

# Effect of LX4211 on Glucose Homeostasis and Body Composition in Preclinical Models<sup>§</sup>

David R. Powell, Christopher M. DaCosta, Melinda Smith, Deon Doree, Angela Harris, Lindsey Buhning, William Heydorn, Amr Nouraldeen, Wendy Xiong, Padmaja Yalamanchili, Faika Mseeh, Alan Wilson, Melanie Shadoan, Brian Zambrowicz, and Zhi-Ming Ding

*Lexicon Pharmaceuticals, Inc., The Woodlands, Texas (D.R.P., C.M.D., M.Sm., D.D., A.H., L.B., A.N., W.X., F.M., A.W., M.Sh., B.Z., Z.-M.D.); and Lexicon Pharmaceuticals, Inc., Princeton, New Jersey (W.H., P.Y.)*

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## ABSTRACT

Treatments that lower blood glucose levels and body weight should benefit patients with type 2 diabetes mellitus (T2DM). We developed LX4211 [(2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-3-(4-ethoxybenzyl)phenyl)-6-(methylthio)tetrahydro-2*H*-pyran-3,4,5-triol], an orally available small molecule that decreases postprandial glucose excursions by inhibiting intestinal sodium/glucose cotransporter 1 (SGLT1) and increases urinary glucose excretion (UGE) by inhibiting renal SGLT2. In clinical studies of patients with T2DM, LX4211 appears to act through dual SGLT1/SGLT2 inhibition to improve glycemic control and promote weight loss. Here, we present preclinical studies that explored the ability of LX4211 to improve glycemic control and promote weight loss. We found that 1) LX4211 inhibited *in vitro* glucose transport mediated by mouse, rat, and dog SGLT1 and SGLT2; 2) a single daily LX4211 dose markedly increased UGE for >24 hours in mice, rats, and dogs;

and 3) in the KK.Cg-Ay/J heterozygous (KKA<sup>Y</sup>) mouse model of T2DM, LX4211 lowered A1C and postprandial glucose concentrations while increasing postprandial glucagon-like peptide 1 concentrations. Also, long-term LX4211 treatment 1) decreased oral glucose tolerance test (OGTT) glucose excursions, increased OGTT 30-minute insulin concentrations and increased pancreatic insulin content in KKA<sup>Y</sup> mice; and 2) decreased weight gain in dogs and rats but not in KKA<sup>Y</sup> mice while increasing food consumption in dogs, rats, and KKA<sup>Y</sup> mice; in these KKA<sup>Y</sup> mice, calories lost through UGE were completely offset by calories gained through hyperphagia. These findings suggest that LX4211 improves glycemic control by dual SGLT1/SGLT2 inhibition in mice as in humans, and that the LX4211-mediated weight loss observed in patients with T2DM may be attenuated by LX4211-mediated hyperphagia in some of these individuals.

## Introduction

An estimated 26 million people in the United States have diabetes, with the prevalence projected to increase to 44 million by 2034 (Huang et al., 2009). Patients with type 1 and type 2 diabetes mellitus (T2DM) are at increased risk for the microvascular complications of renal failure and retinopathy, and for macrovascular complications, such as myocardial infarction and stroke. Available data suggest that lowering blood glucose and/or body weight decreases the risk of microvascular complications and may decrease the risk of macrovascular complications in individuals with T2DM (National Institutes of Health, 1998; Reusch And Wang, 2011; American Diabetes Association, 2013).

T2DM develops due to a defect in the ability of  $\beta$  cells to compensate for insulin resistance with sufficient glucose-stimulated

insulin secretion to normalize blood glucose levels. The natural history of T2DM is a progressive decline in  $\beta$ -cell function. Among other factors, high glucose levels appear to be a significant contributor to impaired  $\beta$ -cell function, a process referred to as glucotoxicity (Wajchenberg, 2007; DeFronzo, 2009).

The above observations suggest that treatments that lower blood glucose levels and body weight should be of value to patients with T2DM. Such effects are observed in patients treated with Roux-en-Y gastric bypass surgery; this treatment often reverses T2DM, even in individuals with long-standing disease (Cohen et al., 2012; Kashyap et al., 2013). Marked calorie restriction can duplicate this effect in patients with T2DM of shorter duration (Lim et al., 2011). In contrast to Roux-en-Y gastric bypass surgery and caloric restriction, the orally available glucose-lowering agents that have been mainstays of antidiabetic therapy for years, including metformin, sulfonylureas, thiazolidinediones, and dipeptidylpeptidase

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**ABBREVIATIONS:** AUC, area under the curve; CL, clearance;  $C_{max}$ , plasma peak concentration; ED<sub>50</sub>, dose causing 50% maximal UGE in the first 24 hours following dosing; %F, oral bioavailability; GLP-1, glucagon-like peptide 1; HEK293, human embryonic kidney 293 cells; HFD, high-fat diet; KKA<sup>Y</sup>, KK.Cg-Ay/J heterozygous; LX4211, (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-3-(4-ethoxybenzyl)phenyl)-6-(methylthio)tetrahydro-2*H*-pyran-3,4,5-triol; OGTT, oral glucose tolerance test; OP-CD, Obese Prone CrI:CD; QMR, quantitative magnetic resonance; S-D, Sprague-Dawley; SGLT1, sodium/glucose cotransporter 1; SGLT2, sodium/glucose cotransporter 2; T-1095, 3-(benzo[b]furan-5-yl)-2',6'-dihydroxy-4'-methylpropiofenone 2'-O-(6-O-methoxycarbonyl- $\beta$ -D-glucopyranoside); T2DM, type 2 diabetes mellitus;  $T_{max}$ , time of plasma peak concentration; UGE, urinary glucose excretion.

4 inhibitors, do not promote significant weight loss and many promote weight gain in patients with T2DM (Nathan et al., 2009; American Diabetes Association, 2013).

The diabetes-mediated impairment in  $\beta$ -cell insulin secretion can be reversed by lowering blood glucose levels. This was first demonstrated using phlorizin, an inhibitor of sodium/glucose cotransporter 2 (SGLT2)-mediated renal glucose reabsorption, to increase urinary glucose excretion (UGE) in partially pancreatectomized rats (Rossetti et al., 1987). Phlorizin was not developed as an antidiabetic agent due to lack of efficacy when given orally, but orally available compounds that increase UGE by inhibiting renal SGLT2 have been developed; these compounds lower blood glucose and body weight while potentially protecting pancreatic  $\beta$  cells (Abdul-Ghani et al., 2012; Musso et al., 2012). In addition, we have developed LX4211 [(2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-3-(4-ethoxybenzyl)phenyl)-6-(methylthio)tetrahydro-2*H*-pyran-3,4,5-triol], a dual SGLT1/2 inhibitor (Goodwin et al., 2009; Zambrowicz et al., 2012) that has some structural similarity to phlorizin (Zambrowicz et al., 2012). In mice and humans, LX4211 not only increased UGE but also inhibited intestinal SGLT1, resulting in higher circulating glucagon-like peptide 1 (GLP-1) levels and lower postprandial glucose excursions (Powell et al., 2013a,b; Zambrowicz et al., 2013a,b). This dual inhibition, which resulted in improved glycemic control and weight loss in clinical studies of patients with T2DM (Rosenstock et al., 2012; Zambrowicz et al., 2012), provides two complementary mechanisms, each of which is insulin-independent, for improving glycemic control while potentially lowering demands on  $\beta$  cells in individuals with T2DM. The studies presented here provide further evidence that LX4211 is a dual SGLT1/SGLT2 inhibitor, examine the effect of LX4211 on UGE, food consumption, and weight gain in multiple species, and demonstrate the ability of LX4211 to improve glycemic control while increasing pancreatic  $\beta$ -cell insulin stores in the KKA<sup>y</sup> mouse model of T2DM.

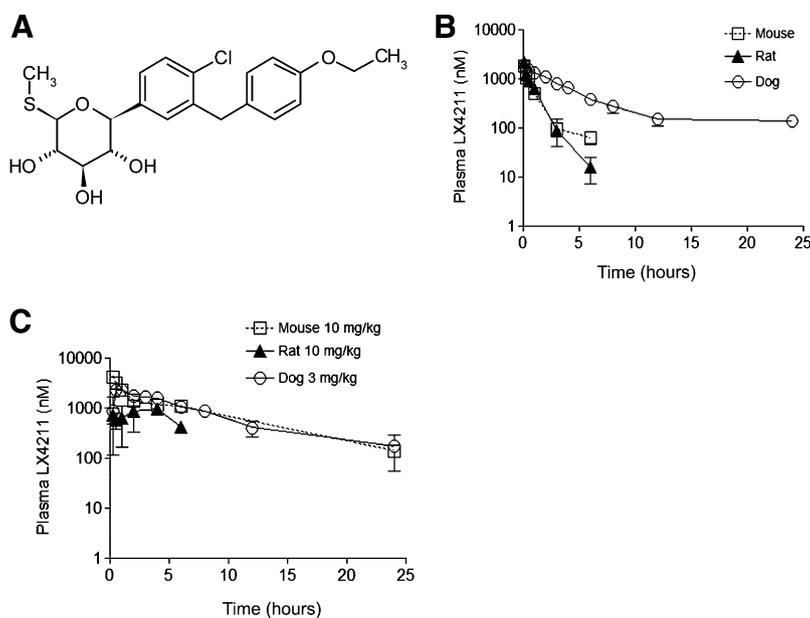
## Materials and Methods

**Animals.** All reported studies were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols for all studies performed at Lexicon Pharmaceuticals were approved by the Lexicon Institutional Animal Care and Use Committee (OLAW Assurance Number, A4152-01; AAALAC International Accreditation Number, 001025). The protocols for all studies performed at Covance Laboratories were approved by the Covance Institutional Animal Care and Use Committee (Animal Welfare Assurance No. A3218-01; USDA Registration No. 35-R-0030).

