Effects of Sustained Treatment With Lixisenatide on Gastric Emptying and Postprandial Glucose Metabolism in Type 2 Diabetes: A Randomized Controlled Trial

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OBJECTIVE

Tachyphylaxis for slowing of gastric emptying is seen with continuous exposure to glucagon-like peptide 1 (GLP-1). We therefore aimed to establish whether prolonged use of a "short-acting" GLP-1 receptor agonist, lixisenatide, achieves sustained slowing of gastric emptying and reduction in postprandial glycemia.

RESEARCH DESIGN AND METHODS

A total of 30 patients with metformin-treated type 2 diabetes underwent assessment of gastric emptying (scintigraphy) and glucose metabolism (dual tracer technique) after a 75-g glucose drink, before and after 8 weeks' treatment with lixisenatide (20 μg subcutaneously daily) or placebo, in a double-blind randomized parallel design.

RESULTS

Gastric retention of the glucose drink was markedly increased after lixisenatide versus placebo (ratio of adjusted geometric means for area under the curve [AUC] over 240 min of 2.19 [95% CI 1.82, 2.64], $P < 0.001$), associated with substantial reductions in the rate of systemic appearance of oral glucose ($P < 0.001$) and incremental AUC for blood glucose ($P < 0.001$). Lixisenatide suppressed both glucagon ($P = 0.003$) and insulin ($P = 0.032$), but not endogenous glucose production, over 120 min after oral glucose intake. Postprandial glucose lowering over 240 min was strongly related to the magnitude of slowing of gastric emptying by lixisenatide ($r = -0.74$, $P = 0.002$) and to the baseline rate of emptying ($r = 0.52$, $P = 0.048$) but unrelated to β-cell function (assessed by β-cell glucose sensitivity).

CONCLUSIONS

Eight weeks' treatment with lixisenatide is associated with sustained slowing of gastric emptying and marked reductions in postprandial glycemia and appearance of ingested glucose. Short-acting GLP-1 receptor agonists therefore potentially represent an effective long-term therapy for specifically targeting postprandial glucose excursions.

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Early intervention to achieve good glycemic control in type 2 diabetes can reduce the risk of micro- and potentially macrovascular complications but entails risks, particularly for insulin therapy, of weight gain and hypoglycemia (1). Recent guidelines recommend an individualized approach to the selection of second-line pharmacological treatment after metformin (2). In the majority of patients with type 2 diabetes in the community, who have relatively modest elevation of glycated hemoglobin (HbA_{1c}) (3), postprandial rather than fasting blood glucose plays an important role in overall glycemic control (4), which is not surprising given that humans are predominantly in the postprandial state. It is now apparent that postprandial glycemia must be specifically targeted in patients with diabetes, particularly in those who have only moderately elevated HbA_{1c} with minimal fasting hyperglycemia (5).

The gut-derived incretin hormone, glucagon-like peptide 1 (GLP-1), has several actions that lower glycemia, including slowing of gastric emptying, glucose-dependent stimulation of insulin, and suppression of glucagon (6–8), all of which potentially impact on the systemic appearance of orally ingested carbohydrate and the rate of endogenous glucose production. The rate at which nutrients empty from the stomach is a particularly important determinant of postprandial glycemia in health and type 2 diabetes (9), and pharmacological agents that slow gastric emptying result in a lowering of the postprandial blood glucose excursion (10).

A number of GLP-1 receptor agonists have been developed for the treatment of type 2 diabetes; these differ in being "short acting" (e.g., lixisenatide or exenatide BD) or "long acting" (e.g., liraglutide or exenatide QW). There is recent evidence that continuous exposure to GLP-1 is associated with tachyphylaxis for its effects on gastric emptying (11,12). Consistent with this concept, postprandial glycemia appears to be better controlled after several weeks' treatment with short-acting versus long-acting GLP-1 receptor agonists, although typically in such studies, either gastric emptying has not been measured (13) or a suboptimal technique has been used—such as paracetamol absorption (14) or a stable isotope breath test involving a meal different from that used to assess the glycemic response (15).

The evolution of type 2 diabetes is characterized by progressive β-cell failure, which entails a loss of insulin secretory capacity and, eventually, a reduction in β-cell mass (16). A therapy that targets postprandial glycemia, without the requirement to stimulate insulin secretion, is an attractive therapeutic option, and there is recent evidence that lixisenatide maintains its efficacy for lowering HbA_{1c} across the spectrum of β-cell function in patients with type 2 diabetes (17).

We aimed to evaluate the effects of sustained use of lixisenatide on gastric emptying, using the most accurate technique of scintigraphy, and on postprandial glucose metabolism, using a dual glucose tracer technique (18), in patients with relatively well-controlled type 2 diabetes treated with metformin alone. We hypothesized that, as a short-acting GLP-1 receptor agonist, lixisenatide would have persisting effects to slow gastric emptying with sustained use and that this would be a dominant mechanism by which it achieves postprandial blood glucose control.

RESEARCH DESIGN AND METHODS

Subjects

We recruited, by advertisement, patients with a history of type 2 diabetes of ≥2 years' duration, treated with metformin for ≥3 months. Responders who met eligibility criteria attended our facility for a screening visit, when fasting blood samples were collected for blood picture, iron studies, biochemistry, and HbA_{1c}; autonomic function was evaluated by standardized cardiovascular tests (19); and gastrointestinal symptoms were evaluated by questionnaire (20). Those with HbA_{1c} <6.5% (48 mmol · mol^{-1}) or >9.0% (75 mmol · mol^{-1}), estimated glomerular filtration rate <30 mL · min^{-1}, definite autonomic neuropathy, elevated liver enzymes, hospital admission for cardiovascular disease within the previous 6 months, previous exposure to GLP-1 receptor agonists, or prominent gastrointestinal symptoms or using medications known to affect gastrointestinal motility were excluded. All participants gave written informed consent, and the protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12616001059459).

Study Design

After enrollment, participants were randomized to treatment with lixisenatide or placebo in a double-blind parallel design. Patients attended our Clinical Research Facility for a baseline study where a glucose drink was administered (day 0) before commencing treatment with daily subcutaneous injections from day 1 to day 56. They attended the laboratory for a second study on the last day of treatment (day 56), when they received their final dose of lixisenatide or placebo 30 min before the glucose test drink.

