Effects of Lixisenatide Versus Liraglutide (Short- and Long-Acting GLP-1 Receptor Agonists) on Esophageal and Gastric Function in Patients With Type 2 Diabetes

Daniel R. Quast,[1] Nina Schenker,[1] Björn A. Menge,[1] Michael A. Nauck,[1] Christoph Kapitza,[2] and Juris J. Meier[1]

OBJECTIVE

Short-acting glucagon-like peptide 1 receptor agonists (GLP-1 RAs) decelerate gastric emptying more than long-acting GLP-1 RAs. Delayed gastric emptying is a risk factor for gastroesophageal reflux disease. We aimed to measure esophageal reflux and function as well as gastric emptying and acid secretion during treatment with short-acting (lixisenatide) and long-acting (liraglutide) GLP-1 RAs.

RESEARCH DESIGN AND METHODS

A total of 57 subjects with type 2 diabetes were randomized to a 10-week treatment with lixisenatide or liraglutide. Changes from baseline in the number of reflux episodes during 24-h pH registration in the lower esophagus, lower esophagus sphincter pressure, gastric emptying (^{13}C-sodium octanoate acid breath test), and gastric acid secretion (^{13}C-calcium carbonate breath test) were analyzed.

RESULTS

Gastric emptying half-time was delayed by 52 min (Δ 95% CI 16, 88) with lixisenatide ($P = 0.0065$) and by 25 min (3, 46) with liraglutide ($P = 0.025$). There was no difference in the number of reflux episodes (mean ± SEM 33.7 ± 4.1 vs. 40.1 ± 5.3 for lixisenatide and liraglutide, respectively, $P = 0.17$) or the extent of gastroesophageal reflux (DeMeester score) (35.1 ± 6.7 vs. 39.7 ± 7.5, $P = 0.61$), with similar results for the individual GLP-1 RAs. No significant changes from baseline in other parameters of esophageal motility and lower esophageal sphincter function were observed. Gastric acidity decreased significantly by −20.7% (−40.6, −0.8) ($P = 0.042$) with the GLP-1 RAs.

CONCLUSIONS

Lixisenatide exerted a more pronounced influence on gastric emptying after breakfast than liraglutide. Neither lixisenatide nor liraglutide had significant effects on esophageal reflux or motility. Gastric acid secretion appears to be slightly reduced by GLP-1 RAs.

[1]Diabetes Division, Department of Medicine I, St Josef-Hospital Bochum, Ruhr-University Bochum, Bochum, Germany
[2]Profil, Neuss, Germany

Corresponding author: Juris J. Meier, juris.meier@ruhr-uni-bochum.de

Glucagon-like peptide 1 receptor agonists (GLP-1 RAs) can be categorized into long-acting (e.g., liraglutide, exenatide once weekly, dulaglutide, albiglutide, semaglutide) and short-acting (e.g., exenatide b.i.d. [unretarded], lixisenatide) compounds (1). Both subclasses of GLP-1 RAs differ markedly from each other with respect to their pharmacokinetic and pharmacodynamic properties (2,3). Mainly, short-acting GLP-1 RAs lead to short-lived peaks of drug concentrations after subcutaneous injection, the duration of which are several hours, with ensuing troughs with near-zero drug concentrations (4). Long-acting GLP-1 RAs provide circulating drug concentrations that are constantly elevated, with minor fluctuations over a 24-h or 7-day period (5,6). Consequently, premeal administration of short-acting GLP-1 RAs elicits a significant delay of gastric emptying even after prolonged treatment periods (weeks/months), thereby primarily reducing postprandial glucose elevations (7). In contrast, long-acting GLP-1 RAs initially delay gastric emptying, but their pharmacokinetic profile leads to desensitization for this effect over a period of weeks to months, known as tachyphylaxis (8). Therefore, during longer-term treatment, long-acting GLP-1 RAs influence postprandial glucose rises predominantly by their effects on insulin and glucagon secretion and have greater effects on fasting plasma glucose due to greater exposure during the night hours (7). Earlier studies with acute intravenous infusion of native human GLP-1 have also indicated an inhibition of gastric acid secretion, but whether this is also the case with GLP-1 RAs has not yet been determined (9).

Delayed gastric emptying and gastric distension are well-established risk factors in the pathophysiology of gastroesophageal reflux disease (GERD) (10). Thus, delayed gastric emptying may temporarily increase the intragastric content of both solids and acidic liquids, thereby increasing the likelihood of reflux into the lower esophagus (11). Several studies have reported higher frequencies of GERD symptoms in people with type 2 diabetes (12–14). So far, little information is available on the effects of GLP-1 RAs on the incidence of GERD (15). Treatment with GLP-1 RAs may theoretically further increase the incidence of gastroesophageal reflux in these patients

(16). However, the effect of GLP-1 RA treatment on gastroesophageal reflux and esophageal motility has not yet been determined.

Therefore, in the current study the effects of a short- and a long-acting GLP-1 RA (lixisenatide and liraglutide, respectively) on gastroesophageal reflux, esophageal motility, gastric emptying, and gastric acid secretion were compared over 10 weeks of treatment.

RESEARCH DESIGN AND METHODS

Study Design

The current study was a randomized, bicentric, investigator-blinded, parallel-group study in subjects with type 2 diabetes. The study was performed at the Diabetes Division, St Josef-Hospital, and at Profil. Informed consent was obtained from all participants. Patients were randomized using the program RANCODE Professional 3.6 (idv Datenanalyse & Versuchsplanung, Gauting, Germany). The randomization and blinding process is described in detail in Supplementary Material. The study was approved by the ethics committee of Ruhr-University Bochum (registration no. 14-5177 FF), and investigations were carried out in accordance with the principles of the Declaration of Helsinki as revised in 2008.

