Effects of LX4211, a Dual Sodium-Dependent Glucose Cotransporters 1 and 2 Inhibitor, on Postprandial Glucose, Insulin, Glucagon-like Peptide 1, and Peptide Tyrosine Tyrosine in a Dose-Timing Study in Healthy Subjects

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ABSTRACT

Background: LX4211 is a first-in-class dual inhibitor of sodium-dependent glucose cotransporters 1 and 2 (SGLT1 and SGLT2). SGLT1 is the primary transporter for glucose absorption from the gastrointestinal tract, and SGLT2 is the primary transporter for glucose reabsorption in the kidney. SGLT1 inhibition reduces postprandial glucose (PPG) levels and increases the release of gastrointestinal peptides such as glucagon-like peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY), whereas SGLT2 inhibition results in increased urinary glucose excretion (UGE).

Objectives: This study evaluated how timing of dose relative to meals changes the pharmacodynamic (PD) effects of LX4211 treatment, including effects on UGE, fasting plasma glucose, PPG, insulin, total and active GLP-1, and PYY. The safety and tolerability of LX4211 in healthy subjects were also assessed.

Methods: This was a randomized, double-blind, placebo-controlled, multiple-dose study to determine the PD effects of LX4211 dose timing relative to meals in 12 healthy subjects. Blood and urine were collected for the analysis of PD variables.

Results: Twelve healthy subjects 30 to 51 years of age were enrolled and treated. Treatment with LX4211 resulted in significant elevation of total and active GLP-1, and PYY while significantly decreasing PPG levels relative to placebo, likely by reducing SGLT1-mediated intestinal glucose absorption. Comparisons performed among the dosing schedules indicated that dosing immediately before breakfast maximized the PD effects of LX4211 on both SGLT1 and SGLT2 inhibition. The comparative results suggested distinct SGLT1 effects on GLP-1, PYY, glucose, and insulin, which were separate from SGLT2-mediated effects, indicating that SGLT1 inhibition with LX4211 may be clinically meaningful. All treatments were well tolerated with no evidence of diarrhea with LX4211 treatment.

Conclusions: This clinical study indicates that dosing of LX4211 immediately before breakfast maximized the PD effects of both SGLT1 and SGLT 2 inhibition and provided a convenient dosing schedule for future trials. LX4211 was safe and well tolerated and, due to its SGLT1 inhibition, produced strong PPG reductions and low UGE relative to selective SGLT2 inhibitors. LX4211 may provide a promising new therapy for patients with type 2 diabetes mellitus. The potential long-term clinical benefits and safety of LX4211 treatment will need to be confirmed in large clinical trials. ClinicalTrials.gov identifier: NCT01334242. (*Clin Ther*. 2013;35:1162–1173)

Key words: Diabetes, GLP-1, SGLT1, SGLT2, PYY, urinary glucose excretion.

INTRODUCTION

LX4211 is a dual inhibitor of sodium-dependent glucose cotransporters 1 and 2 (SGLT1 and SGLT2). SGLT1 is the major glucose and galactose transporter in the gastrointestinal (GI) tract, responsible for uptake of glucose from the diet.[1] Inhibition of SGLT1 results in

These data were presented, in part, as abstracts at the 2011 meeting of the American Diabetes Association (June 24–28, 2011, San Diego, Calif) and the European Association for the Study of Diabetes (September 12–16, 2011, Lisbon, Portugal).

Accepted for publication June 14, 2013.

http://dx.doi.org/10.1016/j.clinthera.2013.06.011

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delayed intestinal glucose absorption and the release of beneficial peptides such as glucagon-like peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY), likely by triggering GI nutrient sensing mechanisms.[2–5] The primary effect of SGLT1 inhibition is the lowering of postprandial glucose (PPG), as demonstrated in preclinical and clinical studies with selective SGLT1 inhibitors.[6,7] Humans with inactivating mutations in SGLT1 have glucose/galactose malabsorption (GGM).[8] SGLT2 is the major cotransporter for reabsorption of glucose in the kidney, with SGLT2 inhibition resulting in increased urinary glucose excretion (UGE). Humans with inactivating mutations in SGLT2 have familial glucosuria.[1]

In a previous Phase II study of patients with type 2 diabetes mellitus (T2DM), LX4211 treatment 2 hours before breakfast resulted in enhanced glycemic control, with improvements in fasting plasma glucose (FPG) and PPG resulting in a 0.76% reduction of glycosylated hemoglobin (HbA_{1C}), relative to placebo, after 4 weeks of dosing (5). LX4211 treatment resulted in additional cardiovascular and metabolic benefits, including a reduction in triglycerides and a trend toward reduction in body weight and blood pressure. LX4211 was well tolerated with no indications of elevated GI side effects relative to placebo. In an additional dose-ranging study, 400 mg LX4211 once daily was the most efficacious dose, with a placebo subtracted reduction in HbA_{1c} of 0.86% ($P < 0.001$) at week 12.[9] Significant reductions were also observed for FPG (–27.1 mg/dL, $P < 0.001$), body weight (–1.8 kg, $P < 0.001$), and blood pressure (–5.7 mm systolic blood pressure, $P < 0.001$).

The purpose of the present study was to examine the effects of a variety of LX4211 dosing schedules on the pharmacodynamic (PD) effects for both SGLT1 and SGLT2 inhibition in healthy subjects. The inhibition of SGLT2 in the kidney follows a simple systemic pharmacokinetic (PK):PD relationship; however, because systemic exposure with LX4211 is insufficient to inhibit SGLT1, luminal inhibition of SGLT1 must be tested empirically by tracking the PD effects resulting from its inhibition, including the release of GLP-1 and PYY. The present study was designed to identify a dosing schedule that would maximize the PD effects of both SGLT1 and SGLT2 inhibition throughout the day, with a particular emphasis on sustaining SGLT1-related effects. Notably, selective SGLT2 inhibitors, at doses relevant to those evaluated in Phase III studies, have been reported to produce only modest decreases in PPG in healthy subjects.[10–12] The present study provided an opportunity to examine the effects of dual SGLT1 and SGLT2 inhibition on PPG in healthy subjects.

METHODS

This study was registered with www.clinicaltrials.gov (NCT01334242). It was a Phase I, randomized, double-blind, placebo-controlled, multiple-dose study to determine the PD effects of LX4211 dose timing relative to meals in healthy subjects. The study was conducted at a

Table I. Baseline demographics.

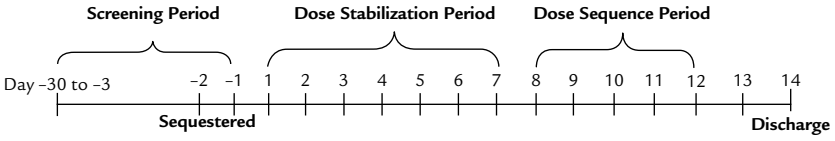
Characteristic	Placebo (n = 2)	LX4211 400 mg (n = 10)	Overall (N = 12)
Age, y, mean (SD)	40.0 (15.56)	42.1 (5.32)	41.8 (6.77)
Male sex, no. (%)	2 (100.0)	10 (100.0)	12 (100.0)
Race, no. (%)			
White	1 (50.0)	8 (80.0)	9 (75.0)
Black or African American	0 (0.0)	2 (20.0)	2 (16.7)
Native Hawaiian or other Pacific Islander	1 (50.0)	0 (0.0)	1 (8.3)
Ethnicity, no. (%)			
Hispanic or Latino	1 (50.0)	4 (40.0)	5 (41.7)
Non-Hispanic or non-Latino	1 (50.0)	6 (60.0)	7 (58.3)
BMI, kg/m^2, mean (SD)	26.05 (0.778)	27.10 (2.844)	26.93 (2.615)

BMI = body mass index.

single center (ICON Development Solution Phase I Center, San Antonio, Tex) between March 7, 2011, and April 9, 2011, and included healthy subjects aged 30 to 51 years of age. Subject demographic characteristics are shown in Table I, and a complete list of inclusion and exclusion criteria is included in the Supplemental material in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011.

The primary objective of the study was to evaluate how the timing of a dose, relative to meals, affected the PD of LX4211 treatment, including effects on UGE, FPG, PPG, insulin, total GLP-1 (tGLP-1) and active GLP-1 (aGLP-1), and PYY. The secondary objective of this study was to evaluate the safety and tolerability of LX4211 in healthy subjects.