On each of the two study days (days 0 and 56), patients attended our facility in the morning (0800 h) after an overnight fast, having consumed a standardized beef lasagne meal the previous evening. An intravenous (IV) cannula was inserted in each forearm: one for blood sampling and the other for IV infusion of glucose tracer (bolus of 28 μmol · kg^{-1} 6,6-[$^2H_2$]glucose, followed by continuous infusion at a rate of 0.28 μmol · min^{-1} · kg^{-1} from $t = -180$ until $t = 240$ min). At $t = -5$ min, a 300-mL drink was given while the subject sat against a gamma camera, consisting of 75 g glucose, labeled with 20 MBq ^{99m}Tc-calcium phytate, and also containing 1.5 g [U-^{13}C]glucose. The drink was consumed within 5 min, and scintigraphic imaging continued for 240 min, with correction of data for subject movement, radionuclide decay, and γ-ray attenuation (21). Gastric retention (expressed as % of the maximum content of the total stomach) was calculated at 15-min intervals for the first 120 min and then hourly until $t = 240$ min. Venous blood was sampled at frequent intervals for measurement of blood glucose and plasma concentrations of glucose tracers, insulin, C-peptide, and glucagon. A fasting sample was also collected on day 56 to measure HbA_{1c}.

Intervention

On days 1–56 inclusive, each subject self-administered lixisenatide or matching placebo (saline) subcutaneously once daily, 30 min before breakfast. The dose of lixisenatide was stepped up from 5 μg on days 1–7 to 10 μg on days 8–14 and 20 μg on days 15–56. Blinding and randomization were coordinated by the hospital pharmacy, and subjects were educated in injection technique at the

day 0 visit. In addition to the study visits on day 0 and day 56, subjects attended our facility on day 7 to collect their medication supply for the following week and on day 14 to receive supplies for the remainder of the intervention. At each visit, participants were weighed and completed a gastrointestinal symptom questionnaire (20). They also completed questionnaires at days 28, 42, and 56. Compliance was monitored at each of these visits by recording used and unused medication, and subjects also recorded each dose of study medication in a daily diary.

Measurements

Blood glucose concentrations were determined by portable glucometer (Optium Xceed; Abbott Laboratories, Chicago, IL) (mean of two measurements at each time point). The remainder of each blood sample was placed into ice-chilled EDTA-treated tubes, and plasma was separated by centrifugation and stored at −80°C until assayed. Insulin was measured by ELISA (10-1113; Mercodia, Uppsala, Sweden), with sensitivity 1.0 mU · L^{-1} and intra- and interassay coefficients of variation (CVs) 2.9% and 6.7%, respectively. C-peptide was measured by ELISA (10-1136-01; Mercodia), with sensitivity 15 pmol · L^{-1} and intra- and interassay CVs 7.7% and 3.7%, respectively. Glucagon was measured by radioimmunoassay (GL-32 K; Millipore, Billerica, MA), with sensitivity 20 pg · mL^{-1} and intra- and interassay CVs 6.4% and 3.2%, respectively. Glucose tracers were measured using gas chromatography–mass spectrometry and glucose fluxes were calculated from the time course of the plasma tracer-to-tracee ratio of 6,6-[$^2H_2$]glucose and [U-^{13}C]glucose (22).

Statistical Analysis

The prespecified primary outcome measure was change in gastric half emptying time from baseline to day 56 for lixisenatide versus placebo. A sample size of 30 patients (15 in each group) was calculated to provide >90% power to detect a 50% increase in gastric half emptying time in the lixisenatide group (23). Gastric emptying was also evaluated by area under the curve (AUC) for retention over 0–240 and 0–120 min, calculated using the trapezoidal rule. Secondary end points included incremental AUC (iAUC) for blood glucose, and plasma insulin, C-peptide, glucagon, R_a of orally ingested glucose, and endogenous glucose production, as well as changes in HbA_{1c}. Parameters of β-cell function (fasting and total insulin secretion, glucose sensitivity, and rate sensitivity and potentiation) were resolved from mathematical modeling of oral glucose tolerance data as previously described (24). Additional exploratory end points were changes in fasting blood glucose and plasma insulin, C-peptide, and glucagon concentrations and body weight.

Analysis was undertaken on a per-protocol basis. The effect of lixisenatide was analyzed using ANCOVA, with fixed effects for treatment group (placebo or lixisenatide) and the baseline value of the outcome parameter. The baseline-adjusted treatment difference at day 56, 95% CI, and *P* value for the treatment effect are reported. Residuals for each model were examined for normality, linearity, and constant variance. Gastric emptying AUC was transformed using the natural log due to nonconstant variance, and the treatment effect is reported as the ratio of the geometric means. A ratio of 1 indicates equal means in the two groups, while values >1 indicate a higher mean for lixisenatide than for placebo and values <1 indicate a lower mean for lixisenatide than placebo. Change over time was calculated as day 56 minus day 0, so negative values indicate a decrease and positive values an increase over time. Differences between groups were calculated as lixisenatide minus placebo, so negative values indicate that lixisenatide had a lower mean than placebo, and positive values indicate that lixisenatide had a higher mean.

Within the lixisenatide group, relationships between changes in postprandial glucose, gastric retention, and average oral glucose R_a are presented as restricted cubic splines (with three knots at 0.1, 0.5, and 0.9). When linearity appeared reasonable, the Pearson correlation was calculated. Multiple linear regression was used to determine the independent associations of change in postprandial glycemia with change in gastric retention and change in β-cell glucose sensitivity. The regression model was assessed for normality, linearity, constant variance, and multicollinearity. The unstandardized regression coefficient (b), the standardized regression coefficient (β), and the partial correlation ($r_{partial}$) are presented; the last represents the correlation between the independent variable and outcome after removal of the effect of the other independent variable.

Data are reported as means and SDs or 95% CIs or as median and interquartile ranges (IQRs) if not normally distributed. Statistical significance was set as $P < 0.05$.

