Injection Site Effects on the Pharmacokinetics and Glucodynamics of Insulin Lispro and Regular Insulin

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OBJECTIVE — The pharmacokinetics and glucodynamics of a new insulin analog, insulin lispro, and regular human insulin were compared and contrasted after subcutaneous administrations in femoral, deltoid, and abdominal injection sites.

RESEARCH DESIGN AND METHODS — Single 0.2 U/kg doses of insulin lispro and regular insulin were administered to 12 healthy subjects in a six-way randomized crossover fashion. Each dose was given after an overnight fast in one of three injection sites: abdominal, deltoid, or femoral. Study drugs were given during a manual euglycemic glucose clamp. Blood samples were collected over the 12-h clamp for measurement of insulin-reactive components, with pharmacokinetic and glucodynamic measurements derived from these serum insulin and clamp measurements.

RESULTS — Glucodynamic comparisons between insulin lispro and regular insulin showed a greater maximum infusion rate (R_{max}) at an earlier time (TR_{max}), regardless of injection site. The total glucose infused (G_{tot}) showed nearly identical values between sites for insulin lispro. Regular insulin showed greater G_{tot} values from deltoid and femoral injections. When comparisons were made between drugs, regular insulin produced significantly greater G_{tot}, primarily driven by the increased G_{tot} from deltoid and femoral injections. Greater maximum serum insulin concentrations (C_{max}) were experienced with insulin lispro at earlier times (t_{max}), regardless of the injection site ($P < 0.001$). Abdominal administrations produced the greatest C_{max} values at the earliest t_{max} for both regular insulin and insulin lispro. Deltoid and femoral injections had lower C_{max} values for both compounds. Shifts also occurred with t_{max}, although these shifts were much greater with regular insulin than with insulin lispro. Equivalent area under the curve (AUC) values were found when compared between compounds.

CONCLUSIONS — Slower absorption from deltoid and femoral administrations resulted in an increased duration of action for both regular insulin and insulin lispro when compared to abdominal administration. However, notable increases in the onset of action were only apparent with regular insulin. The consistency with insulin lispro response from abdominal and extremity injection sites allows more potential sites for subcutaneous injection with an assured rapid response.

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ANOVA, analysis of variance; AUC, area under the curve; C_{max}, maximum insulin concentration; CV, coefficient of variation; GIR, glucose infusion rates; G_{tot}, total glucose infused; RIA, radioimmunoassay; R_{max}, maximum glucose infusion rate; t_{max}, time to maximum glucose concentration; TR_{max}, time to maximum glucose infusion rate.

The site of subcutaneous injection has been recognized as a factor that may influence insulin absorption, and hence its glucodynamic profile, for some time (1–3). Besides physicochemical properties of the insulin formulation used and miscellaneous other factors, absorption of insulin from the subcutaneous tissue is determined by local blood flow, which may markedly differ between various anatomical sites of injection (4). The abdomen is generally accepted as the most rapid and reliable site of injection. Nonetheless, some patients prefer other injection sites for a variety of practical reasons. Reversal of the lysine and proline amino acid sequence at the 28 and 29 positions of the insulin B-chain produced an insulin analog with a large reduction in insulin self-association yet nearly identical insulin activity (5). This compound, insulin lispro, exhibits more rapid absorption and elimination when compared to regular insulin, producing an insulin profile more closely imitating the physiological response in insulin secretion to a meal (6,7).

This study was performed in healthy volunteers to determine whether differences between injection sites would affect insulin lispro activity as they do regular insulin, given the more rapid absorption of insulin lispro (6). We compared both the pharmacokinetic and glucodynamic behavior of insulin lispro and regular insulin after abdominal, femoral, and deltoid administrations.

Study subjects

Twelve healthy men participated in this study. Their age and BMI (mean ± SD) were 24 ± 2.5 years and 22.8 ± 2.1 kg/m^2, respectively. There was no evidence of illness or chronic medication in the subjects.