No formal sample size calculation was made, but the number of required subjects (N = 12) was consistent with other trials with similar objectives and design.[4,5] A randomization schedule was generated before the first dosing period by ICON Development Solutions, LLC, the contract research organization performing data management for the study. The study consisted of a screening period (including an initial 2-day run-in period for diet stabilization and baseline testing), 12 successive days of dosing with no washout between dosing schedules, and a 2-day follow-up period (Figure 1). All subjects checked into the clinic on day –2 for verification of eligibility, safety assessments, diet stabilization, and sequestration. On day –1, qualified subjects were randomly assigned to either LX4211 or placebo and had baseline assessments.



Dosing regimens:

A = dosing 1 hour before breakfast (OBB)

B = dosing 0.5 hour before breakfast (HBB)

C = dosing immediately before breakfast (IBB)

D = dosing immediately before lunch (IBL)

E = dosing 1 hour before breakfast and dinner, split dose (SD)

Subject	Study Day				
	8	9	10	11	12
1	E	D	C	B	A
2	D	C	B	E	A
3	E	C	A	D	B
4	B	D	A	C	E
5	E	A	C	B	D
6	C	D	E	B	A
7	B	A	D	E	C
8	A	E	B	C	D
9	C	E	D	A	B
10	A	B	C	D	E
11	A	C	E	D	C
12	D	B	C	A	E

Figure 1. Study schema and Latin square crossover design.

A solid tablet formulation (200-mg tablets, 400 mg total) or matching placebo was dosed once daily 2 hours before breakfast on days 1 through 7 until LX4211 was at or near expected steady-state levels. The selected 400-mg/d dose of LX4211 produced a maximal HbA_{1C} reduction in a Phase IIb dose-ranging study.[9] Beginning on day 8 and continuing through day 12, subjects were dosed on variable schedules of LX4211 relative to meal time. Subjects on active drug were randomized to a Latin square crossover design[13] (Figure 1) to allow for orthogonal partitioning of the treatment, period, and subject effects. The study design also balanced for first-order carryover effects. Within this schema, each subject received (in a randomized sequence) 5 dosing schedules of LX4211 over the next 5 days: 400 mg 1 hour before breakfast (OBB), 400 mg one-half hour before breakfast (HBB), 400 mg immediately before breakfast (within 5 minutes [IBB]), 400 mg immediately before lunch (within 5 minutes [IBL]), or a split dose (SpD) of 200 mg 1 hour before breakfast and 200 mg 1 hour before dinner. LX4211-assigned subjects were exposed to all 5 dose schedules. The placebo-assigned subjects took study drug (placebo) on all days, with days 8 through 12 using random sequences that included the same dose schedules as those used for the LX4211 assignments, but in varying order. Subjects remained in residence in the clinical research unit until completion of the study. Standardized meals were provided on day –2, baseline (day –1), on days 1 through 12 of dosing, and day 13. Breakfast was provided at the appropriate time point relative to each dosing schedule, lunch 3.5 hours after the completion of breakfast, dinner 8.5 hours after the completion of breakfast, and a snack 8.75 hours after completion of breakfast. All meals were 0.5 hours in duration with instructions to consume the entire meal. For all days, consumption of the evening snack was optional and was given after all PD measurement samples were obtained for the day.

The meal compositions were varied over the course of the study to better mimic a real-world diet and examine the robustness of drug effects under these conditions. Meal plan 1 was used on days –2, 4, and 9 and consisted of a 534-calorie breakfast (11% protein, 82% carbohydrate, 11% fat), a 736-calorie lunch (30% protein, 36% carbohydrate, 34% fat), a 736-calorie dinner (20% protein, 28% carbohydrate, 52% fat),and 167-calorie evening snack (5% protein, 82% carbohydrate, 13% fat).

Meal plan 2 was used on days –1, 5, and 10 and consisted of a 477-calorie breakfast (13% protein, 66% carbohydrate, 21% fat), a 717-calorie lunch (19% protein, 52% carbohydrate, 29% fat), an 866-calorie dinner (17% protein, 50% carbohydrate, 33% fat), and a 135-calorie evening snack (3% protein, 85% carbohydrate, 12% fat).

Meal plan 3 was used on days 1, 6, and 11 and consisted of a 539-calorie breakfast (19% protein, 52% carbohydrate, 29% fat), a 652-calorie lunch (22% protein, 35% carbohydrate, 43% fat), a 781-calorie dinner (14% protein, 59% carbohydrate, 27% fat), and a 200-calorie evening snack (18% protein, 62% carbohydrate, 20% fat).

Meal plan 4 was used on days 2, 7, and 12 and consisted of a 449-calorie breakfast (11% protein, 52% carbohydrate, 37% fat), a 779-calorie lunch (19% protein, 46% carbohydrate, 35% fat), an 851-calorie dinner (23% protein, 35% carbohydrate, 42% fat), and a 105-calorie evening snack (21% protein, 79% carbohydrate, 0% fat).

Meal plan 5 was used on days 3, 8, and 13 and consisted of a 622-calorie breakfast (18% protein, 47% carbohydrate, 35% fat), a 728-calorie lunch (16% protein, 52% carbohydrate, 32% fat), a 675-calorie dinner (15% protein, 41% carbohydrate, 44% fat), and a 206-calorie evening snack (23% protein, 35% carbohydrate, 42% fat).

This clinical study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by an institutional review board with jurisdiction over the site (IntegReview Ethical Review Board, Austin, Tex), and all subjects provided written informed consent before enrollment in the study.

Assessments

The PD end points measured at baseline (day –1) and on days 7 through 12 of dosing were 24-hour UGE, FPG, PPG, insulin, aGLP-1 and tGLP-1, and PYY. Baseline measures were obtained 2 hours before breakfast and 3 minutes before dosing for IBB and IBL dosing, and 2 hours before breakfast and 3 minutes before dinner dosing for the SpD schedule. Plasma tGLP-1 and aGLP-1, total PYY, FPG, PPG, and insulin were measured by Pacific Biomarkers, Inc (Seattle, Wash).

Safety assessments were performed throughout the duration of the study included clinical laboratory tests (biochemistry, hematology, and urinalysis), vital sign measurements (blood pressure, heart rate, respiratory rate, and oral temperature), 12-lead electrocardiograms, weight and physical examinations, and monitoring of

adverse events. Adverse events were coded and listed by body system and preferred term based on the Medical Dictionary for Regulatory Activities, version 12.0.

Statistics

Continuous variables were summarized descriptively by the number of subjects with nonmissing data, mean, SD, median, minimum, and maximum values. Categorical variables were summarized descriptively by their counts and associated percentages.

Maximum likelihood methods were used to calculate point and interval estimates of treatment effect. Statistical tests of dose-timing effects were 2 sided, and significance was to be based on an α level ≤ 0.05. CIs were calculated using 2-sided criteria with a 95% confidence coefficient. Least-squares adjusted statistics were derived to summarize differences among the different doses.

The PD measures were summarized at each individual time point of sample collection using AUC estimates made at various time points over the course of a day's sampling. These latter statistics included calculation for AUC values from time 0 to last observation (AUC_{0-11}) and AUCs values for each meal period. The linear trapezoidal rule was applied to calculate the different AUC estimates. Single time point data were used to summarize UGE and FPG.

The PD end points were to be adjusted by the day −1 data (ie, matching time point from day −1 was to be subtracted and then the statistic was to be calculated), where appropriate. A mixed-effect linear model was used to test treatment differences for the PD data. To satisfy the primary study objective, only data from the Latin square component of the study was used in this analysis. All pairwise dose contrasts were derived from the dose sums of squares to test differences among the LX4211 dose schedules.

Exploratory analyses of differences between placebo and LX4211 for the PD data were assessed by use of a mixed-effects repeated-measures linear model. Simple dose effects were tested at each time point, as appropriate, by abstraction of effects from the dose × time interaction term.