Study Population

The study included male and female patients aged 18–70 years with a range of BMI of 18–40 kg/m^2 (inclusive) who had been diagnosed with type 2 diabetes for at least 3 months. Treatment with a stable regimen of metformin and/or a sulfonylurea or metformin plus thiazolidinedione for at least 1 month or treatment with a stable regimen of insulin with or without additional oral antidiabetes drug treatment (metformin, sulfonylureas, thiazolidinediones) for at least 1 month was obligatory.

Exclusion criteria included contraindications (including known or suspected hypersensitivity) to GLP-1 RAs, current use of GLP-1 RAs or inhibitors of dipeptidyl-peptidase 4, intake of medication that may influence gastric acid secretion or gastrointestinal motility, decompensated glycemic control (HbA$_{1c}$ ≥10%), preexisting significant concomitant diseases (except those typically associated with type 2 diabetes, e.g., arterial hypertension, dyslipidemia), or prominent diabetes complications affecting parameters

to be measured in the current study (including history of GERD or overt gastroparesis), as judged by the investigator. Furthermore, clinically relevant impairment of hepatic, renal, or cardiac function as indicated by alanine aminotransferase, bilirubin, and/or alkaline phosphatase greater than threefold the upper limit of normal at screening, estimated glomerular filtration rate <60 mL/min/1.73 m^2 (estimate according to the MDRD study equation) at screening, clinically relevant electrocardiogram findings, or symptoms of heart failure (New York Heart Association class III–IV) as judged by the investigator were defined as exclusion criteria. Smokers or participants not able or willing to refrain from smoking were not included.

Safety Assessment

Monitoring of adverse events included symptomatic and severe hypoglycemic episodes and gastrointestinal disorders using a structured questionnaire that will be published separately. Laboratory monitoring included pancreatic, renal, and hepatic parameters. Laboratory analysis was performed at MLM Medical Laboratories Moenchengladbach GmbH and the laboratory of St Josef-Hospital.

Study Procedures

Screening Visit

At a screening visit, participants had their medical history taken and underwent a clinical examination. In addition, the following anthropometric variables were assessed: age, height, weight, heart rate, blood pressure, and waist and hip circumference. Venous blood samples were taken in the fasting state for the measurement of standard hematological and clinical chemistry variables.

Interventions

After screening, participants were randomized to receive either lixisenatide or liraglutide. Participants randomized to lixisenatide were administered 10 μg once daily for 1 week, followed by a 20-μg once-daily maintenance dose for the remainder of the trial. Participants randomized to receive liraglutide were administered 0.6 mg once daily for week one after initiation and 1.2 mg once daily for week two, followed by 1.8 mg once daily for the remaining period of the trial. In both groups, the GLP-1 RA was administered 30 min before breakfast. Subjects were asked to attend the clinical unit in the morning in the fasting

condition (only still water was allowed after 8:00 P.M. the evening before the visit) for screening and all other experimental visits.

Experimental Visits

24-h Ambulatory Esophageal pH Measurement.
Following a high-resolution esophageal manometry (HRM), an esophageal monitoring tube for a 24-h ambulatory manometric pH measurement was inserted. This tube contained a pH electrode that was placed 5 cm above the upper border of the lower esophageal sphincter (LES), as previously determined through HRM. Assessment of gastroesophageal reflux was based on the number and duration of episodes with pH <4.0 in the lower third of the esophagus and the DeMeester score (17,18).

HRM.
HRM was performed using a solid-state catheter with 36 circumferential pressure sensors spaced at 1-cm intervals (Unisensor, Attikon, Switzerland). A series of 10 swallows with 5 mL still water at room temperature was registered per manometry. The topographical plots and manometric parameters were analyzed using the software ViMeDat (Standard Instruments GmbH, Karlsruhe, Germany), which analyzes the manometry results based on the Chicago Classification of esophageal disorders (19).

Gastric Emptying of Solid Meal Components: ^{13}C-Sodium Octanoate Breath Test.
The ^{13}C-sodium octanoate breath test is a non-invasive test to measure gastric emptying (20). Study medication was injected 1 h before the meal. After an overnight fast of ~12 h, baseline breath samples were taken into gas-tight sampling bags. Subjects were then served a meal with a total caloric amount of ~1,175.7 kJ with 24% carbohydrate, 16% protein, and 60% fat. This meal was composed of egg, white bread, margarine, and still water. It included 91 mg ^{13}C-octanoic acid, mixed into scrambled eggs to label the meal, and had to be consumed within 10 min. Further breath samples were collected every 15 min after the test meal for 3 h, followed by every 30 min for another 3 h. Gastric emptying and gastric emptying half-time were analyzed using the Wagner-Nelson method (16,21,22). While the study drugs were taken before breakfast, all other morning medication was taken after completion of the gastric emptying test.

Noninvasive Determination of Gastric Acid Secretion: ^{13}C-Calcium Carbonate Breath Test.
The ^{13}C-calcium carbonate breath test (^{13}C-CC-BT) has been designed to estimate gastric acid secretion in a non-invasive manner (23). Study medication was injected 1 h before ingestion of the tracer. After an overnight fast of ~12 h, baseline breath samples were taken. Thereafter, subjects ingested 0.2 g ^{13}C-labeled calcium carbonate dispersed in still water. Breath samples were taken twice at baseline and every 15 min over the test duration of 90 min. ^{13}C-CC-BT was analyzed as described by Shinkai et al. (24). Maximum ^{13}C-CaCO$_3$–derived [13]CO$_2$ exhalation was chosen for estimation of gastric acid secretion, as it is reported to show a good correlation with the total secretion of gastric acid (24).