RESULTS

Fifty-three patients with type 2 diabetes attended for a screening visit, of whom 33 were randomized (Supplementary Fig. 1). Of the latter, 30 patients commenced treatment, all of whom completed the study and were included in the analysis: 9 female and 21 male, mean (SD) age 67.1 (6.0) years and BMI 32.1 (5.1) kg/m^2, median duration of known diabetes 5.5 years (IQR 3.0–10.0), and mean HbA_{1c} 7.1% (0.6%) (53 [6.6] mmol/mol). One had evidence of peripheral neuropathy, and two had a history of ischemic heart disease, but no others had evidence of micro- or macrovascular complications of diabetes. The characteristics of patients in each of the two groups (placebo $n = 15$, lixisenatide $n = 15$) are presented (Supplementary Table 1). The oral glucose tracer was inadvertently omitted from the glucose drink in one patient in the placebo group, so glucose tracer data were only available for 14 placebo-treated patients.

The intervention was well tolerated, and compliance was excellent; patients in both the placebo and lixisenatide groups took a median 98.2% (IQR 98.2–100) of scheduled doses. Seven patients treated with placebo reported adverse effects (including abdominal pain in three, constipation in one, diarrhea in one, and vomiting in one), while eight patients treated with lixisenatide reported adverse effects (including abdominal pain in one, constipation in two, diarrhea in five, vomiting in one, nausea in four, bloating in two, and acid reflux in two). All adverse effects were transient and resolved spontaneously. Gastrointestinal symptom questionnaires administered at screening and on days 7, 14, 28, 42, and 56 revealed low scores in both groups for poor appetite, nausea, fullness, abdominal discomfort, vomiting, abdominal pain, dysphagia, heartburn, and acid regurgitation (data not shown).

Mean body weight declined slightly in both groups, but there was no significant adjusted group difference at day 56 ($P = 0.714$).

Gastric Emptying

The prespecified primary outcome measure of gastric half emptying time could not be used, as 4 of 15 patients treated with lixisenatide (and none treated with placebo) had >50% retention of the glucose drink in the stomach at 240 min on day 56. With evaluation instead of AUC for gastric retention over 0–240 min, the ratio of adjusted geometric means for lixisenatide and placebo at day 56 was 2.19 (95% CI 1.82, 2.64; $P < 0.001$), indicating marked slowing of gastric emptying following 8 weeks' treatment with lixisenatide. Similarly, with evaluation of AUC for gastric retention over 0–120 min, the ratio of adjusted geometric means for lixisenatide and placebo at day 56 was 1.55 (95% CI 1.37, 1.74; $P < 0.001$) (Table 1 and Fig. 1).

Blood Glucose Concentrations

Fasting blood glucose declined slightly in the lixisenatide group on day 56 (mean [SD] −0.9 [0.8] mmol · L^{-1}, ratio of geometric means for differences after treatment 0.89 [95% CI 0.82, 0.97]). There was marked flattening of the postprandial blood glucose curve, such that both the iAUC (0–240 min) and iAUC (0–120 min) showed substantial adjusted group differences at day 56 ($P < 0.001$ for each) (Table 1 and Fig. 2*A*).

R_a of Oral Glucose

The R_a of oral glucose was greatly reduced in the lixisenatide group on day 56, such that there were large adjusted group differences for average R_a over both 0–240 min and 0–120 min ($P < 0.001$ for each) (Table 1 and Fig. 2*B*).

Rate of Endogenous Glucose Production

Endogenous glucose production was not significantly altered after lixisenatide treatment, such that there were no group differences in the average rate of endogenous glucose production at day 56 for either 0–240 min ($P = 0.452$) or 0–120 min ($P = 0.152$) (Table 1 and Fig. 2*C*).

Plasma Insulin Concentrations

There was no significant change in fasting insulin ($P = 0.137$ for adjusted group difference at day 56). However, there was flattening of the postprandial insulin curve in the lixisenatide group on day 56, such that the iAUC (0–120 min) showed a significant adjusted reduction ($P = 0.032$), although the difference was not significant for iAUC (0–240 min) ($P = 0.209$) (Table 1 and Fig. 2*D*).

Plasma C-Peptide Concentrations

Similar to insulin, fasting C-peptide was not altered, but there was flattening of the postprandial C-peptide curve in the lixisenatide group on day 56, although adjusted group differences did not reach statistical significance for either iAUC (0–120 min) ($P = 0.090$) or iAUC (0–240 min) ($P = 0.519$) (Table 1 and Fig. 2*E*).

Plasma Glucagon Concentrations

Fasting glucagon concentrations were not altered with lixisenatide ($P = 0.165$ for adjusted group difference at day 56), but there was suppression of the postprandial glucagon curve in the lixisenatide group on day 56, with a statistically significant adjusted group difference for iAUC (0–120 min) ($P = 0.003$) but not iAUC (0–240 min) ($P = 0.151$) (Table 1 and Fig. 2*F*).

HbA_{1c} and β-Cell Function

HbA_{1c} was reduced after lixisenatide versus placebo (adjusted group difference at day 56 of −0.48% [95% CI −0.70, −0.26] [−5.2 mmol · mol^{-1} (−7.7, −2.8)], $P < 0.001$). At day 56, fasting insulin secretion rate was slightly increased after lixisenatide versus placebo, while total insulin secretion over 240 min was not different between the two groups. β-Cell glucose sensitivity was almost doubled following lixisenatide treatment (Supplementary Fig. 2), while rate sensitivity and potentiation were not affected significantly (Table 1).

Relationships Between Variables

The change in postprandial glucose iAUC after 8 weeks' treatment with lixisenatide was strongly related to the change in gastric retention AUC for both 0–240 min (Pearson $r = -0.74$, $P = 0.002$) and 0–120 min (Pearson $r = -0.89$, $P < 0.001$) (Fig. 3), indicating that patients who achieved greater slowing of gastric emptying with lixisenatide treatment had a more substantial reduction in postprandial glycemia. The change in average R_a of oral glucose was also related to the change in gastric retention AUC over 0–240 min (Pearson $r = -0.66$, $P = 0.007$), indicating that the reduction in systemic appearance of oral glucose was closely related to the slowing of gastric emptying with lixisenatide.