RESULTS

Effects of LX4211 on UGE

Twelve healthy males were enrolled and randomized to receive LX4211 or placebo (10:2). All study subjects who were randomly assigned completed the study. The PD effect of LX4211 inhibition of SGLT2 was examined using UGE (**Figure 2 and Supplemental Table I** in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). Baseline (day −1) 24-hour UGE was 0.15 g, consistent with expectations for healthy subjects who do not have elevated blood glucose levels. The least-squares mean change from baseline for 24-hour UGE was 35.48 g with LX4211 and 0.08 g with placebo. Change from baseline with LX4211 treatment ranged from 33.6 to 37.2 g for all dosing schedules. Changes from baseline were significant for LX4211 treatment ($P < 0.001$) and significantly different from placebo ($P < 0.001$); however, there were no significant differences for between-dosing schedule comparisons. There was a residual effect for SGLT2 inhibition from the previous day's dosing, due to the long half-life ($t_{1/2}$ = 20.7 hours) of LX4211,

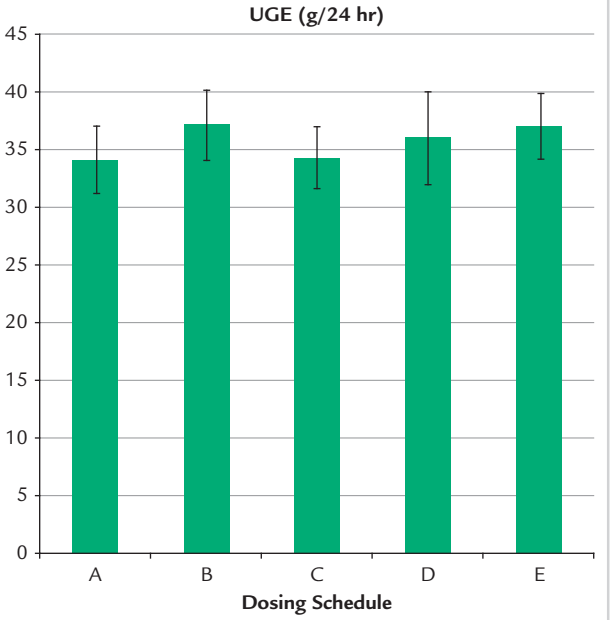


Figure 2. Least-squares mean 24-hour urinary glucose excretion (UGE). (A) Dosing 1 hour before breakfast. (B) Dosing 0.5 hour before breakfast. (C) Dosing immediately before breakfast. (D) Dosing immediately before lunch. (E) Dosing a split dose 1 hour before breakfast and dinner. Error bars represent SEM. UGE was significantly elevated for all doses relative to baseline (day −1) and for the pooled LX4211 doses relative to placebo ($P < 0.001$ for all comparisons).

resulting in sustained systemic exposure. This was illustrated by the 0 to 8-hour UGE for the IBL dosing schedule. Although 0 to 8-hour UGE ranged from 14.5 to 15.8 g for all other dosing schedules, the IBL dosing schedule achieved 0 to 8-hour UGE of 12.6 g despite not dosing until hour 4 of the 8-hour collection period. Despite the residual effects, the data indicated that, after 7 days of daily dosing with LX4211, dose timing appeared to have little impact on the PD effect of SGLT2 inhibition.

Effects of LX4211 Relative to Placebo on GLP-1, PYY, Glucose, and Insulin

Unlike SGLT2 inhibition, blood levels of LX4211 are insufficient to inhibit SGLT1 systemically, whereas luminal levels of LX4211 are available to inhibit SGLT1 in the GI tract. Therefore, the PD effects of SGLT1 inhibition were measured through the secondary events triggered by delayed glucose absorption in the GI tract, including increases of tGLP-1, aGLP-1, and PYY. Measures for these parameters were pooled for all 5 LX4211 treatment groups and compared with placebo measures by analyzing the total AUC after breakfast (0–4 hours) (Figure 3 and Supplemental Table II in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). Total and active GLP-1 were significantly elevated with LX4211 treatment after breakfast ($P = 0.014$ and $P = 0.013$, respectively). PYY levels were also significantly elevated, with LX4211 treatment relative to placebo after breakfast ($P < 0.001$). PPG was significantly reduced after treatment with LX4211 compared with placebo throughout the day ($P < 0.001$ for $AUC_{0–11}$), whereas insulin was not significantly decreased (Figure 4 and Supplemental Table II in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). LX4211 treatment resulted in a significant reduction in FPG relative to placebo (–6.46 vs –0.60 mg/dL, $P = 0.005$) (Supplemental Table III in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). Thus, LX4211 elevated aGLP-1, tGLP-1, and PYY while reducing FPG and PPG in healthy subjects.

Dose Schedule Comparisons

The different dosing schedules were compared for their effects on GLP-1 and PYY (Figure 3 and Supplemental Table IV in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). Of the 3 dosing schedules before breakfast (OBB, HBB, and IBB), IBB dosing (Figure 3A) produced significant tGLP-1 elevations relative to OBB dosing after breakfast ($P = 0.041$). There were no significant differences between OBB, HBB, and IBB dosing on aGLP-1 or PYY after breakfast (Figures 3B and 3C). Thus, IBB dosing may be preferable to OBB dosing to maximize the PD effects for SGLT1-mediated elevations of tGLP-1.

Dosing before breakfast was next compared with IBL and SpD dosing for effects on GI peptides (Figures 3D–3I). IBB and HBB dosing produced significant tGLP-1 elevations after breakfast relative to IBL dosing ($P = 0.023$ and $P = 0.003$, respectively) (Figures 3A and 3D and Supplemental Table IV in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). Comparisons of IBB dosing versus IBL and SpD dosing demonstrated significant elevations of aGLP-1 with IBB dosing relative to IBL or SpD dosing ($P = 0.011$ and $P = 0.027$, respectively) (Figures 3E and 3H and Supplemental Table IV in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). Cross-dose comparisons also showed that PYY levels were significantly elevated after breakfast for OBB, HBB, and IBB dosing, relative to IBL dosing ($P = 0.033$, $P = 0.005$, and $P = 0.005$, respectively) (Figures 3C and 3F and Supplemental Table IV in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). There were no significant differences between before breakfast and SpD dosing schedules for tGLP-1 or PYY (Figures 3G and 3I and Supplemental Table IV in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). Dose comparisons indicated that IBL dosing resulted in reduced SGLT1-mediated PD effects on GLP-1 and PYY relative to IBB dosing.

Between-dosing comparisons showed no significant differences for glucose reductions (Figures 4A, 4C, and 4E and Supplemental Table IV in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011); however, they did reveal significant reductions in insulin after breakfast with IBB dosing relative to OBB and SD dosing ($P = 0.03$ and $P = 0.019$, respectively) (Figures 4B and 4F and Supplemental Table IV in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011). IBB dosing appeared to produce a decrease in insulin after breakfast relative to IBL dosing (Figure 4D and Supplemental Table IV in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011), but this difference was not statistically significant ($P = 0.087$).

LX4211 was well tolerated in healthy subjects with repeated dosing of 400 mg daily for 12 days (Table II) with no deaths, serious adverse events, or discontinuations due