End Points
Primary end point was defined as change from baseline (week −1) in the number of reflux episodes over 24 h after 10 weeks of treatment with lixisenatide versus liraglutide. Secondary end points (each after 10 weeks of treatment with liraglutide versus lixisenatide) were change from baseline (week −1) in the time characterized by a pH <4.0 in the lower third of the esophagus, change from baseline of peristaltic tone in the esophagus and pressure of the LES, change from baseline of gastric emptying, and change from baseline of the amount of nonstimulated, stimulated, and steady-state gastric acid secretion.

Statistical Analyses and Sample Size
Subject characteristics are expressed as means ± SD or number (proportion of total). Results are expressed as means ± SEM or Δ (95% CI). Data were analyzed using Student t test for paired samples or Student t test with Welch correction for unpaired samples. Categorical parameters (contingency tables) were assessed with Fisher exact test. Gastric emptying was analyzed using repeated-measures ANOVA with gastric content (as % of the initial value) over 360 min as the dependent variable, treatment (after versus before initiation of GLP-1 RA treatment) as fixed variable, and subject as random independent variable. Differences after 10 weeks of treatment with lixisenatide and liraglutide versus baseline are expressed as Δ (with or without 95% CI). Statistical significance was defined as $P < 0.05$. For statistical analyses and figures, GraphPad Prism, version 8.0.0

for Windows (GraphPad Software, San Diego, CA) (www.graphpad.com), and Statistica (data analysis software system), version 13.5 for Windows (TIBCO Software, Palo Alto, CA) (http://tibco.com), were used.

Based on the assumption of a mean of 30 reflux episodes per 24 h and an SD of 35, $n = 50$ subjects would provide 90% power (at an α-level of 0.05) to detect a difference by 15, e.g., a 50% change after treatment with a GLP-1 RA. For differences in changes from baseline between lixisenatide and liraglutide treatment, $n = 25$ per groups would provide 90% power to detect a difference of 15 reflux episodes per 24 h assuming an SD of 15.

RESULTS

Baseline Data
A total of 110 male or female patients with type 2 diabetes were screened for the study. Of these, 57 (51.8%) subjects (all Caucasians) were enrolled. Seven (12.3%) participants did not complete the study (two [28.7%] participants withdrew consent before receiving study medication, two [28.7%] were incompliant with the study protocol, and three [42.9%] had unsuccessful esophageal manometry or pH measurements). Of the participants that completed the study, 26 (52%) received liraglutide and 24 (48%) received lixisenatide. Baseline characteristics of completers are depicted in Table 1. Aside from pulse rate, there was no significant difference between the groups at baseline (mean ± SD 68.7 ± 8.6 bpm in lixisenatide and 63.5 ± 8.1 bpm in liraglutide, $P = 0.035$) (Supplementary Table 1). Participants treated with liraglutide experienced more weight loss ($P = 0.0078$) and a greater reduction in BMI ($P = 0.020$) compared with those treated with lixisenatide (Supplementary Table 1). There was no significant difference in the concomitant use of other glucose-lowering agents between the groups (Supplementary Table 2). In consideration of the study period of 10 weeks, changes in HbA$_{1c}$ from baseline were not measured.

Esophageal pH Measurement
Baseline measurements were similar in both treatment groups (Table 2). Overall, there were no significant differences in the parameters of pH measurement (Fig. 1A–C). The number of reflux episodes did not change significantly after 10 weeks of

Table 1—Patients' characteristics at baseline

	All participants	Treated with lixisenatide	Treated with liraglutide	Significance of differences (P)
n female/n male (% female)	18/32 (36)	10/14 (41.7)	8/18 (30.8)	
Age, years	60.4 ± 7.4	60.7 ± 7.6	60.2 ± 7.3	0.81
Duration of type 2 diabetes, years	13.5 ± 1.0	12.6 ± 1.1	14.4 ± 1.7	0.38
Arterial hypertension	32 (64)	15 (62.5)	18 (69.2)	0.77
Duration of arterial hypertension, years	10.4 ± 1.5	15.6 ± 7.5	11.9 ± 1.9	0.64
Dyslipidemia	19 (38)	6 (25)	13 (50)	0.087
Diabetic neuropathy*	4 (8)	0	4 (15.4	0.11
Diabetic retinopathy	7 (14)	1 (4.2)	6 (23.1)	0.10
HbA$_{1c}$, % (mmol/mol)	7.5 ± 0.8 (58.8 ± 8.3)	7.5 ± 0.8 (58.7 ± 8.2)	7.5 ± 0.8 (58.9 ± 8.6)	0.93
Fasting plasma glucose, mmol/L	8.8 ± 2.3	9.0 ± 1.7	8.7 ± 2.7	0.61
Participants smoking	5 (10)	3 (12.5)	2 (7.7)	0.66
Participants consuming alcohol	32 (64)	15 (62.5)	17 (65.4)	>0.99
Alcohol consumption of participants consuming alcohol, units/week	3.7 ± 4.3	3.5 ± 4.2	3.9 ± 4.5	0.82
Height, cm	172.0 ± 10.6	169.5 ± 12.0	174.2 ± 8.7	0.12

Data are expressed as means ± SD or n (%) of patients unless otherwise indicated. P values were calculated from Student t test with Welch correction or Fisher exact test. *Based on vibration perception threshold and medical history.

treatment with lixisenatide (mean ± SEM 27.26 ± 5.21 to 32.11 ± 5.09, P = 0.23) or liraglutide (39.43 ± 6.14 to 47.29 ± 8.88, P = 0.34) (Table 2). There also were not significant changes in DeMeester score and time with pH <4.0 during the 24-h measurement period after 10 weeks of treatment (Table 2). At baseline, a DeMeester scoring indicating increased esophageal reflux (>14.7) was present in 7 (36.8%) participants in the lixisenatide and in 14 (66.7%) patients in the liraglutide group. There were numerical increases in those with abnormal results after 10 weeks of treatment for both GLP-1 RAs (from 21 [52.5%] to 26 [65.0%], P = 0.36), with lixisenatide treatment (from 7 [36.8%] to 11 [57.9%], P = 0.33) or with liraglutide treatment (from 14 [66.7%] to 15 [71.4%], P > 0.99), all of which were not significant. pH measurement and analysis were completed in 19 (79.2%) patients in the lixisenatide group and 21 (80.8%) patients of the liraglutide group (Supplementary Table 3).

