Original Research

Pharmacokinetics and pharmacodynamics of insulin glargine–insulin glulisine basal-bolus and twice-daily premixed analog insulin in type 1 diabetes mellitus patients during three standardized meals

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A R T I C L E   I N F O

A B S T R A C T

Aims: To evaluate the pharmacokinetics and pharmacodynamics of basal insulin glargine with meal-time insulin glulisine or twice daily 75/25 premixed neutral protamine insulin lispro and insulin lispro in individuals with type 1 diabetes during three standardized meals over a 24 hour duration and compare to physiologic insulin and glucose responses in healthy non-diabetic individuals.
Methods: Twelve healthy (4 male/8 female) and thirteen individuals with type 1 diabetes (8 male/5 female) were studied during three sequential standardized meals. Individuals with type 1 diabetes received either glargine and glulisine injected 5 minutes subcutaneously before each meal or premixed insulin lispro injected 5 minutes before breakfast and dinner in a randomized fashion separated by eight weeks.
Results: The incremental systemic insulin AUC, maximal insulin concentration, and rate of rise of systemic insulin (0–30 minutes) during all three meal intervals were similar between glargine/glulisine and healthy controls. Incremental glucose AUC with glargine/glulisine was similar to controls at lunch and dinner. With premix 75/25 insulin, insulin AUC was lower and incremental glucose AUC was greater at lunch compared to the healthy and glargine/glulisine. Hypoglycemic events before lunch were greater with premix insulin group than with glargine/glulisine (p < 0.0001).
Conclusions: Glargine/glulisine pharmacokinetics in type 1 diabetes can closely approximate physiologic insulin responses in healthy individuals during a day in which three standardized meals are consumed. Additionally, when glulisine is dosed only five minutes pre-meal, systemic insulin concentration rises as rapidly as prandial endogenous insulin levels. This present study compared glargine and glulisine administered in an approximate 50/50 proportion. Future studies of alternate meal times, meal content and differing premixed insulin preparations are indicated.

Introduction

In healthy individuals, low levels of circulating insulin are maintained during interprandial periods, while during a meal, insulin levels rise rapidly and then taper to fasting concentrations as glucose levels return to baseline [1]. In order to improve glycemic control and reduce microvascular complications in patients with diabetes, substantial effort has been directed toward the development of insulin analog formulations, delivery methods and dosing schedules that more closely match physiologic insulin profiles [2].

One common insulin replacement regimen involves multiple daily injections (basal-bolus therapy), consisting of a longer-acting insulin (insulin glargine, degludec, detemir, or NPH insulin injected once or twice daily) and rapid-acting insulin (glulisine, aspart or lispro) injected prior to a meal to provide prandial insulin coverage. Another option for insulin replacement is premixed analog insulin, typically injected twice daily, which contains either neutral protamine lispro and insulin lispro (as a 75:25 ratio) or protamine crystalline aspart and insulin aspart (as a 70:30 ratio). The protamine portion of premix insulin is similar to NPH insulin and functions as intermediate-acting insulin to cover basal insulin requirements. The rapid-acting component is aimed to cover breakfast and dinner prandial insulin requirements [3].

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While pharmacokinetic (PK) and pharamacodynamic (PD) studies of long-acting and rapid-acting insulin analogs have been published [4,5], previous studies often involved injection of these insulins under fasting conditions or involved injection prior to only one standardized meal challenge. Comparison groups usually consisted of patients receiving regular human insulin and/or NPH insulin as part of their basal-bolus therapy. To further examine the PK and PD of insulin analogs used in contemporary basal-bolus regimens in the treatment of type 1 diabetes mellitus, the aim of this study was to determine an entire day-long profile of insulin and glucose levels over the course of three standardized meal challenges. Insulin glargine (glargine) was injected prior to breakfast, and insulin glulisine (glulisine) was used as the pre-meal bolus insulin. A comparison group of non-diabetic healthy individuals served as a control group to demonstrate normal physiologic insulin and glucose responses during the three meals. A second comparison group consisted of patients with type 1 diabetes who consumed all three standardized meals and received pre-mixed injections of neutral protamine lispro and insulin lispro prior to breakfast and dinner.