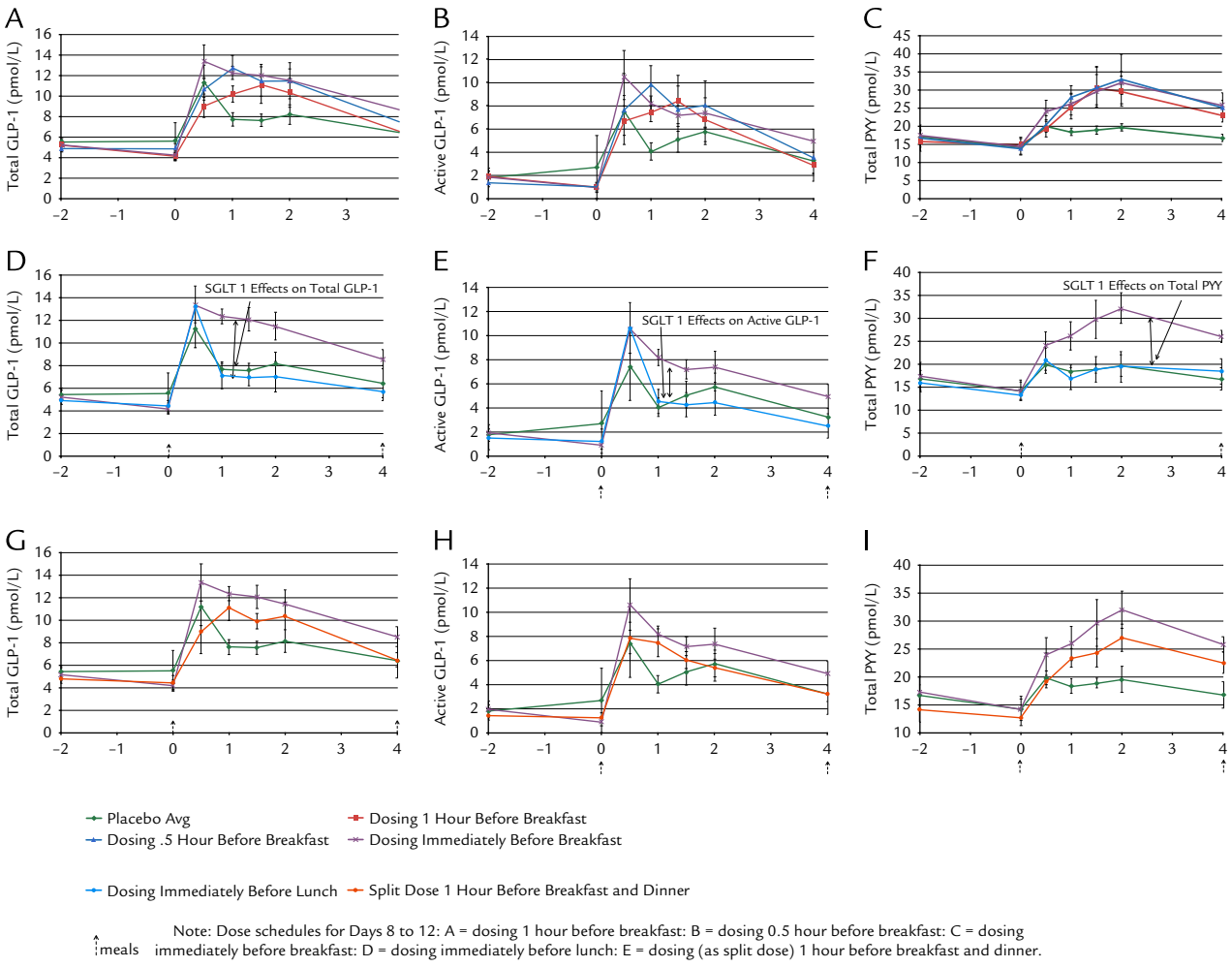


Figure 3. Pharmacodynamic effects of LX4211 dosing schedules on gastrointestinal peptides relative to placebo. (A) Before breakfast dosing relative to placebo for total glucagon-like peptide 1 (GLP-1). (B) Before breakfast dosing relative to placebo for active GLP-1. (C) Before breakfast dosing relative to placebo for peptide tyrosine tyrosine (PYY). (D) Immediately before breakfast versus immediately before lunch dosing relative to placebo for total GLP-1. (E) Immediately before breakfast versus immediately before lunch dosing relative to placebo for active GLP-1. (F) Immediately before breakfast versus immediately before lunch dosing relative to placebo for PYY. (G) Immediately before breakfast versus split dosing relative to placebo for total GLP-1. (H) Immediately before breakfast versus split dosing relative to placebo for active GLP-1. (I) Immediately before breakfast versus split dosing relative to placebo for PYY. SGLT-1 = sodium-dependent glucose cotransporter 1. Error bars represent SEM.

to adverse events. Among the 12 study subjects (10 active, 2 placebo), 4 subjects (3 active, 1 placebo) reported adverse events. All events were mild in intensity, unrelated to the study treatment and resolved quickly. None of the adverse events were reported in >1 subject. No clinically meaningful trends were observed in clinical laboratory parameters, vital signs, physical examinations, or electrocardiographic parameters. One LX4211-treated subject reported a change in bowel habits, described as a decrease in frequency without constipation.

DISCUSSION

In the Phase IIa study (LX4211.1-201-DM), LX4211 was dosed 2 hours before breakfast.[5] The goal of the

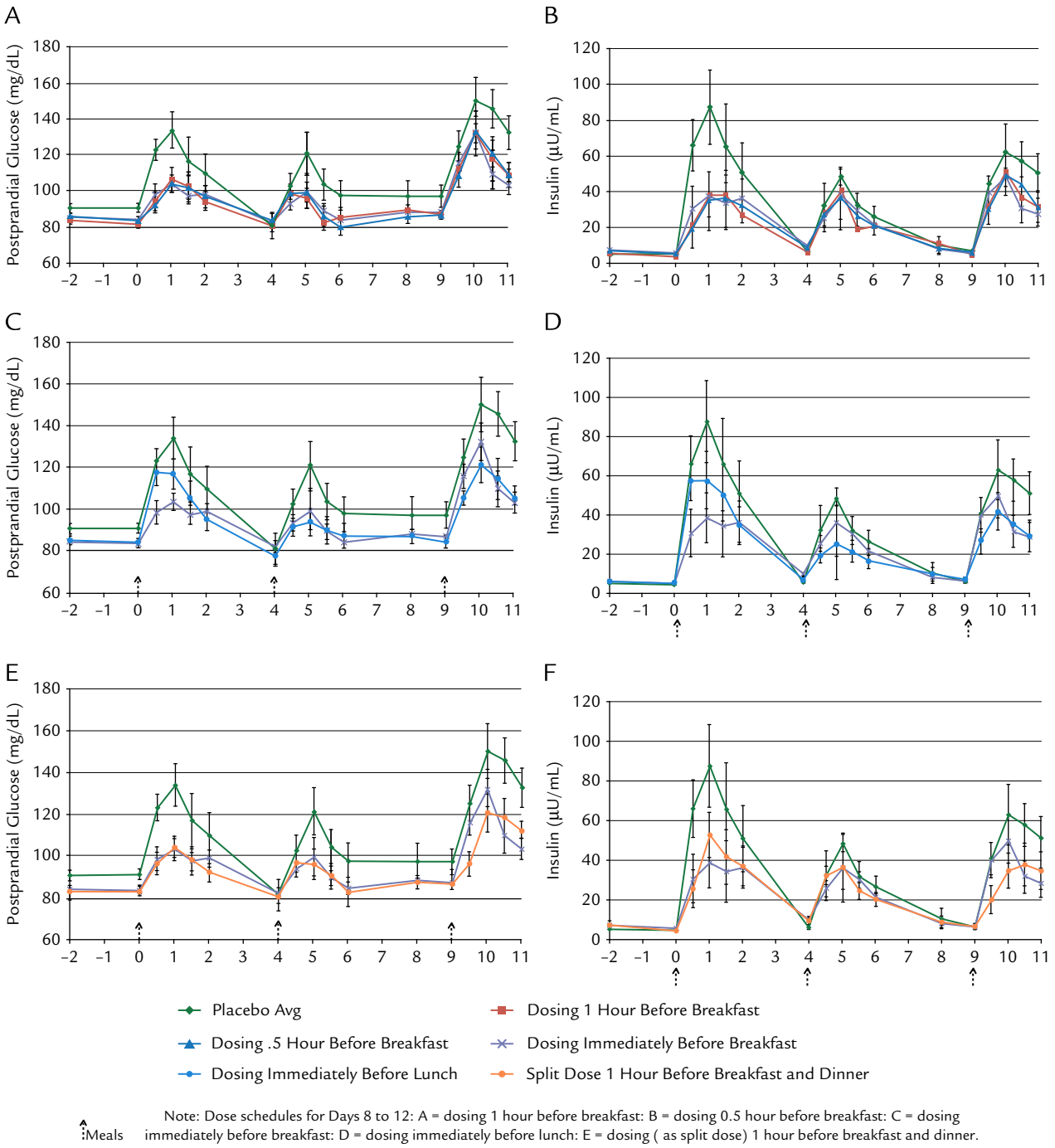


Figure 4. Pharmacodynamic effects of LX4211 dosing schedules on glucose and insulin relative to placebo. (A) Before breakfast dosing relative to placebo for glucose. (B) Before breakfast dosing relative to placebo for insulin. (C) Immediately before breakfast versus immediately before lunch dosing relative to placebo for glucose. (D) Immediately before breakfast versus immediately before lunch dosing relative to placebo for insulin, (E) Immediately before breakfast versus split dosing relative to placebo for glucose. (F) Immediately before breakfast versus split dosing relative to placebo for insulin. Error bars represent SEM.