High-Resolution Esophageal Manometry

Among the parameters examined, no difference was found for treatment with GLP-1 RAs in general (Fig. 1E–H) or for each GLP-1 RA studied individually (Table 2). Baseline measurements were similar. Neither lixisenatide nor liraglutide led to a significant change in resting pressure of the LES (Fig. 1E), mean distal pressure amplitude (Fig. 1F), maximum distal pressure amplitude (Fig. 1G), or the relaxation of the LES after the act of swallowing (Fig. 1H). HRM was successful in 23 (88.5%) participants of the liraglutide group and 19 (79.2%) of the lixisenatide group (Supplementary Table 3).

Gastric Emptying

After 10 weeks of treatment, gastric emptying was significantly delayed with lixisenatide (P < 0.0001) (Fig. 2A) and liraglutide (P < 0.0002) (Fig. 2B). Gastric emptying half-time was delayed, with a mean ± SEM increase by 25 ± 10 min (P = 0.025) in the liraglutide group and by 52 ± 17 min (P = 0.0065) in the lixisenatide group. The breath testing for gastric emptying was successful in 21 (87.5%) participants in the lixisenatide group and in 26 (100%) participants in the liraglutide group (Supplementary Table 3).

At baseline, three (5.3%) patients exhibited a prolongation of gastric emptying of >2 SD (216.8 ± 4.6 min). None of these patients reported symptoms of gastroparesis at screening. The results of the study were not altered by exclusion of these patients from analysis (data not shown).

Gastric Acid Secretion

There was no significant baseline difference between the lixisenatide group and the liraglutide group (P = 0.88). The pooled analysis showed a significant reduction of gastric acid production with the GLP-1 RAs, by −20.7 ± 9.9% (P = 0.042) (Fig. 1D). For the individual agents, no significant difference was found (Table 2). In five (10%) participants of the total study cohort, no ^{13}C excursion was registered at both study visits (one [20%] in the lixisenatide group and four [80%] in the liraglutide group, respectively). These participants were excluded from the primary analysis. However, with inclusion of these participants, the overall changes between baseline and 10 weeks were still significant (P = 0.044) and similar for lixisenatide (P = 0.19) and liraglutide (P = 0.11). The ^{13}C-CC-BT yielded results in 26 (100%) participants in the liraglutide group and in 24 (100%) participants in the lixisenatide group (Supplementary Table 3).

Safety

No significant difference in hypoglycemic or gastrointestinal adverse events was found between the groups (Supplementary Table 4). All patients with hypoglycemia were under treatment with insulin or sulfonylurea. No severe hypoglycemic episodes occurred during the trial.

CONCLUSIONS

The current study was designed to examine the effects of lixisenatide and liraglutide on gastroesophageal reflux, esophageal motility, gastric emptying, and gastric acid secretion in subjects with type 2 diabetes. We report that

Table 2—Parameters of upper gastrointestinal motility, gastroesophageal reflux, gastric emptying, and gastric acid secretion at baseline and after 10 weeks of treatment with lixisenatide or liraglutide

	Treatment with lixisenatide				Treatment with liraglutide				Difference between the groups	
	Baseline	After 10 weeks of treatment	Difference vs. baseline	P	Baseline	After 10 weeks of treatment	Difference vs. baseline	P	Difference between treatments	P
Esophageal reflux										
Episodes/24 h	27.3 ± 5.2	32.1 ± 5.1	4.8 (−3.4, 13.1)	0.23	39.4 ± 6.1	47.3 ± 8.9	7.9 (−9.1, 24.8)	0.34	−3.0 (−21.9, 15.9)	0.75
DeMeester score	23.2 ± 6.0	27.4 ± 6.4	4.2 (−10.9, 19.2)	0.57	45.8 ± 11.1	50.8 ± 12.8	5.0 (−28.9, 38.9)	0.76	−0.8 (−38.1, 36.5)	0.97
% time pH <4.0	2.9 ± 1.5	1.9 ± 0.7	−1.0 (−3.2, 1.2)	0.34	5.1 ± 2.0	8.4 ± 3.1	3.3 (−2.8, 9.4)	0.27	−4.3 (−10.8, 2.2)	0.19
Esophageal manometry										
Resting pressure of the LES (mmHg)	26.1 ± 2.6	26.1 ± 2.5	0.0 (−4.1, 4.1)	0.99	31.1 ± 3.0	32.9 ± 3.5	1.9 (−3.8, 7.6)	0.50	−1.9 (−8.7, 5.0)	0.58
Mean distal pressure amplitude (mmHg)	64.9 ± 7.9	64.4 ± 6.1	−0.6 (−12.7, 11.6)	0.93	81.3 ± 7.4	83.2 ± 7.5	1.9 (−7.1, 10.9)	0.66	−2.5 (−17.1, 12.2)	0.74
Maximum distal pressure amplitude (mmHg)	176.4 ± 16.7	177.1 ± 20.8	0.7 (−29.9, 31.2)	0.96	197.0 ± 15.6	180.6 ± 9.2	−16.4 (−43.2, 10.5)	0.22	17.0 (−22.4, 56.5)	0.39
Postdeglutitive esophageal relaxation (%)	66.3 ± 3.7	61.8 ± 3.9	−4.4 (−11.9, 3.1)	0.23	63.9 ± 3.0	60.1 ± 3.6	−3.8 (−10.2, 2.6)	0.23	−0.6 (−10.2, 8.9)	0.89
Gastric emptying half-time (min)	88.4 ± 7.7	135.9 ± 16.0	52.0 (16.3, 87.8)	0.0065	97.5 ± 9.0	122.1 ± 12.8	24.6 (3.4, 45.7)	0.025	27.5 (−13.2, 68.1)	0.17
Gastric acid secretion: maximum ^{13}C excretion ($\Delta^{13}C_{maximum}$) (‰)	138.9 ± 24.4	107.5 ± 11.9	−31.4 (−79.3, 16.4)	0.19	145.2 ± 15.7	118.0 ± 9.5	−27.3 (−60.6, 6.0)	0.10	−4.2 (−61.3, 53.0)	0.88

