Mechanisms of Action of Liraglutide in Patients With Type 2 Diabetes Treated With High-Dose Insulin

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Context: The mechanisms of action of incretin mimetics in patients with long-standing type 2 diabetes (T2D) and high insulin requirements have not been studied.

Objective: To evaluate changes in β-cell function, glucagon secretion, and fat distribution after addition of liraglutide to high-dose insulin.

Design: A single-center, randomized, double-blind, placebo-controlled trial.

Setting: University of Texas Southwestern and Parkland Memorial Hospital clinics.

Patients: Seventy-one patients with long-standing (median, 17 years) T2D requiring high-dose insulin treatment (>1.5 U/kg/d; average, 2.2 ± 0.9 U/kg/d).

Intervention: Patients were randomized to liraglutide 1.8 mg/d or matching placebo for 6 months.

Main Outcome Measures: We measured changes in insulin and glucagon secretion using a 4-hour mixed-meal challenge test. Magnetic resonance-based techniques were used to estimate sc and visceral fat in the abdomen and ectopic fat in the liver and pancreas.

Results: Glycosylated hemoglobin improved significantly with liraglutide treatment, with an end-of-trial estimated treatment difference between groups of −0.9% (95% confidence interval, −1.5, −0.4%) ($P = .002$). Insulin secretion improved in the liraglutide group vs placebo, as measured by the area under the curve of C-peptide ($P = .002$) and the area under the curves ratio of C-peptide to glucose ($P = .003$). Insulin sensitivity (Matsuda index) and glucagon secretion did not change significantly between groups. Liver fat and sc fat decreased in the liraglutide group vs placebo ($P = .0006$ and $P = .01$, respectively), whereas neither visceral nor pancreatic fat changed significantly.

Conclusions: Treatment with liraglutide significantly improved insulin secretion, even in patients with long-standing T2D requiring high-dose insulin treatment. Liraglutide also decreased liver and sc fat, but it did not alter glucagon secretion. **(*J Clin Endocrinol Metab* 101: 1798–1806, 2016)**

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Abbreviations: AUC, area under the curve; AUC_C, AUC for C-peptide; AUC_G, AUC for glucose; CI, confidence interval; ETD, estimated treatment difference; GLP-1 RA, glucagon-like peptide-1 receptor agonists; HbA1c, glycosylated hemoglobin; iAUC, incremental AUC; MMCT, mixed-meal challenge test; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; SAT, subcutaneous adipose tissue; T2D, type 2 diabetes; TE, echo time; VAT, visceral adipose tissue.

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Progression of type 2 diabetes (T2D) is hallmarked by β-cell dysfunction and insulin resistance. The etiology of these events in diabetes is not completely understood, but one unifying theory is glucolipotoxicity (1). Weight gain, lipotoxicity, and unsuppressed glucagon secretion are some of the important mechanisms contributing to worsening hyperglycemia. Chronic overfeeding, possibly along with an abnormality in fat storage, leads to an abundant and constant supply of free fatty acids, which can be stored in ectopic places where they fuel chemical reactions deleterious to these organs (2). It has been demonstrated that intramyocellular fat accumulation is strongly correlated with peripheral insulin resistance, and intrahepatic lipid accumulation leads to hepatic insulin resistance (3). In addition to the classical effect of peripheral insulin resistance, hepatic insulin resistance, and β-cell dysfunction, unopposed unregulated α-cell hypersecretion of glucagon worsens hepatic gluconeogenesis and ketone production (4, 5).

In patients requiring high insulin doses, these pathophysiological abnormalities are further fueled, leading to a vicious cycle that perpetuates hyperglycemia. Insulin infusion has been shown to increase hepatocellular lipids by 18%, and hepatocellular fat content has been shown to correlate with insulin dosage in patients with T2D (6). Insulin also promotes weight gain, which in turn drives the lipotoxicity process and might help explain progressive β-cell dysfunction with high insulin doses. To achieve good glycemic control in patients treated with high insulin doses, it is imperative to interrupt this vicious cycle.

Treatment with glucagon-like peptide-1 receptor agonists (GLP-1 RA) may protect β-cells against lipotoxicity-induced apoptosis by achieving oxidative balance and inhibiting islet cell inflammation (7, 8). GLP-1 RA have also shown the ability to activate AMP-activated protein kinase in liver and muscle with resultant improvements in insulin sensitivity (9), and there is growing evidence that GLP-1 has direct metabolically desirable effects on the liver that could improve hepatic steatosis (10–15), including suppression of hepatic lipogenesis, stimulation of lipid oxidation, and improvement of hepatic sensitivity (16).

