
Running title: Short-Acting Insulin Analogue Pharmacology

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**Objective.** Injected volume and subcutaneous adipose tissue blood flow (ATBF) affect insulin absorption. Pharmacokinetics of short-acting insulin analogues were established by assessing injection of small doses in lean subjects, healthy or with type 1 diabetes. In obese patients however, daily dosages are larger and ATBF is decreased. This study assessed the kinetics of a short-acting insulin analogue in obese subjects with type 2 diabetes.

**Research Design and Methods.** Euglycemic clamps following subcutaneous lispro injections were performed. Six healthy control subjects received 10 U. Seven obese (BMI 38.3±7.0 kg/m²) subjects with type 2 diabetes received 10, 30, and 50 U. Plasma lispro was measured by specific radioimmunoassay and ATBF by $^{133}$Xe-washout technique.

**Results.** ATBF was 64% lower in subjects with type 2 diabetes than in control subjects. Following 10 U injection, time to lispro plasma peak ($T_{\text{max}}$) was similar (48.3 vs. 55.7 min; controls vs. subjects with type 2 diabetes) although maximal concentration ($C_{\text{max}}$/dose was 41% lower in subjects with type 2 diabetes, with lower and delayed maximal glucose infusion rate (GIR$_{\text{max}}$: 9.0 vs. 0.6 mg/kg/min, p<0.0001, 69 vs. 130 min, p<0.0001, respectively). Following 30 and 50 U injections, $T_{\text{max}}$ (88.6 and 130.0 min, respectively) and time to GIR$_{\text{max}}$ (175 and 245 min) were further delayed and dose related ($r^2=0.51$; p=0.0004 and $r^2=0.76$; p<0.0001, respectively).

**Conclusions.** Absorption and hypoglycemic action of increasing dosages of lispro are critically delayed in obese subjects with type 2 diabetes.

The general purpose of intensive insulin regimens is achieve postprandial glucose control. Short-acting insulin analogues, were originally designed to fit this premise by synchronizing plasma insulin increase and food absorption (1). They are indeed absorbed more quickly than regular human insulin. However, this was demonstrated in normal weight healthy subjects or lean subjects with type 1 diabetes in studies assessing small i.e. 4-12 U subcutaneous injections (2-4). Paradoxically, few studies (5,6) have either assessed such dosages or been conducted in overweight subjects with or without type 2 diabetes although clinical practice shows that most patients with type 2 diabetes on insulin are distinctly overweight or obese and on much larger insulin dosages (7,8).

Multiple factors affect insulin absorption including its physicochemical properties, excipients, concentration, dosage as well as the clinical conditions under which it is injected e.g. orthostatic position, injection site, depth, exercises, massage, temperature and smoking (1,4,9,10). Injected volume (11) and subcutaneous adipose tissue blood flow (ATBF) are two other major absorption factors (12,13). Although it is well recognized that ATBF is dramatically altered in obese insulin-resistant individuals and in subjects with type 2 diabetes, baseline values are 50-70% lower than for lean healthy subjects and physiological postprandial doubling is blunted (14,15), most studies have nevertheless been conducted in lean subjects.

In view of the above, we hypothesized that absorption rate and activity of short-acting insulin analogues would be substantially lower in obese subjects with type 2 diabetes than pharmacokinetics reported in the literature. This study thus assessed the...
pharmacokinetic and pharmacodynamic responses of obese subjects with type 2 diabetes to subcutaneous injections of lispro at incrementally larger dosages (10, 30, and 50 U) during euglycemic clamps.

RESEARCH DESIGN AND METHODS

Study Design. This single dose, single blinded, controlled, 3-way randomized sequential study in subjects with type 2 diabetes was conducted at the Clinical Research Center. Patients aged 18-75 years, BMI ≥ 30 kg/m², A₁c ≤ 10%, taking over 100 U of insulin daily, with or without oral hypoglycemic agents, non smoker, were recruited. All were asked to maintain a stable diet and physical activity level between experiments and to refrain from strenuous exercise, alcohol, and caffeine intake for 48 hours before each experiment. The experimental protocol was duly approved by the Research Ethics Committee, conducted according to the Declaration of Helsinki principles, and all subjects signed the consent form.