Data are presented as means ± SEM or, for difference vs. baseline (Δ), as mean Δ (95% CI). Difference between treatments, as mean Δ (95% CI), is displayed for changes from baseline. P values were calculated from Student t test for paired samples or Student t test with Welch correction. Postdeglutitive, after the act of swallowing.
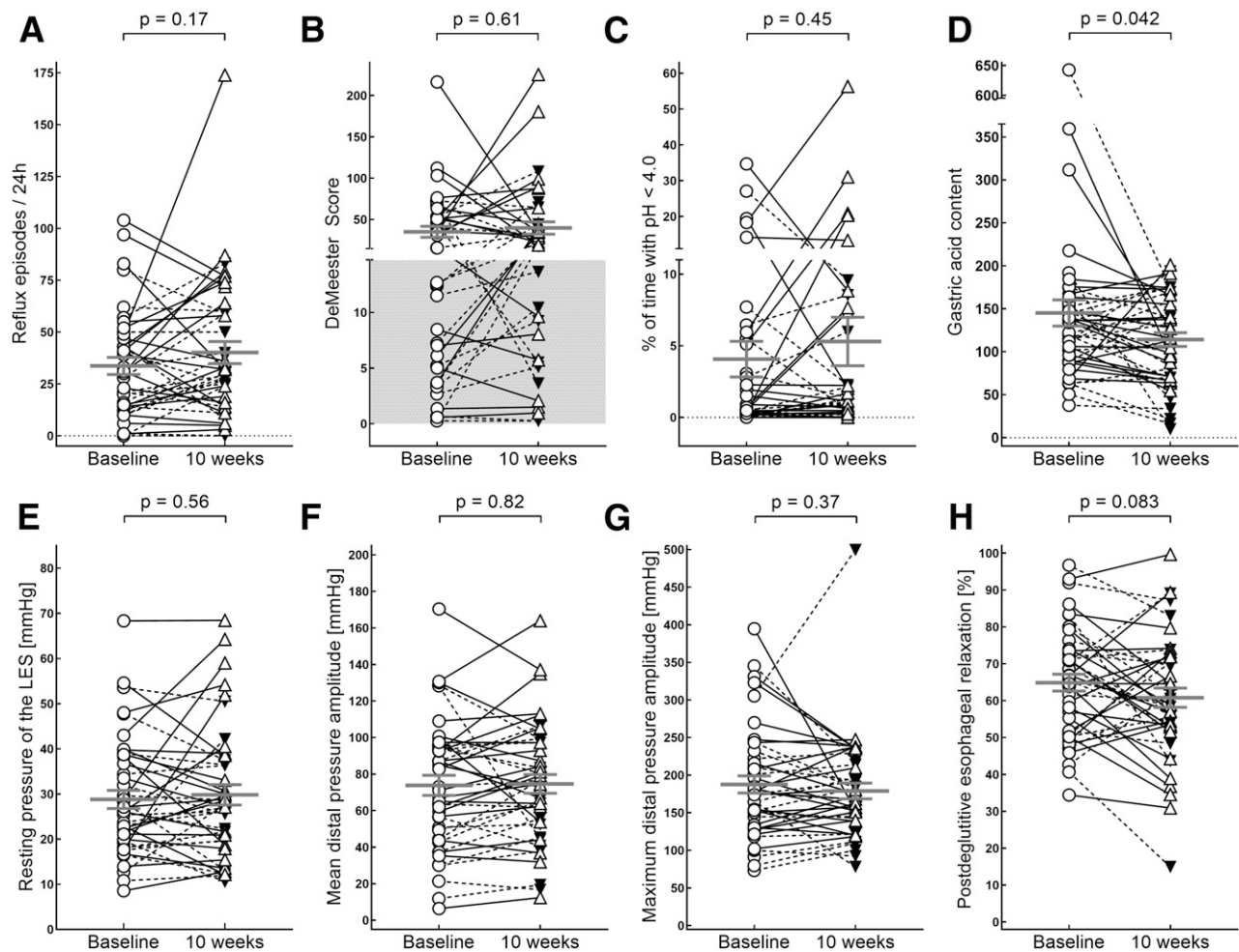
Figure 1—Various parameters before and after treatment with lixisenatide (▼) and liraglutide (△). Data are presented as individual values and means ± SEM. P values were calculated from Student t test for paired samples. For differences between lixisenatide and liraglutide treatments, see Table 2. A–C: Parameters derived from 24-h pH measurements. Measurements were performed 5 cm above the LES (identified through manometry). A: Number of reflux episodes per 24 h. B: DeMeester score. The gray bottom segment indicates the physiologic range (DeMeester score of up to 14.7). C: Percentage time with pH <4.0 during the registration period. D: Gastric acid content in the fasting state, 60 min after injection of the study drug. E–H: Results of the high-resolution manometry. Visualized are resting pressure of the LES (E), mean distal pressure amplitude (F), maximum distal pressure amplitude (G), and postdeglutitive esophageal relaxation (H).

neither lixisenatide nor liraglutide treatment had significant effects on the number of gastroesophageal reflux episodes per 24-h period. Likewise, esophageal motility was not affected. After 10 weeks of treatment, both GLP-1 RAs elicited a significant delay in gastric emptying of solid components of a test breakfast. Lixisenatide and liraglutide led to a minor suppression of gastric acid secretion, which only reached statistical significance when analyses for both GLP-1 RAs were combined.