Prospective, placebo-controlled clinical trials attempting to understand the glucose-lowering effect of GLP-1 RA have focused primarily on their use as add-on therapy in patients relatively early in their progression of T2D, typically only on oral antihyperglycemic medications or basal insulin (17–20). Although GLP-1 RA have been found to reduce postprandial glucose and glucagon levels and improve β-cell function (21), it is not known to what extent these same mechanisms explain the glycemic improvements found in patients with long-standing diabetes treated with high-dose insulin. The purpose of this report was to investigate the mechanisms that might be responsible for the observed glycemic improvement when the GLP-1 RA liraglutide is added to high-dose insulin regimens in patients with uncontrolled T2D (22), specifically by assessing insulin secretion, glucagon dynamics, adipose distribution, and ectopic (hepatic and pancreatic) fat deposition.

Patients and Methods

Overall design

This was a single-center, randomized, double-blinded, placebo-controlled trial that evaluated the effectiveness, safety, and mechanisms of action of liraglutide in patients with uncontrolled T2D requiring high doses of insulin (Clinical Trials.gov; NCT01505673). The primary outcome (changes in glycemic control) was reported previously (22). We are reporting here the prespecified secondary outcomes of insulin secretion, glucagon secretion, and fat distribution, including hepatic fat content.

Eligible patients had T2D, were using a total daily dose of insulin of >1.5 U/kg/d, had glycosylated hemoglobin (HbA1c) between 7.5% and 11%, age ≥18 years, and stable doses of all hypoglycemic agents (including insulin dose) for ≥3 months before enrollment. The University of Texas (UT) Southwestern Research Ethics Board approved the protocol, and all participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization guidelines.

Eligible patients were randomized after a 2-week placebo-only run-in period to receive liraglutide or matching placebo. We performed a computer-generated, stratified blocked randomization using HbA1c (8.5%) and weight (104 kg) as stratification variables. The study drug (liraglutide or matching placebo) was initiated at a dose of 0.6 mg/d and administered sc daily (any time of the day at the same time). The dose was increased weekly to 1.2 mg/d and to the final dose of 1.8 mg/d. Follow-up clinic visits occurred at 1, 2, 4, and 6 months after randomization. All background medications, including insulin, remained stable during the study. Insulin was decreased by 20% at randomization in patients with a baseline HbA1c <8% and thereafter was only titrated for safety (hypoglycemia or symptomatic hyperglycemia).

Study measurements

Mixed-meal challenge test

Evaluation of β-cell function was performed in a 4-hour mixed-meal challenge test (MMCT), using high-protein Boost 1 g/kg carbohydrate equivalent ingested over 5 minutes. The MMCT was performed at randomization and at end of study (after 6 months of treatment). Patients fasted for 12 hours before each test. The study drug, oral antidiabetic agents, and basal insulin were withheld for >24 hours before each testing, whereas short-acting insulin was withheld for >12 hours before each testing. HbA1c and routine chemistry were measured in the fasting state at time "0" (baseline), before ingestion of the test meal. Plasma glucose, glucagon, and C-peptide were measured at baseline (before ingestion of the mixed meal) and six more times over the 4-hour test (30, 60, 90, 120, 180, and 240 minutes). Glucose, chemistry, and HbA1c were measured at a commercial laboratory (Quest Diagnostics). C-peptide was

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measured using the Mercodia C-peptide ELISA per the manufacturer's instructions at the MD Anderson Cancer Research Center, Houston, Texas. The cross-reactivity of the ELISA has been determined as 2% for proinsulin, 2% for proinsulin (split 32–33), 3% for proinsulin (des 31–32), 10% for proinsulin (split 64–65), 74% for proinsulin (des 64–65), and less than .0006% for insulin. The minimal limit of C-peptide detection for this ELISA is 15 pmol (0.045 mg/L) ± 2 SD, with a total coefficient of variation of 5.07 ± 1.92 (within assay, 3.6 ± 1.0; and between assays, 3.27 ± 2.32, respectively). Glucagon was determined by RIA (Millipore) per the manufacturer's instructions in the Clinical Diabetes Laboratory at UT Southwestern. The radioactivity was measured in a Wizard 1470 Automatic Gamma Counter (PerkinElmer). This RIA has a specificity of 100% for glucagon and <0.1% for oxyntomodulin and no cross-reactivity to insulin, proinsulin, C-peptide, somatostatin, or pancreatic polypeptide. The lowest detection limit of the assay is 18.45 pg/mL ± 2 SD (for sample volume of 100 μL). The coefficient of variation within the same assay is 4.85 ± 1.33, and between assays it was 11.73 ± 2.97. All samples were processed immediately after collection and stored at −80°C until analysis. All samples from the same patient were analyzed in the same batch.