Subjects

All healthy subjects had normal liver, kidney, electrolyte and blood count values and had a normal response to an oral glucose tolerance test. Individuals with type 1 diabetes also had normal liver, kidney, electrolyte and blood count values. Apart from one subject with mild background retinopathy, all type 1 diabetes patients were free from tissue complications of diabetes. Twelve healthy subjects [4 males and 8 females, age 27 ± 6 years, BMI 24 ± 2 kg/m^2, hemoglobin A1c (HbA1c) $5.2 \pm 0.3\%$] and thirteen subjects with type 1 diabetes (8 male and 5 female, age 30 ± 11 years, BMI 24 ± 3 kg/m^2, and HbA1c $7.3 \pm 1.1\%$, diabetes duration 9.9 ± 10.2 years) were studied in a single blind randomized fashion (Table 1). Background insulin treatment in the individuals with type 1 diabetes consisted of basal-bolus (glargine/aspart or glargine/lispro) or insulin pump (aspart or lispro) therapy. With the exception of one subject who completed only the lispro premix portion of the study, all participants with type 1 diabetes completed both treatment arms. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board, and all subjects gave written and verbal informed consent.

Materials and methods

Individuals with type 1 diabetes were randomly assigned in a 1:1 ratio to one of two treatment sequences (A or B), with a minimum 8-week washout period between treatment visits. In sequence A, subjects received a basal-bolus insulin regimen of insulin glargine and insulin glulisine (glargine/glulisine) during the first treatment period and received premixed neutral protamine lispro and insulin lispro 75/25 (lispro premix) during the second treat-

Table 1
Baseline demographics and clinical characteristics

Characteristic	Healthy (N = 12)	Type 1 diabetes (N = 13)
Age (years)[a]	26.7 (6.0)	30.3 (10.6)
Sex (n)		
Male	4	8
Female	8	5
Height (cm)	167 (10.8)	176 (10.3)
Weight (kg)	67.9 (13.0)	74.5 (12.0)
BMI (kg/m^2)	24.3 (2.4)	24.1 (2.9)
HbA1c (%)	5.2 (0.3)	7.3 (1.1)

[a] Values are expressed as mean (standard deviation); HbA1c = glycated hemoglobin a1c; BMI = body mass index.

ment period. Those randomized to sequence B received lispro premix and then glargine/glulisine. Healthy subjects did not receive exogenous insulin and participated in only one study visit.

All participants were admitted to the Vanderbilt General Clinical Research Center the evening prior to the study period. Upon admission of patients with type 1 diabetes, a retrograde intravenous cannula (I.V.) was inserted under local anesthesia into the back of a hand for blood sample collection, and a second I.V. was inserted in an antegrade fashion in the forearm for infusions. Upon I.V. insertion, patients with type 1 diabetes suspended their usual insulin regimen (long-acting insulin was taken no later than the morning prior to admission), and intravenous regular human insulin was infused to maintain euglycemia (target blood glucose ~5–6.7 mmol/L). Non-diabetic controls received placement of a retrograde I.V., as above, in the morning preceding the study. Each subject received a standardized dinner meal and then fasted overnight for ≥8 hours.

Meal challenge

On the study day, all subjects received three meal challenges (08:00, 13:00 and 18:00), standardized for total caloric content and tailored to provide a daily total of 30 kcal/kg actual body weight, which is considered standard calorie content based on estimated energy expenditure of moderately active healthy individuals [6]. Meals were consumed within 20 minutes. The total calories per day were divided among meals: 1/6th at breakfast (17%), 2/6th at lunch (33%) and 3/6th at dinner (50%) by convention. Meal calories were approximately 50% carbohydrate, 30% lipid and 20% protein, in accordance with healthy macronutrient meal composition standards [6].

Insulin regimens

In the diabetes groups, overnight intravenous insulin infusion was continued until 07:45 on the morning of the study day. In the glargine/glulisine group, glargine was dosed at 0.35 units/kg administered subcutaneously at 07:00 (1 hour prior to breakfast). Glulisine was dosed at 1 unit/10 g carbohydrate per meal, administered subcutaneously 5 minutes prior to each meal (07:55, 12:55 and 17:55). In the lispro premix group, total lispro premix dose was determined by referencing the total daily dose as calculated for the glargine/glulisine treatment period, with two-thirds administered at 07:55 (5 minutes prior to breakfast) and one-third administered at 17:55 (5 minutes prior to dinner). Thus, an equivalent insulin dosage was administered to each type 1 diabetes patient during both protocols.