RESEARCH DESIGN AND METHODS

— This was a six-way crossover, randomized, open-labeled study. On six separate occasions each subject received a single 0.2 U/kg subcutaneous dose of either insulin lispro or regular insulin (Eli Lilly, Indianapolis, IN). The anatomical site of injection differed with

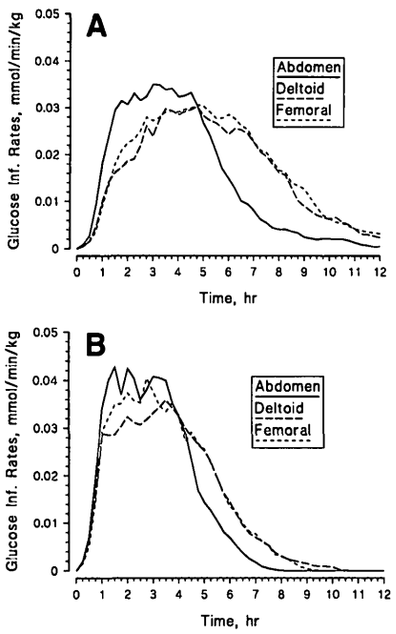

Figure 1—*Mean GIR versus time of all treatments* (n = 12)*: Regular insulin (0.2 U/kg;* A*, insulin lispro (0.2 U/kg;* B*). Appropriate injection sites are defined in the legend.*

each of the three injections given of the same drug. Tested injection sites included abdominal (~10 cm lateral to the umbilicus), femoral (~20 cm below the anterior superior iliac spine), and deltoid (~10 cm above the elbow on the lateral side of the arm) sites, with treatments separated by at least 7 days. All doses were administered subcutaneously (lifting a skinfold prior to injection) by syringe with a 13-mm needle at a 45° angle by the same investigator.

Subjects were admitted to the clinic in the morning after an overnight fast. After injection of the study drug, blood glucose concentrations were kept constant using a manually performed glucose clamp procedure, based on a previously described algorithm (8). The mean glucose level was targeted at 0.5 mmol/l below the fasting level. Whole blood glucose levels were measured at least every 5 min by a glucose analyzer (2300 STAT, YSI, Yellow Springs, Ohio). Glucose infusion rates (GIRs) were adjusted accordingly, intending to keep blood glucose levels within 0.3 mmol/l of the target level. Additionally, blood samples for determination of insulin, insulin lispro, and C-peptide concentrations were collected at 15-min intervals for the 1st hour, 30-min intervals for the 2nd hour, and hourly thereafter. All study procedures were continued until 12 h after injection of the study drug. The subjects remained fasted during the 12-h test period. After 12 h, study procedures were terminated and the subjects received a large meal rich in carbohydrates, as well as an extra intravenous bolus of dextrose, if indicated.

Sample analysis

Blood samples collected for analysis of insulin lispro, insulin, and C-peptide were allowed to clot and then centrifuged and separated. Serum samples were stored frozen at −20°C until analysis. Serum concentrations of insulin and insulin lispro were determined by a radioimmunoassay (RIA) method (9). The detection limit for insulin was <36 pmol/l in this assay; the coefficient of variation (CV) ranged between 5 and 8. Insulin lispro was ~90% cross-reactive with this method. Insulin lispro serum levels were counted using a standard curve prepared with insulin lispro standards. Serum C-peptide levels were measured by RIA in the same laboratory using a commercially available RIA (Novo Cell Tech, Denmark).

Glucodynamic and pharmacokinetic analyses

Data recorded during the clamp procedures (blood glucose values, GIR [millimoles per minute per kilogram, and total cumulative amount of glucose infused [G_{tot}, millimoles per kilogram]) were used to evaluate the glucodynamic response. From the GIR versus time data, the maximum infusion rates (R_{max}, millimole per minute per kilogram) and the times to maximum infusion rate (TR_{max}, minutes) were documented.

C-peptide–corrected insulin measurements (10) were used to estimate the maximum drug concentrations (C_{max}), the times those concentrations occurred after administration (t_{max}), and the area under the insulin or insulin lispro versus time curve (AUC). These values were used as comparative pharmacokinetic indices between treatments. The AUC was determined using trapezoidal methods (11). Half-lives reported from these data were calculated using a least squares regression method. Both pharmacokinetic and glucodynamic measurements were compared statistically between treatments. A relationship between the insulin serum concentrations and the GIR values at each time was constructed using an effect compartment model (12). From this relationship, estimates of onset of action and of duration of action were produced from all treatments.

Statistical analyses

The type of insulin (regular insulin or insulin lispro), the different administration sites (abdominal, deltoid, and femoral), and their interactions were included as factors in a split-split plot analysis of variance (ANOVA). In this analysis, insulin and site effects were tested against the appropriate combination of error sources comprising the subjects-by-insulin, subjects-by-site, and residual error terms. Interactions were tested against the residual error. Specific pairwise comparisons between sites were performed within the ANOVA model, with abdominal administrations considered as a reference. The statistical analyses were similar for all parameters with an α level of 0.05. Missing observations were occasionally encountered, so least squares means were calculated.

RESULTS

— All subjects completed all treatments in this study with no serious adverse events reported.