The area under the curve (AUC) and incremental AUC (iAUC) for glucose (AUC_G), C-peptide (AUC_C), and glucagon ($AUC_{Glucagon}$) were measured using the trapezoidal rule. Insulin secretion was estimated using the AUC_G and AUC_C (total, incremental, and 0-maximal production) and then determining ratios (AUC_C/AUC_G). Insulin sensitivity was calculated using the C-peptide-based Matsuda index (23, 24) adapted to MMCT (25, 26). We used C-peptide instead of insulin levels to eliminate cross-contamination with exogenous insulin and insulin antibodies. The calculation was done according to the following formula: Matsuda index = $500\,000/\sqrt{((C_0 \times G_0 \times 333) \times (C_{mean} \times G_{mean} \times 333))}$. The oral disposition index was measured by multiplying the insulin secretion (AUC_C/AUC_G) by the Matsuda index to estimate the β-cell function adjusted for total body insulin sensitivity (27–29).

Fat distribution

Volumes of abdominal sc adipose tissue (SAT) and visceral adipose tissue (VAT) were determined at baseline and 6 months after randomization from single abdominal axial images at the level between the vertebral L2 and L3 bodies, as previously described (30, 31). A single observer blinded to the volunteer's characteristics and treatment assignment performed all image analyses.

Liver and pancreas fat content was quantified using a 3 Tesla whole-body magnetic resonance imaging (MRI) scanner (Achieva; Philips Healthcare), as previously described (31, 32). MRI data were acquired using a Torso-XL synergy phased-array coil or the build-in body coil for those patients with large body habitus where imaging with the phased-array coil was not feasible. Anatomic single-shot T2-weighted fast spin echo images through the abdomen were collected to locate the liver and pancreas with patients in the supine position holding their breath at end-exhalation to improve reproducibility. Using three perpendicular images of the liver, a volume of 30 × 30 × 30 mm^3 for spectroscopic (proton magnetic resonance spectroscopy [MRS]) testing was selected within the liver, avoiding any perivisceral fat and large intrahepatic vessels. Spectroscopic data were collected as patients breathed freely with magnetic resonance acquisition triggered at exhalation, using a PRESS sequence (PointRESolvedSPectroscopy) with the following acquisition parameters: average number of signals, 16; minimum echo time (TE), 28 milliseconds. Data processing was done using commercial software (LC-Model) (33). Data from the PRESS sequence were back-corrected for T2 decay with values obtained from a five-echo (TE1 = 12 milliseconds; ΔTE = 20 milliseconds) STEAM spectroscopy acquired in a single additional breath hold using a similar-size acquisition volume and anatomic location in the liver as was used for the PRESS acquisition. The T2 values collected from individual subjects were averaged, and the same T2 decay correction was applied to all subjects. Pancreatic fat content was quantified using a breath-held three-dimensional axial spoiled gradient echo acquisition with six echoes and multipeak spectral modeling (mpDixon-Quant pulse sequence; preproduct software at the time of study) (34). This imaging method was chosen to minimize partial volume effects of peripancreatic fat, which are difficult to avoid when applying MRS methods (35). On the 4-mm-thick axial proton density fat fraction images, a representative section through the pancreatic body was selected. On this section, the entire visible pancreas was manually segmented, and the mean pixel proton density fat fraction value was calculated (36, 37). To prevent the retroperitoneal fat intervening the pancreatic lobules from introducing bias, any pixels having >50% fat fraction were excluded in this calculation.

Statistical analysis

The sample size for the overall study was estimated to detect a treatment difference between liraglutide and placebo of 0.5% for HbA1c based on the change from randomization to month 6; the standard deviation of the HbA1c change was estimated at 0.7%. The analysis of this primary outcome showed a significant difference between groups (P = .002). The current report describes the prespecified secondary outcomes of this study.