The change in postprandial glucose iAUC after lixisenatide was also related to gastric retention AUC at baseline for 0–240 min (Pearson $r = 0.52$, $P = 0.048$) and nonsignificantly for 0–120 min (Pearson $r = 0.45$, $P = 0.093$), indicating that patients with more rapid gastric emptying at baseline had greater reductions in postprandial blood glucose with lixisenatide treatment.

The relationship between change in postprandial glucose iAUC after lixisenatide and change in β-cell glucose sensitivity did not reach statistical significance for 0–240 min (Pearson $r = -0.40$, $P = 0.138$) or 0–120 min (Pearson $r = -0.43$, $P = 0.109$). In the multiple regression model, the change in gastric retention remained strongly associated with change in postprandial glucose iAUC ($r_{partial} = -0.87$, $\beta = -0.85$, $P < 0.001$ for 0–120 min) after adjustment for the change in β-cell glucose sensitivity, whereas the association with the change in β-cell glucose sensitivity was not independently significant ($r_{partial} = -0.24$, $\beta = -0.12$, $P = 0.40$). Finally, the change in postprandial glucose iAUC after lixisenatide was not related to β-cell glucose sensitivity at baseline (Pearson $r = 0.25$, $P = 0.379$).

CONCLUSIONS

We have shown that the short-acting GLP-1 receptor agonist lixisenatide induces substantial slowing of gastric emptying that persists over several weeks, associated with marked reductions in postprandial glycemia and systemic appearance of ingested glucose in metformin-treated patients with type 2 diabetes. A short-acting GLP-1 receptor agonist, such as lixisenatide, therefore potentially represents an effective long-term therapy for specifically targeting postprandial blood glucose excursions.

We observed that the more lixisenatide slowed gastric emptying, the greater the lowering of postprandial blood glucose, and also that when gastric emptying was more rapid at baseline, the benefit of lixisenatide in terms of glucose lowering was greater. We recently reported that patients with relatively uncomplicated type 2 diabetes, as a group, have faster gastric emptying than

Table 1—Outcomes

	Placebo ($N = 15$)			Lixisenatide ($N = 15$)			Adjusted group difference (ratio of geometric means)	
	Day 0	Day 56	Change	Day 0	Day 56	Change	Mean (95% CI)	*P*
Gastric retention AUC 0–240 min (% · min)	8,544 (1,255)	8,394 (1,698)	−150 (1,171)	8,339 (1,867)	18,370 (4,858)	10,031 (4,926)	2.19 (1.82, 2.64)^	<0.001
Gastric retention AUC 0–120 min (% · min)	7,454 (944)	7,120 (1,189)	−334 (891)	7,165 (1,092)	10,770 (1,464)	3,605 (1,804)	1.55 (1.37, 1.74)^	<0.001
Fasting blood glucose (mmol · L^{-1})	8.3 (1.2)	8.4 (2.0)	0.1 (1.2)	7.6 (1.7)	6.7 (1.1)	−0.9 (0.8)	0.89 (0.82, 0.97)^	0.013
Blood glucose iAUC 0–240 min (mmol · L^{-1} · min)	1,388 (338)	1,375 (293)	−14 (288)	1,407 (491)	461 (455)	−947 (414)	−925 (−1,156, −693)	<0.001
Blood glucose iAUC 0–120 min (mmol · L^{-1} · min)	777 (162)	802 (154)	26 (212)	842 (299)	239 (233)	−602 (328)	−575 (−723, −426)	<0.001
Average R_aO 0–240 min (μmol · min^{-1} · kg^{-1})	10.5 (2.7)	10.6 (1.9)	0.2 (1.9)	10.9 (2.6)	5.2 (3.5)	−5.7 (3.6)	−5.7 (−7.6, −3.7)	<0.001
Average R_aO 0–120 min (μmol · min^{-1} · kg^{-1})	23.8 (3.3)	23.9 (4.0)	0.1 (3.5)	24.9 (5.9)	7.6 (6.6)	−17.3 (6.7)	−16.9 (−20.7, −13.1)	<0.001
Average EGP 0–240 min (μmol · min^{-1} · kg^{-1})	5.79 (1.76)	5.70 (2.48)	−0.09 (1.86)	6.74 (2.10)	6.58 (1.21)	−0.17 (2.45)	0.54 (−0.91, 1.98)	0.452
Average EGP 0–120 min (μmol · min^{-1} · kg^{-1})	3.35 (1.93)	3.97 (2.35)	0.62 (2.41)	4.34 (2.18)	5.27 (1.38)	0.93 (2.44)	1.06 (−0.42, 2.54)	0.152
Fasting insulin (mU · L^{-1})	5.8 (3.4)	6.2 (4.1)	0.4 (1.4)	5.4 (4.0)	6.6 (4.2)	1.2 (1.5)	0.8 (−0.3, 1.9)	0.137
Insulin iAUC 0–240 min (mU · L^{-1} · min)	3,359 (2,023)	3,822 (2,638)	463 (943)	5,365 (4,380)	3,068 (2,885)	−1,791 (2,876)	−1,301 (−3,377, 775)	0.209
Insulin iAUC 0–120 min (mU · L^{-1} · min)	1,964 (1,329)	2,431 (2,052)	468 (879)	3,107 (2,377)	1,316 (1,575)	−2,297 (4,997)	−1,506 (−2,868, −143)	0.032
Fasting C-peptide (pmol · L^{-1})	752 (352)	744 (317)	−8.0 (145)	678 (285)	711 (234)	33 (138)	25 (−70, 119)	0.597
C-peptide iAUC 0–240 min (nmol · L^{-1} · min)	225 (74)	253 (134)	28 (94)	325 (166)	220 (164)	−104 (254)	−39 (−16, 84)	0.519
C-peptide iAUC 0–120 min (nmol · L^{-1} · min)	98 (41)	117 (85)	19 (55)	139 (66)	78 (62)	−61 (103)	−51 (−111, 9)	0.090
Fasting glucagon (pg · mL^{-1})	66 (26)	62 (24)	−4 (15)	63 (25)	66 (21)	3 (14)	7 (−3, 16)	0.165
Glucagon iAUC 0–240 min (pg · mL^{-1} · min)	648 (657)	426 (497)	−223 (612)	476 (616)	159 (369)	−317 (712)	−233 (−557, 91)	0.151
Glucagon iAUC 0–120 min (pg · mL^{-1} · min)	581 (668)	350 (352)	−231 (585)	458 (618)	38 (71)	−420 (627)	−296 (−479, −113)	0.003
HbA_{1c} (%)	7.3 (0.6)	7.2 (0.6)	−0.13 (0.29)	6.9 (0.4)	6.3 (0.5)	−0.58 (0.25)	−0.48 (−0.70, −0.26)	<0.001
HbA_{1c} (mmol · mol^{-1})	56 (6.6)	55 (6.6)	−1.4 (3.2)	52 (4.4)	45 (5.5)	−6.3 (2.7)	−5.2 (−7.7, −2.8)	<0.001
Fasting insulin secretion rate (pmol · m^{-2} · min^{-1})	93.2 (39.7)	93.7 (36.8)	0.5 (14.9)	84.5 (28.4)	102.8 (33.0)	18.3 (16.8)	17.0 (5.0, 29.0)	0.007
Total insulin secretion (nmol · m^{-2})	53.5 (18.2)	57.7 (23.9)	4.2 (12.5)	64.8 (26.5)	59.2 (23.3)	−5.5 (35.8)	−2.3 (−19.9, 15.2)	0.789
β-Cell glucose sensitivity (pmol · min^{-1} · m^{-2} · mmol · L^{-1})	20.2 (11.4)	22.8 (13.5)	2.6 (6.9)	29.4 (20.7)	53.7 (28.6)	24.3 (27.3)	2.10 (1.48, 2.98)^	<0.001
Potentiation factor (ratio)*	1.0 (0.3)	1.1 (0.4)	−0.02 (0.5)	1.0 (0.8)	1.2 (0.7)	0.1 (0.9)	0.4 (−0.9, 0.1)	0.138
Rate sensitivity (pmol · mmol · L^{-1} · m^{-2})*	133 (219)	158 (373)	25 (342)	150 (237)	265 (1,209)	106 (1,105)	3.3 (0.0, 70,242)^	0.811
Body weight (kg)	92.2 (17.0)	91.2 (17.1)	−1.0 (1.6)	88.5 (14.4)	87.3 (14.2)	−1.3 (2.0)	−0.3 (−1.6, 1.1)	0.714