The lack of effect of both GLP-1 RAs on gastroesophageal reflux and on various measures of esophageal motility is a novel and somewhat unexpected finding, since any persisting delay in gastric emptying induced especially with a short-acting

GLP-1 RA might have been expected to promote gastroesophageal reflux events (10). The small increase of participants with a DeMeester scoring indicating significant reflux seen in the lixisenatide did not show statistical significance. In the liraglutide group, no such effect was seen. The difference may also be due to the higher percentage of participants with a pathological DeMeester scoring at baseline in the liraglutide group compared with the lixisenatide group (66.7% vs. 36.8%, respectively). Nonetheless, none of the described results were significant. Thus, even though gastric emptying is significantly delayed in both agents, no significant effects on gastroesophageal reflux were observed. For lixisenatide, the absence of such effects might reflect

the transient nature of GLP-1 receptor activation with lixisenatide. For liraglutide, a less pronounced effect on gastric emptying may further reduce the risk of reflux.

Another important end point of the current study was the impact of lixisenatide and liraglutide on esophageal motility. Esophageal motility is regulated in a complex manner, and multiple aspects ranging from propulsion and relaxation to the resting tone of the esophagus and the LES need to be considered (25). Because of the inhibitory effect of GLP-1 RAs on gastric emptying, a missing influence on the resting pressure of the LES was rather unexpected. A prolonged gastric filling could have been expected to show at least a minor influence on the
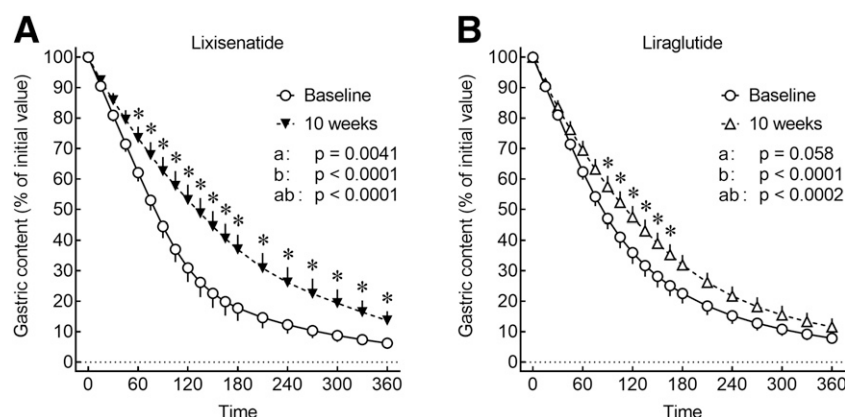
Figure 2—Gastric emptying of solid components in lixisenatide (*A*) and liraglutide (*B*) before and after 10 weeks of treatment. Gastric content was calculated with the Wagner-Nelson equation. Data are expressed as means ± SEM. Data were analyzed using repeated-measures ANOVA with gastric content (as % of the initial value) over 360 min as the dependent variable, treatment (after vs. before initiation of GLP-1 RA treatment) as fixed variable, and subject as random independent variable. The results are presented with *P* values marked 'a' representing the overall effect of the intervention, *P* values marked 'b' representing the effect of time, and *P* values marked 'ab' representing the interaction of intervention and time on gastric emptying. If a or ab were significant ($P < 0.05$), post hoc (Duncan) tests were performed to locate significant differences to single time points. For gastric emptying half-time, see Table 2.

resting pressure due to a more pronounced upper gastric distension. However, neither a short-acting nor a long-acting GLP-1 RA showed a significant effect on any of the esophageal pressure parameters examined. It should be noted that in clinical practice, the evaluation of HRM involves not only the quantitative analyses conducted in the current study but also a visual analysis of the respective pressure changes over time in more qualitative terms. In the current study, numeric analysis was favored due to its better reproducibility and lower dependence on investigators' experience.

Inhibition of gastric acid secretion has previously been reported during intravenous GLP-1 administration after pentagastrin stimulation (26). In the current study, an indirect measure of gastric acid secretion based on calcium carbonate metabolism was employed. Notably, unlike in earlier studies, this assessment was performed not after stimulation (i.e., by pentagastrin) but, rather, under baseline conditions. This approach was used successfully to evaluate acid suppression after acid blocker treatment, but no use of this method in evaluation of GLP-1 RAs has been reported so far (23). Interestingly, a small, but significant (mean ± SEM −20.7 ± 9.9%, *P* = 0.042), reduction in gastric acid secretion was found in the combined analysis of effects of either lixisenatide or liraglutide

treatment. A significant effect was not observed in the individual analysis of lixisenatide's or liraglutide's effects, perhaps because of low statistical power. However, compared with the effect of proton pump inhibitors the effect is minor (23). The minimal inhibition of gastric acid secretion observed with both our study drugs and previously with native GLP-1 (27) is compatible with a reduction in gastrin secretion as recently indicated (28), as well as with the expression of GLP-1 receptors on parietal cells (29).

Five participants (four in the liraglutide group) showed no significant gastric acid production on both visits. Since the intake of proton pump inhibitors or other medication that may influence gastric acidity or gastrointestinal motility was not allowed in the current study, the lack of gastric acid production suggests an underlying condition such as atrophic gastritis, which is known to reduce acid production and ^{13}C excursion in ^{13}C-CC-BT (24).