An intention-to-treat analysis is reported, consisting of all randomized subjects who received study medication and had at least one postrandomization study visit. For continuous endpoints, treatment responses were compared with linear mixed-effects repeated-measures models. These models included a between-treatment group factor, a repeated factor for study evaluation visits, and a group × visit interaction term; the study participant was modeled as a random effect. The difference in response between treatment groups was assessed via the interaction effect. Pairwise comparisons were made using the least-square contrasts derived from these mixed-effects models. An additional repeated factor was included in the mixed model to analyze the MMCT time course. Variables with positively skewed distributions were log-transformed before analysis. Results are presented as mean and standard deviation, unless otherwise specified. Univariate associations were assessed using Spearman rank correlation (*rho*). A two-sided P value <.05 was considered statistically significant. Statistical analyses were conducted with SAS 9.4 software (SAS Institute).

Results

We screened 98 patients, of which 71 were randomized (35 to the liraglutide group, and 36 to the placebo group).

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Table 1. Baseline Characteristics According to Treatment Group Assignment

	Liraglutide	Placebo
n	35	36
Age, y	52.8 (8.1)	55.5 (6.6)
Gender, %		
Men	34.3	38.9
Women	65.7	61.1
BMI, kg/m^2	40.7 (6.7)	41.6 (10.4)
Weight, kg	114.6 (21.4)	116.1 (26.6)
Diabetes duration, y	16 [12–23]	18 [13–27]
Background medications, %		
Metformin	80.0	63.9
Statins	80.0	88.9

Abbreviation: BMI, body mass index. Results are expressed as mean (SD) or median [25th percentile–75th percentile] unless otherwise noted.

Completion rates were high: 91% in the liraglutide group, and 94% in the placebo group. Baseline characteristics were similar between the two groups (Table 1). Patients had long-standing uncontrolled T2D with a median time of insulin use of 8 (range, 4–13) years and total daily dose of insulin of 2.2 ± 0.9 U/kg/d. The insulin regimens were as follows: premixed human insulin (48% of patients), basal-bolus regimen with analog insulins (40%), human insulin NPH and regular combination (8%), and U500 regular human insulin (4%), all equally distributed between the two groups; these remained the same throughout the study.

As previously reported (22), the decrease in HbA1c was significantly greater in the liraglutide group (estimated treatment difference [ETD], −0.9%; 95% confidence interval [CI], −1.5, −0.4%); $P = .002$) (Table 2). Furthermore, patients in the liraglutide group had a greater reduction in body mass index (ETD, −0.9%; 95% CI, −1.6, −0.2%; $P = .01$), and weight (ETD, −2.34 kg; 95% CI, −4.32, −0.36 kg; $P = .02$). Patients in the liraglutide group had a 11.5% (95% CI, −21.8, −1.1%; $P = .2$) reduction in daily insulin dose requirement.

Table 2. MMCT Results and Fat Distribution at Randomization and After 6 Months of Treatment With Liraglutide or Placebo