Data are mean (SD) unless otherwise indicated. EGP, rate of endogenous glucose production; R_aO, R_a of oral glucose. *Data are median (IQR). ^Difference between groups is presented as the ratio of geometric means.

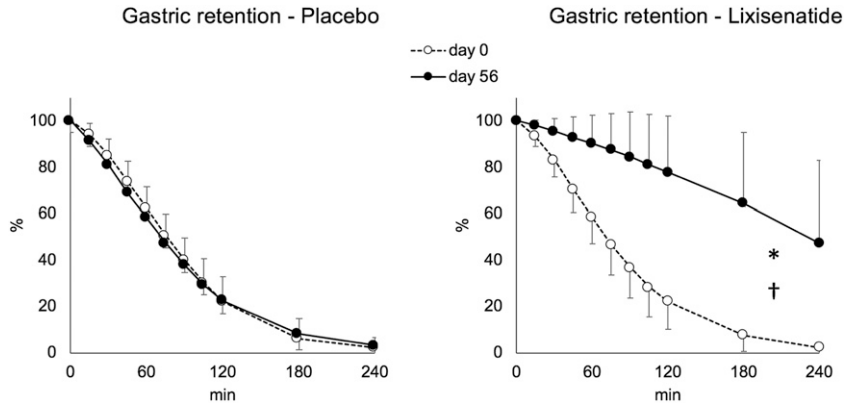

Figure 1—Gastric retention (%), measured by scintigraphy, of a 75-g glucose drink at baseline (day 0) and after 8 weeks' treatment with placebo ($n = 15$) or lixisenatide ($n = 15$) (day 56) in 30 metformin-treated patients with type 2 diabetes. Data are means (SD). The ratio of adjusted geometric means for AUC (0–240 min) for lixisenatide and placebo at day 56 was 2.19 (95% CI 1.82, 2.64) (*$P < 0.001$) and for AUC (0–120 min) was 1.55 (95% CI 1.37, 1.74) (†$P < 0.001$).

age-matched control subjects (25), which would make such patients particularly suitable for a short-acting GLP-1 receptor agonist. Conversely, patients with slow gastric emptying at baseline—which is more common in those with multiple complications of diabetes and poor overall glycemic control (20)—would be less likely to achieve substantial postprandial glucose lowering.

As well as slowing gastric emptying, lixisenatide suppressed glucagon secretion after the glucose drink, which represents an additional mechanism of postprandial glycemic control. Likewise, postprandial plasma insulin concentrations were lower with lixisenatide, especially during the initial 2 h postprandially. Although endogenous glucose production is suppressed by GLP-1 in fasting pancreatic clamp experiments (26), its postprandial regulation is more complex (27). Concurrent suppression of both glucagon and insulin, maintaining a relatively constant ratio between them, may explain why postprandial endogenous glucose production was similar with placebo and lixisenatide at day 56. Additionally, the lack of change in endogenous glucose production despite lower plasma insulin concentrations could indicate that hepatic insulin sensitivity was enhanced by lixisenatide.

Lixisenatide treatment had marked effects on β-cell function. Fasting insulin secretion rates were slightly increased, while β-cell glucose sensitivity was greatly improved by lixisenatide as compared with placebo. With regard to the mechanisms underlying these effects, in the perfused rat pancreas, lixisenatide directly stimulates both first- and second-phase insulin release (28). Likewise, in patients with type 2 diabetes, lixisenatide enhances both first- and second-phase insulin response to an IV glucose challenge (29). In addition, chronically lower fasting and postprandial glycemia reduces the impact of hyperglycemia/glucose toxicity on β-cell function. Of note is that baseline β-cell glucose sensitivity (~20 pmol · min^{-1} · m^{-2} · mmol · L^{-1}) was already profoundly impaired as compared with the median value (105 pmol · min^{-1} · m^{-2} · mmol · L^{-1}) in a historical group of subjects without diabetes ($n = 96$, age = 55 years, BMI = 30.3 kg/m^2) receiving 75 g oral glucose (23). Thus, lixisenatide remains effective even in patients with β-cell dysfunction (17). Therefore, the improved glycemic control induced by lixisenatide can be ascribed to both an indirect mechanism (slower gastric emptying) and a direct action on the β-cell (30), although the multiple regression analysis indicates that the effect on gastric emptying was predominant.

