SUMMARY - To compare the postprandial glucodynamics of Humalog® Mix25™, (Humalog® Mix75/25 in the US, Mix25), to human insulin 30/70 (Humulin® 70/30 in the US, 30/70) in patients with type 1 or type 2 diabetes. Ninety-three patients with type 1 diabetes and 84 patients with type 2 diabetes were evaluated in two separate but identical protocols using a randomized, multicenter, double-blind, crossover design. Patients consumed test meals 5 minutes after equal doses of Mix25 or 30/70. Plasma glucose was measured at baseline and 15 minute intervals for 4 hours after the meal. Two-hour postprandial glucose (2pp), 2-hour glucose excursion (2ppex), glucose versus time area under the curve 0 to 4 hours (AUC0-4) and glucose excursion area under the curve 0 to 2 and 0 to 4 hours (AUCex0-2, AUCex0-4) were calculated. For the combined patient population, Mix25 resulted in significantly lower lower 2pp (12.45 ± 3.59 vs. 13.47 ± 3.62 mmol/L; p < 0.001), AUC0-4 (44.45 ± 12.20 vs. 47.25 ± 11.97 mmolh/L; p < 0.001), and glucose excursion parameters: 2ppex (8.37 ± 2.72 vs. 9.40 ± 2.81 mmol/L; p < 0.001), and AUCex0-4 (5.17 ± 3.15 vs. 6.60 ± 3.13 mmolh/L; p < 0.001), compared to 30/70. Further analysis of the treatment by type of diabetes indicated that Mix25 provided nearly identical glucose excursion responses in type 1 and type 2 diabetes up to 2 hours after the test meal, in contrast to 30/70. Pre-meal injection of Mix25 resulted in lower postprandial blood glucose levels compared to 30/70. The postprandial blood glucose response following Mix25 was similar in patients with either type 1 or type 2 diabetes.

Key-words: pre-meal Humalog Mix25, type 1 and type 2 diabetes mel-litus.

HUMALOG® MIX25™ OFFERS BETTER MEALTIME GLYCEMIC CONTROL IN PATIENTS WITH TYPE 1 OR TYPE 2 DIABETES


RÉSUMÉ - Humalog® Mix25™ permet un meilleur contrôle glycémique prandial chez les patients atteints de diabète de type 1 et de type 2. Comparer les cinétiques postprandiales de l’Humalog® Mix25™ et de l’Umuline Profil 30 chez des patients diabétiques de type 1 et de type 2. Quatre-vingt-treize patients diabétiques de type 1 et 84 patients diabétiques de type 2 ont été évalués dans deux protocoles distincts mais identiques dans le cadre d’une étude randomisée multicentrique en double insu avec crossover. Un repas test était ingéré 5 minutes après l’injection de doses égales de Mix25 ou de 30/70. La glycémie était mesurée à l’état basal et à des intervalles de 15 minutes dans les 4 heures suivant le repas. La glycémie post-prandiale de 2h (2pp), l’excursion glycémique des 2h (2ppex), l’aire sous la courbe glycémique de 0 à 4 h (AUC0-4) et l’aire sous la courbe d’excursion glycémique de 0 à 22h et de 0 à 4h (AUCex0-2, AUCex0-4) ont été déterminées. Pour l’ensemble de la population, la Mix25 a permis l’obtention de valeurs significativement plus basses pour lower 2pp (12,45 ± 3,59 vs. 13,47 ± 3,62 mmol/L; p < 0.001), AUC0-4 (44,45 ± 12,20 vs. 47,25 ± 11,97 mmolh/L; p < 0.001), et pour les paramètres d’excursion glycémique: 2ppex (3,20 ± 2,72 vs. 4,00 ± 2,81 mmol/L; p < 0,001), AUCex0-4 (5,45 ± 3,15 vs. 6,60 ± 3,13 mmolh/L; p < 0,001), and AUCex0-4 (7,57 ± 8,37 vs. 11,02 ± 6,47 mmolh/L; p < 0,001) par rapport à la 30/70. Une analyse complémentaire en fonction du type de diabète a montré que la Mix25 permet des réponses glycémiques pratiquement identiques dans le diabète de type 1 et de type 2 jusqu’à 2 heures suivant le repas-test, contrairement à la 30/70. L’injection pré-prandiale de Mix25 permet l’obtention de glycémies post-prandiales plus basses par rapport à la 30/70. La réponse glycémique postprandiale avec la Mix25 est similaire chez les patients qu’ils soient diabétiques de type 1 ou de type 2.