	Liraglutide		Placebo		*P* Value Between Groups
	Baseline	6 Months	Baseline	6 Months	
n	35	32	36	34	
HbA1c, %	9.0 (1.2)	7.9 (1.1)	8.9 (1.0)	8.9 (1.3)	.002
MMCT measurements					
Fasting glucose, mg/dL	217 (69)	179 (75)	213 (77)	197 (98)	.36
Fasting C-peptide, μg/L	2.05 (0.96)	2.48 (1.18)	2.11 (1.42)	1.75 (1.19)	.006
Fasting glucagon, pg/mL	104.8 (44.3)	107.3 (44.5)	93.5 (42.2)	93.8 (37.8)	.98
AUC_G, mg/dL/min	82 256 (22 598)	71 747 (22 141)	83 049 (18 948)	79 278 (29 416)	.34
AUC_C, μg/L/min	1001.2 (429.4)	1234.6 (588.6)	1065 (577.7)	922.9 (470.5)	.002
$AUC_{Glucagon}$, pg/mL	32 989 (11 547)	32 290 (10 691)	29 415 (13 092)	30 195 (13 339)	.60
Ratio AUC_C/AUC_G	0.013 (0.007)	0.019 (0.010)	0.014 (0.009)	0.013 (0.008)	.003
Ratio $AUC_C/AUC_{Glucagon}$	0.035 (0.018)	0.044 (0.027)	0.041 (0.023)	0.037 (0.027)	.03
$iAUC_G$, mg/dL/min	30 128 (11 635)	28 772 (11 160)	32 012 (12 143)	31 987 (11 460)	.76
$iAUC_C$, μg/L/min	509.4 (294.2)	639.2 (445.6)	558.1 (308.3)	502.0 (315.9)	.05
$iAUC_{Glucagon}$, pg/mL	7830.4 (7596.9)	6527.8 (6003.1)	6974.6 (6960.2)	7691.5 (8955.4)	.42
Ratio $iAUC_C/iAUC_G$	0.02 (0.013)	0.026 (0.019)	0.0197 (0.013)	0.017 (0.011)	.04
G_{max}, mg/dL	345.5 (93.6)	308.4 (86.2)	347.0 (77.5)	335.2 (120.6)	.39
C_{max}, μg/L	4.78 (1.96)	5.93 (2.93)	4.88 (2.61)	4.34 (2.13)	.004
$Glucagon_{max}$, pg/mL	160.2 (58.7)	158.0 (55.1)	143.8 (66.5)	140.7 (63.1)	.99
$\Delta G_{0\text{-}max}$, mg/dL	128.3 (58.2)	129.3 (50.3)	134.3 (61.9)	138.2 (55.0)	.97
$\Delta C_{0\text{-}max}$, μg/L	2.73 (1.50)	3.45 (2.43)	2.77 (1.60)	2.58 (1.54)	.08
$\Delta Glucagon_{0\text{-}max}$, pg/mL	55.3 (44.9)	50.6 (35.6)	50.3 (44.2)	46.9 (48.7)	.95
Ratio $(\Delta C/\Delta G)_{0\text{-}max}$	0.03 (0.02)	0.03 (0.02)	0.02 (0.02)	0.02 (0.01)	.23
Matsuda index	2.18 [1.65, 3.31]	2.88 [1.67, 3.70]	2.28 [1.48, 4.07]	3.12 [1.78, 9.70]	.17
Disposition index	0.17 (0.14, 0.22)	0.27 (0.21, 0.33)	0.19 (0.15, 0.23)	0.24 (0.17, 0.34)	.48
Abdominal fat distribution					
Subcutaneous, cm^2	333.2 (118.5)	311.9 (104.9)	353.3 (176.9)	376.6 (188.6)	.01
Visceral, cm^2	375.6 (138.3)	355.0 (139.9)	338.9 (142.2)	339.0 (120.1)	.11
Ratio visceral/total	52.5 (13.5)	52.53 (12.7)	49.7 (17.1)	48.9 (15.0)	.70
Liver fat, %	15.7 (8.5)	12.3 (5.8)	10.9 (5.8)	12.2 (7.8)	.0006
Pancreatic fat, %	13.44 (6.39)	12.53 (8.13)	14.02 (7.66)	14.64 (9.25)	.24

Abbreviations: G, glucose; C, C-peptide; max, highest data point after baseline; iAUC, incremental over baseline AUC; Δ, change; 0-max, difference between the value at baseline and the maximum level post-baseline. Data are expressed as mean (SD) unless otherwise noted. Matsuda index is expressed as median [25th percentile–75th percentile]. Disposition index is expressed as geometric mean ± 95% CI. Matsuda index, disposition index, and pancreatic fat data were log-transformed before analysis.