Protocol. Experiments were performed three weeks apart in randomized order (10, 30, or 50 U). Subjects with type 2 diabetes were admitted at 8:00 PM on the evening preceding each experimental day, after having their evening meal and their usual insulin injection. An intravenous antecubital cannula was inserted into each arm, one for venous sampling and glucose measurements (Beckman Instruments Inc. Diagnostic Systems Group, Brea, CA), the other for dual administration of human insulin (Toronto R, Novo Nordisk Canada Inc., Mississauga, ON) and dextrose as needed. Plasma glucose level was brought progressively into the normal target range i.e. 5-6 mmol/l overnight. Experiments started at 8:00 AM. Anthropometric data were recorded: height, weight and body composition by bioelectrical impedance (TANITA Corporation of America Inc., Arlington Heights, IL). Subject were kept fasting (drinking water permitted) during the entire 8-hour clamp study. A venous catheter was retrogradely inserted into the hand of the same arm used for night-time blood samplings with the hand kept warm in a heating pad. Euglycemic clamp was performed following subcutaneous injection of lispro, 20 minutes after interruption of the overnight insulin infusion. Lispro was administered with a pen device (HumaPenErgo, Eli Lilly Canada Inc. Toronto, ON) with an 8 mm needle (30G×0.3×8mm) into subcutaneous adipose tissue 8 cm above umbilicus and 10 cm from the medial line. Plasma glucose was measured every 5 min in order to clamp glucose levels between 5 and 6 mmol/l with a 20% dextrose infusion via the antecubital catheter already in place. Blood samples were collected at 10-min intervals for the first 3 hours and at 20-min intervals thereafter. Study procedures ended at 4:00 PM. Subjects received a meal and their usual dose of insulin. They were discharged once glucose stabilized over 6 mmol/l.

Control healthy subjects were admitted on the experimental day at 7:30 AM; fasting from 8:00 PM the prior evening. Each received a single dose 10 U lispro; all other procedures were identical to those described above.

ATBF was measured once, on the first experimental day, in each subject using the gold standard method i.e. $^{133}$Xe wash-out technique, a routinely-used technique in our hands (16). Briefly, $^{133}$Xe (Bristol-Myers Squibb Canada Co., Dorval, QC) was injected in the subcutaneous adipose tissue of the abdomen, at the opposite side of the insulin injection site. ATBF was measured quantitatively using a Mediscint System (John Caunt Scientific Ltd., Oxford, England).

Sample Analysis. Blood samples were collected in tubes containing sodium-citrate and a protease inhibitor cocktail (Complete, EDTA-free, Roche Diagnostics, Mannheim, Germany). Blood was promptly centrifuged at
4°C and the resultant plasma aliquots were frozen immediately in liquid nitrogen and stored at -80°C until assaying. Plasma lispro was measured in duplicate with a specific radioimmunoassay kit (Linco Research Inc., St-Charles, MO).

Calculations and Statistical Analyses. Plasma lispro measurements were used to estimate absorption rate constant (ka), maximum plasma concentration (C_{max}), time to maximal concentration (T_{max}), area under lispro plasma concentration curve (AUC_{0-\infty}), C_{max} to dose ratio (C_{max}/D), AUC_{0-\infty} to dose ratio (AUC_{0-\infty}/D), volume of distribution (V_z), clearance (Cl), half-life (t_{1/2}), and mean residence time (MRT). Calculations were performed assuming a non-compartmental distribution using the WinNonlin 5.2 software (Pharsight Co., Mountain View, CA, USA).