Glucodynamic results

Blood glucose concentrations were targeted between 4.0 and 4.2 mmol/l and maintained at the target level with a CV of 6% or less during the course of the clamp procedure in all subjects. The mean GIR versus time curves for regular insulin and insulin lispro (Fig. 1) showed a slower absorption of regular insulin from the deltoid and femoral sites. For regular insulin, time to the peak effect (TR_{max}) was significantly increased by >75 min for both deltoid and femoral administrations when compared to the abdominal administration (Table 1). The peak GIR (R_{max}) for regular insulin was not significantly different between the sites. For insulin lispro, the TR_{max} was only nominally increased for the deltoid administration (mean, 8 min) and increased by ~50 min for the femoral site (Table 1). Neither increase was significant when compared to the abdominal site. Insulin lispro R_{max} was reduced for both the femoral and deltoid sites, but did not reach a level of significance with the femoral site. The cumulative glucose infused (G_{tot}) did not significantly increase with either deltoid or femoral injections when compared to abdominal injections for either regular insulin or insulin lispro (Table 1). Nonetheless, significant differences did exist between all regular insulin

Table 1—*Summary of glucodynamic and pharmacokinetic parameters*

		Glucodynamic measurements						Pharmacokinetic measurements					
		R_{max}		TR_{max}		G_{tot}		C_{max}		t_{max}		AUC_{0}^{∞}	
Drug	Site	mmol · min^{-1} · kg^{-1}	*P*	min	*P*	mmol/kg	*P*	pmol/l^{-1}	*P*	min	*P*	pmol · min · l^{-1}	*P*
Regular insulin	Abdomen	0.0421	—	201	—	10.6	—	281	—	78.8	—	71,660	—
Regular insulin	Deltoid	0.0372	0.185	301	0.001	11.8	0.226	192	0.055	229	<0.001	62,830	0.213
Regular insulin	Femoral	0.0368	0.156	280	0.007	12.6	0.058	162	0.013	185	0.004	62,700	0.174
Insulin lispro	Abdomen	0.0539	—	155	—	9.94	—	589	—	46.3	—	83,260	—
Insulin lispro	Deltoid	0.0403	<0.001	163	0.749	10.3	0.752	395	<0.001	62.5	0.612	78,800	0.490
Insulin lispro	Femoral	0.0474	0.079	203	0.081	10.7	0.448	458	0.005	58.8	0.696	76,630	0.309
Injection site comparisons			0.002		0.030		0.066		<0.001		0.003		0.106
Insulin vs. insulin lispro			0.002		0.001		0.014		<0.001		<0.001		0.002

Data are least squares means corrected for C-peptide. *P* values are pairwise comparisons within drug, using abdominal site as reference.

and insulin lispro administrations, with regular insulin inducing a greater overall glucose demand. This difference was driven primarily by the increased G_{tot} from deltoid and femoral injections.

Pharmacokinetic results

Mean C_{max} and t_{max} data demonstrated a faster peak action of both regular insulin and insulin lispro from the abdominal site of injection compared to the deltoid and femoral sites (Table 1). Moreover, insulin lispro maintained a higher peak and more rapid onset of action when compared to regular insulin for all three injection sites (Table 1). For regular insulin, t_{max} was significantly shorter for abdominal injections than for deltoid or femoral injections. For insulin lispro, a significantly shorter t_{max} for abdominal injections could not be demonstrated ($P < 0.7$; Table 1). Total absorption of the insulin dose (using AUC values as an index) was nearly consistent between all three injection sites for either insulin lispro or regular insulin (Table 1). Absorption was not consistent between the two drugs, with insulin lispro exhibiting greater AUC values from all injection sites (ANOVA, $P = 0.002$).

Determination of onset of action and duration of action

Results from the determination of onset of action and duration of action are given in Table 2. The duration of action increased by 2–3 h for regular insulin and 1–1.5 h for insulin lispro after deltoid and femoral administrations compared to the abdominal administrations. The onset of action was delayed by 20–40 min for insulin, with the average onset calculated as 45, 76, and 62 min from abdominal, deltoid, and femoral injections, respectively. Conversely, deltoid or femoral administrations of insulin lispro did not result in a delay in the onset of action, with average values of 27, 34, and 33 min from abdominal, deltoid, and femoral sites.

CONCLUSIONS — The pharmacokinetic and glucodynamic results of this study show that insulin lispro was more rapidly absorbed than insulin at each of the separate injection sites. These results support previous observations in healthy volunteers at a variety of doses (6,7). The response was more rapid and shorter lasting for insulin lispro at each injection site when compared to regular insulin, reflective of the more rapid absorption of insulin lispro.