Mots-clés : humalog Mix25 pré-prandiale, diabète de type 1 et de type 2.
INTRODUCTION

Insulin lispro (Humalog®, Eli Lilly and Company) is a rapid-acting insulin analog with a lower tendency for self-association than regular human insulin, resulting in a more rapid absorption rate and a shorter duration of activity than regular human insulin. [1] These characteristics produce a more physiological mealtime insulin profile than regular human insulin [2] and result in better postprandial glucose control with a lower risk of hypoglycemia [3, 4].

Pre-mixed insulin preparations containing insulin lispro recently became commercially available. The intermediate-acting insulin within these mixtures consists of a crystalline suspension of lispro-protamine crystals referred to as NPL [5, 6]. Clinical data have shown that the time-activity profiles of NPL are consistent with an intermediate-acting insulin and that NPL can be safely substituted for NPH without major differences in glycemic control [6]. Insulin lispro mixtures, which combine the convenience and improved dosing accuracy of manufactured mixtures while maintaining the desired activity profile of insulin lispro, have been approved for use in the European Union, Canada, the U.S., and several other countries. Insulin lispro Mix25 (Mix25; Humalog® Mix25™ known as Humalog®, Mix75/25™ in the US) is the first available insulin formulation in which both the rapid-acting and the basal components are insulin analogs. Insulin lispro mixtures, such as Mix25, are often used in a twice daily regimen, administered prior to morning and evening meals. The rapid-acting component of Mix25 (insulin lispro) should prove particularly beneficial in preventing postprandial hyperglycemia when compared with human insulin mixtures [7].

There has been increasing evidence in the recent literature that postprandial glucose elevations, independent of HbA1c, can cause metabolic abnormalities that may lead to an increased risk in diabetes complications. Thus, the availability of new insulin products that specifically target postprandial control would appear to benefit patients with either type 1 or type 2 diabetes.

Two studies reported here were performed to compare the postprandial glucodynamic response following Humalog Mix25 or human insulin 30/70. These studies were identical in design and were conducted simultaneously in the same study sites, differing only in patient populations: type 1 or type 2 diabetes. The results of the type 2 study have been previously reported [8]. Here we present the results of the pooled type 1 and type 2 studies.

MATERIALS AND METHODS

Patient Selection

Ninety-three patients with type 1 diabetes and 84 patients with type 2 diabetes 24 to 74 years of age were enrolled in these studies. Patients were in stable glycemic control, as determined by a hemoglobin A1c ≤1.5 times the upper limit of normal by the local laboratory. For at least 30 days prior to entering the studies, patients with type 2 diabetes were using a manufactured or self-prepared human insulin mixture (short-acting and intermediate-acting insulin) in the morning, a short-acting insulin at dinner, and a second NPH insulin dose given either at dinner or at bedtime. Patients with type 1 diabetes were using a similar insulin regimen or intensive insulin therapy (multiple daily injections or subcutaneous insulin infusion device). The total daily insulin dose was ≤2.0 U/kg.

Both studies were approved by the institutional review boards of the participating centers. Patients were required to complete and sign an informed consent document to participate in accordance with the Declaration of Helsinki and good clinical practice guidelines.

Study Design

The studies were conducted simultaneously at 7 sites in Canada using identical study designs, but in two patient populations (type 1 and type 2 diabetes). Both studies were randomized, double-blind, crossover studies comparing the postprandial glucodynamic response to either Mix25 or 30/70 after receiving a standardized morning test meal. Two test meals were administered for each insulin therapy.