Table II. Summary of treatment-emergent adverse events by system organ class and preferred term.

MedDRA System Organ Class Preferred Term	No. (%) of Subjects	
	LX4211 400 mg (n = 10)	Placebo (n = 2)
No. of subjects with at least 1 TEAE	3 (30)	1 (50)
Gastrointestinal disorders	1 (10)	0
Change of bowel habit	1 (10)	0
Psychiatric disorders	1 (10)	0
Anxiety	1 (10)	0
Renal and urinary disorders	0	1 (50)
Micturition urgency	0	1 (50)
Respiratory, thoracic, and mediastinal disorders	1 (10)	0
Cough	1 (10)	0
Vascular disorders	2 (20)	0
Phlebitis	1 (10)	0
Phlebitis superficial	1 (10)	0

MedDRA = Medical Dictionary for Regulatory Affairs; TEAE = treatment-emergent adverse event.

present study was to test different dosing schedules of LX4211, with the intent of identifying more convenient dosing regimens while still maximizing the PD effects for both SGLT1 and SGLT2 inhibition throughout the day and after all 3 meals. After 7 days of daily dosing 2 hours before breakfast (days 1–7), all 5 dosing schedules assessed on days 8 to 12 produced significant elevations of 24-hour UGE from 33.6 g to 37.2 g with no significant differences in 24-hour UGE when comparing dose schedules. This suggests that dose timing is not an important factor for maximizing the PD effects of SGLT2 inhibition with LX4211.

Systemic exposure of LX4211 is low and insufficient to inhibit SGLT1,[5] which occurs in the lumen of the GI tract and, unlike SGLT2 inhibition, does not follow a systemic PK:PD relationship. The PD effects of SGLT1 inhibition were assessed via secondary events resulting from delayed glucose absorption, including release of the GI peptides GLP-1 and PYY.[2–5] It was hypothesized that inhibiting SGLT1 in the GI tract delays glucose absorption and triggers the natural nutrient sensing mechanisms of the GI tract[5] similar to the effects caused by Roux-en-Y gastric bypass surgery or ingestion of dietary-resistant starch.[14–17] This mechanism of enhanced GI peptide release with SGLT1 inhibition has been confirmed in animal studies[2,3] and in previous studies in patients with T2DM.[4,5] The present study is the fourth clinical trial to demonstrate this LX4211-mediated increase in GLP-1 and PYY release from the GI tract. The tGLP-1 elevations were maximized with IBB dosing relative to OBB dosing, providing a convenient dosing schedule for future studies with LX4211.

The elevation of postprandial GLP-1 secretion after LX4211 treatment contrasts with reports from 2 preclinical studies in mice describing the short-term suppression of postprandial GLP-1 release after blocking SGLT1 action.[18,19] Co-injection of glucose and phloridzin, an SGLT inhibitor, into the upper small intestine suppressed the normal glucose-mediated GLP-1 response in mice at 5 minutes post-injection.[18] Additionally, SGLT1 knockout mice exhibited a reduced GLP-1 response relative to wild-type mice 5 minutes after an oral glucose challenge.[19] These short-term studies indicate SGLT1 may be important for glucose sensing in the upper small intestine and immediate GLP-1 release within 5 minutes of a meal, but they are not indicative of the longer term effects of SGLT1 inhibition on GI peptide release after meals throughout the course of the day. Elevations of GLP-1 have been observed for hours in mice after LX4211 treatment and a meal challenge,[3] and after breakfast, lunch, and dinner in patients with T2DM treated with LX4211.[4,5] These extended effects may result from glucose and short-chain fatty acid sensing by L cells in the distal small intestine and colon and are indicative of

the overall effects of SGLT1 inhibition on GI peptide release throughout the day.

IBL dosing of LX4211 results in nearly complete loss of increases in tGLP-1, aGLP-1, and PYY after breakfast (Figures 3D–3F). For IBL dosing relative to IBB dosing aGLP-1, tGLP-1, and PYY were all significantly reduced after breakfast ($AUC_{0–4}$), suggesting that SGLT1 inhibition and its concomitant PD effects were largely lost by breakfast the day after the previous dose of LX4211. Due to the long systemic half-life of LX4211,[5] a nearly full inhibition of SGLT2 was maintained after breakfast the day after a dose. This is supported by the lack of change in 24-hour UGE between IBL and IBB dosing, with these dosing schedules producing 35.72 g and 34.06 g of UGE, respectively. Thus, glucose and insulin benefits remaining after breakfast on the days of IBL dosing are likely mediated primarily by the SGLT2 mechanism of LX4211 (Figures 4C and 4D). This provides an opportunity to distinguish the SGLT1 effects from the SGLT2 effects of LX4211. By comparing placebo with IBL and IBB dosing of LX4211, one can get some idea of a comparison of the effects of no inhibition, SGLT2 inhibition, and dual SGLT1 and SGLT2 inhibition for GLP-1, PYY, glucose, and insulin (Figures 3D–3F and 4C and 4D). These data provided support that the SGLT1 effect could be clinically meaningful but this will need to be proved in large long-term clinical trials that examine the effects of LX4211 on HbA_{1C} in patients with diabetes.

Also interesting in the present study were the combined PD effects of dual SGLT1 and SGLT2 inhibition on glucose and insulin because these measures provide insight into what long-term clinical benefits might be achieved with LX4211 dosing (Figure 4). LX4211 produced significant reductions in plasma glucose after breakfast, lunch, and overall, with no significant decreases in insulin relative to placebo. Reductions in PPG were most pronounced after breakfast and lunch (Figure 4A), with PPG barely increasing out of the normal FPG range at these 2 meals. This is in contrast to selective SGLT2 inhibitors that, at doses relevant to those studied in Phase III, have reported minimal effects on PPG or in oral glucose tolerance tests (OGTTs) in healthy subjects.[10–12] Single doses of 100 and 200 mg canagliflozin produced only modest reductions in PPG after breakfast, despite larger 24-hour UGEs, than those achieved with LX4211.[12] Doses of 100 and 200 mg canagliflozin produced 24-hour UGE of ~45 g and ~50 g, respectively, in healthy subjects. At doses of 400 mg and higher, canagliflozin produced ~70 g of UGE and a slightly greater PPG decrease. The authors speculate that the higher doses may be having a direct effect on intestinal glucose absorption. More recently, a 300-mg dose of canagliflozin was tested for its effect on PPG and the rate of appearance of oral glucose (RaO) in healthy subjects during a meal challenge immediately after dosing.[11] Canagliflozin again reduced PPG and insulin for the first 2 hours after the meal; however, from 2 to 6 hours after the meal, there was compensatory glucose uptake and insulin release, resulting in an overall decrease in PPG ($AUC_{0–6}$) of 26%. This rebound glucose uptake appears to result from a loss of SGLT1 inhibition after 2 hours as indicated by RaO rising above placebo from hours 2 to 6 for canagliflozin-treated subjects. The result is absorption of glucose that had been in the lumen of the GI and a compensatory increase in insulin release to deal with the increase in blood glucose. It is questionable whether the delay in glucose absorption that resulted in a 6% decrease in RaO (AUC) after breakfast will translate into a clinically meaningful benefit.

A loss of SGLT1 inhibition at 2 hours was also observed in the original canagliflozin study in healthy subjects,[12] and a similar loss of SGLT1 inhibition and compensatory glucose absorption and insulin release at 2 hours was also reported for GSK-1614235, an apparently short-acting, selective SGLT1 inhibitor, in healthy subjects.[6] Additionally empagliflozin was reported to produce no change in blood glucose AUC in healthy subjects during an OGTT administered after dosing.[10] By contrast, there was no indication of loss of SGLT1 inhibition (Figures 4A and 4B) at any point throughout the day after LX4211 dosing.