Despite inducing profound slowing of gastric emptying, lixisenatide was well tolerated, and compliance was excellent. The presence of gastrointestinal adverse effects was specifically sought using a standardized questionnaire, and while these did occur in the early stages of treatment, they were mild and transient, as observed in previous analyses of self-reported adverse effects from trials involving GLP-1 receptor agonists (31). This highlights the fact that slowing of gastric emptying per se is unlikely to be the major driver of gastrointestinal symptoms induced by GLP-1 receptor agonists and is consistent with the recognition that, in general, there is a weak relationship between symptoms and delayed gastric emptying (32).

The strengths of the current study are that optimal techniques were used to measure both gastric emptying and postprandial glucose metabolism, in relation to the same glucose drink. The effects of lixisenatide on gastric emptying, systemic appearance of oral glucose, and postprandial glycemia were marked, and the dose and timing of administration of lixisenatide conformed to standard clinical practice. One limitation of the study was that the patients in general had relatively good glycemic control and few complications, which may limit the generalizability of the observations. However, in more severely hyperglycemic patients, achieving good control of fasting glycemia would be a higher priority than would optimizing postprandial glycemia, which was the target of interest in the current study. Another limitation was that the study was of only 8 weeks' duration and was not powered to examine relationships between changes in gastric emptying and HbA_{1c}. Ideally, the findings should be confirmed with a more physiological mixed solid and liquid meal; nonetheless, a 75-g oral glucose load is a well-accepted standard for evaluation of glucose tolerance, and probably helped reduce heterogeneity in this proof-of-concept study. Furthermore, we compared lixisenatide with placebo rather than an active comparator; it would now be of interest to examine effects on gastric emptying after prolonged exposure to a long-acting GLP-1 receptor agonist. Also, we measured the effects of lixisenatide on gastric emptying after 8 weeks' treatment but not acutely, so we cannot be certain that there was not a slight decline in its effect over time. However, in our recent study evaluating 15 patients with type 2 diabetes by scintigraphy after a single 10-μg dose of lixisenatide and a 75-g glucose drink (21), gastric emptying over the first 120 min showed a ~50% reduction with lixisenatide compared with placebo (mean 0.75 vs. 1.57 kcal · min^{-1}). A similar calculation in the current study indicates mean gastric emptying of 0.56