The patients also completed two morning practice test meals at home using 30/70 to determine the appropriate study insulin dose for the actual test-meal visits, and recorded blood glucose (BG) values following the meal until dinnertime. The test meal consisted of approximately 500 kcal (50% carbohydrate, 20% protein, and 30% fat).

Each patient was randomly assigned to one of two sequences, Mix25 followed by 30/70 or the opposite sequence, with the test meals separated by 3 to 11 days. The study insulin (Mix25 or 30/70) was injected in the abdominal region 5 minutes prior to serving the test meal. Individual patients received the same insulin dose for each test meal. Patients continued to use their prestudy insulin between visits.

On the morning of each test meal visit, the patients checked their BG using their own glucose meter. If their fasting BG was >5 or <11 mmol/L, the patients
remained fasting, omitted their usual morning insulin dose, and traveled to the investigative site. At the study site, an intravenous catheter was inserted for blood sampling. The study insulin was injected 5 minutes before the test meal (time 0), that was consumed within 10 minutes. Blood samples were collected at baseline and every 15 minutes up to 4 hours after dosing. The samples were collected in tubes containing sodium fluoride and shipped to a central processing laboratory for determination of plasma glucose concentrations (Covance Laboratories, Indianapolis, IN). Glucose concentrations were measured using a Hitachi 747-200 Chemistry Analyzer (Hitachi Products, Hitachi, Ltd., Tokyo, Japan) with Roche Diagnostics (Indianapolis, Indiana, USA) reagents.

Patients were asked to keep their usual physical activity, meal composition, and evening insulin dose consistent on evenings prior to test meal visits. Bedtime insulin was given before 10:00 PM and subcutaneous insulin infusion pumps were turned off no later than 2 hours before the patient’s appointment at the study site.

Patients who experienced hypoglycemia during a test meal (BG concentrations < 3.5 mmol/L or hypoglycemic symptoms) had a final blood sample collected and were treated for hypoglycemia with appropriate measures. These patients were allowed to remain in the study and were scheduled for the remaining test meals.

Glucodynamic Evaluations

Several parameters were calculated from the plasma glucose measurements, including the 2-hour postprandial glucose concentration (2pp), and the area under the glucose concentration versus time curve from 0-4 hours (AUC0-4). Additional parameters based upon glucose excursions from baseline were also calculated. Excursions from baseline were defined as the baseline (time = 0) plasma glucose concentration subtracted from each of the measured plasma glucose concentrations. From the excursion data, the 2-hour (2ppex), and the area under the plasma glucose excursion versus time curve from 0-2 and 0-4 hours (AUCex0-2, AUCex0-4) were determined. For patients who experienced hypoglycemia, all subsequent BG values were assumed to be 3.5 mmol/L during the remaining test meal period.

Test Materials

Humalog® Mix25™ and Humulin® 30/70 were provided by Eli Lilly and Company (Indianapolis, Indiana, USA) in blinded 10 mL vials containing 100 U/mL.

Statistical Methods

Mean on therapy method was used to analyze glucodynamic parameters. A crossover model suggested by Grizzle [9] was used to test the main effects of treatment, diabetes type, sequence, investigator, and treatment period effects for the combined data. First order interactions of treatment by type, investigator by sequence, investigator by treatment, and investigator by period were included in the model. A general linear model (GLM) approach was used to implement the crossover analyses for all glucodynamic parameters. PROC GLM of SAS® (Cary, North Carolina, USA) was used to perform the statistical analysis on all data. The effects, including interaction terms (with the exception of sequence effect), were tested at the 0.05 level of significance. A similar analysis was conducted for type 1 and type 2 patients separately. The incidence (percentage of patients) of hypoglycemic episodes during the studies was analyzed using a method suggested by Nagelkerke et al. [10]

RESULTS

Patients Characteristics

Table I summarizes the patient’s baseline characteristics by diabetes type. Eighty six of 93 patients with type 1 diabetes and 79 of 84 patients with type 2 diabetes completed the study.