Sample collection

The hand with the retrograde I.V. was placed in a heated box (55–60 °C) during the study so that arterialized blood could be obtained for measurement of glucose and insulin [7]. Samples were collected hourly from 06:00 to 24:00 and every 15 minutes for 2 hours directly following each meal.

Bioanalytical methods

Blood glucose concentrations were measured using a point-of-care glucometer (Ascensia Elite XL, Bayer, Leverkusen, Germany) and were subsequently confirmed by measuring plasma glucose levels using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Total serum insulin concentrations were measured using radioimmunoassay, based on guinea pig polyvalent anti-rat insulin antibody and radioactive iodine-labeled human insulin, validated for appropriate cross-reactivity to human insulin and each

insulin analog studied. All insulin concentration values reported were derived from the human insulin standard curves, irrespective of whether the sample contained human insulin, insulin lispro, insulin glargine, insulin glulisine or some mixture of the aforementioned insulins. The calibration range was 2.15–276 μU/mL for insulin assays. The lower and upper limits of quantification were 4.3 and 138 μU/mL, respectively (Farmovs-Parexel, Bloemfontein, South Africa).

Statistical analysis

PK and PD variables were derived from systemic insulin and glucose concentration-time data, respectively, using standard non-compartmental techniques (i.e., trapezoidal summation for positive-peak area-under-the-curve [AUC]). Meal-specific analysis was conducted using mean pre-meal baseline-normalized serum insulin and serum glucose data from mealtime intervals. Each meal interval was defined as all samples obtained between the pre-meal baselines through 5 hours postprandial. Therefore, the breakfast, lunch and dinner intervals consisted of samples drawn from 8:00 to 13:00, 13:00 to 18:00, and 18:00 to 23:00, respectively. Comparison of baseline parameters between patients with diabetes and healthy subjects was based on unpaired, two-tailed *t* test ($\alpha = 0.05$), with 95% confidence intervals (CIs). Estimates of mean response by treatment regimen and treatment comparisons were based on one-way analysis of variance followed by Tukey's multiple comparison post-test.

Results

Insulin pharmacokinetics

The mean total systemic insulin dose for glargine/glulisine was 56 ± 9 units (47.6% glargine, 52.4% glulisine) and was equivalent to lispro premix (54 ± 9 units, 75% lispro protamine, 25% lispro).

The total $AUC_{[i]}$ over the duration of the study was not statistically significant between any groups (Table 2). The incremental systemic insulin area under-the-curve ($AUC_{[i]}$) during all three meal intervals was similar between glargine/glulisine and healthy controls (Table 2 and Fig. 1). Incremental $AUC_{[i]}$ for the breakfast interval was greater with lispro premix treatment compared to both healthy controls and glargine/glulisine treatment ($p < 0.05$). Lunchtime incremental $AUC_{[i]}$ with lispro premix treatment was reduced compared to healthy controls and glargine/glulisine treatment ($p < 0.001$). Incremental $AUC_{[i]}$ during the dinner interval in the lispro premix group was similar to healthy controls but reduced compared to the glargine/glulisine group ($p < 0.001$).

The mean time to maximal insulin concentration ($Tmax_{[i]}$) was similar between the healthy control group and the glargine/glulisine group for all three meals. $Tmax_{[i]}$ was longer in the lispro premix group compared to the other two groups at breakfast, lunch and dinner ($p < 0.05$–0.001).

The maximal change in insulin concentration from the start of each meal ($\Delta Cmax_{[i]}$) was similar in the healthy controls and glargine/glulisine. Lispro premix $\Delta Cmax_{[i]}$ was similar to healthy controls and the glargine/glulisine group at breakfast. Lunch time lispro premix $\Delta Cmax_{[i]}$ (1.4 ± 1.9 μU/ml) was reduced at lunch compared to healthy (63.4 ± 11.7 μU/ml; $p < 0.001$) and glargine/glulisine (52.2 ± 6.1 μU/ml; $p < 0.001$). Insulin $\Delta Cmax_{[i]}$ at dinner was also reduced in the premix group (29.6 ± 3.7 μU/ml) versus healthy (versus 76.1 ± 17.5 μU/ml; $p < 0.05$) and glargine/glulisine (78.4 ± 7.0 μU/ml; $p < 0.01$).