Whether there is a clinical impact of the reduction of gastric acid secretion with GLP-1 RAs is unclear. Theoretically, any reduction in gastric acid secretion might reduce the subsequent risk for reflux events or damage caused thereby, e.g., reflux esophagitis or duodenal ulcers. Recently, the administration of GLP-1 was shown to reduce gastrin secretion

in healthy individuals and in individuals with type 2 diabetes, suggesting a possible mechanism for the observed reduction in acidity (28). However, given the small magnitude of this effect, a major clinical impact appears unlikely.

Lixisenatide and liraglutide significantly inhibited gastric emptying after 10 weeks of treatment. The short-acting GLP-1 RA lixisenatide showed a more pronounced effect, with a delay of the gastric emptying half-time of more than twice that with the long-acting GLP-1 RA liraglutide. Previous studies reported a large difference in the inhibition of gastric emptying with short-acting versus long-acting GLP-1 RAs, which cannot be reported in the current study (30,31). Indeed, the delay in nutrient absorption subsequent to delayed gastric emptying is believed to represent the main mechanism of action reducing postprandial glucose excursions with short-acting GLP-1 RAs but applies mainly to meals before which such medications have been injected (32). Nevertheless, with long-acting GLP-1 RAs, the effects on gastric emptying are largely diminished during chronic treatment because of tachyphylaxis (2). In contrast with this and in line with the present results, a recent study reported a persistent effect of the long-acting formulation of exenatide on gastric emptying after a treatment period of 8 weeks (33). Hence, it remains unclear how much time it takes to reach a steady state in therapy with short-acting versus long-acting GLP-1 RAs and after which period tachyphylaxis can be observed.

Since recruitment was a major challenge during this study, the study protocol was amended to allow the inclusion of patients pretreated with dipeptidyl-peptidase 4 inhibitors. In total, four patients with sitagliptin and one patient with vildagliptin (lixisenatide group) were recruited (Supplementary Table 2). A washout period of 4 weeks preceded the inclusion. However, previous studies have shown that both sitagliptin and vildagliptin have no impact on gastric emptying (34,35). Furthermore, all reported parameters maintained their statistical significance when these participants were excluded from the analyses.

A couple of limitations need to be considered: The assessment of gastric emptying and gastric acid secretion was performed using noninvasive breath

tests, which allowed for the estimation of the respective parameters without radioactive exposure. However, only semiquantitative estimates can be generated using these measurements. While we acknowledge that gastric emptying scintigraphy represents the gold standard, the octanoate breath test has previously been validated and used extensively in previous studies with GLP-1 RAs (36,37). Indeed, the Wagner-Nelson approach for deriving gastric emptying from $^{13}CO_2$ exhalation data gives similar results compared with scintigraphy (22). Also, aspiration of gastric juice after pentagastrin stimulation may be considered the gold standard for the determination of (maximum) gastric acid secretion, but such an approach appears further away from physiological conditions than the currently employed calcium carbonate breath test. A placebo arm and a third visit after 7 days may have added further information. Due to the study design, no conclusion about early effects can be drawn. The study was powered to exclude an increase in reflux episodes of >50%, but no firm conclusions regarding less pronounced changes in the number of reflux events can be drawn. The study design was investigator blinded, but open-label for patients. Finally, improper placement of the pH measurement tube caused by using only pH variation for placement is susceptible for artifacts and can influence the DeMeester score. To avoid this, the proper positioning of the pH measurement tube was ascertained by prior manometric determination of the LES, thereby providing additional validation. It is also theoretically possible that changes in meal size or composition induced by the GLP-1 RAs might have reduced the frequency of reflux episodes, thereby potentially obscuring an induction of GERD.

In conclusion, the current study has demonstrated that despite the delay in gastric emptying, neither lixisenatide nor liraglutide leads to significant alterations in gastroesophageal reflux events or esophageal motility. These findings provide additional reassurance as to the overall gastrointestinal safety of GLP-1 RAs. The minor reduction of gastric acid secretion might protect against the risk of reflux or gastritis.