All mouse and some rat studies were performed at Lexicon Pharmaceuticals. KK.Cg-Ay/J heterozygous mice (KKA<sup>y</sup> mice; The Jackson Laboratory, Bar Harbor, ME) were obtained at 4 weeks of age and fed a high-fat diet (HFD) containing 45% kcal as fat (45% HFD, D12451; Research Diets, New Brunswick, NJ). C57BL/6-Tyrc-Brd (C57) weanling mice from an in-house colony were fed either 45% HFD for at least 20 weeks before the study to induce obesity, or were fed a standard rodent chow diet (5010, LabDiet; PMI Nutrition International, St. Louis, MO) containing 23% calories as fat. Obese Prone Crl:CD rats (OP-CD rats; Charles River Laboratories International, Wilmington, MA) were obtained at 4 weeks of age and fed HFD containing 60% kcal as fat (60% HFD, D12492; Research Diets). Crl:CD Sprague-Dawley (S-D) rats (Charles River Laboratories) were obtained at 4 weeks of age and fed either chow diet or low-fat diet containing 10% kcal as fat (D12450B; Research Diets). All mice and rats were maintained in a temperature-controlled environment on a fixed 12-hour light/dark cycle with free access to water and food.

Some rat and all dog studies were performed at Covance Laboratories (Madison, WI). During the 4- and 13-week rat toxicology studies, S-D rats were fed Teklad Certified Rodent Diet 8728C (Harlan Laboratories, Indianapolis, IN). During the 4-week dog toxicology study, beagle dogs were fed Teklad Certified Canine Diet 2027C (Harlan Laboratories). During the short-term dog pharmacokinetic/UGE studies, beagle dogs were fed noncertified Canine Diet 5L03 (PMI Nutrition International) and were provided with certified canine treats, as appropriate. Rats and dogs were housed in temperature-controlled environments on a fixed 12-hour light/dark cycle with free access to water and food.

**Body Composition Measurements.** Body composition of mice and rats was measured using quantitative magnetic resonance (QMR; ECHO Medical Systems, Houston, TX) technologies.



**Fig. 1.** Pharmacokinetic studies of LX4211 in three pre-clinical species. (A) Chemical structure of LX4211. Plasma concentrations of LX4211 (mean  $\pm$  S.D.) were measured at various times after (B) an intravenous dose of 1 mg/kg to each species, and (C) an oral dose of 10 mg/kg to mice and rats, and an oral dose of 3 mg/kg to dogs.

**Glucose Tolerance Tests.** Oral glucose tolerance tests (OGTTs) were performed on conscious, unanesthetized mice. After an overnight fast, mice were bled at baseline and then received 2 g/kg glucose by oral gavage. Whole-blood samples obtained from the retro-orbital plexus at 0, 30, 60, 90, and 120 minutes were directly assayed for glucose levels by ACCU-CHEK Aviva glucometer (Roche, Indianapolis, IN); serum obtained at 0 and 30 minutes was used to measure insulin levels (Ultra Sensitive Rat Insulin ELISA Kit, Cat. 90060; Crystal Chem, Downers Grove, IL).

**Glucose Levels and pH of Gastrointestinal Contents.** For fed OP-CD rats, glucose concentration, pH, and volume of cecal contents were measured as described previously elsewhere (Powell et al., 2013a).

**Pancreatic Insulin Content.** At necropsy, the pancreas was isolated, weighed, and snap-frozen with glass beads in bead beater tubes. At the time of assay, 295 ml 95% EtOH was mixed with 5 ml 12N HCl (37.5%) to make acidified ethanol. Chilled (4°C) acidified EtOH was added to each tube at the ratio of 1 g pancreas/10 ml (1:10 w/v). After homogenizing for 3 minutes at 4°C, the mixture was incubated at 4°C for 48 hours and then centrifuged at 3000g for 30 minutes at 4°C. The supernatant was then assayed for insulin (Ultra Sensitive Rat Insulin ELISA Kit), and total pancreatic insulin content was calculated.

**24-Hour UGE.** For mouse studies, 24- to 35-week-old male C57 mice with diet-induced obesity (30–51 g) were individually housed in metabolic cages (Nalge Nunc International, Rochester, NY) and fed 45% HFD homogenized in water at a 2:1 (wt:wt) ratio; this paste diet prevented contamination of urine with crumbled diet. After an acclimatization period, each mouse received, by oral gavage, a single dose of either vehicle or vehicle containing LX4211. For this and all other studies, the gavage volume was 5 ml/kg and the vehicle was aqueous 0.1% v/v Tween 80 unless stated otherwise. Complete 24-hour urine samples were collected during the 24 hours after dosing (day 1) and, for some mice, on days 2 and 3. In a separate study, 12- to 13-week-old female (38–55 g) KKA<sup>y</sup> mice had UGE measured by the same protocol except that they received LX4211 daily.

For rat studies, 11- to 15-week-old male (367–531 g) S-D rats were individually housed in metabolic cages (Nalge Nunc International) and fed low-fat diet homogenized in water at a 1:1 (wt:wt) ratio. After an acclimatization period, each rat received, by oral gavage, a single dose of either vehicle or LX4211. Complete 24-hour urine samples were collected on days 1 and 2 and, for some rats, on days 3 and 4. In separate studies, 9-week-old male OP-CD rats had UGE measured by the same protocol except that the rats received daily doses of either vehicle or LX4211.

For the above mouse and rat studies, the volume of each 24-hour urine collection was recorded and then the urine sample was centrifuged and analyzed for glucose concentration (Cobas Integra 400 Clinical Chemistry Autoanalyzer; Roche).

Adult, non-naïve male (8–12 kg) beagle dogs were fasted overnight and then individually housed in cages designed for separation of urine and feces and for collection of urine. Each dog received, by oral gavage, a single dose of either vehicle or LX4211. All dogs were provided free access to food at 4 hours after dosing. Complete 24-hour urine samples were collected on day 1 and, for some dogs, on day 2. After the volume of each 24-hour urine collection was recorded, the urine sample was centrifuged and frozen at –70°C for later batch analysis of glucose concentrations (Cobas Integra 400 Clinical Chemistry Autoanalyzer). For all species, the absolute quantity of glucose excreted per day per animal was calculated based on urine volume and urine glucose concentration.

**Pharmacokinetics.** Adult male C57 mice (25–35 g) and male S-D rats (250–450 g) were maintained on chow diet. Adult male beagle dogs are the same animals used in the UGE studies described previously; they were fasted overnight before dosing and were fed 6 hours after dosing. All animals had free access to water and were conscious through the study.

TABLE 1  
LX4211 inhibits SGLT-mediated glucose transport

Species	SGLT1		SGLT2	
	IC <sub>50</sub>	N	IC <sub>50</sub>	N
	<i>nM</i>		<i>nM</i>	
Human <sup>a</sup>	36 ± 9	9	1.8 ± 0.4	8
Mouse <sup>b</sup>	62 ± 26	8	0.6 ± 0.2	8
Rat	104 ± 38	11	0.5 ± 0.3	10
Dog	86 ± 14	8	1.4 ± 0.5	8

IC<sub>50</sub>, concentration causing half-maximal inhibition; N, number of determinations.

<sup>a</sup>Data are from Zambrowicz et al., 2012.

<sup>b</sup>Data are from Powell et al., 2013b.

For intravenous administration, LX4211 was dissolved in 15% Captisol to form a clear solution and was administered at a dose of 1 mg/kg to all species. For oral administration, LX4211 was gavaged to mice and rats at a dose of 10 mg/kg and to dogs at 3 mg/kg. After intravenous injection, serial blood samples were collected in EDTA-containing tubes through 6 hours in rodents and up to 48 hours in dogs. After oral administration, serial blood samples were collected through 24 hours in rodents and up to 48 hours in dogs. The plasma fraction was immediately separated by centrifugation at 4°C, and then stored at –20°C until sample analysis. Bioanalysis of plasma samples for quantitating plasma LX4211 concentrations was conducted using liquid chromatography/mass spectroscopy.