<table>
<thead>
<tr>
<th>Type 1</th>
<th>Type 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>41F/52M</td>
<td>31F/53M</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.07 ± 12.24</td>
<td>81.10 ± 13.28</td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.80 ± 9.45</td>
<td>59.87 ± 8.14</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.18 ± 3.06</td>
<td>29.16 ± 3.76</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>15.74 ± 10.46</td>
<td>15.14 ± 7.87</td>
</tr>
<tr>
<td>Duration of insulin therapy (years)</td>
<td>15.36 ± 10.48</td>
<td>5.88 ± 5.26</td>
</tr>
</tbody>
</table>

Data are presented as Mean ± SD.
tion parameters were significantly lower for Mix25 compared with 30/70 (Table II), with the glucose excursion curves showing a clear separation between the two treatments for the combined patient population (Fig. 1).

For all patients, a mean ± SD insulin dose of 29.9 U ± 16.1 (0.38 U/kg ± 0.19) was given for Mix25 treatment, while a dose of 30.1 U ± 16.2 (0.39 U/kg ± 0.19) was given for 30/70 treatment. The mean insulin dose for Mix25 was 24.97 U ± 13.65 (0.34 U/kg ± 0.17) for type 1 patients and 35.44 U ± 16.9 (0.43 U/kg ± 0.19) for type 2 patients. The mean insulin dose for 30/70 was 25.15 U ± 13.85 (0.35 U/kg ± 0.17) for type 1 patients and 35.58 U ± 16.9 (0.44 U/kg ± 0.19) for type 2 patients.

The glucose excursion curves by diabetes type and treatment are shown in Figure 2. Mean glucose excursions for Mix25 were lower than those noted for 30/70 at all time-points beyond 1 hour after the meal for both type 1 and type 2 patients. The postprandial plasma glucose excursion response to Mix25 was nearly identical between type 1 and type 2 patients, in contrast to the differing responses after 30/70 (Fig. 2). Type 1 patients treated with 30/70 had a higher postprandial glycemic response than patients with type 2 diabetes.

An analysis of the combined data showed significant differences for 2pp, AUC_{0-4}, and AUC_{ex0-2} (P < 0.001). Significant type and treatment interactions were observed for 2pp (p = 0.011), AUC_{0-4} (p = 0.020), 2pp_{ex} (p = 0.035), and AUC_{ex0-2} (p = 0.038) (Table II). This indicates that there are differences in these parameters between type 1 and type 2 diabetes for the two study insulins. Because AUC_{ex0-2} represents a more robust measure of the 2-hour postprandial glycemic response compared to an isolated 2 hour blood glucose measurement, diabetes type and treatment interaction was further analyzed by comparing the adjusted means of AUC_{ex0-2} for each insulin by diabetes type. The AUC_{ex0-2} for 30/70 was

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**Table II. Glucodynamic Values for the Combined Treatment Group (Mean ± SD).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mix25 n = 173</th>
<th>30/70 n = 168</th>
<th>p-value Treatment</th>
<th>p-value Type</th>
<th>p-value Treatment and Type Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>9.22 ± 2.61</td>
<td>9.07 ± 2.25</td>
<td>0.357</td>
<td>&lt; 0.001</td>
<td>0.237</td>
</tr>
<tr>
<td>2pp, mmol/L</td>
<td>12.45 ± 3.59</td>
<td>13.47 ± 3.62</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.011</td>
</tr>
<tr>
<td>AUC_{0-4}, mmol·h/L</td>
<td>44.45 ± 12.20</td>
<td>47.25 ± 11.97</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.020</td>
</tr>
<tr>
<td>Excursion parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2pp_{ex}, mmol/L</td>
<td>3.20 ± 2.72</td>
<td>4.40 ± 2.81</td>
<td>&lt; 0.001</td>
<td>0.790</td>
<td>0.035</td>
</tr>
<tr>
<td>AUC_{ex0-2}, mmol·h/L</td>
<td>5.45 ± 3.15</td>
<td>6.60 ± 3.13</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.038</td>
</tr>
<tr>
<td>AUC_{ex0-4}, mmol·h/L</td>
<td>7.57 ± 8.37</td>
<td>11.02 ± 8.47</td>
<td>&lt; 0.001</td>
<td>0.854</td>
<td>0.129</td>
</tr>
</tbody>
</table>

