 66 mg/kg; $P = 0.04$).

CONCLUSIONS—These data demonstrate that mixing aspart with detemir insulin markedly lowers the early PD action of aspart and prolongs its time-action profile as compared with the separate injection of these analogs. These changes in insulin PD should be weighed against the added convenience of mixing when considering such unlicensed use of these insulins in youth with type 1 diabetes.

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Basal-bolus insulin therapy given as either multiple daily injections or by an insulin pump is a mainstay of diabetes treatment for achieving optimal glycemic control in type 1 diabetes. The relatively flat and prolonged duration of action of insulin detemir and glargine (1,2) make them better options for basal insulin replacement in multiple daily injection regimens in comparison with NPH insulin. In children and adolescents, a negative aspect of both long-acting insulin analogs is that they increase the number of daily insulin injections due to warnings against mixing long- and rapid-acting insulins together (insulin glargine, Lantus; sanofi-aventis;

sanofi-aventis, available from <http://www.lantus.com/hcp/closing.aspx>; insulin detemir rDNA origin, brand name Levemir drug insert; Novo Nordisk, Bagsvaerd, Denmark). Despite these warnings, some patients and pediatric practitioners continue to mix both types of insulin analogs in order to improve compliance by reducing the number of daily injections. This practice has been supported by clinical trials that failed to demonstrate an adverse impact of mixing insulins on A1C levels or on continuous glucose monitoring profiles (3–5).

In keeping with labeled warnings against mixing, we recently used the glucose

clamp technique to demonstrate that the pharmacodynamic (PD) time-action profile of lispro insulin is markedly blunted and delayed when it is mixed with glargine insulin prior to injection (6). This study was undertaken to examine whether mixing of insulin aspart with insulin detemir causes a similar alteration in aspart's PD action.

RESEARCH DESIGN AND METHODS

Subjects

Eight subjects with type 1 diabetes (four female) who attended the Yale Children's Type 1 Diabetes Clinic were studied. Subjects with a clinical diagnosis of type 1 diabetes for at least 1 year's duration, age ranging from 11–21 years, continuous subcutaneous insulin infusion therapy for at least 3 months, A1C $<9.0\%$, BMI $<95\%$ for age and sex, and the ability to comprehend written and spoken English were eligible for enrollment. Exclusion criteria included any other medical disease aside from type 1 diabetes or treated hypothyroidism, use of medications that might affect glycemic control, pregnancy or breast-feeding, not consistently using barrier methods or abstinence as contraception, or any other condition that in the judgment of the investigators would interfere with the subject's or parent's ability to provide informed consent or the investigator's ability to perform the study. The Yale University Human Investigation Committee approved the study.

Study personnel explained the risks and benefits of the study to the subjects and parents, obtained informed consent from the parents and informed assent from the subjects, performed history taking and physical examinations, and measured HbA_{1c} during the enrollment visit. Each subject was randomized to the mixed versus separate injection during the first versus second admission by the flip of a coin.

Procedures

Subjects were admitted to the Yale-New Haven Hospital Research Unit on the evening prior to the euglycemic clamp to

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monitor blood glucose levels. An intravenous catheter was placed to measure blood glucose levels hourly overnight, and insulin dose was adjusted via insulin pump to achieve glucose levels between 80 and 120 mg/dL on the morning of the euglycemic clamp. A second intravenous catheter was placed in the contralateral arm in the morning for infusion of 20% dextrose.

All subjects underwent two euglycemic clamp procedures on separate days in random order within 4 weeks of one another, as previously described (7,8). Subjects received 0.2 units/kg aspart and 0.4 units/kg detemir as a single mixed injection or two separate injections. Subjects who received the mixed injection before the first clamp were given separate injections prior to the second euglycemic clamp performed within 4 weeks of the first clamp and vice versa. Insulin aspart and detemir were mixed in the same syringe (BD insulin syringe with ultra-fine needle, 8 mm, 31 gauge; BD Biosciences, Franklin Lakes, NJ) at room temperature immediately before the injection into the deep subcutaneous tissue of the left arm through a two-finger pinch of skin at a 45–90° angle. Subjects were given detemir insulin in the left and aspart insulin in the right arm on the day that they were randomized to receive insulins separately. Neither the subject nor the investigator was blinded to mixing versus separate insulin injection. The infusion of insulin via the insulin pump was suspended just prior to the administration of aspart and detemir.