Using glucose infusion rate (GIR) vs. time data, the maximum glucose infusion rate (GIR_{max}), time to maximum glucose infusion rate (t(GIR_{max})), and total glucose infusion from injection to end of clamp (GI_{tot}) were calculated. The study comprised one experiment in healthy subjects and three in obese subjects with type 2 diabetes; in the latter subjects, 10 U experiments were used as control for comparison with larger dosages. Results not normally distributed, based on the Normal Quintile Plot, were log-transformed for all statistical analyses and reported back-transformed in their original units. Values of p<0.05 were considered significant.

Fisher’s exact tests, for categorical variables, and unpaired t-tests, for continuous variables, were used to compare characteristics between groups. Unpaired t-tests were used for comparison between groups of pharmacokinetic and pharmacodynamic variables with 10 U injections. Repeated measures ANOVA tests were used to compare differences in pharmacokinetic and pharmacodynamic variables at different dosages in subjects with type 2 diabetes, with Tukey HSD tests for post-hoc multiple comparisons.

RESULTS

Six healthy subjects and seven obese subjects with type 2 diabetes (A_1c 8.1±1.2%, duration of diabetes 20.2±8.6 years, insulin therapy 5.1±4.2 years) were enrolled. Subjects with type 2 diabetes participated in all three experiments (10, 30 and 50 U). Their age, BMI, weight and adiposity indices were higher although ATBF was blunted (Table 1). Heart rate, BP and ATBF remained stable in both groups during experiments (data not shown).

Following the 10 U injection, the ratio C_{max}/D was 41% lower (p<0.001) in subjects with type 2 diabetes than in healthy subjects, but C_{max}, T_{max}, AUC_{0-\infty}, AUC_{0-\infty}/D, ka and Cl were similar in both groups. MRT, V_z and t_{1/2} tended to be greater in subjects with type 2 diabetes than in controls (Figure 1, Table 2). Following the 30 and 50 U injections, ka dropped by 60% (p=0.035) and T_{max} was delayed by 33 (p=0.118) and 74 min (p<0.001), respectively. C_{max}/D, Cl, V_z and t_{1/2} were not affected by the dose, although MRT tended to be greater. T_{max} (r^2=0.51; p=0.0004), C_{max} (r^2=0.90; p<0.0001) and AUC_{0-\infty} (r^2=0.94; p<0.0001) were associated with dosage.

The glucodynamic differences between healthy and subjects with type 2 diabetes following 10 U of lispro were considerable (Figure 2, Table 2). GIR_{max} and GI_{tot} were respectively 7% (p<0.0001) and 4%
(p<0.0001) of the value measured in healthy subjects, and tGIRmax was prolonged by one hour (p<0.0001). Following the 30 and 50 U injections, GIRmax and GItot were different from the 10 U values (p<0.0001 for both). Following 50 U lispro injection, tGIRmax was longer than after the 10 and 30 U injections (p=0.002). GIRmax (r²=0.67, p<0.0001), GItot (r²=0.73, p<0.0001) and tGIRmax (r²=0.76, p<0.0001) were strongly correlated with dosage. Following 10 U injection, the average difference between T max and tGIRmax was 19 min in healthy subjects and 74 min (p<0.0007) in subjects with type 2 diabetes. The gap increased further when subjects received 30 and 50 U (86 and 115 min respectively).

When GIR was plotted as a function of lispro plasma concentrations, the sequential response-concentration relationship depicted a counter-clockwise hysteresis for both healthy and subjects with type 2 diabetes (Figure 3). In healthy subjects receiving 10 U of insulin, an initial GIR response, of 2.22 mg/kg/min, was seen with insulin concentrations nearing 40 pmol/l. Thereafter, large increases of insulin concentrations were required to increase GIR, although once the response was triggered, it was maintained while plasma concentrations decreased to 20% of the C max.