Rates of rise of meal time insulin from 0 to 30 minutes (0–30) appear similar between healthy controls and glargine/glulisine at each meal. Rate of insulin rise was significantly slower in lispro premix compared to healthy controls during breakfast and lunch ($p < 0.05$ and $p < 0.001$, respectively) and was significantly slower in lispro premix compared to glargine/glulisine at lunch and dinner ($p < 0.01$).

Glucose pharmacodynamics

At breakfast, incremental glucose area under-the-curve ($AUC_{[g]}$) was less in healthy controls versus glargine/glulisine ($p < 0.01$; Table 2, Fig. 2 and Supplemental Fig. S1). Incremental $AUC_{[g]}$ for the lunch interval was similar between healthy controls and glargine/glulisine groups but was significantly greater with lispro premix treatment as compared to healthy controls ($p < 0.001$) and glargine/glulisine ($p < 0.01$). Positive incremental $AUC_{[g]}$ for the dinner interval was similar among all groups.

The mean time to maximal glucose concentration ($Tmax_{[g]}$) at the breakfast interval was similar in all three groups. At lunch, $Tmax_{[g]}$ was similar between glargine/glulisine and the other two groups but delayed in the lispro premix group compared to healthy controls ($p < 0.01$). There were no differences in $Tmax_{[g]}$ among the groups at the dinner time interval.

At breakfast, the maximal change in glucose concentration ($\Delta Cmax_{[g]}$) was significantly greater in both insulin treatment groups compared to the healthy group ($p < 0.05$ and $p < 0.001$ glargine/glulisine and lispro premix, respectively). At lunch, the blood glucose excursion was significantly greater in lispro premix than in healthy controls or glargine/glulisine ($p < 0.01$). No differences were observed during the dinner meal.

Table 2
Incremental AUC values for insulin and glucose in healthy controls and in patients with type 1 diabetes

| | Insulin mean AUC (μU/ml*min) (SD) | | | *p*-Values | | |
	Healthy	Glargine/ Glulisine	Lispro Premix	Healthy vs Glargine/Glulisine	Healthy vs Lispro Premix	Glargine/Glulisine vs Lispro Premix
Breakfast	**4,189.8** (3,492.7)	**4,366.6** (1,656.2)	**7,656.1** (3,299.2)	ns	<0.05	<0.05
Lunch	**6,939** (4,613.7)	**8,437.6** (2,386.6)	**1,708.4** (1,104.7)	ns	<0.001	<0.001
Dinner	**8,301.7** (6,349.7)	**11,915.0** (2,344.7)	**4,822.6** (1,809.1)	ns	ns	<0.001
Total	**19,808** (4,247)	**27,381** (2,309)	**20,313** (2,054)	ns	ns	ns

| | Glucose mean AUC$_g$ (mmol/L*min) (SD) | | | *p*-Values | | |
	Healthy	Glargine/ Glulisine	Lispro Premix	Healthy vs Glargine/Glulisine	Healthy vs Lispro Premix	Glargine/Glulisine vs Lispro Premix
Breakfast	**225.6** (186.8)	**746.5** (439.4)	**532.6** (343.4)	<0.01	ns	ns
Lunch	**314.3** (150.0)	**577.9** (422.7)	**1,394.8** (745.2)	ns	<0.001	<0.01
Dinner	**364.3** (252.6)	**346.7** (338.9)	**436.3** (375.4)	ns	ns	ns