**Pharmacokinetic Data Analysis.** All plasma concentration versus time data for LX4211 were analyzed using noncompartmental model 200 (for oral administration) and 201 (for intravenous administration) of WinNonlin (version 5.0; Pharsight, Inc. Mountain View, CA). The half-life during the terminal phase was calculated from the elimination rate constant ( $\lambda$ ) determined by the linear regression analysis of the log-linear part of the plasma concentration curve. The area under the curve (AUC<sub>0–t</sub>) was calculated using linear up/log down trapezoidal method up to the last measured concentration at time *t*. AUC<sub>0–∞</sub> was calculated as AUC<sub>0–t</sub> + C<sub>t</sub>/λ. Clearance (CL) was calculated by dose/AUC<sub>0–∞</sub>. Other pharmacokinetic parameters included plasma peak concentration (C<sub>max</sub>), the time of C<sub>max</sub> (T<sub>max</sub>), and volume of distribution at steady state (V<sub>ss</sub>). Oral bioavailability (%F) was calculated by the following equation:

$$\%F = (\text{AUC}_{0-\infty} \text{ p.o.} / \text{AUC}_{0-\infty} \text{ i.v.}) \times (\text{Dose i.v.} / \text{Dose p.o.})$$

**KKA<sup>y</sup> Diabetic Mouse Studies.** Female KKA<sup>y</sup> mice were individually housed and fed 45% HFD. In study 1, 10-week-old mice were acclimatized for 2 weeks and then, based on A1C and OGTT glucose AUC values, were randomized into two groups that began to receive, as dietary admixture, either vehicle or LX4211 at a dose of 1 mg/kg per day. In study 2, 6- to 7-week-old mice were acclimatized for 1 week and then, based on A1C and body weight data, were randomized into three groups that began to receive as a dietary admixture either vehicle or LX4211 dosed at 1 or 10 mg/kg per day. Measurements performed on the mice included UGE while in metabolic cages, body composition by QMR, body weight, and food consumption. Glycemic control was evaluated by A1C levels (study 1:

TABLE 2  
Pharmacokinetic parameters after a 1 mg/kg i.v. dose of LX4211

Species	N	Half-Life	AUC <sub>0–∞</sub>	CL	V <sub>ss</sub>
		<i>h</i>	<i>nM</i> * <i>h</i>	<i>ml/min/kg</i>	<i>l/kg</i>
Dog	5	7.27 ± 1.39	8849 ± 1796	4.6 ± 0.9	1.98 ± 0.34
Rat	4	0.90 ± 0.09	1847 ± 132	21.3 ± 1.5	1.40 ± 0.07
Mouse	4	1.39 ± 0.45	1940 ± 331	20.6 ± 3.0	2.07 ± 0.14

CL, clearance; N, number of animals; V<sub>ss</sub>, volume at steady state.

TABLE 3  
Pharmacokinetic parameters after a 3 or 10 mg/kg oral dose of LX4211

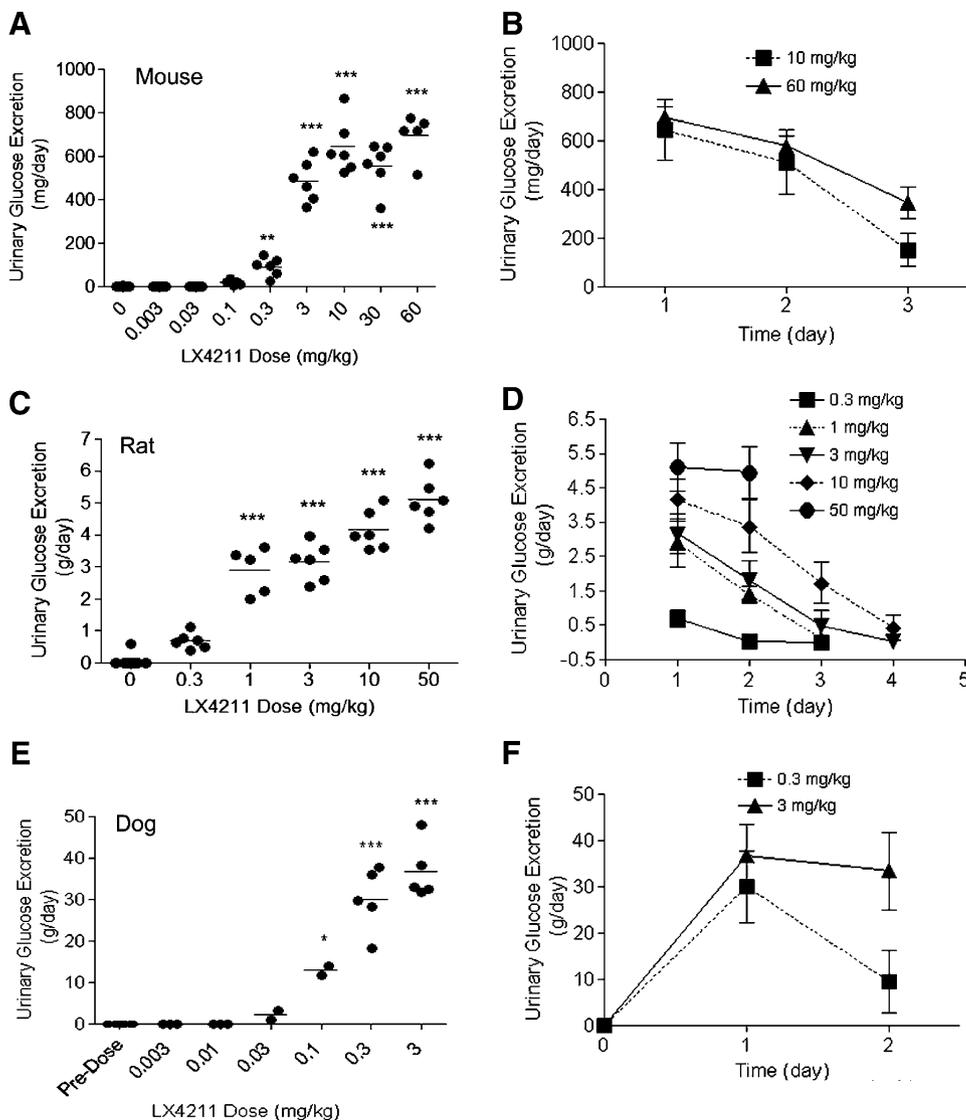
Species	N	Dose	Half-Life	$T_{max}$	$C_{max}$	$AUC_{0-\infty}$	%F
		mg/kg	h	h	nM	nM*h	
Dog	5	3	7.38 ± 1.17	0.65 ± 0.34	2502 ± 325	18775 ± 4132	71 ± 16
Rat	4	10	ND	3.00 ± 1.15	1116 ± 378	ND	ND
Mouse	4	10	6.52 ± 0.82	0.31 ± 0.13	4373 ± 408	19079 ± 1580	98 ± 8

%F, % bioavailable; N, number of animals; ND, not determined.

Cobas Integra 400; study 2: A1c Now+ System; Bayer HealthCare, Tarrytown, NY), fed glucose levels (Cobas Integra 400), and OGTT glucose excursions. The  $\beta$ -cell insulin reserves were evaluated in both studies at necropsy by measuring pancreatic insulin content. On the final day of study 2, the mice received an oral 2 g/kg glucose bolus and then, 2 hours later at necropsy, plasma was obtained to measure total GLP-1 concentrations (Glucagon-Like Peptide-1 Total ELISA Kit, catalog no. EZGLP1T-36K; Millipore Corp., Billerica, MA), as described previously elsewhere (Powell et al., 2013b).

**OP-CD Obese Rat Study.** Beginning at 8 weeks of age, male OP-CD rats were individually housed in metabolic cages and fed 60% HFD. Food consumption and body weight were measured daily

Monday through Friday. Starting at 9 weeks of age, rats were dosed by oral gavage once daily with either vehicle or 30 mg/kg LX4211. Complete 24-hour urine samples were collected on dosing days 2–4, and 24-hour fecal collections on dosing day 4, after which the rats were removed from the metabolic cages. Urine samples were used to calculate UGE as described earlier. Fecal samples were weighed, homogenized with MilliQ water (2 g water to 1 g feces) using a bead beater at 4°C, and then centrifuged at 4°C; the supernatant was analyzed for pH and glucose concentration, and fecal glucose content was calculated from the product of glucose concentration and volume of supernatant. The rats were necropsied in the fed state on day 25 of dosing; glucose levels and pH were measured in cecal contents, and



**Fig. 2.** LX4211 increases UGE in mice, rats, and dogs. UGE was measured over 24-hour intervals as described in *Materials and Methods*. In (A) mice, (C) rats, and (E) dogs, UGE was measured during the first 24 hours after treatment with vehicle or increasing doses of LX4211. After animals received a single dose of LX4211 on day 1, UGE was also measured in (B) mice during three successive 24-hour intervals; (D) rats during two to four successive 24-hour intervals; and (F) dogs during two successive 24-hour intervals. (A, C, and E) \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  versus the vehicle-treated group.

carcasses were analyzed for body composition by QMR, as described earlier.