In obese subjects with type 2 diabetes, following a 10 U injection, much greater concentrations of insulin were required to produce even a minimal effect; e.g. 273 pmol/l of insulin elicited a GIR of 0.1 mg/kg/min. The response later increased abruptly to attain GIRmax when plasma concentrations of insulin were already dropping; once GIRmax was attained, the response decreased linearly with insulin plasma concentrations. The same pattern was observed for 30 and 50 U injections.

CONCLUSIONS
This study characterizes the pharmacokinetic and pharmacodynamic properities of short-acting insulin analogue lispro in obese subjects with type 2 diabetes. Following low dose injection (10 U), lispro absorption in subjects with type 2 diabetes was as comparable as in control subjects although the hypoglycaemic effect was blunted. However, both absorption and activity were severely delayed and blunted at higher dosages (30 and 50 U) in subjects with type 2 diabetes, featuring a dose-response effect. Kinetic and dynamic parameters estimated in control subjects confirmed those published elsewhere (2-4) and support the value of our findings.

It has been repeatedly proposed, from correlations with pharmacokinetic parameters, that subcutaneous fat thickness, obesity and low ATBF reduce insulin absorption (12,13). Conversely, the present study does not confirm these facts when small dosages are administered. Insulin Vz and Cl are dependent upon fat free mass (17). Conversely, adipose tissue is essentially water free. Therefore, higher fat free mass and total body water in our subjects with type 2 diabetes could explain the increment tendency in Vz which should account for the decrease in C max and C max/D when comparing with control subjects.

Within our obese subjects with type 2 diabetes presenting high daily insulin needs, we indeed expected to observe a blunted pharmacodynamic profile compared to control subjects. Moreover, we showed a dose-dependent delay of T max and GIRmax at high doses in obese type 2 diabetic subjects. Similar results were found in healthy subjects, at lower dose, with lispro (18) and with inhaled insulin in subjects with type 1 diabetes (19). These effects observed at lower dose were expected to be more pronounced with higher doses in insulin-resistant subjects. Interindividual variation in insulin requirements was evaluated in overweight subjects with type 2 diabetes (20). The 8-hour clamp period was not long enough to determine the entire absorption and action profile of 36 U of regular human insulin.
Authors attributed these results to the possible slow insulin absorption in obese subjects with type 2 diabetes and to decreasing insulin absorption with increasing doses. They also correlated the absorbed insulin amount to daily insulin requirements. Herein, at low dosage, we did not observe a slower absorption of lispro in obese subjects with type 2 diabetes, but indeed confirmed that higher doses have a reduced effect. Thus, in obese subjects with type 2 diabetes, as ours, high insulin needs may account in part for low absorption efficiency with high doses.

As shown in Figure 3, both groups exhibited a counter-clockwise hysteresis although the magnitude was severely blunted in subjects with type 2 diabetes following 10, 30 and 50 U injections. Meanwhile, GIR remained very low as compared with control subjects. These findings illustrate the insulin resistance expected in our obese subjects with type 2 diabetes.

Fast prandial rise in plasma and fast action of insulin are both key to adequate postprandial metabolic control. The importance of determining whether short-acting insulin analogues are efficient was recently brought into question (21-23). Several studies (reviewed in 22) have noted no or few benefits for these analogues relatively to human insulin in patients with type 2 diabetes, as opposed to type 1 diabetes. Recent large studies (24,25) provided no evidence supporting the use of preprandial insulins as compared to basal insulins. The prolonged time-action profile of short-acting insulin analogues shown in this study could provide an explanation to why preprandial insulins have not had the expected benefits. In daily life, the delay in pharmacodynamic responses following short-acting analogues injections may hamper postprandial metabolic control, especially when large dosages are used.

The limitation of this study relates to the impossibility to distinguish between group and dose effect as high dosages were not tested in the control group; testing high dosages in control subjects would indeed require intensive care management.

In summary, this study shows that absorption and hypoglycemic action of short-acting insulin analogues are critically delayed at incrementally larger dosages in obese subjects with type 2 diabetes.