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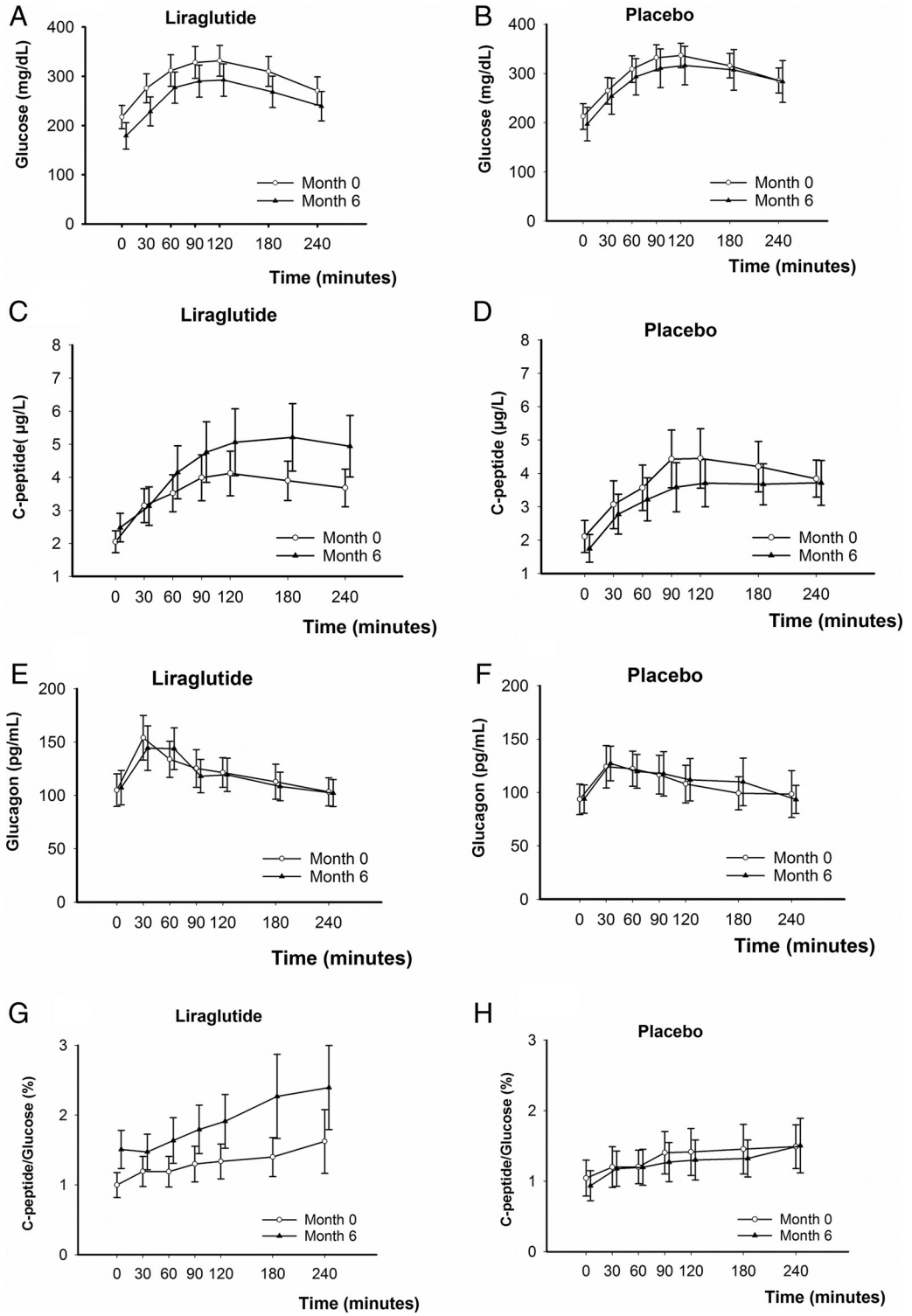

Figure 1. Changes in the biological parameters, measured during a 4-hour MMCT, at baseline, and after 6 months of treatment with liraglutide (A, C, E, and G) or placebo (B, D, F, and H) in patients with long-standing T2D requiring high-dose insulin treatment. Data are expressed as mean and 95% CI of the mean.

Glucose and β-cell function

The results of the MMCT along with the calculated insulin secretion, insulin sensitivity, and β-cell function indices are shown in Table 2. Fasting glucose decreased significantly within the liraglutide group ($P = .03$), but no significant difference between groups was found ($P = .36$). Fasting C-peptide increased significantly within the liraglutide group ($P = .04$), but not in the placebo group ($P = .07$) ($P = .006$ between groups).

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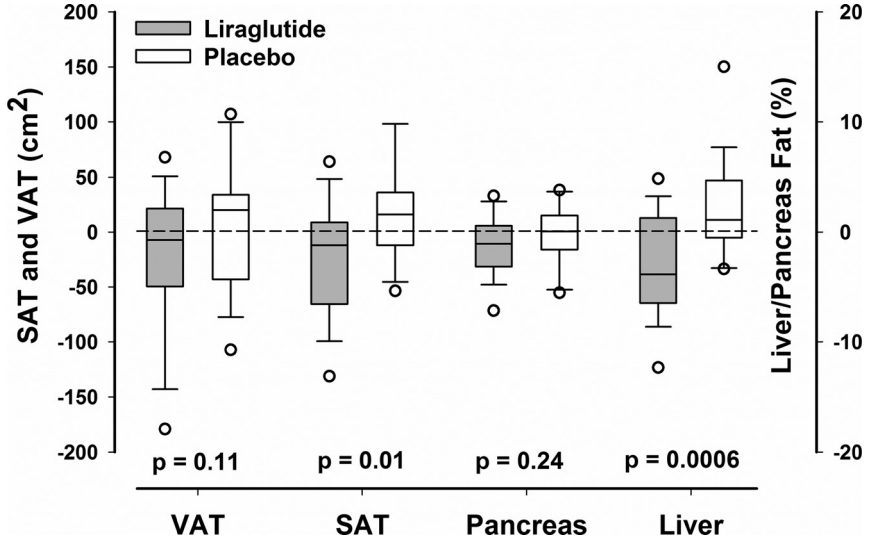

Figure 2. Changes in the distribution of fat content over 6 months of treatment with liraglutide compared to placebo in patients with T2D requiring high-dose insulin treatment. Data are expressed as median and interquartile range.