References

1. Nauck MA, Meier JJ. Management of endocrine disease: are all GLP-1 agonists equal in the treatment of type 2 diabetes? Eur J Endocrinol 2019;181:R211–R234
2. Meier JJ. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. Nat Rev Endocrinol 2012;8:728–742
3. Abd El Aziz MS, Kahle M, Meier JJ, Nauck MA. A meta-analysis comparing clinical effects of short- or long-acting GLP-1 receptor agonists versus insulin treatment from head-to-head studies in type 2 diabetic patients. Diabetes Obes Metab 2017;19:216–227
4. Trujillo JM, Goldman J. Lixisenatide, a once-daily prandial glucagon-like peptide-1 receptor agonist for the treatment of adults with type 2 diabetes. Pharmacotherapy 2017;37:927–943
5. Jacobsen LV, Flint A, Olsen AK, Ingwersen SH. Liraglutide in type 2 diabetes mellitus: clinical pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 2016;55:657–672
6. Geiser JS, Heathman MA, Cui X, et al. Clinical pharmacokinetics of dulaglutide in patients with type 2 diabetes: analyses of data from clinical trials. Clin Pharmacokinet 2016;55:625–634
7. 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
8. Jelsing J, Vrang N, Hansen G, Raun K, Tang-Christensen M, Knudsen LB. Liraglutide: short-lived effect on gastric emptying – long lasting effects on body weight. Diabetes Obes Metab 2012;14:531–538
9. Wettergren A, Wøjdemann M, Meisner S, Stadil F, Holst JJ. The inhibitory effect of glucagon-like peptide-1 (GLP-1) 7-36 amide on gastric acid secretion in humans depends on an intact vagal innervation. Gut 1997;40:597–601
10. Bor S. Reflux esophagitis, functional and non-functional. Best Pract Res Clin Gastroenterol 2019;40-41:101649
11. Wang KY, Chen YW, Wang TN, et al. Predictor of slower gastric emptying in gastroesophageal reflux disease: survey of an Asian-Pacific cohort. J Gastroenterol Hepatol 2019;34:837–842
12. Sun XM, Tan JC, Zhu Y, Lin L. Association between diabetes mellitus and gastroesophageal reflux disease: a meta-analysis. World J Gastroenterol 2015;21:3085–3092
13. Rouf MA, Khan M, Sharif JU, et al. Prevalence of GERD in type II diabetes mellitus patients admitted in a tertiary care hospital of Bangladesh. Mymensingh Med J 2017;26:710–715
14. Lluch I, Ascaso JF, Mora F, et al. Gastroesophageal reflux in diabetes mellitus. Am J Gastroenterol 1999;94:919–924
15. Noguchi Y, Katsuno H, Ueno A, et al. Signals of gastroesophageal reflux disease caused by incretin-based drugs: a disproportionality analysis using the Japanese adverse drug event report database. J Pharm Health Care Sci 2018;4:15
16. Phillips LK, Rayner CK, Jones KL, Horowitz M. Measurement of gastric emptying in diabetes. J Diabetes Complications 2014;28:894–903
17. Johnson LF, Demeester TR. Twenty-four-hour pH monitoring of the distal esophagus. A quantitative measure of gastroesophageal reflux. Am J Gastroenterol 1974;62:325–332
18. Gyawali CP, Kahrilas PJ, Savarino E, et al. Modern diagnosis of GERD: the Lyon Consensus. Gut 2018;67:1351–1362
19. Kahrilas PJ, Bredenoord AJ, Fox M, et al.; International High Resolution Manometry Working Group. The Chicago Classification of esophageal motility disorders, v3.0. Neurogastroenterol Motil 2015;27:160–174
20. Choi MG, Camilleri M, Burton DD, Zinsmeister AR, Forstrom LA, Nair KS. [^{13}C]octanoic acid breath test for gastric emptying of solids: accuracy, reproducibility, and comparison with scintigraphy. Gastroenterology 1997;112:1155–1162

21. Sanaka M, Nakada K, Nosaka C, Kuyama Y. The Wagner-Nelson method makes the [^{13}C]-breath test comparable to radioscintigraphy in measuring gastric emptying of a solid/liquid mixed meal in humans. Clin Exp Pharmacol Physiol 2007;34:641–644

22. Trahair L, Nauck M, Wu T, et al. Measurement of gastric emptying using scintigraphy and a ^{13}C-octanoic acid breath test with Wagner-Nelson analysis in type 2 diabetes (54(th) EASD Annual Meeting of the European Association for the Study of Diabetes: Berlin, Germany, 1 - 5 October 2018). Diabetologia 2018;61:1–620

23. Miyamoto S, Tsuda M, Kato M, et al. Evaluation of gastric acid suppression with vonoprazan using calcium carbonate breath test. J Clin Biochem Nutr 2019;64:174–179

24. Shinkai H, Iijima K, Koike T, et al. Calcium carbonate breath test for non-invasive estimation of gastric acid secretion. Tohoku J Exp Med 2014;232:255–261

25. Tolone S, Savarino E, Docimo L. Is there a role for high resolution manometry in GERD diagnosis? Minerva Gastroenterol Dietol 2017;63:235–248

26. Meier JJ, Nauck MA, Kask B, et al. Influence of gastric inhibitory polypeptide on pentagastrin-stimulated gastric acid secretion in patients with type 2 diabetes and healthy controls. World J Gastroenterol 2006;12:1874–1880

27. Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W. Lack of effect of synthetic human gastric inhibitory polypeptide and glucagon-like peptide 1 [7-36 amide] infused at near-physiological concentrations on pentagastrin-stimulated gastric acid secretion in normal human subjects. Digestion 1992;52:214–221

28. Rehfeld JF, Knop FK, Asmar M. Gastrin secretion in normal subjects and diabetes patients is inhibited by glucagon-like peptide 1: a role in the gastric side effects of GLP-1-derived drugs? Scand J Gastroenterol 2019;54:1448–1451

29. Pyke C, Heller RS, Kirk RK, et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. Endocrinology 2014;155:1280–1290

30. Horowitz M, Flint A, Jones KL, et al. Effect of the once-daily human GLP-1 analogue liraglutide on appetite, energy intake, energy expenditure and gastric emptying in type 2 diabetes. Diabetes Res Clin Pract 2012;97:258–266

31. 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

32. Becker RH, Stechl J, Steinstraesser A, Golor G, Pellissier F. Lixisenatide reduces postprandial hyperglycaemia via gastrostatic and insulinotropic effects. Diabetes Metab Res Rev 2015;31:610–618

33. Jones KL, Huynh LQ, Hatzinikolas S, et al. Exenatide once weekly slows gastric emptying of solids and liquids in healthy, overweight people at steady-state concentrations. Diabetes Obes Metab 2020;22:788–797

34. Stevens JE, Buttfield M, Wu T, et al. Effects of sitagliptin on gastric emptying of, and the glycaemic and blood pressure responses to, a carbohydrate meal in type 2 diabetes. Diabetes Obes Metab 2020;22:51–58

35. He YL. Clinical pharmacokinetics and pharmacodynamics of vildagliptin. Clin Pharmacokinet 2012;51:147–162

36. Lorenz M, Pfeiffer C, Steinsträsser A, et al. Effects of lixisenatide once daily on gastric emptying in type 2 diabetes–relationship to postprandial glycemia. Regul Pept 2013;185:1–8

37. Meier JJ, Menge BA, Schenker N, et al. Effects of sequential treatment with lixisenatide, insulin glargine, or their combination on meal-related glycaemic excursions, insulin and glucagon secretion, and gastric emptying in patients with type 2 diabetes. Diabetes Obes Metab 2020;22:599–611