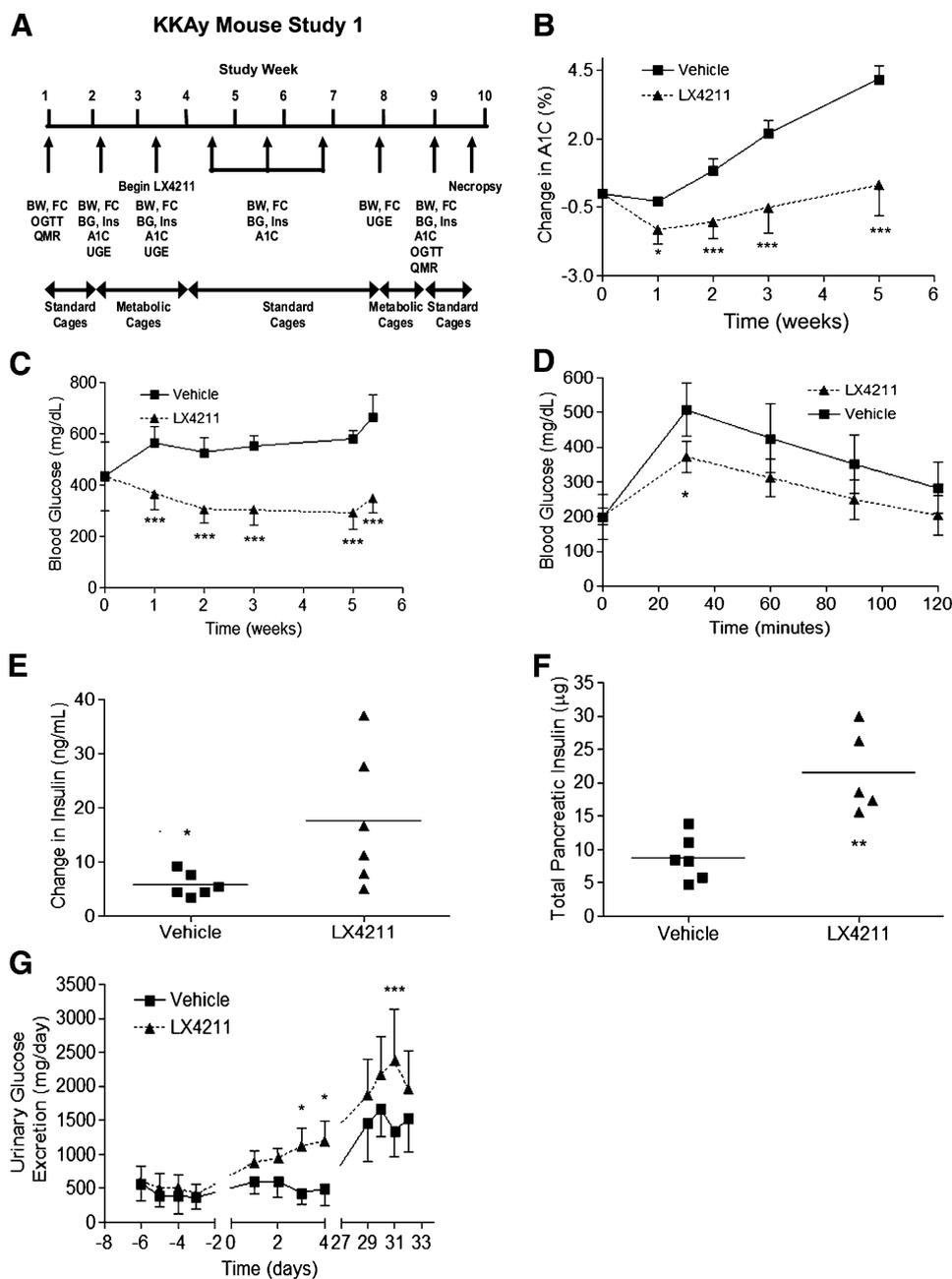
**Toxicology Studies.** Body weight and food consumption data, obtained as part of GLP studies exploring the toxicology of LX4211, were analyzed from dosing groups that showed no significant adverse effects of LX4211 treatment. For all three toxicology studies, the dosing vehicle was 0.25% (w/v) methylcellulose in water.

In the 4-week rat toxicology study, male (220–310 g) S-D rats, 6 to 7 weeks of age at study onset, began to receive single daily 10 ml/kg oral gavage doses of either vehicle (15 rats) or vehicle containing 10 (10 rats), 30 (10 rats) or 100 (15 rats) mg/kg of LX4211. Dosing continued for 4 weeks; during this time, the rats were housed individually in stainless steel cages. Body weights were recorded before the first dose was given on the first day of dosing, and weekly thereafter. Food consumption was measured weekly during the dosing phase of the study.

In the 13-week rat toxicology study, male (310–450 g) S-D rats, 11 to 12 weeks of age at study outset, began to receive single daily 10 ml/kg oral gavage doses of either vehicle (15 rats) or vehicle containing 3 (10 rats), 10 (10 rats), 30 (15 rats), or 100 (15 rats) mg/kg of LX4211. Dosing continued for 13 weeks, and during this time, the rats were housed individually in stainless steel cages. Body weights and food consumption were monitored as in the 4-week rat toxicology study.

In the 4-week dog toxicology study, 13 male (9–10 kg) and 13 female (6–8 kg) purebred beagle dogs, 6.5 to 7.5 months of age at study outset, received single daily oral gavage doses of either vehicle (10 dogs) or vehicle containing 30 (6 dogs) or 100 (10 dogs) mg/kg of LX4211. Dosing continued for 28 days; during this time, the dogs were housed individually in stainless steel cages and were offered diet for at least 6 hrs/d. Body weights and food consumption were monitored as in the 4-week rat toxicology study.

**SGLT2 and SGLT1 Cell Lines.** The full-length coding sequences of rat SGLT2 and SGLT1 (accession numbers NM\_022590.2



**Fig. 3.** LX4211 improves glucose homeostasis in KKA<sup>y</sup> diabetic mice: study 1. (A) Schematic outline of study design. BG, fed blood glucose; BW, body weight; FC, food consumption; Ins, fed serum insulin; UGE, 24-hour UGE measured in metabolic cages. (B) A time course of change in A1C levels. (C) A time course of fed blood glucose levels. (D) Blood glucose excursions from the week 9 OGTT data. (E) Change in serum insulin levels between the 0- and 30-minute time points of the week 9 OGTT. (F) Total pancreatic insulin: at necropsy, the pancreas of each mouse was isolated, and pancreatic insulin was extracted and measured as described in *Materials and Methods*. (G) Multiple 24-hour UGE measurements were made on mice in metabolic cages both before and after the start of LX4211 dosing. (B–G) \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001 versus the vehicle-treated group.

and BC081827) and dog SGLT2 and SGLT1 (accession numbers NM\_001007142 and NM\_001007141) containing hemagglutinin (HA)-tags at the N terminus were cloned into the mammalian expression vector pIRESpuro2 (Clontech, Mountain View, CA). Human embryonic kidney 293 (HEK293) cells (American Type Culture Collection, Manassas, VA) were transfected with the HA-SGLT2-pIRESpuro2 and HA-SGLT1-pIRESpuro2 vectors, and bulk stable cell lines were selected in the presence of 0.5  $\mu\text{g}/\text{ml}$  of puromycin. All HA-SGLT2- and HA-SGLT1-expressing cell lines were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 units penicillin/ml, 0.1 mg/ml streptomycin, and 0.5  $\mu\text{g}/\text{ml}$  of puromycin. These HEK293 cell lines were used in experiments to determine the  $\text{IC}_{50}$  (concentration causing half-maximal inhibition) of LX4211 for SGLT2- and SGLT1-mediated glucose transport.

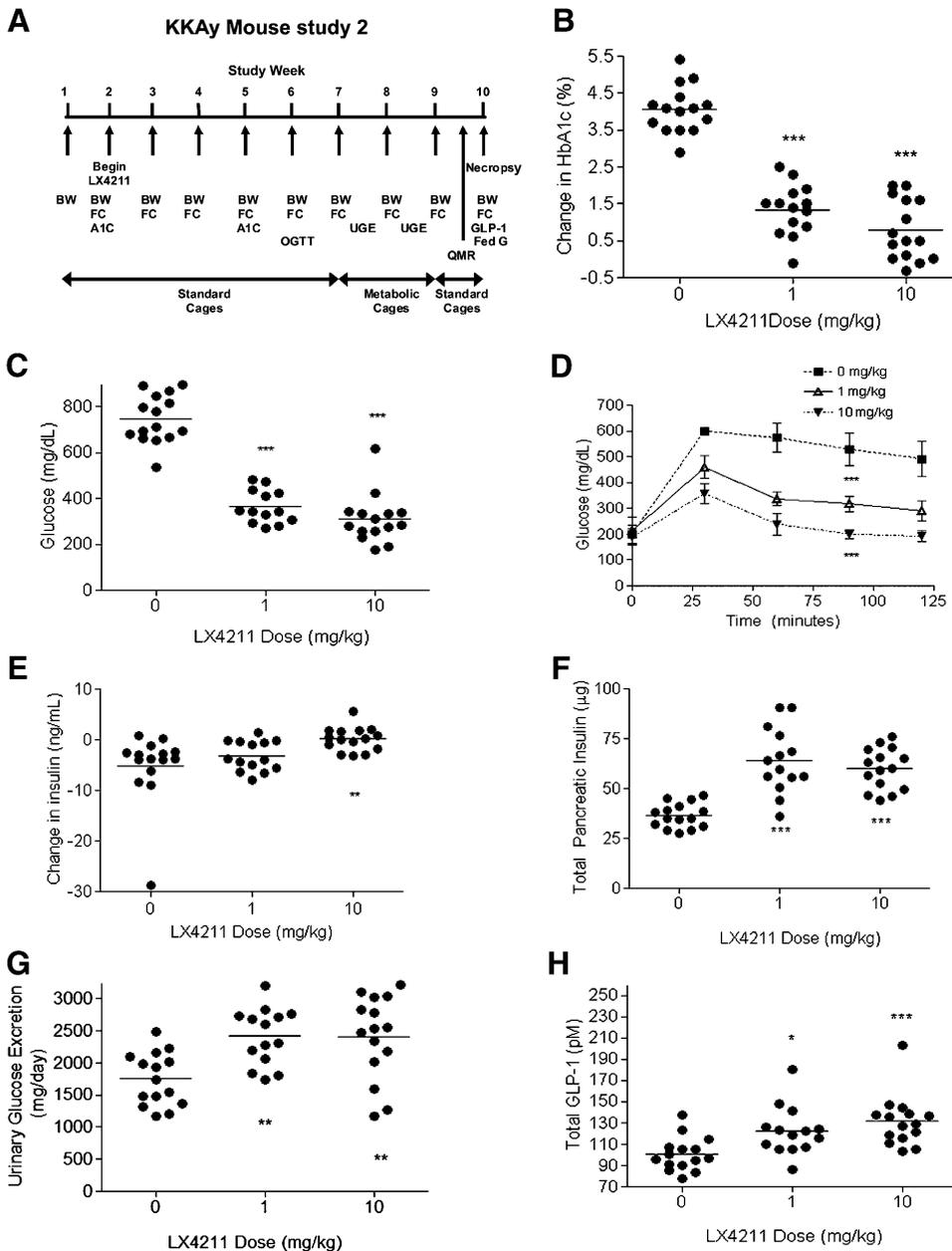
**$\alpha$ -Methylglucopyranoside Uptake Assay.** When expressed in cells, SGLT2 and SGLT1 mediate sodium-coupled uptake of D-glucose or  $\alpha$ -methylglucopyranoside, a nonmetabolizable glucose analog specific for sodium-dependent glucose transporters. The ability of