AUC_G decreased significantly in the liraglutide group ($P = .03$), but there was not a significant difference between groups ($P = .34$) (Table 2 and Figure 1). After the oral meal challenge, there was a significant improvement in insulin secretion between groups as calculated by AUC_C ($P = .002$) and total insulin secretion AUC_C/AUC_G ($P = .003$). The liraglutide group showed a significant increase in β-cell function when adjusted for total body insulin sensitivity (disposition index) ($P = .02$), although the between-group difference was not significant ($P = .48$). There was no significant change in insulin sensitivity (Matsuda index) between groups.

Patients in the liraglutide group had improved incremental insulin secretion with significant increases in $iAUC_C$ ($P = .05$) and $iAUC_C/iAUC_G$ ($P = .04$), although incremental glucose levels were not statistically different either within or between groups. There was a significant increase in C_{max} ($P = .004$) and a nearly significant increase in $\Delta C_{0\text{-}max}$ ($P = .08$).

In the liraglutide group, changes in HbA1c correlated negatively with $iAUC_C$ (rho = −0.43; $P = .014$), AUC_C/AUC_G (rho = −0.396; $P = .027$), and $iAUC_C/iAUC_G$ (rho = −0.37; $P = .04$) and positively with AUC_G (rho = 0.37; $P = .035$).

Glucagon secretion

Fasting glucagon levels did not change over the course of the study in either group (Table 2). Glucagon secretion did not change in either group, with nonsignificant effects on $AUC_{Glucagon}$, $iAUC_{Glucagon}$, $Glucagon_{max}$, and $\Delta Glucagon_{0\text{-}max}$ (Figure 1). $AUC_C/AUC_{Glucagon}$ improved in the liraglutide group and worsened in the placebo group ($P = .03$ between groups).

Liver and pancreatic fat content and adipose tissue distribution

Results of the change in fat distribution are shown in Table 2 and Figure 2. Eighty-four percent of patients underwent MRI/MRS with the Torso XL phased-array coil, and the remainder required the use of the built-in body coil. The liraglutide group, compared to placebo, had a significant reduction in abdominal SAT ($P = .01$ between groups), whereas VAT and the ratio of visceral to total fat did not change significantly between groups. Liver fat content decreased significantly in the liraglutide group compared with the placebo group ($P = .0006$ between groups). Pancreatic fat content improved minimally in the liraglutide group (median change, −1.3; interquartile range, −3.87, 0.6; $P = .056$) and remained unchanged in the placebo group (median change, 0.09; interquartile range, −1.92, 1.82; $P = .36$). The between-group difference in pancreatic fat content was not significant ($P = .24$), as shown in Figure 2.

Discussion

Our findings demonstrate that the known beneficial effect of liraglutide on insulin secretion is present even in patients with long-standing T2D who require treatment with high doses of insulin (>1.5 U/kg/d). In contrast, liraglutide had no effect on glucagon secretion or insulin sensitivity in this population. Furthermore, we observed a significant reduction in hepatic steatosis and sc fat content.

This is the first study to evaluate the changes induced by liraglutide in the underlying disease pathophysiology in patients with long-standing T2D who require high-dose insulin treatment. These results were counterintuitive because one might expect that patients with such long-standing disease would have little to no residual β-cell function and improvements in glycemic control would be driven primarily through suppression of glucagon and improvements in insulin sensitivity, either via weight loss or directly by drug effect. To the contrary, we found that liraglutide exerted its hypoglycemic effect through improving insulin secretion and did not significantly impact α-cell function or insulin sensitivity.

These findings suggest that liraglutide may slow and possibly improve the long-term disease progression via improvements in β-cell function. This effect, previously

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noted in patients with lower insulin doses or with earlier stage diabetes (38–40), was seen even in this population with long-standing disease and minimal baseline C-peptide secretion. It is likely that liraglutide stimulates GLP-1R-dependent mechanisms within the remaining β-cells to enhance their proliferation, cytoprotection, and insulin secretion, thereby disrupting the glucolipotoxic effects of long-standing diabetes and high doses of insulin (41).