The strong PPG reduction after breakfast and lunch produced by LX4211 is likely due to a sustained SGLT1 inhibition. This association of SGLT1 inhibition, with more pronounced PPG control, is supported by animal studies with SGLT inhibitors covalently attached to nonabsorbable polymers.[20,21] These agents remain in the GI tract after oral delivery where they inhibit intestinal SGLT1 and specifically improve glycemic control in an OGTT. Recent studies of SGLT1 knockout mice,[2,3] as well as preclinical[7] and clinical[6] studies with SGLT1-selective inhibitors also indicate a primary effect of SGLT1 inhibition on PPG.

The strong LX4211 effect on PPG could help explain the lower UGE produced by LX4211 in healthy subjects,

relative to selective SGLT2 inhibitors. The amount of glucose spilled in the urine is dependent on both the degree of inhibition of SGLT2 and the amount of glucose in the blood that is filtered in the kidney. Thus, lower PPG levels, as a result of SGLT1 inhibition, could result in lower UGE. In the present study, LX4211 produced ~35 g of glucose over 24 hours. A maximal 24-hour UGE of 45 g was also observed with a 300-mg liquid formulation of LX4211 in the Phase I ascending single-dose study in healthy subjects,[5] which was not further increased at 500 mg despite a dose-dependent increase in systemic pharmacokinetics. Selective SGLT2 inhibitors have produced greater 24-hour UGE in healthy subjects, with dapagliflozin,[22] canagliflozin,[12] and empagliflozin[23] reaching 62 g, 70 g, and 74 g of UGE, respectively, in dose-ranging studies. Washburn and Poucher[24] recently reviewed clinical data for SGLT inhibitors and compared UGE produced by the various agents in both healthy subjects and patients with T2DM, providing further support for the low UGE produced by LX4211 relative to the selective SGLT2 inhibitors. This reduction in 24-hour UGE may be due to the enhanced postprandial glycemic control achieved through SGLT1 inhibition with LX4211, relative to selective SGLT2 inhibitors, resulting in lower plasma glucose levels throughout the day and less glucose available to be filtered and spilled into the urine.

The majority of SGLT inhibitors for diabetes are highly selective for SGLT2 over SGLT1. The focus on SGLT2 selectivity is largely due to theoretical concerns that SGLT1 inhibition could trigger GI side effects, such as diarrhea, and is based on patients with GGM who have a loss of function mutations in SGLT1 resulting in malabsorption of glucose or galactose from their diet.[24] Even small amounts of glucose and galactose trigger GI side effects in these patients[1]; however, most individuals in an Amish cohort of 33 individuals with loss of functional mutations in SGLT1 were reported to tolerate a normal carbohydrate-containing diet before their 20s, perhaps due to a change in gut flora.[25] Pharmacological inhibition of SGLT1 with LX4211 does not appear to be associated with GI side effects in clinical trials.[4,5,9] This may be because LX4211 does not completely block intestinal glucose absorption, as exhibited by glucose excursions after meals or OGTTs in treated patients with T2DM[4,5] or healthy subjects (**Figure 4A**). This finding contrasts with the flat blood glucose excursions during OGTTs in patients with GGM (8) or SGLT1 knockout mice (2). Pharmacological inhibition of SGLT1 with LX4211 allows some intestinal glucose absorption. As in previous clinical trials.[4,5,9], LX4211 was well tolerated in this study, with no evidence of increased hypoglycemia or GI or other adverse events. LX4211 dosing IBB once daily demonstrated favorable effects on glycemic control. Therefore, dual SGLT1 and SGLT2 inhibition may be a viable new approach to the treatment of T2DM, worthy of further investigation in additional clinical trials.

ACKNOWLEDGMENTS

Lexicon Pharmaceuticals, Inc provided the funding for this LX4211 clinical study

The authors thank Johanna Bronner for assuring the integrity of the database, Gui-lan Ye for monitoring safety, and Mark O'Neill for study monitoring. Drs. Zambrowicz, Ogbaa, Mr. Fazier, Mr. Banks, Ms. Turnage, Dr. Freiman, and Dr. Sands were responsible for the study design (clinical). All authors were responsible for the writing of the manuscript and final approval.

CONFLICTS OF INTEREST

Dr. Zambrowicz is the Chief Scientific Officer of Lexicon Pharmaceuticals, Inc, and owns stock. Dr. Sands is the President and Chief Executive Officer of Lexicon Pharmaceuticals, Inc, and owns stock. Dr. Ogbaa, Mr. Banks, Ms. Turnage, Ms. Boehm, and Dr. Powell are employees of Lexicon Pharmaceuticals, Inc and own stock. Mr. Frazier and Dr. Freiman were employees of Lexicon Pharmaceuticals, Inc and own stock. Dr. Ruff has no conflicts to disclose. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

SUPPLEMENTAL MATERIAL

Supplemental material accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clinthera.2013.06.011.

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SUPPLEMENTARY INFORMATION

Complete List of Inclusion/Exclusion Criteria

Inclusion Criteria

Subjects must have met all of the following criteria to be considered eligible to participate in the study:

1. Adults $\geq$18 to $\leq$55 years of age at the time of screening:
   a. Females must have been of nonchild-bearing potential, surgically sterile (documented hysterectomy, tubal ligation, or bilateral salpingo-oophorectomy) or postmenopausal (defined as 12 months of spontaneous amenorrhea). If necessary, follicle-stimulating hormone results $>$35 IU/L at screening were confirmatory in the absence of a clear postmenopausal history.
   b. Males must have agreed to use an adequate method of contraception during the study and for 30 days after the discharge visit. Adequate methods of contraception for subjects or partner included the following: condom with spermicidal gel, diaphragm with spermicidal gel, coil (intrauterine device), surgical sterilization, vasectomy, oral contraceptive pill, depo-progesterone injections, progesterone implant (ie, Implanon), and abstinence. If a subject was not usually sexually active but became active, he or his partner was to use medically accepted forms of contraception.

   Vital signs (after 5 minutes resting in a supine position) at screening, which were within the following ranges:
   a. systolic blood pressure: 90–140 mm Hg
   b. diastolic blood pressure: 50–90 mm Hg
   c. heart rate: 50–100 beat/min or considered not clinically significant if outside this range

   Note: Subjects with vital signs outside the above range could have been eligible for the study (via waiver) if the
2. screening physician thought that the results were not clinically significant and would not have affected study conduct.
3. Body mass index $\geq$18 to $\leq$35 kg/m^2 at screening.
4. Able and willing to provide written informed consent.

Exclusion Criteria

Subjects who met any of the following criteria were to be excluded from participating in the study:

1. Use of any medication, including any prescription, over-the-counter, herbal tea, or other supplements, within 5 days of dosing (before first dose of study medications), with the exception of those approved by the investigator and sponsor.
2. Receipt of any investigational agent or study treatment within 30 days before day 1.
3. Receipt of any protein or antibody-based therapeutic agents (eg, growth hormones or monoclonal antibodies) within 3 months before screening. (Note: Influenza vaccine was allowed if administered $>$21 days before day 1.)
4. Previous exposure to any SGLT inhibitor, including LX4211.
5. Use of cigarettes or any tobacco product within 6 weeks before screening and while participating in the study (day 2 through discharge).
6. History of bariatric surgery or any other gastrointestinal surgery that may have induced malabsorption.
7. History of any major surgery within 6 months before screening.
8. History of any serious adverse reaction or hypersensitivity to any inactive component of LX4211, ie, microcrystalline cellulose, croscarmellose sodium (disintegrant), talc, silicone dioxide, and magnesium stearate (nonbovine), unless reaction was deemed irrelevant to the study by the investigator and sponsor.
9. History of renal disease or significantly abnormal kidney function tests (glomerular filtration rate $<$80 mL/min as calculated using the Cockcroft-Gault equation) at screening.
10. History of hepatic disease or significantly abnormal liver function tests ($>$1.5 times the upper limit of normal). (Note: isolated bilirubin $>$1.5 times the upper limit of normal was acceptable if bilirubin was fractionated and direct bilirubin $<$35%.)
11. History of any clinically relevant psychiatric, renal, hepatic, pancreatic, cardiovascular, neurological, or gastrointestinal abnormality.
12. History of any active infection within 30 days before day 1.