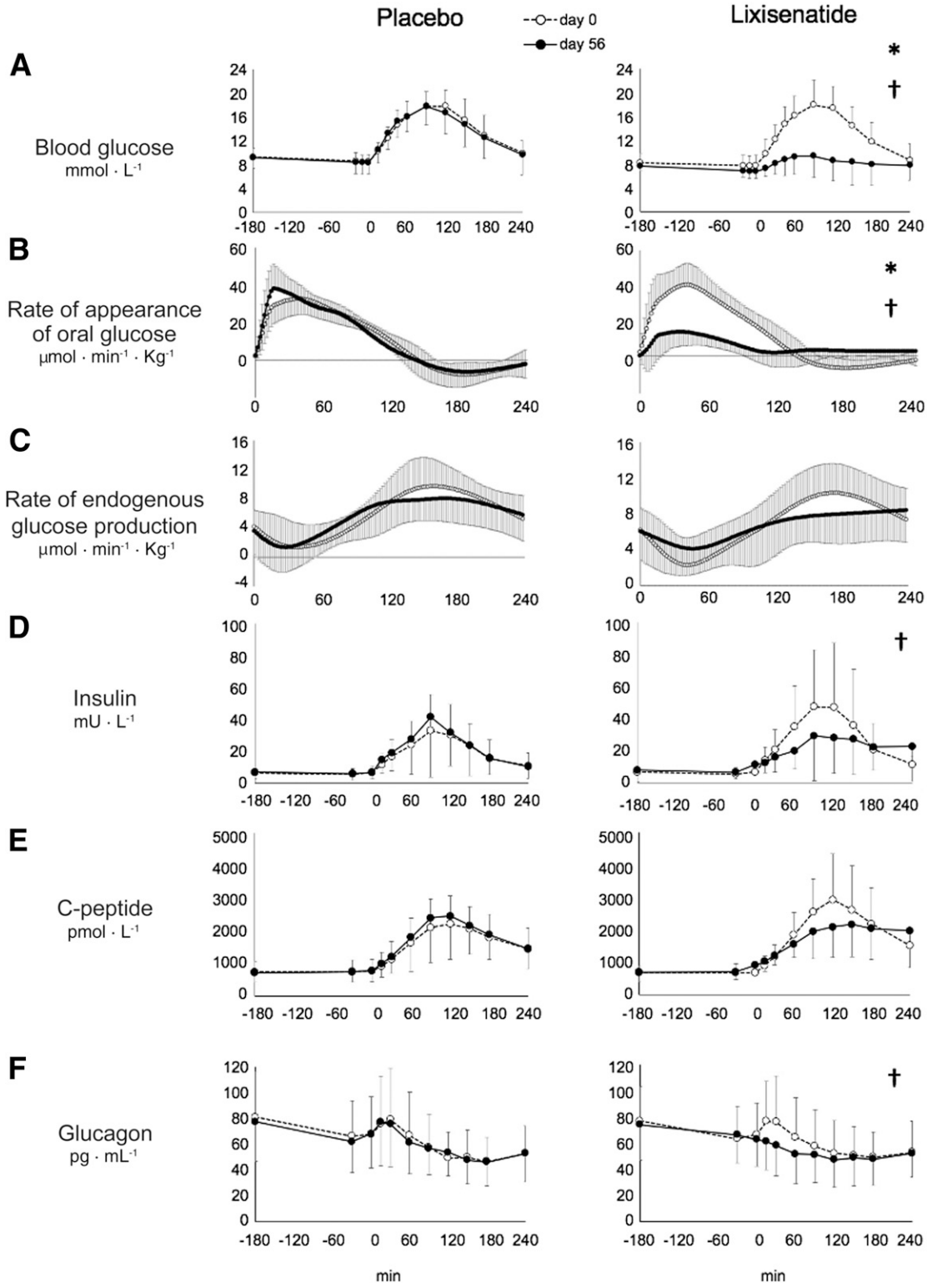

Figure 2—Blood glucose concentration (*A*), R_a of oral glucose (*B*), rate of endogenous glucose production (*C*), and plasma insulin (*D*), C-peptide (*E*), and glucagon (*F*) concentrations before and after a 75-g glucose drink consumed at $t = 0$ min at baseline (day 0) and after 8 weeks' treatment with placebo ($n = 15$) or lixisenatide ($n = 15$) (day 56) in 30 metformin-treated patients with type 2 diabetes. Data are means (SD). *Significant adjusted group differences at day 56 for AUC (0–240 min). †Significant adjusted group differences at day 56 for AUC (0–120 min).

kcal · min^{-1} after lixisenatide and 1.95 kcal · min^{-1} after placebo, i.e., a 70% reduction. Although the lixisenatide dose was higher in the current study, it seems unlikely that there could have been much diminution in effect compared with acute exposure. Finally, it is possible that suppression of small intestinal motility and transit by lixisenatide could have contributed to the reduction in postprandial glycemia by reducing the rate of small intestinal glucose absorption, as we have

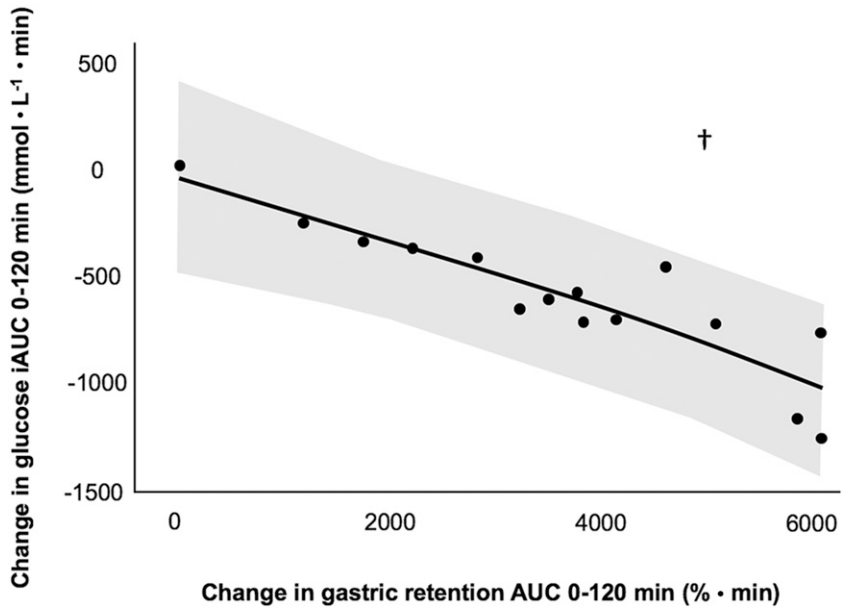

Figure 3—Relationship between the change in postprandial blood glucose iAUC after 8 weeks' treatment with lixisenatide and the change in gastric retention AUC for 0–120 min in 15 patients treated with lixisenatide (Pearson $r = -0.89$, †$P < 0.001$).

previously shown with exenatide (33), but we did not evaluate small intestinal function in the current study. However, the close relationship between the slowing of gastric emptying and reduction in R_a of the ingested glucose tracer suggests that any contribution of small intestinal effects was probably modest.

In summary, we have demonstrated, using optimal techniques, that lixisenatide has profound effects to slow gastric emptying and retard the systemic appearance of orally ingested carbohydrate that persist with sustained use and relate closely to the ability of this medication to achieve marked lowering of postprandial glycemia. Short-acting GLP-1 receptor agonists are likely to be particularly applicable to patients with relatively good overall glycemic control and rapid or at least normal rates of gastric emptying at baseline, irrespective of duration of diabetes. Potentially, such patients could be identified using a screening test for gastric emptying, such as a stable isotope breath test (34), as a means of individualizing the choice of glucose-lowering medications, particularly since we have shown that the rate of gastric emptying remains relatively stable within individual patients when followed over a number of years (35).

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Author Contributions. C.K.R. designed the study, analyzed and interpreted the data, and drafted the manuscript. L.E.W. assisted with study design, recruited patients, and collected and analyzed data. L.K.P. recruited patients and collected and analyzed data. K.L. undertook the statistical analysis. M.J.B. recruited patients and collected and analyzed data. J.G. recruited patients and collected and analyzed data. T.W. analyzed the data and reviewed the manuscript. K.L.J. analyzed scintigraphic data and reviewed the manuscript. M.H. contributed to study design and data interpretation and reviewed the manuscript. E.F. contributed to study design and data interpretation and reviewed the manuscript. D.T. contributed to study design and data interpretation. S.F. contributed to sample analysis and data interpretation. A.M. analyzed and interpreted the data and reviewed the manuscript. A.N. contributed to study design, analyzed and interpreted the data, and reviewed the manuscript. C.K.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837–853

2. Davies MJ, D'Alessio DA, Fradkin J, et al. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia 2018;61:2461–2498

3. Kemp TM, Barr EL, Zimmet PZ, et al. Glucose, lipid, and blood pressure control in Australian adults with type 2 diabetes: the 1999-2000 AusDiab. Diabetes Care 2005;28:1490–1492

4. Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA_{1c}. Diabetes Care 2003;26:881–885

5. Riddle M, Umpierrez G, DiGenio A, Zhou R, Rosenstock J. Contributions of basal and postprandial hyperglycemia over a wide range of A1C levels before and after treatment intensification in type 2 diabetes. Diabetes Care 2011;34:2508–2514