Liraglutide did not have an impact on glucagon secretion in this study. This may be due to the exposure time to liraglutide in this study; recent evidence suggests that GLP-1 RA only suppress glucagon acutely, but with longer-term use (as in our study) this effect is not sustained (38, 42). For example, in the LIraglutide and Beta-cell RepAir (LIBRA) Trial, postchallenge hyperglucagonemia was noted with chronic use of liraglutide, which emerges as early as 12 weeks of treatment (20). Additionally, we instructed our patients to hold liraglutide the morning of the MMCT, which may have further contributed to the lack of effect on α-cell suppression, perhaps an effect that is only seen in the presence of the drug. The increase of $AUC_C/AUC_{Glucagon}$ in the liraglutide group suggests that liraglutide exerts its effect more on β-cells than α-cells. One might speculate that the paracrine effect of increased insulin secretion should have helped to lower postprandial glucagon levels, although in C-peptide-negative subjects with type 1 diabetes, GLP-1 RA (GLP-1 RAs) were also able to inhibit glucagon secretion (43), and the overall mechanism of GLP-1 inhibition of glucagon secretion remains controversial (41).

Liraglutide led to reductions in liver fat content, which is consistent with previous reports by Jendle et al (13) using liraglutide as monotherapy or added to metformin. Furthermore, Armstrong et al (44) conducted a randomized placebo-controlled trial of 52 patients with biopsy-proven nonalcoholic steatohepatitis who were assigned to either 1.8 mg liraglutide or placebo for 48 weeks. Patients underwent liver biopsy before randomization and again after treatment. In this study, the liraglutide group had significant reductions of adipose inflammation and tissue lipolysis, hepatic lipogenesis, and both hepatic and adipose insulin resistance (45). GLP-1 receptors are present in hepatocytes, and GLP-1 RA may directly modulate the insulin signaling pathway (46) or protect hepatocytes via reduction of fatty acid accumulation (47). We did not find insulin sensitivity to improve with reductions in hepatic triglyceride content, an unexpected result given the strong association between nonalcoholic fatty liver disease and metabolic syndrome (48). Liraglutide did induce a significant degree of weight loss and improved β-cell function, which may have been the dominant drivers in reducing hepatic steatosis.

Pancreatic fat content decreased minimally (not statistically significant) with liraglutide and did not change in the placebo group. Although this finding is consistent with studies showing that pancreatic fat content increases slightly with weight and abnormal glucose tolerance (31, 49, 50), the overall treatment effect during the study period was small. It is possible that the removal of ectopic fat from the pancreas (in contrast to liver fat) requires a longer-term or more potent intervention. Furthermore, quantification of pancreatic fat, especially in this very obese population, is still technically very challenging. Although the method we developed (36, 37) is robust and minimizes contamination from the visceral fat depot, it might still lack sufficient precision to reliably quantify relatively small changes in pancreatic fat content.

There were several limitations to this study. We used the Matsuda index as a surrogate for insulin sensitivity, which is not the "gold standard" technique and might have been inadequate in our patient population with long-standing diabetes and high-dose insulin treatment. MMCT-based methods for estimation of both β-cell function and insulin resistance have been validated in patients with T2D (51, 52) and represent a good compromise in this population when both measurements are needed. Additionally, this study lasted only 6 months, and a longer exposure time to liraglutide might be needed to reveal significant effects on insulin resistance and β-cell function. Bunck et al (53) found that sustained improvements to β-cell function with exenatide were achieved only after 3 years of therapy in a subset of patients who failed to find sustained effects after a 1-year treatment period. Our study enrolled patients with advanced disease, preventing discontinuation of treatment (insulin plus study drug) for more than 24 hours. Unfortunately, this may have limited the ability to precisely assess durable changes in β-cell function. Furthermore, the use of such high doses of exogenous insulin by these patients may have contaminated the insulin measurement and influenced the C-peptide-based indices. Because the dose of insulin treatment did not significantly change during the study, we hope that any interference equally affected both the baseline and end-of-study measurements, thus allowing us to reliably quantify the change over time. Lastly, we did not perform statistical adjustment for multiple comparisons for these mechanistic end-points, but all outcomes and analyses were prespecified.

Conclusion

We found that adding liraglutide to high-dose insulin therapy in obese patients with long-standing and uncontrolled T2D improves glycemia primarily though improvement in insulin secretion and not through suppres-

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sion of glucagon secretion. Furthermore, we did not observe significant changes in insulin sensitivity in this cohort, despite significant reductions in SAT and liver fat content.

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