13. History of alcohol or substance abuse within 2 years before day 1.
14. Known history of hepatitis B surface antigen, hepatitis C antibody, human immunodeficiency virus type 1 or human immunodeficiency virus type 2.
15. Existence of any surgical or medical condition that, in the judgment of the investigator, might have interfered with the absorption, distribution, metabolism, or excretion of LX4211.
16. Presence of clinically significant physical, laboratory, or ECG findings (including QT interval corrected using Fridericia's formula >450 msec or PR interval >210 msec) at screening that, in the opinion of the investigator and/or sponsor may have interfered with any aspect of study conduct or interpretation of results.
17. Concurrent conditions that could have interfered with safety and/or tolerability measurements.
18. Donation or loss of >400 mL of blood or blood product within 3 months of day 1.
19. Positive urine glucose at screening.
20. A positive pregnancy test at screening (females only).
21. Positive urine screen for drugs of abuse at screening.
22. Positive breath test for alcohol at screening.
23. Need for dietary restrictions, unless the restrictions were approved by the investigator and sponsor.
24. Inability or difficulty swallowing whole tablets or capsules.
25. Unable or unwilling to communicate or cooperate with the investigator for any reason.

Supplemental Table I. Statistical Analysis Summary of 24-Hour Urinary Glucose Excretion (g/24 h) UGE (PD Population).

	LX4211 Dose Schedule									
Statistic	A		B		C		D		E	
N	10		10		10		10		10	
Least-squares mean change from baseline (95% CI)	33.62 (30.68–36.55)		37.19 (34.25–40.12)		34.06 (31.12–37.00)		35.72 (32.7–38.66)		36.80 (33.87–39.74)	
P	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
Between-group treatment comparisons	A vs B	A vs C	A vs D	A vs E	B vs C	B vs D	B vs E	C vs D	C vs E	D vs E
Least-squares mean change from baseline (95% CI)	−3.57 (−7.74 to 0.61)	−0.44 (−4.62 to 3.73)	−2.11 (−6.28 to 2.07)	−3.19 (−7.36 to 0.99)	−3.12 (−1.05 to 7.30)	−1.46 (−2.71 to 5.64)	−0.38 (−3.79 to 4.56)	−1.66 (−5.84 to 2.51)	−2.74 (−6.92 to 1.43)	−1.08 (−5.26 to 3.09)

	Pooled LX4211-treated subjects versus placebo from baseline (day −1) to the averaged response over days 8–12		
	LX4211	Placebo	LX4211 vs Placebo
Least-squares mean change from baseline (95% CI)	35.48 (32.99–37.97)	0.08 (5.49–5.64)	35.40 (29.30–41.50)
P	< 0.001	0.98	< 0.001

A = dosing 1 hour before breakfast; B = dosing 0.5 hour before breakfast; C = dosing immediately before breakfast; D = dosing immediately before lunch; E = dosing a split dose 1 hour before breakfast and dinner.

Supplemental Table II. Statistical Analysis of Baseline (Day -1) Adjusted Plasma PD Parameters of PPG, Insulin, GLP-1 and PYY; Pooled LX4211 Compared to Placebo.

(PD Population)

Parameter	Treatment	N	LS Mean (95% CI)	*P* for Testing LS Mean = 0	Treatment Comparison	LS Mean Difference (95% CI)	*P* of Treatment Comparison
PPG (mg*hr/dL)	LX4211	50	−84.79 (−101.57 to −68.00)	<0.001	LX4211 vs Placebo	−97.59 (−138.71 to −56.47)	<0.001
AUC_{0-last}	Placebo	10	12.80 (−24.73 to 50.34)	0.497			
Insulin (uU*hr/mL)	LX4211	50	−169.86 (−204.51 to −135.22)	<0.001	LX4211 vs Placebo	−25.47 (−110.34 to 59.39)	0.550
AUC_{0-last}	Placebo	10	−144.39 (−221.86 to −66.92)	<0.001			
Total GLP-1	LX4211	50	8.58 (5.78 to 11.38)	<0.001	LX4211 vs Placebo	9.16 (1.97 to 16.35)	0.014
(pmol*hr/L)	Placebo	9	−0.58 (−7.20 to 6.05)	0.862			
AUC_{0-4}							
Active GLP–1	LX4211	50	5.22 (2.51 to 7.93)	<0.001	LX4211 vs Placebo	8.89 (1.92 to 15.85)	0.013
(pmol*hr/L)	Placebo	9	−3.67 (−10.09 to 2.75)	0.257			
AUC_{0-4}							
Total PYY	LX4211	50	41.33 (32.19 to 50.47)	<0.001	LX4211 vs Placebo	42.16 (19.77 to 64.55)	<0.001
(pmol*hr/L)	Placebo	10	−0.83 (−21.27 to 19.61)	0.936			
AUC_{0-4}							

Note: The analysis is based on an analysis of variance model with fixed effects for treatment (LX4211 versus placebo), period (Days 8, 9, 10, 11, and 12), and treatment-by-period (if $P < 0.10$). The dependent variable is change from baseline (Day −1) of the pharmacodynamic parameter for LX4211 and placebo treatments.

A = dosing 1 hour before breakfast; B = dosing 0.5 hour before breakfast; C = dosing immediately before breakfast; D = dosing immediately before lunch; E = dosing a split dose 1 hour before breakfast and dinner.

Supplemental Table III. Statistical Analysis Summary of Fasting Plasma Glucose (mg/dL) FPG (PD Population).

	LX4211 Dose Schedule				
Statistic	A	B	C	D	E
N	10	10	10	10	10
Least-squares mean change from baseline (95% CI)	−8.19 (−10.3 to −6.06)	−5.17 (−7.30 to −3.03)	−6.12 (−8.26 to −3.98)	−6.14 (−8.28 to −4.01)	−6.68 (−8.81 to −4.54)
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

	Pooled LX4211-treated subjects versus placebo from baseline (day −1) to the averaged response over days 8–12.		
	LX4211	Placebo	LX4211 vs Placebo
Least-squares mean change from baseline (95% CI)	−6.46 (−8.09 to −4.83)	−0.60 (−4.23 to 3.03)	−5.86 (−9.84 to −1.88)
P	< 0.001	0.74	0.005

A = dosing 1 hour before breakfast; B = dosing 0.5 hour before breakfast; C = dosing immediately before breakfast; D = dosing immediately before lunch; E = dosing a split dose 1 hour before breakfast and dinner.

Supplementary Table IV. Statistical Analysis of Baseline (Day -1) Adjusted Plasma PD Parameters of PPG, Insulin, GLP-1 and PYY; LX4211 Compared to Placebo.