LX4211 to inhibit SGLT2- and SGLT1-mediated glucose transport was estimated by measuring SGLT2- or SGLT1-mediated  $^{14}\text{C}$ - $\alpha$ -methylglucopyranoside uptake in the presence of increasing compound concentration, as described previously elsewhere (Powell et al., 2013b).

**Statistics.** Data are presented as mean  $\pm$  S.D. Comparisons between two groups were analyzed by unpaired Student's *t*-test. Comparisons among three or more groups were analyzed by one-way analysis of variance with post-hoc analysis performed by the Bonferroni method, except for the 13-week rat study where post-hoc comparisons between each LX4211-treated group with the vehicle group were made using Dunnett's test.  $P < 0.05$  was considered statistically significant.

## Results

**LX4211 Inhibition of SGLT1 and SGLT2 In Vitro.** The structure of LX4211 is presented in Fig. 1A. LX4211 has been shown to inhibit glucose transport by HEK293 cells overexpressing



**Fig. 4.** LX4211 improves glucose homeostasis in KKAy diabetic mice: study 2. (A) Schematic outline of study design. BW, body weight; FC, food consumption; Fed G, fed serum glucose; UGE, 24-hour UGE measured in metabolic cages. (B) Change in A1C between baseline (week 2) and week 6 of the study. (C) Fed serum glucose levels obtained at necropsy. (D) Blood glucose excursions from the week 6 OGTT data. (E) Change in serum insulin levels between the 0- and 30-minute time points of the week 6 OGTT data. (F) Total pancreatic insulin: at necropsy, the pancreas of each mouse was isolated, and pancreatic insulin was extracted and measured as described in *Materials and Methods*. (G) The mean value of two 24-hour UGE measurements made on consecutive days in mice acclimatized in metabolic cages during study week 7 or 8. (H) Total GLP-1 levels measured at necropsy, 2 hours after a 2 g/kg oral glucose bolus. (B–H) \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  versus the vehicle-treated group.

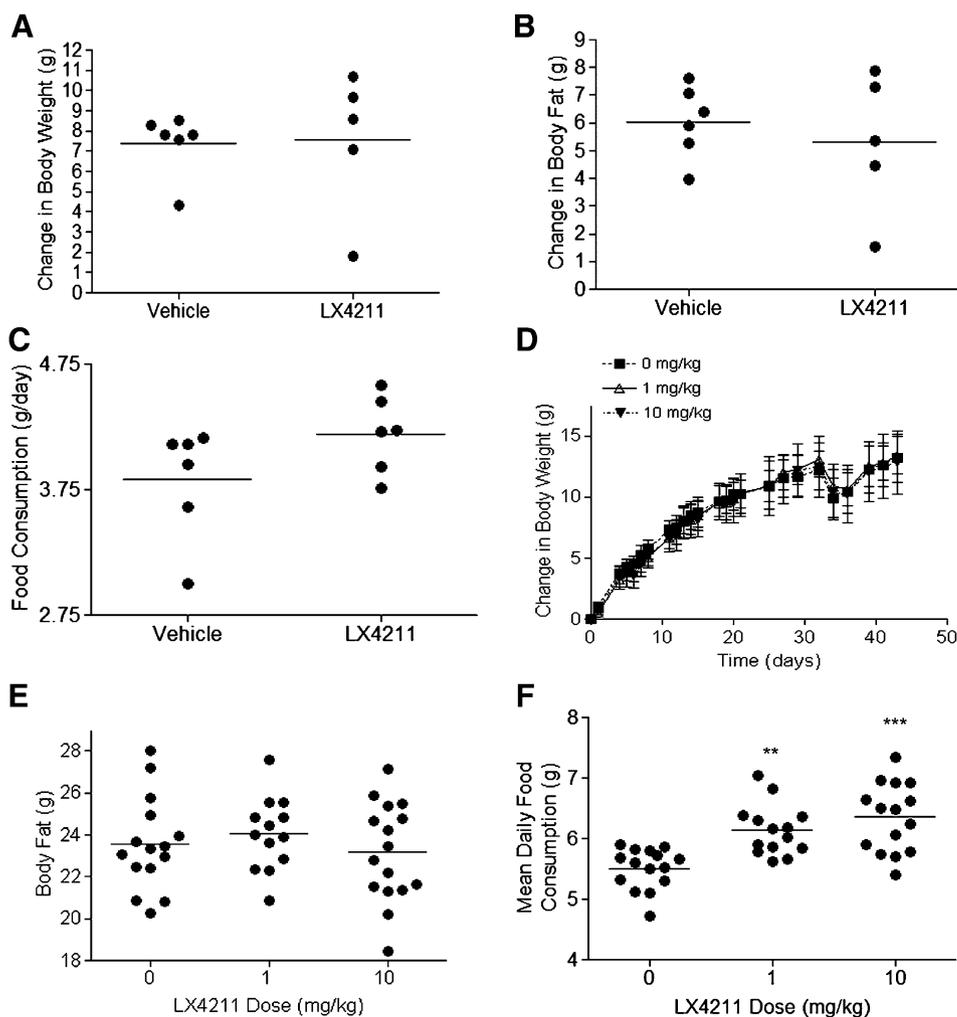
mouse or human SGLT1 or SGLT2 (Zambrowicz et al., 2012; Powell et al., 2013b). As shown in Table 1, LX4211 also inhibits glucose transport mediated by rat and dog SGLT1 and SGLT2.

**LX4211 Pharmacokinetics.** The major pharmacokinetic parameters of LX4211 in the mouse, rat, and dog are summarized in Tables 2 and 3. Plasma concentration-time profiles are presented in Fig. 1, B and C. After an intravenous injection of LX4211, a low plasma CL was observed in all species (Table 2), with the lowest CL in the dog (4.6 ml/min per kilogram). At steady state, the volume of distribution for LX4211 appeared comparable across species (Table 2). After oral administration, LX4211 was extensively absorbed by the mouse and dog, with oral bioavailabilities (%F) of 98 and 71%, respectively (Table 3). The  $T_{max}$  in the rat after oral dosing was approximately 3 hours, and the plasma levels remained high even at 6 hours after dosing. Thus, it was not possible to determine a true elimination rate, or  $AUC_{0-\infty}$ , and therefore the %F could not be accurately determined for the rat. However, it is clear that the bioavailability in the rat is quite high.

**LX4211 Effect on UGE.** For all species tested, LX4211 caused a dose-dependent increase in glucose excretion over the 24 hours after dosing (Fig. 2). In mice, the minimal dose that increased UGE was 0.1 mg/kg, the maximal 24-hour

UGE was 628 mg/d, and the  $ED_{50}$  (daily dose causing 50% maximal glucose excretion in the first 24 hours after dosing) was estimated to be 1.8 mg/kg (Fig. 2A). As shown in Fig. 2B, UGE was close to maximal for 2 days after mice received single 10 mg/kg or 60 mg/kg doses of LX4211. In rats, the lowest dose tested (0.3 mg/kg) increased UGE, the maximal 24-hour UGE was 5.1 g/d, and the  $ED_{50}$  was estimated to be 0.75 mg/kg (Fig. 2C). As shown in Fig. 2D, UGE was close to maximal for at least 2 days after rats received a single 50 mg/kg dose of LX4211. In dogs, the minimal dose that increased UGE was 0.1 mg/kg, the maximal 24-hour UGE was 36.7 g/d, and the  $ED_{50}$  was estimated to be 0.2 mg/kg (Fig. 2E). As shown in Fig. 2F, UGE was close to maximal for at least 2 days after dogs received a single 3 mg/kg dose of LX4211.