**Author contributions:** M.G.A. contributed to study concept, discussion, researched data, and wrote the manuscript. P.duS. contributed to discussion, data analysis, and reviewed/edited the manuscript. J.P.B. contributed to discussion, data analysis, statistics and reviewed/edited the manuscript. E.M. researched ATBF data. P.B. contributed to discussion, data analysis, and wrote the manuscript. J.M. contributed to study concept, discussion, and reviewed/edited the manuscript. J.L.A. was the principal investigator and contributed to the study concept, discussion and wrote the manuscript.

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**REFERENCES**


**TABLE 1. Characteristics of study groups.**

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th>Subjects with type 2 diabetes</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (men/women)</td>
<td>6 (3/3)</td>
<td>7 (6/1)</td>
<td>0.266</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>23.7 ± 2.4</td>
<td>60.3 ± 7.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1 ± 1.4</td>
<td>38.3 ± 7.0</td>
<td>0.0002</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.0 ± 7.6</td>
<td>111.0 ± 14.3</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>22.4 ± 7.9</td>
<td>32.6 ± 5.1</td>
<td>0.017</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>15.4 ± 4.5</td>
<td>36.5 ± 9.6</td>
<td>0.0005</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>54.6 ± 10.2</td>
<td>74.5 ± 7.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Total body water (kg)</td>
<td>40.0 ± 7.5</td>
<td>54.5 ± 5.7</td>
<td>0.002</td>
</tr>
<tr>
<td>ATBF(ml/min/100g tissue)</td>
<td>4.2 ± 0.7</td>
<td>1.5 ± 0.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means and standard deviations. BMI, body mass index; ATBF, subcutaneous adipose tissue blood flow.
**TABLE 2.** Pharmacokinetic and pharmacodynamic parameters following subcutaneous injection of lispro (10 U in healthy subjects and 10, 30, and 50 U in obese subjects with type 2 diabetes).

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (10 U)</th>
<th>Subjects with type 2 diabetes (10 U)</th>
<th>Subjects with type 2 diabetes (30 U)</th>
<th>Subjects with type 2 diabetes (50 U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_a$ (min$^{-1}$)</td>
<td>0.0531 ± 0.0236</td>
<td>0.0455 ± 0.0242</td>
<td>0.0184 ± 0.0076 $^\S$</td>
<td>0.0179 ± 0.0091 $^\S$</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (min)</td>
<td>48.3 ± 4.1</td>
<td>55.7 ± 14.0</td>
<td>88.6 ± 21.9</td>
<td>130.0 ± 46.0 $^\S,^\I$</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (pmol/l)</td>
<td>523 ± 42</td>
<td>310 ± 28</td>
<td>808 ± 218</td>
<td>1313 ± 346 $^\S,^#$</td>
</tr>
<tr>
<td>$C_{\text{max}}/D$ (l$^{-1}$)</td>
<td>0.0091 ± 0.0007</td>
<td>0.0054 ± 0.0005 $^\D$</td>
<td>0.0047 ± 0.0012</td>
<td>0.0046 ± 0.0012</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (pmol·min/l)</td>
<td>68462 ± 17346</td>
<td>60683 ± 15191</td>
<td>192155 ± 46873 $^\I$</td>
<td>372571 ± 59578 $^\I,^#$</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}/D$ (min/l)</td>
<td>1.190 ± 0.302</td>
<td>1.056 ± 0.264</td>
<td>1.140 ± 0.188</td>
<td>1.296 ± 0.208</td>
</tr>
<tr>
<td>$V_z$ (l)</td>
<td>67 ± 16</td>
<td>118 ± 34</td>
<td>104 ± 53</td>
<td>107 ± 46</td>
</tr>
<tr>
<td>$C_l$ (l/min)</td>
<td>0.88 ± 0.21</td>
<td>0.99 ± 0.22</td>
<td>0.90 ± 0.14</td>
<td>0.79 ± 0.13</td>
</tr>
<tr>
<td>$t^{1/2}$ (min)</td>
<td>67 ± 15</td>
<td>100 ± 34</td>
<td>97 ± 38</td>
<td>136 ± 72</td>
</tr>
<tr>
<td>$\text{MRT}$ (min)</td>
<td>119 ± 21</td>
<td>180 ± 65</td>
<td>196 ± 30</td>
<td>236 ± 49</td>
</tr>
<tr>
<td>$t_{\text{GIR}_{\text{max}}}$ (min)</td>
<td>69 ± 12</td>
<td>130 ± 23 $^\D$</td>
<td>175 ± 21</td>
<td>245 ± 64 $^\S,^#$</td>
</tr>
<tr>
<td>$\text{GIR}_{\text{max}}$ (mg/kg/min)</td>
<td>9.0 [7.1, 11.4]</td>
<td>0.6 [0.4, 0.9] $^\D$</td>
<td>2.0 [1.4, 2.7] $^\I$</td>
<td>2.5 [1.7, 3.7] $^\I$</td>
</tr>
<tr>
<td>$\text{GI}_{\text{tot}}$ (mg/kg)</td>
<td>2299 [1881, 2811]</td>
<td>92 [49, 174] $^\D$</td>
<td>364 [249, 533] $^\I$</td>
<td>678 [462, 994] $^\I$</td>
</tr>
</tbody>
</table>