Plasma glucose levels were measured every 5 min, and a 20% dextrose infusion was adjusted to clamp plasma glucose concentrations between 80 and 100 mg/dL during 5 h of the study, as previously described (7,8). Blood for measurement of plasma insulin levels was collected every 10 min for the first 90 min, every 15 min for the next 90 min, and every 30 min for the last 120 min.

Biochemical methods

A1C was measured by the DCA Vantage Analyzer (Siemens Medical Equipment, Malvern, PA) and plasma glucose by the YSI Glucose Analyzer (YSI Life Sciences, Yellow Springs, OH). The very high plasma levels of albumin-bound insulin that are achieved after detemir injection precluded measurements of plasma aspart insulin levels due to cross-reactivity with the Mercodia iso-insulin ELISA assay (Mercodia Iso-Insulin ELISA Technical Note and insert, 2007; Mercodia Inc., Uppsala, Sweden).

Statistical analyses

Data are expressed as means \pm SEM. Rate of exogenous glucose infusion (GIR) analyzed every 10 min was adjusted for changes in the glucose space, as previously described (9). The PD parameters that were calculated for each clamp study included area under the curve of the glucose infusion rate (AUC_{GIR}), maximum glucose infusion rate (GIR_{max}), and time to reach maximum glucose infusion rate (T_{GIRmax}). Statistical comparisons between the two study conditions (mixed versus separate injections) were performed using GraphPad Prism version 5.0 (GraphPad Software Inc., La Jolla, CA). Paired *t* tests were used to compare GIR_{max} , GIR_{300min} , T_{GIRmax} , $AUC_{GIR 0-300min}$, and $AUC_{GIR 0-180min}$ between control and mixed injection days. Each subject acted as its own control for the paired *t* test analysis of plasma glucose and glucose clamp data.

RESULTS—Eight subjects (four female) with type 1 diabetes, age 17.3 ± 0.6 years and A1c of $7.3 \pm 0.3\%$, were enrolled and completed both clamp studies. Plasma glucose levels were similar for the mixed and separate injection studies during the 5 h of the clamp (100 ± 5 vs. 100 ± 5 mg/dL, respectively; $P = 0.5$) with an intraindividual plasma glucose concentration coefficient of variation of $5.3 \pm 0.2\%$ vs. $4.7 \pm 0.1\%$ (mean \pm SD) for separate and mixed injections, respectively.

The overall time-action profiles and PD parameters following the separate and mixed injections are shown in Fig. 1 and Table 1. As can be seen in Fig. 1, when aspart and detemir were given as separate injections, there was a rapid and sharp increase in GIR, which decreased to $\sim 25\%$ of peak values at the end of the study at 300 min. In contrast, the early rise in GIR was blunted, but late insulin action was increased following the mixed compared with separate injections. As shown in Table 1, mixing aspart with detemir significantly reduced the GIR_{max} but increased the GIR_{300min} as compared with separate injections. Although there were no significant differences between T_{GIRmax} and $AUC_{GIR 0-300min}$, the $AUC_{GIR 0-180min}$ was decreased with mixing.