**LX4211 Effect on Glucose Homeostasis.** The  $KKA^y$  mouse model of T2D was used in two independent studies to test the effects of LX4211. In study 1 (Fig. 3A), LX4211 at a dose of 1 mg/kg significantly lowered both A1C (Fig. 3B) and fed glucose levels (Fig. 3C) relative to vehicle-treated control mice. During an OGTT, LX4211 also significantly lowered glucose excursions (Fig. 3D) while significantly increasing insulin concentrations between 0 and 30 minutes (Fig. 3E). At necropsy, the total pancreatic insulin content was significantly greater in the LX4211-treated mice (Fig. 3F). As



**Fig. 5.** The effect of LX4211 on body composition and food consumption in  $KKA^y$  diabetic mice. In  $KKA^y$  study 1, mice were evaluated for (A) change in body weight between the start of LX4211 treatment and necropsy; (B) change in body fat between QMR measurements made in week 1 and week 9; and (C) food consumption, reported as the mean daily intake measured during the 8 days that the mice were maintained in metabolic cages while receiving LX4211 or vehicle. In  $KKA^y$  study 2, mice were evaluated for (D) change in body weight between measurements made on the first day of LX4211 or vehicle treatment and on multiple days during the first 6 weeks of treatment; (E) body fat measured by QMR during study week 9; and (F) food consumption, reported as the mean daily intake during the first 6 weeks of treatment with LX4211 or vehicle. (F) \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  versus the vehicle-treated group.

expected, UGE was significantly increased by LX4211 (Fig. 3G). In study 2 (Fig. 4A), LX4211 at doses of 1 and 10 mg/kg significantly lowered both A1C (Fig. 4B) and fed glucose levels (Fig. 4C). During an OGTT, each LX4211 dose significantly lowered glucose excursions (Fig. 4D), and insulin concentrations were better maintained in the group receiving 10 mg/kg of LX4211 relative to vehicle-treated controls (Fig. 4E). Total pancreatic insulin content was significantly greater in both LX4211 treatment groups relative to vehicle-treated controls (Fig. 4F). UGE was significantly and comparably increased by the two LX4211 doses (Fig. 4G).

**LX4211 Effect on Body Composition.** Long-term treatment with LX4211 had no effect on either body weight or body fat of KKA<sup>y</sup> mice from the two independent studies (Fig. 5, A, B, D, and E; Supplemental Table 1). However, LX4211 increased food consumption in these mice (Fig. 5, C and F). In study 2, which showed a significant increase in food consumption, the estimated mean increase in energy intake by LX4211-treated mice (11.8 kJoules/mouse) was comparable to the estimated mean energy lost as urinary glucose by these mice (10.6 kJoules/mouse).

Lean rats and dogs treated with LX4211 for 4 weeks gained significantly less body weight despite consuming significantly more food (Fig. 6, A–D). To examine this effect more closely, lean rats were treated with increasing doses of LX4211 for 13

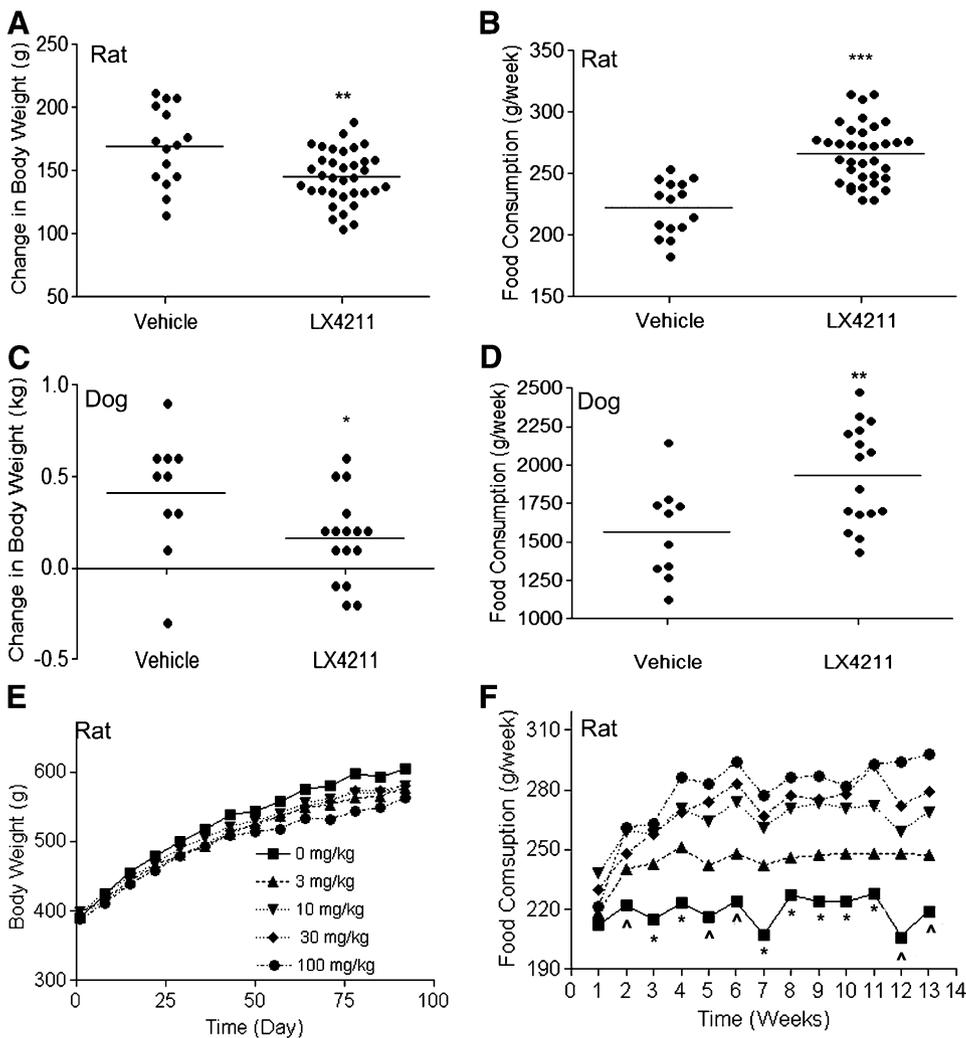
weeks. Body weight gain in these rats was impaired by LX4211 treatment (Fig. 6E; Supplemental Table 2); although the differences in body weight gain did not achieve statistical significance, they were accompanied by significant, dose-dependent increases in food consumption (Fig. 6F; Supplemental Table 2).

In a study using OP-CD obese rats (Fig. 7A), treatment with 30 mg/kg LX4211 induced a robust increase in UGE (Fig. 7B). After 4 weeks of treatment, LX4211-treated rats gained significantly less body weight, body fat, and lean body mass relative to vehicle-treated controls (Fig. 7, C–E; Supplemental Table 1) despite comparable food intake (Fig. 7F).

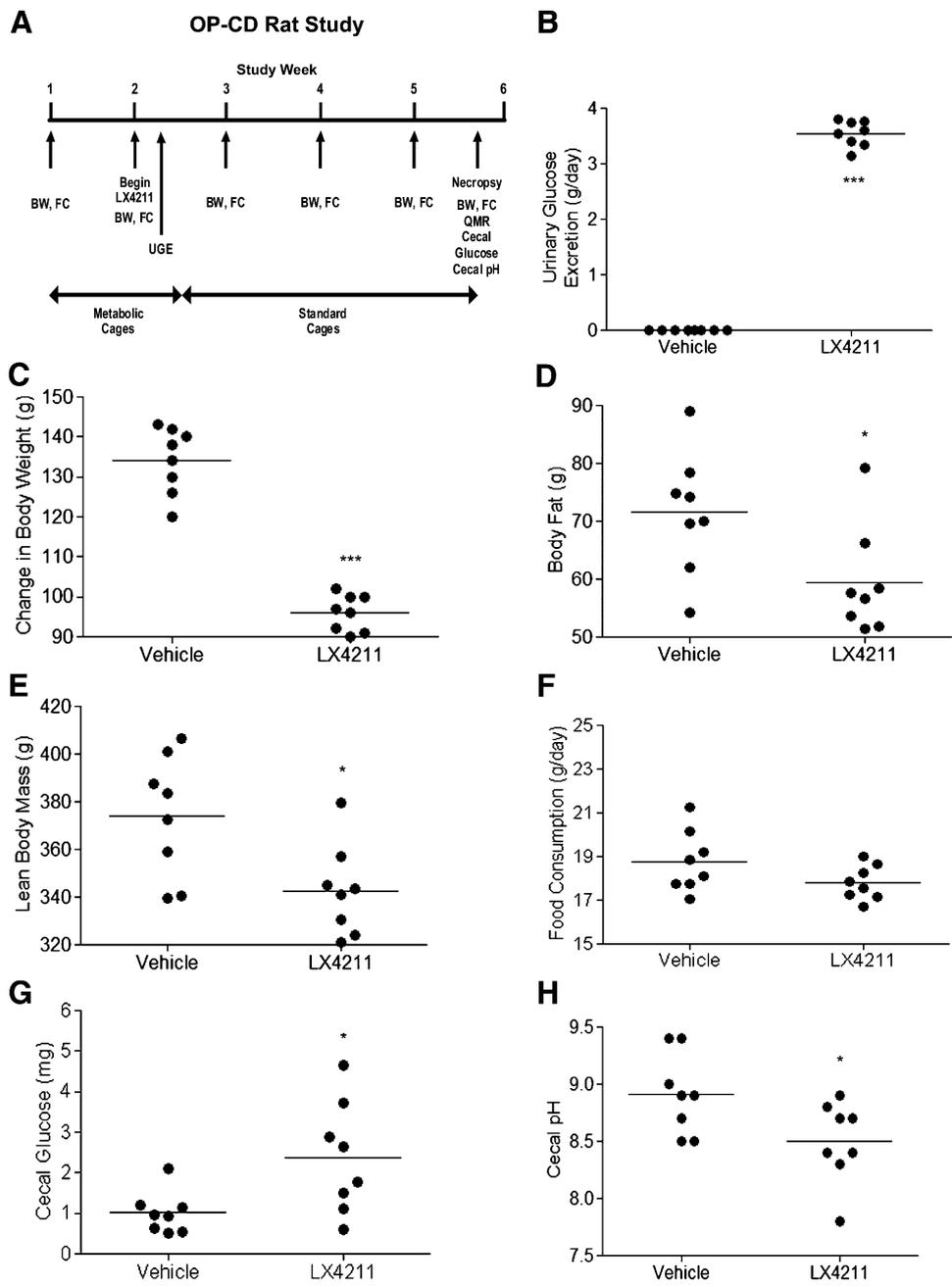
**LX4211 Effect on Intestinal Glucose Absorption.** After 4 weeks of LX4211 treatment, OP-CD obese rats showed a significant increase in cecal glucose content and a decrease in cecal pH (Fig. 7, G–H). Although GLP-1 levels were not measured in these rats, total GLP-1 levels were significantly increased in LX4211-treated KKA<sup>y</sup> mice 2 hours after an oral challenge with 2 g/kg glucose (Fig. 4H).