All data are presented as Mean ± SD; pp = postprandial; 2pp_{ex} = 2-hour postprandial excursion; AUC_{ex0-2} = area under the curve plasma glucose excursion 0-2 hours; AUC_{ex0-4} = area under the curve plasma glucose excursion 0-4 hours.
significantly different between patients with type 1 and type 2 diabetes (7.05 and 5.98 mmol•h/L; \(p < 0.001\)), but similar for type 1 and type 2 diabetes for Mix25 (5.43 and 5.26 mmol•h/L; \(p = 0.569\)). Additionally, a separate analysis for type 1 and type 2 diabetes showed Mix25 resulted in significantly lower glucose excursion parameters compared with 30/70 (Tables III and IV).

### Hypoglycemia

Forty patients (23%) experienced hypoglycemic episodes (BG < 3.5 mmol/L or symptoms of hypoglycemia) during the Mix25 test meal days (time of test meal until the evening meal), while 50 patients (29%) experienced hypoglycemic episodes during the 30/70 test meal days (\(p = 0.203\)). Sixty two patients reported a total of 73 hypoglycemic episodes during the 4 hours of monitoring following the test meal period (Mix25: 28 patients, 33 episodes; 30/70: 34 patients, 40 episodes). Between lunch and dinner, 38 patients reported a total of 52 hypoglycemic episodes after the test meal periods, (Mix25: 16 patients, 21 episodes; 30/70: 22 patients, 31 episodes). Most of these episodes occurred in the patients with type 1 diabetes. No patient required assistance, intravenous glucose, or glucagon injection for treatment of hypoglycemia. The distribution of hypoglycemic episodes was similar for the two treatment groups (Fig. 3).

### DISCUSSION

In the present study, the postprandial glycemic response of two types of pre-mixed insulins were compared in patients with type 1 or type 2 diabetes. The two mixtures differed in the active insulin component, with Mix25 containing insulin lispro and 30/70 containing human insulin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mix25</th>
<th>30/70</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>9.48 ± 2.91</td>
<td>9.46 ± 2.48</td>
<td>0.943</td>
</tr>
<tr>
<td>Excursion parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2pp&lt;sub&gt;x&lt;/sub&gt;, mmol/L</td>
<td>3.07 ± 3.07</td>
<td>4.64 ± 3.23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;exc&lt;/sub&gt;&lt;sub&gt;0-2&lt;/sub&gt;, mmol•h/L</td>
<td>5.17 ± 2.66</td>
<td>5.90 ± 2.61</td>
<td>0.009</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;exc&lt;/sub&gt;&lt;sub&gt;0-4&lt;/sub&gt;, mmol•h/L</td>
<td>8.01 ± 7.02</td>
<td>10.6 ± 6.47</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**Table III.** Glucodynamic Values by Treatment for Type 1 Diabetes (Mean ± SD).

**Table IV.** Glucodynamic Values by Treatment for Type 2 Diabetes (Mean ± SD).
Treatment with Humalog® Mix25™ prior to a standard test meal resulted in significantly lower postprandial blood glucose values compared to equal doses of human insulin 30/70 in patients with type 1 or type 2 diabetes. In addition, Mix25 provided a more consistent postprandial glucose response between type 1 and type 2 diabetes in contrast to the differing postprandial response observed with human insulin 30/70.

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