The values for insulin mean AUC and glucose mean AUC are listed in bold font, and the standard deviations are in parentheses. AUC = area under the curve; SD = standard deviation.
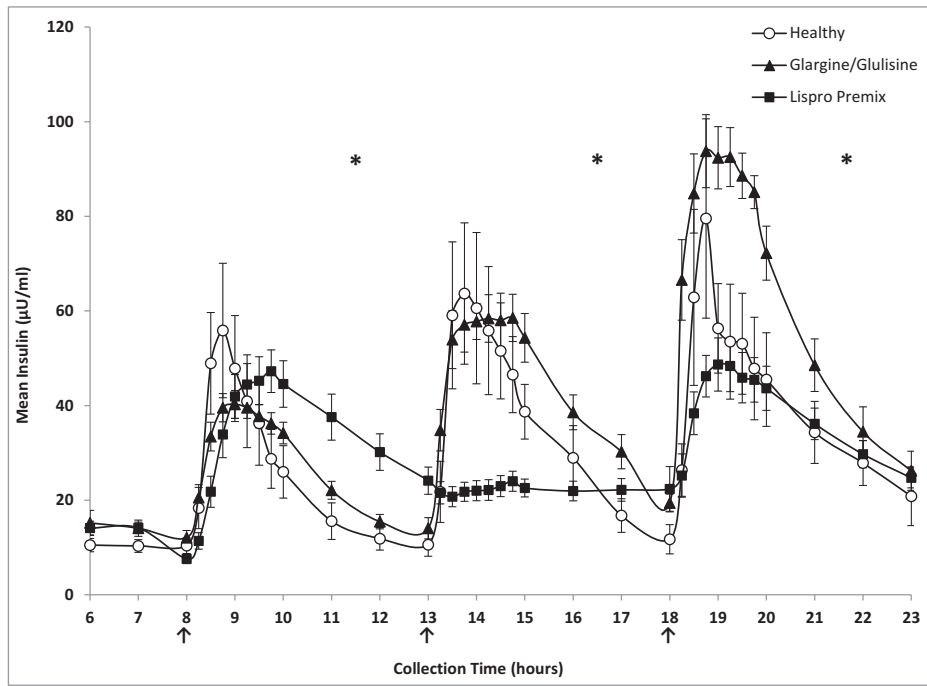
Figure 1. Mean insulin levels over time during the study period. *Insulin AUC was significantly different between healthy controls and lispro premix at breakfast ($p < 0.05$) and lunch ($p < 0.001$) and significantly different between glargine/glulisine and lispro premix at breakfast ($p < 0.05$), lunch and dinner ($p < 0.001$). Arrows below the x-axis indicate the times at which the standardized meals were consumed. Open circles = healthy controls; black triangles = glargine/glulisine group; black squares = lispro premix group.
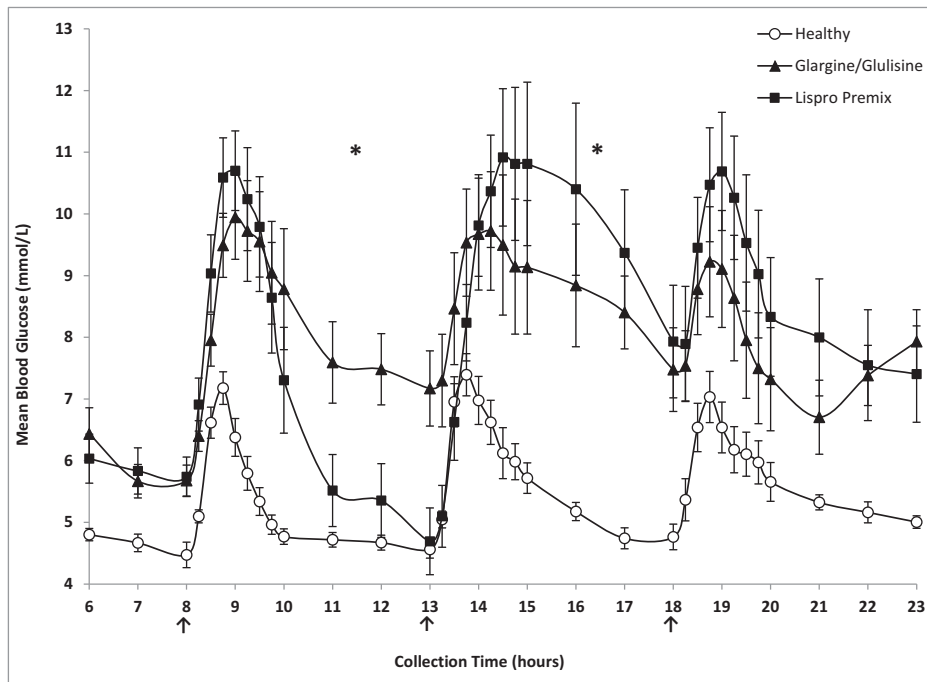
Figure 2. Mean blood glucose levels over time during the study period. *Glucose AUC was significantly different between healthy controls and glargine/glulisine at breakfast ($p < 0.01$). At lunch, glucose AUC was significantly different between healthy controls and lispro premix ($p < 0.001$) and between glargine/glulisine and lispro premix ($p < 0.01$). Arrows below the x-axis indicate the times at which the standardized meals were consumed. Open circles = healthy controls; black triangles = glargine/glulisine group; black squares = lispro premix group.