CONCLUSIONS—Our data demonstrate that when aspart and detemir insulins are given at the same time as separate injections, the time-action profile in the 300 min following the injection

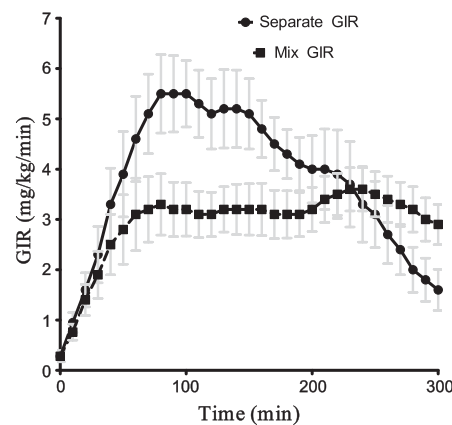


Figure 1—Mean glucose infusion rate with SEM for separate (circles with solid line) and mixed (squares with dashed line) injections of aspart and detemir insulins.

is very similar to that we observed in previous studies following the same 0.2 units/kg dose of insulin aspart alone (7,8). Specifically, in the 5 h following a bolus of aspart alone, the GIR_{max} and T_{GIRmax} were similar to corresponding values in this study following the separate injection. Because previous studies have shown that detemir does not reach 50% maximum action until 4–14 h after injection (10), it is unlikely that it made a major contribution to the early stimulation of glucose metabolism following the separate injection in this study.

In contrast to the results following separate injections, our data demonstrate that mixing insulin aspart with insulin detemir diminishes the peak action of aspart insulin and shifts the time-action curve to the right, as evidenced by the lower GIR_{max} and greater GIR_{300min} in the mixed compared with the separate injections. Overall insulin action reflected by $AUC_{GIR 0-300min}$ over the whole 5 h of the study was only slightly reduced with mixing because the decreased AUC during the first 3 h ($AUC_{GIR 0-180min}$) was offset by the increase in AUC during the following hours. The number of subjects in our study was small but sufficient to demonstrate statistically significant differences between the PD measures of insulin for mixed and separate injection groups. However, failure to detect statistically significant differences, as in $AUC_{GIR 0-300min}$, must be interpreted with caution in view of the small sample size in this study. These changes are qualitatively similar to what we have previously reported with mixing of insulin lispro and glargine (6).

In a recent clinical trial, Nguyen et al. (5) examined the effects of mixing aspart

Table 1—PD summary measures after subcutaneous injection of aspart insulin and detemir insulin in separate or mixed injections

PD parameters	Separate injection	Mix injection	P value
GIR _{max} (mg/kg/min)	6.1 ± 0.7	4.5 ± 0.5	0.03
GIR _{300min} (mg/kg/min)	1.6 ± 0.4	2.9 ± 0.4	0.01
T _{GIRmax} (min)	110 ± 18	130 ± 23	0.3
AUC _{GIR 0–300min} (mg/kg)	1,137 ± 149	888 ± 112	0.2
AUC _{GIR 0–180min} (mg/kg)	757 ± 105	491 ± 66	0.04

Data are mean ± SEM. P values in boldface refer to the significance of differences between separate and mixed injections.

and detemir during outpatient treatments of 14 pediatric patients with type 1 diabetes. No significant differences in overall sensor glucose AUC or mean amplitude of glycemic excursions were observed on separate and mixed injection days during this randomized, crossover, open-label study that used 48-h continuous glucose monitoring as its primary outcome measure. Although these results might appear to be inconsistent with our findings, they are not incompatible with our results. We only studied a fixed aspart to detemir unit dose ratio of 1:2 (equivalent to a molar ratio of 1:8), whereas in clinical practice, the fraction of aspart can be increased to compensate for the delayed and diminished peak action of aspart when it is mixed with detemir. Moreover, our study did not assess the impact of other factors such as previously administered albumin-bound detemir that could influence the effect of mixing on insulin action in the clinical setting, because all of our subjects were receiving insulin pump therapy prior to this study.

It is noteworthy that after mixing, the peak postdinner sensor glucose values exceeded 200 mg/dL in almost all of Nguyen's subjects, and the nighttime sensor glucose values in the borderline to frankly hypoglycemic range were observed in 9 of the 14 subjects (5). Given the alterations in the time-action profiles observed in our study, we speculate that postdinner hyperglycemia and increased risk of nocturnal hypoglycemia in the study by Nguyen et al. (5) were due, at least in part, to the mixed dose

of aspart and detemir that was given at dinnertime.

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