6. Deane AM, Nguyen NQ, Stevens JE, et al. Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. J Clin Endocrinol Metab 2010;95:215–221

7. Little TJ, Pilichiewicz AN, Russo A, et al. Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. J Clin Endocrinol Metab 2006;91:1916–1923

8. Nauck MA, Niedereichholz U, Ettler R, et al. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol 1997;273:E981–E988

9. Jones KL, Horowitz M, Wishart MJ, Maddox AF, Harding PE, Chatterton BE. Relationships between gastric emptying, intragastric meal distribution and blood glucose concentrations

in diabetes mellitus. J Nucl Med 1995;36:2220–2228
10. Gonlachanvit S, Hsu CW, Boden GH, et al. Effect of altering gastric emptying on postprandial plasma glucose concentrations following a physiologic meal in type-II diabetic patients. Dig Dis Sci 2003;48:488–497
11. Nauck MA, Kemmeries G, Holst JJ, Meier JJ. Rapid tachyphylaxis of the glucagon-like peptide 1-induced deceleration of gastric emptying in humans. Diabetes 2011;60:1561–1565
12. Umapathysivam MM, Lee MY, Jones KL, et al. Comparative effects of prolonged and intermittent stimulation of the glucagon-like peptide 1 receptor on gastric emptying and glycemia. Diabetes 2014;63:785–790
13. Kapitza C, Forst T, Coester HV, Poitiers F, Ruus P, Hincelin-Méry A. Pharmacodynamic characteristics of lixisenatide once daily versus liraglutide once daily in patients with type 2 diabetes insufficiently controlled on metformin. Diabetes Obes Metab 2013;15:642–649
14. Drucker DJ, Buse JB, Taylor K, et al.; DURATION-1 Study Group. Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study. Lancet 2008;372:1240–1250
15. Meier JJ, Rosenstock J, Hincelin-Méry A, et al. Contrasting effects of lixisenatide and liraglutide on postprandial glycemic control, gastric emptying, and safety parameters in patients with type 2 diabetes on optimized insulin glargine with or without metformin: a randomized, open-label trial. Diabetes Care 2015;38:1263–1273
16. Marselli L, Suleiman M, Masini M, et al. Are we overestimating the loss of beta cells in type 2 diabetes? Diabetologia 2014;57:362–365
17. Bonadonna RC, Blonde L, Antsiferov M, et al. Lixisenatide as add-on treatment among patients with different β-cell function levels as assessed by HOMA-β index. Diabetes Metab Res Rev 2017; 33:e2897
18. Muscelli E, Casolaro A, Gastaldelli A, et al. Mechanisms for the antihyperglycemic effect of sitagliptin in patients with type 2 diabetes. J Clin Endocrinol Metab 2012;97:2818–2826
19. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. Br Med J (Clin Res Ed) 1982;285:916–918
20. Horowitz M, Maddox AF, Wishart JM, Harding PE, Chatterton BE, Shearman DJ. Relationships between oesophageal transit and solid and liquid gastric emptying in diabetes mellitus. Eur J Nucl Med 1991;18:229–234
21. Jones KL, Rigda RS, Buttfield MDM, et al. Effects of lixisenatide on postprandial blood pressure, gastric emptying and glycaemia in healthy people and people with type 2 diabetes. Diabetes Obes Metab 2019;21:1158–1167
22. Mari A, Stojanovska L, Proietto J, Thorburn AW. A circulatory model for calculating nonsteady-state glucose fluxes. Validation and comparison with compartmental models. Comput Methods Programs Biomed 2003;71:269–281
23. Chang J, Russo A, Bound M, Rayner CK, Jones KL, Horowitz M. A 25-year longitudinal evaluation of gastric emptying in diabetes. Diabetes Care 2012;35:2594–2596
24. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419
25. Watson LE, Xie C, Wang X, et al. Gastric emptying in patients with well-controlled type 2 diabetes compared with young and older control subjects without diabetes. J Clin Endocrinol Metab 2019;104:3311–3319
26. Seghieri M, Rebelos E, Gastaldelli A, et al. Direct effect of GLP-1 infusion on endogenous glucose production in humans. Diabetologia 2013;56:156–161
27. Camastra S, Astiarraga B, Tura A, et al. Effect of exenatide on postprandial glucose fluxes, lipolysis, and ß-cell function in non-diabetic, morbidly obese patients. Diabetes Obes Metab 2017;19:412–420
28. Riz M, Pedersen MG, Toffolo GM, et al. Minimal modeling of insulin secretion in the perfused rat pancreas: a drug effect case study. Am J Physiol Endocrinol Metab 2014;306:E627–E634
29. Meier JJ, Schenker N, Kahle M, Schliess F, Kapitza C, Menge BA. Impact of insulin glargine and lixisenatide on β-cell function in patients with type 2 diabetes mellitus: a randomized open-label study. Diabetes Obes Metab 2017; 19:1625–1629
30. Meier JJ, Kemmeries G, Holst JJ, Nauck MA. Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. Diabetes 2005;54:2212–2218
31. Horowitz M, Aroda VR, Han J, Hardy E, Rayner CK. Upper and/or lower gastrointestinal adverse events with glucagon-like peptide-1 receptor agonists: incidence and consequences. Diabetes Obes Metab 2017;19:672–681
32. Jones KL, Russo A, Stevens JE, Wishart JM, Berry MK, Horowitz M. Predictors of delayed gastric emptying in diabetes. Diabetes Care 2001; 24:1264–1269
33. Thazhath SS, Marathe CS, Wu T, et al. The glucagon-like peptide 1 receptor agonist exenatide inhibits small intestinal motility, flow, transit, and absorption of glucose in healthy subjects and patients with type 2 diabetes: a randomized controlled trial. Diabetes 2016;65:269–275
34. Szarka LA, Camilleri M, Vella A, et al. A stable isotope breath test with a standard meal for abnormal gastric emptying of solids in the clinic and in research. Clin Gastroenterol Hepatol 2008; 6:635–643.e1
35. Watson LE, Phillips LK, Wu T, et al. Longitudinal evaluation of gastric emptying in type 2 diabetes. Diabetes Res Clin Pract 2019;154:27–34