Safety/hypoglycemia

No healthy patients experienced a hypoglycemic event (serum glucose concentration <3.3 mmol/L). Ten individuals with type 1 diabetes experienced a total of 18 hypoglycemic events. During treatment with glargine/glulisine, 4 patients experienced 8 hypoglycemic events, 2 of whom experienced 3 events each. Two, five and one of the hypoglycemic events occurred after the breakfast, lunch and dinner meals, respectively. During treatment with lispro premix, 9 patients experienced 10 hypoglycemic events; all of these events occurred prior to the lunch interval. The number of hypoglycemic events that occurred in the lispro premix group at lunch was statistically greater than the number of events that occurred in the glargine/glulisine group during the same meal interval (p < 0.0001). There were no episodes of severe hypoglycemia in any group.

Discussion

The current study examined the effects of three standardized meals (breakfast, lunch and dinner) on glucose and systemic insulin levels over a 24 hour period in individuals with type 1 diabetes administered glargine/glulisine basal-bolus therapy or 75/25 lispro premixed insulin twice daily and age and weight matched healthy controls. This study demonstrates that, acutely, glargine/glulisine more closely mimics physiologic insulin responses to meals and that pre-lunch hypoglycemic episodes were less frequent compared to premixed insulin.

Reviewing the available literature, we identified four other published studies which described the use of three sequential meals over the course of a day while measuring insulin and glucose levels [1,8–10]. In one, responses to standardized test meals were studied in healthy and obese participants [1]. The other three compared responses in type 1 diabetes subjects following a treatment period with NPH insulin and mealtime insulin lispro, insulin aspart or regular human insulin [8–10]. One of the type 1 diabetes studies also included a non-diabetic comparator group [10]. The novelty of this study lies in the comparison of a widely used combination of a contemporary basal insulin (insulin glargine) and the rapid acting analog insulin glulisine versus a premixed insulin formulation.

A number of similarities were observed between the glargine/glulisine and healthy control groups. The incremental $AUC_{[i]}$ responses were similar to healthy individuals during all three meal intervals. Moreover, in the glargine/glulisine and healthy controls the peak systemic insulin level ($\Delta Cmax_{[i]}$) increased similarly between both groups at each meal. This indicates that the increased endogenous insulin response to the larger carbohydrate load in healthy subjects could be adequately-matched by the larger glulisine dose received by subjects with type 1 diabetes with each successively larger meal. Systemic insulin levels were measured and do not distinguish between insulin components and may not be reflective of the portal concentration of insulin in the healthy controls. In healthy individuals, insulin is secreted into the portal vein and is predominantly cleared by the liver and less so by the kidney. This creates a portal-peripheral gradient whereby portal vein insulin levels in healthy individuals are 3–4 fold higher than in the systemic circulation. However, in individuals with type 1 diabetes, the portal vein insulin concentration will be dependent on the release of insulin from the subcutaneous depot. Thus, in type 1 diabetes mellitus the portal-peripheral insulin gradient can be reversed from the physiologic norm with higher peripheral as compared to hepatic vein insulin levels. This of course will depend upon the rate and pattern of insulin release from the subcutaneous depot.

When glulisine was administered subcutaneously only 5 minutes before each meal, the rate of rise of insulin during the first 30 minutes of each meal appears similar to healthy controls. The rate of rise of insulin data is most germane to the glargine/glulisine group as glargine would not be expected to contribute in a significant manner to the rate of rise. The rate of rise data in the premix groups will be reflective of both the intermediate and rapid acting components. Studies have examined the effects of administering insulin glulisine either 0–15 minutes prior to a meal or 15 minutes following a meal [11,12]. Pre-meal dosing of glulisine appears to be most advantageous, with regard to reductions in post-meal glucose excursion and 12-week HbA1c levels. Dosing guidelines currently advise that insulin glulisine should be administered 15 minutes prior to or within 20 minutes of beginning a meal [13]. Surveys indicate that patients tend to inject insulin within 15 minutes prior to a meal and prefer shorter intervals between injection and mealtime as a matter of ease and convenience and as an effort to avoid hypoglycemia and improve glycemic control [14,15]. Nearly half of type 1 diabetes patients studied in a recent report did not employ a delay between injection and mealtime [16]. The findings from the current study suggest that injecting glulisine only 5 minutes prior to a meal is sufficient to produce increases in prandial insulin levels in people with type 1 diabetes that are indistinguishable from age and weight-matched healthy controls.