The injection site has long been recognized as a factor affecting insulin absorption (1–3,13). It is generally agreed that insulin absorption from femoral sites is slower than that from abdominal sites (2,3). In this study, deltoid and femoral sites were quite similar, but the separation of abdominal absorption from both deltoid and femoral injections was striking. This was true for both regular insulin and insulin lispro. For regular insulin the glucodynamic changes observed between the injection sites were in agreement with the serum concentration versus time profiles. The GIR versus time profiles (Fig. 1) portray the prolonged absorption from the deltoid and femoral sites, reflected by an increase in TR_{max} and by extended glucose infusions relative to abdominal administrations. Differences between the glucodynamic results of the various injection sites were not as notable with insulin lispro. The insulin lispro glucodynamic profiles for the three sites (Fig. 1) show considerable overlap, with only a significant reduction in R_{max} from deltoid injections, despite significant reductions in C_{max} after both deltoid and femoral administrations. No change was noted in TR_{max} between the three sites, reflecting the similar t_{max} values noted with insulin lispro. Differences between the two drugs and the injection sites were also apparent in the calculated onset of action and duration of action (Table 2). The prolonged absorption from the deltoid and femoral areas was manifested by an increased duration of action for both drugs. Onset of action was also delayed for these sites, but the magnitude of these changes appears to be drug specific with only minor effects with insulin

Table 2—*The 95% CIs for onset and duration of action*

Drug	Injection site	Onset interval (min)	Duration interval (h)
Regular insulin	Abdomen	30–70	6.5–8.5
Regular insulin	Deltoid	60–110	8.75–11.75
Regular insulin	Femoral	50–110	8.75–12.0
Insulin lispro	Abdomen	20–40	5.25–6.5
Insulin lispro	Deltoid	25–50	6.5–8.0
Insulin lispro	Femoral	25–50	6.25–7.75

lispro. Increases in insulin lispro duration of action were also less than those of regular insulin. The magnitude of the shifts in onset and duration of action are dose specific, with smaller doses expected to produce smaller shifts (12). However, since regular insulin and insulin lispro exhibit linear pharmacokinetic and glucodynamic disposition up to 0.2 U/kg (7), the changes would be expected to be proportional. Thus a rapid onset of action can still be expected with smaller insulin lispro doses.

The total glucose infused was significantly greater after regular insulin administrations when compared to insulin lispro (P = 0.014, Table 1). Although no significant differences were observed in total glucose infused between sites (P = 0.066), a consistent trend was apparent toward a greater amount of glucose infused with deltoid and femoral injections, especially with regular insulin. The G_{tot} differences between regular insulin and insulin lispro have been observed previously (7) and relate to the hysteresis between the serum concentrations and glucodynamic response. The hysteresis describes the time delay between observed serum insulin concentrations and the transport of that insulin to the effect site. The hysteresis is smaller with a longer absorption; a reduction in the hysteresis curve reflects less transit time to an effect site and better correlation between insulin concentrations and response. The same reasoning supports the greater total effect observed with deltoid and femoral injections when compared to abdominal injections.

The observed differences in absorption rate are a factor of physiologic changes between the various sites: differences in the subcutis and blood flow. These changes are known to dramatically affect subcutaneous absorption (13). Upper arm and femoral sites have a reduced blood flow and result in a prolonged insulin absorption (13).

The site of injection does not appear to appreciably affect the rate of absorption of insulin lispro but does appear to affect the duration of action. The lack of the injection site effects upon the rate of absorption are likely related to the loose hexameric formation of insulin lispro and its rapid dissociation into monomers and dimers within the subcutaneous depot; this does not become a rate-limiting effect, as it is for regular insulin.

In summary, this study shows that insulin lispro exhibits a more rapid time-action profile, a shorter duration of action, and a more intense maximal effect compared to regular insulin as assessed by pharmacokinetic and glucodynamic measurements during euglycemic clamping. For both compounds, abdominal administrations are absorbed faster than deltoid or femoral injections, although persistent absorption from the injection site is more pronounced with regular insulin. Insulin lispro's onset of action is only minimally affected by the injection site, unlike regular insulin, with insulin lispro's duration of action prolonged by about an hour from deltoid or femoral injections. We believe this study supports the abdomen as the preferable site of premeal subcutaneous injections for both regular insulin and insulin lispro to promote the most rapid response. Should extremity administrations become necessary, less deviation from optimal administration time (abdominal administrations) is expected with insulin lispro.

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