## Discussion

LX4211 is a nonselective dual SGLT1/2 inhibitor in all species studied to date, and it is orally bioavailable in these same species (Zambrowicz et al., 2012; Powell et al., 2013b;



**Fig. 6.** Effects of LX4211 on body weight of lean rats and dogs. In rats, (A) change in body weight and (B) food consumption were measured during a 4-week toxicology study and then compared between the vehicle-treated group ( $N = 15$ ) and pooled data from groups receiving LX4211 at doses of 10 ( $N = 10$ ), 30 ( $N = 10$ ), or 100 ( $N = 15$ ) mg/kg per day. In dogs, (C) change in body weight and (D) food consumption were also measured during a 4-week toxicology study and then compared between the vehicle-treated group ( $N = 10$ ) and pooled data from groups receiving LX4211 at doses of 30 ( $N = 6$ ) or 100 ( $N = 10$ ) mg/kg per day. In rats, (E) body weight and (F) food consumption were measured during a 13-week toxicology study and then compared between the vehicle-treated group ( $N = 15$ ) and individual groups receiving LX4211 at doses of 3 ( $N = 10$ ), 10 ( $N = 10$ ), 30 ( $N = 15$ ) or 100 ( $N = 15$ ) mg/kg/d. The key for all groups in E and F is found in E. No statistically significant adverse effects of LX4211 treatment were observed at these doses in any of the three studies. (A–D)  $*P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$  versus the vehicle-treated group. (F)  $*P < 0.05$  for vehicle versus all other groups;  $^{\wedge}P < 0.05$  for vehicle versus the 10, 30, and 100 mg/kg per day groups.



**Fig. 7.** Effects of LX4211 on OP-CD obese rats. (A) Schematic outline of study design. BW, body weight; FC, food consumption; UGE, 24-hour UGE measured in metabolic cages. (B) UGE, presented as mean 24-hour UGE values estimated from collections made on three consecutive days in metabolic cages. (C) Change in body weight between the start of LX4211 treatment and necropsy. (D) Body fat and (E) lean body mass, measured by QMR at the end of the study. (F) Food consumption, reported as the mean daily intake during the 25 days of treatment with LX4211 or vehicle. Cecal contents were isolated from fed rats at the time of necropsy and used to measure (G) cecal glucose, reported here as the total amount of glucose recovered, and (H) cecal pH, which represents a direct pH measurement of the cecal contents. (B–E, G, and H) \**P* < 0.05; \*\*\**P* < 0.001 versus the vehicle-treated group.

data presented here). The prolonged exposure observed after oral administration of LX4211 to mice, rats, and dogs is consistent with what has been reported in humans, as is the prolonged increase in UGE (Zambrowicz et al., 2012). These observations support the relevance of LX4211 studies in mouse, rat, and dog preclinical models.

KKA<sup>Y</sup> mice are quite hyperglycemic despite hyperinsulinemia and  $\beta$ -cell insulin degranulation, indicating that their  $\beta$  cells cannot fully compensate for the increased demand created by severe insulin resistance (Iwatsuka et al., 1970). We used this T2DM model to test the effect of LX4211 on glycemic control. We found that glycemic control was improved in two independent studies where KKA<sup>Y</sup> mice received LX4211; A1C and fed glucose values were much lower in LX4211-treated mice, similar to findings in patients

with T2DM (Zambrowicz et al., 2012), and glucose excursions during OGTTs were significantly decreased during LX4211 treatment in mice, as they were in humans (Zambrowicz et al., 2012).

Despite their decreased glucose excursions, the LX4211-treated mice had significantly greater glucose-stimulated insulin release compared with vehicle-treated control mice. In each of the two studies, this was associated with a significant increase in pancreatic insulin content; the mice were in the fed state in both instances, and, in fact, the mice from study 2 had also received an oral glucose challenge 2 hours before necropsy.

The combination of decreased glucose excursions, increased insulin response, and increased  $\beta$ -cell insulin reserves in multiple cohorts of LX4211-treated postprandial mice

suggests that LX4211 is having a beneficial effect on  $\beta$ -cell function. This beneficial effect may be due, in part, to the ability of LX4211 to lower blood glucose by increasing UGE and by delaying intestinal glucose absorption, two insulin-independent mechanisms of action that should unload  $\beta$  cells by decreasing the demand for insulin. Thiazolidinediones also lower blood glucose by insulin-sparing mechanisms, raising the possibility that, like thiazolidinediones, LX4211 may preserve  $\beta$ -cell function during long-term treatment (Wajchenberg, 2007; DeFronzo, 2009; Kahn et al., 2011). Consistent with these observations, 1) SGLT2 deficiency preserved  $\beta$ -cell function in *db/db* mice (Jurczak et al., 2011); 2) phlorizin-mediated SGLT2 inhibition markedly improved multiple measures of  $\beta$ -cell function in partially pancreatectomized rats (Rossetti et al., 1987); and 3)  $\beta$ -cell function and islet morphology were significantly improved in Zucker diabetic fatty rats treated with the SGLT2 inhibitor dapagliflozin (Macdonald et al., 2010).

Improved glycemic control in LX4211-treated KKA<sup>y</sup> mice may be due not only to increased UGE but also to other mechanisms. In healthy mice, LX4211 treatment is associated with blunted OGTT glucose excursions along with increased cecal glucose and circulating GLP-1 levels, and with lower cecal pH. This is due to LX4211-mediated inhibition of intestinal SGLT1, resulting in delayed absorption of intestinal glucose and subsequent appearance of glucose in the cecum, where it undergoes fermentation to short-chain fatty acids, which are powerful mediators of GLP-1 release (Powell et al., 2013b; Zambrowicz et al., 2013a). Healthy humans and patients with T2DM also show LX4211-mediated decreases in postprandial glucose excursions accompanied by increases in circulating GLP-1 levels (Zambrowicz et al., 2012, 2013a,b). In the data presented here, postprandial OP-CD obese rats treated with LX4211 also show greater cecal glucose content and lower cecal pH, further supporting the likelihood that this is a general mechanism across species. In addition, the finding that LX4211-treated KKA<sup>y</sup> mice had blunted glucose excursions and increased GLP-1 levels after oral glucose challenge suggests that this mechanism is active in diabetic mice as it is in diabetic humans, and may have contributed to the overall improvement in glycemic control observed in the KKA<sup>y</sup> mice. Further, the increased GLP-1 levels that accompanied LX4211 treatment may also help to preserve  $\beta$ -cell function (Wajchenberg, 2007; DeFronzo, 2009; Bunck et al., 2011).

Body weight and body fat values were comparable between LX4211-treated and untreated KKA<sup>y</sup> mice in two independent studies. Although mice receiving LX4211 lost large amounts of glucose in their urine, the caloric losses were offset by an increase in food consumption. These results were consistent with our findings in SGLT2 knockout mice (Powell et al., 2013a). In comparison, KKA<sup>y</sup> mice treated with the SGLT2 inhibitors T-1095 [3-(benzo[b]furan-5-yl)-2',6'-dihydroxy-4'-methylpropiphenone 2'-O-(6-O-methoxycarbonyl- $\beta$ -D-glucopyranoside)] or sergliflozin showed a slight but significant decrease in weight gain, which was associated with a slight increase in food consumption in the sergliflozin-treated mice (Oku et al., 1999; Katsuno et al., 2009). In general, our studies of LX4211-treated rats and dogs show less weight gain despite increased food consumption, results that are consistent with studies of rats with diet-induced obesity where weight loss associated with long-term dapagliflozin treatment was partially

offset by increased food consumption (Devenny et al., 2012).