Previous work has provided conflicting results regarding whether lispro, aspart, and glulisine have comparable PK and PD [17]. In some PK/PD studies, absorption of insulin glulisine appeared to be faster than absorption of lispro or aspart. Morrow et al. [18] found that time to 50% of maximal concentration within the first hour was 21 minutes for glulisine versus 31 and 32 minutes for lispro and aspart, respectively. Arnolds et al. [19] found that the time to 10 and 20% of maximum insulin concentration was shorter for glulisine versus aspart, and correspondingly, the AUC for glucose infusion rate (to maintain euglycemia) was 30 mg/kg in the glulisine group versus 16 mg/kg in the aspart group. Similarly, Heise et al. [20] found that the time to 10% of total AUC insulin was shorter with a 0.2 U/kg dose glulisine compared to an equivalent dose of lispro. Additionally, the AUC for the glucose infusion rate in the first hour (102.3 mg/kg versus 83.1 mg/kg) was greater following glulisine as compared to lispro. The apparent increased rate of absorption of glulisine may be attributable to the fact that glulisine is prepared as a zinc-free injection, whereas the presence of zinc in the aspart and lispro injections may promote hexamer formation at the injection site [21].

Lispro premix insulin was dosed as two-thirds of the total daily dose injected prior to breakfast in an effort to cover both the breakfast and lunch meals and the final one-third prior to dinner. Thus, while glucose exposure, as measured by incremental $AUC_{[g]}$, was similar in the glargine/glulisine and lispro premix groups during the breakfast period, the lispro premix insulin dose resulted in a significantly larger incremental $AUC_{[i]}$ compared to both the healthy group and the glargine/glulisine group. Additionally, in the lispro premix group, blood glucose levels fell below baseline prior to lunch, and all ten of the hypoglycemic events in the lispro premix group occurred in the late breakfast period. Despite the exaggerated breakfast time $AUC_{[i]}$ in the lispro premix group, insulin concentrations in this group were not sufficient at lunch. The $AUC_{[i]}$ following lispro premix was approximately one-quarter of the $AUC_{[i]}$ observed in healthy controls and nearly one-fifth of the $AUC_{[i]}$ in the glargine/glulisine group. Correspondingly, the lunch interval $AUC_{[g]}$ was substantially greater than observed in the healthy and glargine/glulisine groups. Blood glucose also did not return to pre-breakfast levels in the lispro premix after lunch or dinner. Therefore, the lispro premix increased the risk of hypoglycemia between breakfast and lunch and failed to provide lunch time meal coverage, exposing patients to a large, long-duration postprandial glucose excursion.

For patients with type 1 diabetes, premix insulin is not included as an option within recommendations cited by guidelines

provided by the American Diabetes Association or the American Association of Clinical Endocrinologists [22–24], as intensive insulin therapies (basal-bolus therapy with three or more injections or continuous subcutaneous insulin infusion therapy) provide superior glycemic control [25]. Despite the lack of recommendations, providers may still elect to prescribe this treatment plan, as it negates the need to count carbohydrates at meals and could be advantageous in situations of low literacy or poor adherence [26]. Furthermore, use of premix insulin for type 1 diabetes is more prevalent in nations outside of the USA [27–29]. In an analysis of 75 non-Western countries identified as members of the International Diabetes Federation, 25 countries provided guidelines for use of pharmacotherapy for type 1 diabetes, of which 60% included basal-bolus and premix regimens as first-line therapeutic options [30].

A smaller proportion of subjects in the glargine/glulisine treatment group (4/12) experienced hypoglycemic events than in the lispro premix treatment group (9/13). Notably, this difference occurred despite the fact that lunch and dinner time insulin exposure was statistically greater in the glargine/glulisine group compared to the lispro premix group. Because this was a one-day study and the mealtime glulisine:carbohydrate dose was fixed, some subjects may have received a larger insulin:carbohydrate ratio than needed. This may have been the case in two subjects in the glargine/glulisine group who each experienced three hypoglycemic events. Clinically, the basal-bolus regimen allows for flexibility in real time, and the glulisine:carbohydrate ratio could be adjusted for patients' needs at each meal to avoid postprandial hypoglycemia.