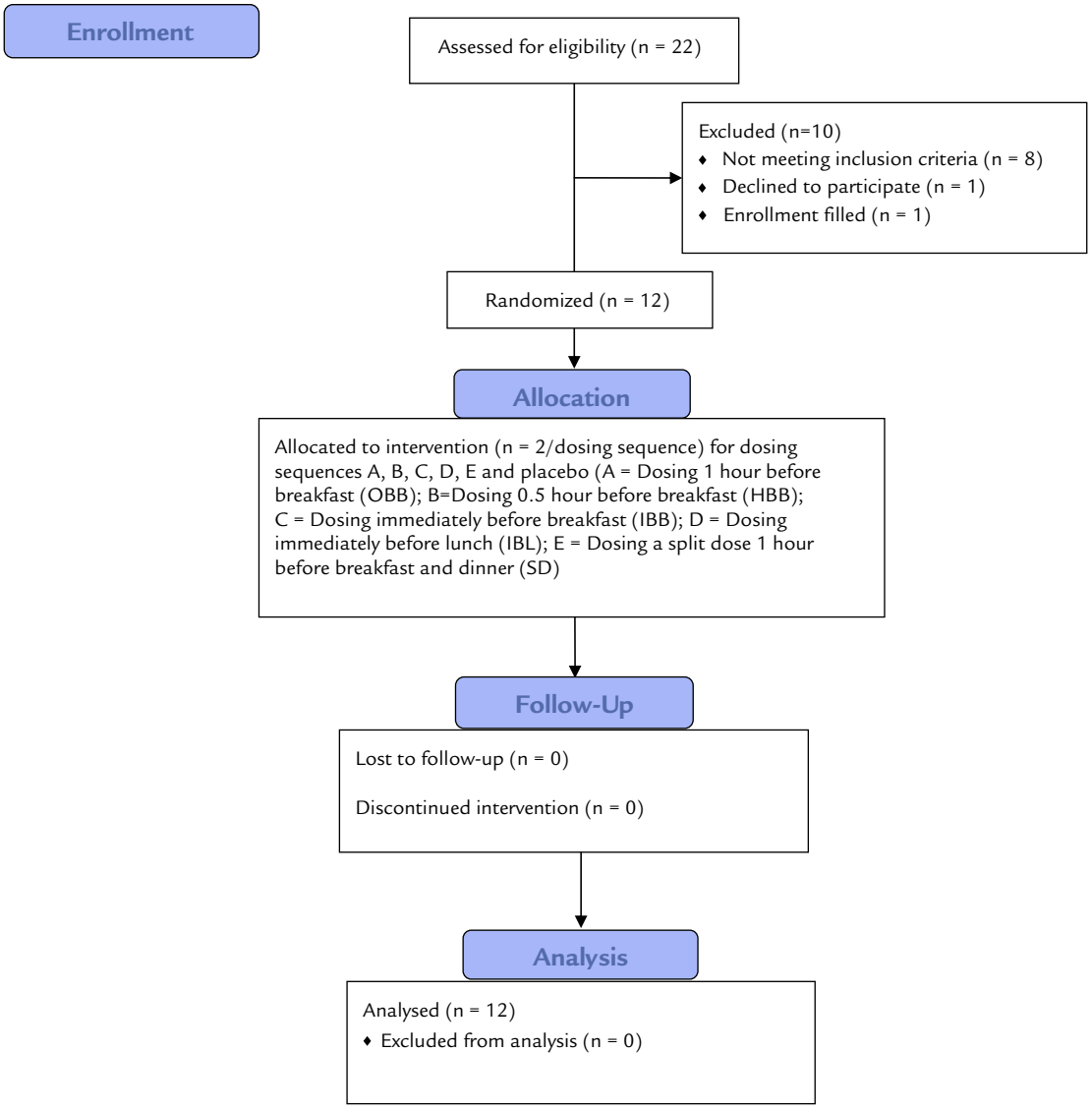
Parameter	Dose Sch.	N	LS Mean (95% CI)	*P* for Testing LS Mean = 0	Dose Schedule Comparison	LS Mean Difference (95% CI)	*P* Dose Schedule Comparison
PPG (mg*hr/dL) AUC_{0-4}	A	10	−55.33 (−68.27 to −42.38)	< 0.001	A vs B	−5.84 (−24.25 to 12.56)	0.521
	B	10	−49.49 (−62.43 to −36.54)	< 0.001	A vs C	−11.07 (−29.47 to 7.34)	0.228
	C	10	−44.26 (−57.21 to −31.31)	< 0.001	A vs D	−11.70 (−30.10 to 6.71)	0.204
	D	10	−43.63 (−56.57 to −30.68)	< 0.001	A vs E	4.30 (−14.10 to 22.71)	0.636
	E	10	−59.63 (−72.57 to −46.68)	< 0.001	B vs C	−5.23 (−23.63 to 13.18)	0.566
					B vs D	−5.86 (−24.26 to 12.55)	0.520
					B vs E	10.14 (−8.26 to 28.55)	0.269
					C vs D	−0.63 (−19.04 to 17.77)	0.944
					C vs E	15.37 (−3.04 to 33.77)	0.098
					D vs E	16.00 (−2.40 to 34.41)	0.086
Insulin (uU*hr/mL) AUC_{0-4}	A	10	−81.33 (−100.82 to −61.83)	< 0.001	A vs B	8.58 (−19.14 to 36.30)	0.531
	B	10	−89.91 (−109.40 to −70.41)	< 0.001	A vs C	30.96 (3.24 to 58.67)	0.030
	C	10	−112.29 (−131.78 to −92.79)	< 0.001	A vs D	6.94 (−20.78 to 34.66)	0.612
	D	10	−88.27 (−107.77 to −68.78)	< 0.001	A vs E	−2.68 (−30.40 to 25.03)	0.844
	E	10	−78.64 (−98.14 to −59.15)	< 0.001	B vs C	22.38 (−5.34 to 50.09)	0.109
					B vs D	−1.64 (−29.35 to 26.08)	0.905
					B vs E	−11.26 (−38.98 to 16.45)	0.412
					C vs D	−24.02 (−51.73 to 3.70)	0.087
					C vs E	−33.64 (−61.36 to −5.92)	0.019
					D vs E	−9.63 (−37.34 to 18.09)	0.483
Total GLP-1 (pmol*hr/L) AUC_{0-4}	A	10	6.72 (1.24 to 12.19)	0.018	A vs B	−4.89 (−12.67 to 2.89)	0.209
	B	10	11.61 (6.13 to 17.08)	< 0.001	A vs C	−8.13 (−15.91 to −0.35)	0.041
	C	10	14.85 (9.37 to 20.32)	< 0.001	A vs D	4.26 (−3.53 to 12.04)	0.272
	D	10	2.46 (−3.02 to 7.93)	0.366	A vs E	−0.57 (−8.36 to 7.21)	0.881
	E	10	7.29 (1.81 to 12.76)	0.011	B vs C	−3.24 (−11.02 to 4.54)	0.401
					B vs D	9.15 (1.36 to 16.93)	0.023
					B vs E	4.32 (−3.47 to 12.10)	0.265
					C vs D	12.39 (4.61 to 20.17)	0.003
					C vs E	7.56 (−0.22 to 15.34)	0.057

(continued)

Supplementary Table IV. (continued).

Parameter	Dose Sch.	N	LS Mean (95% CI)	*P* for Testing LS Mean = 0	Dose Schedule Comparison	LS Mean Difference (95% CI)	*P* Dose Schedule Comparison
					D vs E	−4.83 (−12.61 to 2.95)	0.214
Active GLP–1 (pmol*hr/L)	A	10	4.39 (0.19 to 8.59)	0.041	A vs B	−3.59 (−9.56 to 2.38)	0.228
AUC_{0-4}	B	10	7.98 (3.78 to 12.18)	< 0.001	A vs C	−5.12 (−11.09 to 0.85)	0.090
	C	10	9.51 (5.31 to 13.71)	< 0.001	A vs D	2.87 (−3.10 to 8.84)	0.333
	D	10	1.52 (−2.68 to 5.72)	0.464	A vs E	1.70 (−4.27 to 7.67)	0.565
	E	10	2.69 (−1.51 to 6.89)	0.200	B vs C	−1.53 (−7.50 to 4.44)	0.605
					B vs D	6.46 (0.49 to 12.43)	0.035
					B vs E	5.29 (−0.68 to 11.26)	0.080
					C vs D	7.99 (2.01 to 13.96)	0.011
					C vs E	6.81 (0.84 to 12.78)	0.027
					D vs E	−1.17 (−7.14 to 4.80)	0.691
Total PYY (pmol*hr/L) AUC_{0-4}	A	10	44.00 (29.00 to 59.00)	< 0.001	A vs B	−8.74 (−30.07 to 12.59)	0.408
	B	10	52.74 (37.74 to 67.74)	< 0.001	A vs C	−8.25 (−29.58 to 13.08)	0.435
	C	10	52.25 (37.25 to 67.25)	< 0.001	A vs D	23.34 (2.01 to 44.67)	0.033
	D	10	20.66 (5.66 to 35.66)	0.009	A vs E	6.99 (−14.34 to 28.31)	0.508
	E	10	37.01 (22.01 to 52.01)	< 0.001	B vs C	0.49 (−20.84 to 21.82)	0.963
					B vs D	32.08 (10.75 to 53.41)	0.005
					B vs E	15.73 (−5.60 to 37.05)	0.142
					C vs D	31.59 (10.26 to 52.92)	0.005
					C vs E	15.24 (−6.09 to 36.56)	0.155
					D vs E	−16.35 (−37.68 to 4.97)	0.127

Note: The analysis is based on an analysis of variance model with fixed effects for subject, dose schedule, carryover and period (Days 8, 9, 10, 11, and 12). The dependent variable is change from baseline (Day -1) of the PD parameter for LX4211 treatment.
A = dosing 1 hour before breakfast; B = dosing 0.5 hour before breakfast; C = dosing immediately before breakfast; D = dosing immediately before lunch; E = dosing a split dose 1 hour before breakfast and dinner. The asterisk in these units is "times."



Supplemental Figure 1. CONSORT Diagram