Taken together, these results suggest that the ability of LX4211 to lower body weight and body fat in preclinical models depends on the extent to which LX4211-mediated hyperphagia compensates for the increased loss of glucose calories in urine. Weight loss is consistently reported in clinical trials evaluating patients with T2DM who received LX4211 (Rosenstock et al., 2012; Zambrowicz et al., 2012) or other SGLT2 inhibitors (Abdul-Ghani et al., 2012). The effect of LX4211 on food consumption in patients with T2DM has not yet been carefully monitored; such studies will be required to determine whether hyperphagia attenuates the degree of weight loss in some LX4211-treated patients as it does in some LX4211-treated preclinical models.

In summary, data presented here show that LX4211 is a dual SGLT1/SGLT2 inhibitor in multiple preclinical species as it is in humans; this suggests that the response to LX4211 in these species may be predictive of the response to LX4211 in humans. This hypothesis is supported by the ability of LX4211 to markedly lower A1C and postprandial glucose excursions while increasing UGE and GLP-1 levels in diabetic mice as in humans. Based on these considerations, our data suggest that LX4211 may maintain  $\beta$ -cell insulin reserves in patients with T2DM, and that the LX4211-mediated weight loss observed in these patients may be attenuated by LX4211-mediated hyperphagia in at least some of these individuals.

#### Authorship Contributions

*Participated in research design:* Powell, DaCosta, Smith, Heydorn, Nouraldeen, Yalamanchili, Mseeh, Wilson, Shadoan, Zambrowicz, Ding.

*Conducted experiments:* DaCosta, Smith, Doree, Harris, Buhning, Xiong, Mseeh, Shadoan.

*Contributed new reagents or analytic tools:* Mseeh.

*Performed data analysis:* Powell, DaCosta, Smith, Doree, Heydorn, Nouraldeen, Yalamanchili, Mseeh, Wilson, Shadoan, Ding.

*Wrote or contributed to the writing of the manuscript:* Powell, Mseeh, Wilson, Zambrowicz, Ding.

#### References

- Abdul-Ghani MA, Norton L, and DeFronzo RA (2012) Efficacy and safety of SGLT2 inhibitors in the treatment of type 2 diabetes mellitus. *Curr Diab Rep* **12**:230–238.
- American Diabetes Association (2013) Standards of medical care in diabetes—2013. *Diabetes Care* **36** (Suppl 1):S11–S66.
- Bunck MC, Cornér A, Eliasson B, Heine RJ, Shaginian RM, Taskinen MR, Smith U, Yki-Järvinen H, and Diamant M (2011) Effects of exenatide on measures of  $\beta$ -cell function after 3 years in metformin-treated patients with type 2 diabetes. *Diabetes Care* **34**:2041–2047.
- Cohen RV, Pinheiro JC, Schiavon CA, Salles JE, Wajchenberg BL, and Cummings DE (2012) Effects of gastric bypass surgery in patients with type 2 diabetes and only mild obesity. *Diabetes Care* **35**:1420–1428.
- DeFronzo RA (2009) Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* **58**: 773–795.
- Devenny JJ, Godonis HE, Harvey SJ, Rooney S, Cullen MJ, and Pellemounter MA (2012) Weight loss induced by chronic dapagliflozin treatment is attenuated by compensatory hyperphagia in diet-induced obese (DIO) rats. *Obesity (Silver Spring)* **20**:1645–1652.
- Goodwin NC, Mabon R, Harrison BA, Shadoan MK, Almstead ZY, Xie Y, Healy J, Buhning LM, DaCosta CM, and Bardenhagen J, et al. (2009) Novel L-xylose derivatives as selective sodium-dependent glucose cotransporter 2 (SGLT2) inhibitors for the treatment of type 2 diabetes. *J Med Chem* **52**:6201–6204.
- Huang ES, Basu A, O'Grady M, and Capretta JC (2009) Projecting the future diabetes population size and related costs for the U.S. *Diabetes Care* **32**: 2225–2229.
- Iwatsuka H, Shino A, and Suzuoki Z (1970) General survey of diabetic features of yellow KK mice. *Endocrinol Jpn* **17**:23–35.
- Jurczak MJ, Lee HY, Birkenfeld AL, Jornayvaz FR, Frederick DW, Pongratz RL, Zhao X, Moeckel GW, Samuel VT, and Whaley JM, et al. (2011) SGLT2 deletion improves glucose homeostasis and preserves pancreatic beta-cell function. *Diabetes* **60**:890–898.
- Kahn SE, Lachin JM, Zinman B, Haffner SM, Aftiring RP, Paul G, Kravitz BG, Herman WH, Viberti G, and Holman RR; ADOPT Study Group (2011) Effects of

- rosiglitazone, glyburide, and metformin on  $\beta$ -cell function and insulin sensitivity in ADOPT. *Diabetes* **60**:1552–1560.
- Kashyap SR, Bhatt DL, Wolski K, Watanabe RM, Abdul-Ghani M, Abood B, Pothier CE, Brethauer S, Nissen S, and Gupta M, et al. (2013) Metabolic effects of bariatric surgery in patients with moderate obesity and type 2 diabetes: analysis of a randomized control trial comparing surgery with intensive medical treatment. *Diabetes Care* **36**:2175–2182.
- Katsuno K, Fujimori Y, Ishikawa-Takemura Y, and Isaji M (2009) Long-term treatment with sergliflozin etabonate improves disturbed glucose metabolism in KK-A(y) mice. *Eur J Pharmacol* **618**:98–104.
- Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, and Taylor R (2011) Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* **54**:2506–2514.
- Macdonald FR, Peel JE, Jones HB, Mayers RM, Westgate L, Whaley JM, and Poucher SM (2010) The novel sodium glucose transporter 2 inhibitor dapagliflozin sustains pancreatic function and preserves islet morphology in obese, diabetic rats. *Diabetes Obes Metab* **12**:1004–1012.
- Musso G, Gambino R, Cassader M, and Pagano G (2012) A novel approach to control hyperglycemia in type 2 diabetes: sodium glucose co-transport (SGLT) inhibitors: systematic review and meta-analysis of randomized trials. *Ann Med* **44**:375–393.
- Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, and Zinman B; American Diabetes Association; European Association for Study of Diabetes (2009) Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* **32**:193–203.
- National Institutes of Health (1998) Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults—the evidence report. *Obes Res* **6** (Suppl 2):51S–209S.
- Oku A, Ueta K, Arakawa K, Ishihara T, Nawano M, Kuronuma Y, Matsumoto M, Saito A, Tsujihara K, and Anai M, et al. (1999) T-1095, an inhibitor of renal  $\text{Na}^+$ -glucose cotransporters, may provide a novel approach to treating diabetes. *Diabetes* **48**:1794–1800.
- Powell DR, DaCosta CM, Gay J, Ding Z-M, Smith M, Greer J, Doree D, Jeter-Jones S, Mseeh F, and Rodriguez LA, et al. (2013a) Improved glycemic control in mice lacking SglT1 and SglT2. *Am J Physiol Endocrinol Metab* **304**:E117–E130.
- Powell DR, Smith M, Greer J, Harris A, Zhao S, DaCosta C, Mseeh F, Shadoan MK, Sands A, and Zambrowicz B, et al. (2013b) LX4211 increases serum glucagon-like peptide 1 and peptide YY levels by reducing sodium/glucose cotransporter 1 (SGLT1)-mediated absorption of intestinal glucose. *J Pharmacol Exp Ther* **345**:250–259.
- Reusch JE and Wang CC (2011) Cardiovascular disease in diabetes: where does glucose fit in? *J Clin Endocrinol Metab* **96**:2367–2376.
- Rosenstock J, Zambrowicz B, Lapuerta P, Ogbaa I, Banks P, Freiman J, Frazier K, Kelly M, Boehm K, and Sands A (2012) Impact of LX4211, a dual inhibitor of sodium glucose transporter 1 and 2, on cardiovascular risk factors in type 2 diabetes (Abstract). *Circulation* **126**:2780 DOI: 10.1161/CIR.0b013e318278c90d.
- Rossetti L, Shulman GI, Zawulich W, and DeFronzo RA (1987) Effect of chronic hyperglycemia on in vivo insulin secretion in partially pancreatectomized rats. *J Clin Invest* **80**:1037–1044.
- Wajchenberg BL (2007)  $\beta$ -cell failure in diabetes and preservation by clinical treatment. *Endocr Rev* **28**:187–218.
- Zambrowicz B, Freiman J, Brown PM, Frazier KS, Turnage A, Bronner J, Ruff D, Shadoan M, Banks P, and Mseeh F, et al. (2012) LX4211, a dual SGLT1/SGLT2 inhibitor, improved glycemic control in patients with type 2 diabetes in a randomized, placebo-controlled trial. *Clin Pharmacol Ther* **92**:158–169.
- Zambrowicz B, Ding Z-M, Ogbaa I, Frazier K, Banks P, Turnage A, Freiman J, Smith M, Ruff D, and Sands A, et al. (2013a) Effects of LX4211, a dual SGLT1/SGLT2 inhibitor, plus sitagliptin on postprandial active GLP-1 and glycemic control in type 2 diabetes. *Clin Ther* **35**:273–285, e7.
- Zambrowicz B, Ogbaa I, Frazier K, Banks P, Turnage A, Freiman J, Boehm KA, Ruff D, Powell DR, and Sands A (2013b) 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. *Clin Ther* **35**:1162–1173.e8.

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**Address correspondence to:** Dr. David R. Powell, Lexicon Pharmaceuticals, Inc., 8800 Technology Forest Place, The Woodlands, TX 77381. E-mail: dpowell@lexpharma.com

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