A healthy control group was included as a means of assessing the degree to which the two insulin therapies studied matched physiologic responses to meals. Overall, insulin sensitivity was expected to be similar between the individuals with type 1 diabetes and the healthy controls. The individuals with type 1 diabetes had a normal BMI and good metabolic control with a mean HbA1c value of 7.3%. For control purposes, insulin dosing was standardized by administering a set ratio of insulin to carbohydrates. Glulisine was dosed in a conventional manner of 1 unit for every 10 gm carbohydrate, which approximated the 450-rule (450/total daily insulin) for dosing recommendations for the meal-time insulin to carbohydrate ratio [31]. This represented an applicable starting ratio typically used in clinical practice. The conventional strategy for dosing with lispro premix insulin is to split the injection into two doses, two-thirds in the morning and one-third in the evening [26,32,33], as was done in this study.

Limitations of this study include the fact that it was a small study that entailed only one day of monitoring for each insulin regimen. As a set ratio of insulin to carbohydrate was used, the effects of a different carbohydrate load or insulin to carbohydrate ratio or the addition of a pre-meal correction scale cannot be predicted based on the current data. Additionally, individualization of insulin dosing parameters (i.e., insulin to carbohydrate ratios or addition of a correction factor) may have further demonstrated the ability of basal bolus therapy to mimic normal healthy physiology to a greater extent. Premix insulin dosing was fixed for the purposes of this study; however, dosing of premix could be adjusted based on the meal content. Other experimental designs such as comparing the PK/PD effects of the two insulin regimens before a single standardized meal (e.g. breakfast) may have been more favorable toward the use of 75:25 premix analog insulin. One could consider that using a 50/50 analog premix formulation would be another comparator to this study's distribution of glulisine to glargine which was approximately 50/50. However, the presence of additional fast-acting preprandial insulin before breakfast in the premix insulin might also contribute to a greater prevalence of late morning/pre-lunch hypoglycemia. Our study of individuals with type 1 diabetes mellitus provides a very useful model to investigate the PK characteristics

of a subcutaneous injected insulin without the confounding effects of changing endogenous insulin leads; however, the study design did not include enough data points to additionally quantify the rate of rise of insulin (i.e. the times to 10%, 20% or 50% max absorption).

Strengths of the study include the three mixed-meal, day-long design with a controlled observation period that included frequent blood sampling, which provided information about insulin pharmacokinetics and pharmacodynamics throughout the day. The crossover study design prevented discrepancies in insulin sensitivity between the two treatment groups and allowed for equal total insulin doses for each individual during both treatment arms. Total dose and total AUC[i] were similar, demonstrating internal consistency of the study design. The participants with type 1 diabetes were metabolically well controlled (HbA1c 7.3%) and blood glucose was tightly controlled during the night before the study, indicating that glucotoxicity was not a confounding factor in observations.

Conclusion

In summary, this study was designed to approximate the typical daily meal intake of a patient with type 1 diabetes who consumes breakfast, lunch and dinner. The PK of the glargine/glulisine treatment mimicked patterns of endogenous insulin levels in healthy controls, as evidenced by similarities in incremental $AUC_{[i]}$, $Tmax_{[i]}$, and $\Delta Cmax_{[i]}$ in the two groups. Additionally, upon glulisine injection only 5 minutes before a meal, systemic insulin levels showed a similar rate of rise as endogenous insulin in healthy individuals. While premixed analog insulin allows for a simplified dosing regimen, it is at the expense of an increased risk for hypoglycemia and lunch time postprandial hyperglycemia. This study was hypothesis-generating and allows opportunities to further study differing approaches of insulin delivery, meal content and dosing of insulin regimens tailored to an individual patient and his/her daily meal intake. This supports the importance of conducting studies that cover an entire day, and provide additional information beyond that observed with a simple one-meal challenge. We conclude that a regimen of basal insulin (glargine) and glulisine (injected 5 minutes before three standard meals) for people with type 1 diabetes can reproduce physiologic insulin responses observed in age and BMI matched healthy controls.

Conflict of interest

EML, LMY and DBT have no conflicts of interest to disclose. SND is a consultant to Sanofi.

Appendix. Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jcte.2015.12.002.

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