Data are means and standard deviations, unless mentioned otherwise; $K_a$, lispro absorption rate constant; $T_{\text{max}}$, time to reach maximum plasma concentration; $C_{\text{max}}$, maximum plasma concentration; D, dose; $\text{AUC}_{0-\infty}$, area under the plasma lispro curve; $V_z$, distribution volume; Cl, clearance; $t^{1/2}$, half-life; MRT, mean residence time; $t_{\text{GIR}_{\text{max}}}$, time to maximum glucose infusion rate; $\text{GIR}_{\text{max}}$, maximum glucose infusion rate; $\text{GI}_{\text{tot}}$, total amount of infused glucose.

*geometric means with 95% confidence intervals; $^\D$ p<0.001 compared with healthy controls using unpaired $t$-tests; $^\S$ p<0.001 compared with healthy controls using unpaired $t$-tests; $^\I$ p<0.04 compared with 10 U in subjects with type 2 diabetes using repeated-measures ANOVA; $^\#$ p<0.05 compared with 30 U in subjects with type 2 diabetes using repeated-measures ANOVA; $^\|$ p<0.002 compared with 30 U in subjects with type 2 diabetes using repeated-measures ANOVA; $^\S,^\I$ p<0.002 compared with 30 U in subjects with type 2 diabetes using repeated-measures ANOVA.

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**FIG. 1.** Mean (±SD) plasma lispro concentration over 480-min euglycaemic clamps following subcutaneous injection of 10 U in healthy subjects (black circles ●) and 10 U (open circles ○), 30 U (open squares □), and 50 U (open triangles △) in obese subjects with type 2 diabetes.

**FIG. 2.** Glucose infusion rate over 480-min euglycaemic clamps following subcutaneous injection of 10 U in healthy subjects (white bars) and 10 U (black bars), 30 U (oblique tight hatched bars), and 50 U (oblique hatched bars) in obese subjects with type 2 diabetes.

**FIG. 3.** Plot of mean glucose infusion rate as a function of insulin plasma concentrations in healthy subjects receiving subcutaneously 10 U of lispro (black circles ●) and in obese subjects with type 2 diabetes receiving 10 U (open circles ○), 30 U (open squares □), and 50 U (open triangles △) of lispro. Data points are connected in chronological order; as depicted by the arrows, the resulting relationship denotes a counter-clockwise hysteresis.
Figure 1

![Figure 1](image1)

Figure 2

![Figure 2](image2)
Figure 3