The Impact of Chronic Liraglutide Therapy on Glucagon Secretion in Type 2 Diabetes: Insight from the LIBRA Trial

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Context: In patients with type 2 diabetes (T2DM), impaired suppression of postprandial glucagonemia is a metabolic defect that contributes to hyperglycemia. Treatment with a glucagon-like peptide-1 agonist can reduce hyperglucagonemia in the acute setting, but little is known about the durability of this effect with long-term treatment.

Objective: To evaluate the impact of chronic liraglutide therapy on glucagon regulation in early T2DM.

Design/Setting/Participants/Intervention: In this double-blind, randomized, placebo-controlled trial, 51 patients with T2DM of 2.6 ±1.9 years duration were randomized to either daily subcutaneous liraglutide or placebo injection and followed for 48-weeks, with serial assessment of the glucose, insulin, C-peptide, and glucagon responses to a 75g oral glucose tolerance test (OGTT) every 12 weeks.

Main Outcome Measures: Glucagon response was assessed with the incremental area-under-the-glucagon-curve ($iAUC_{glucagon}$) from measurements at 0-, 30-, 60-, 90- and 120-minutes on each OGTT.

Results: As expected, compared to placebo, liraglutide induced a robust enhancement of the post-challenge insulin and C-peptide response at each of 12-, 24-, 36- and 48-weeks, with a concomitant reduction in glycemic excursion. However, liraglutide also induced a paradoxical increase in post-challenge glucagonemia that first emerged at 12-weeks and persisted over the 48-week treatment period. Indeed, baseline-adjusted $iAUC_{glucagon}$ was significantly higher in the liraglutide group as compared to placebo at 12-weeks (170.2±34.9 vs 65.4±36.4 pg/ml*2h, P=0.04), 36-weeks (162.2±27.9 vs 55.7±30.4 pg/ml*2h, P=0.01), and 48-weeks (155.5±26.5 vs. 45.7±27.0 pg/ml*2h, P=0.006).

Conclusion: In contrast to its acute glucagon-lowering effect, chronic treatment with liraglutide is associated with increased post-challenge hyperglucagonemia in patients with early T2DM.

In recent years, there has been growing recognition of the role of pancreatic α-cell dysfunction in the pathophysiology of type 2 diabetes (T2DM) (1–3). Whereas an elevation in blood glucose concentration typically suppresses the secretion of glucagon by the α-cells in subjects without diabetes, patients with T2DM have a blunted or absent α-cell response to a glucose load, resulting in paradoxical postchallenge hyperglucagonemia (1, 3, 4). Indeed,

Abbreviations:

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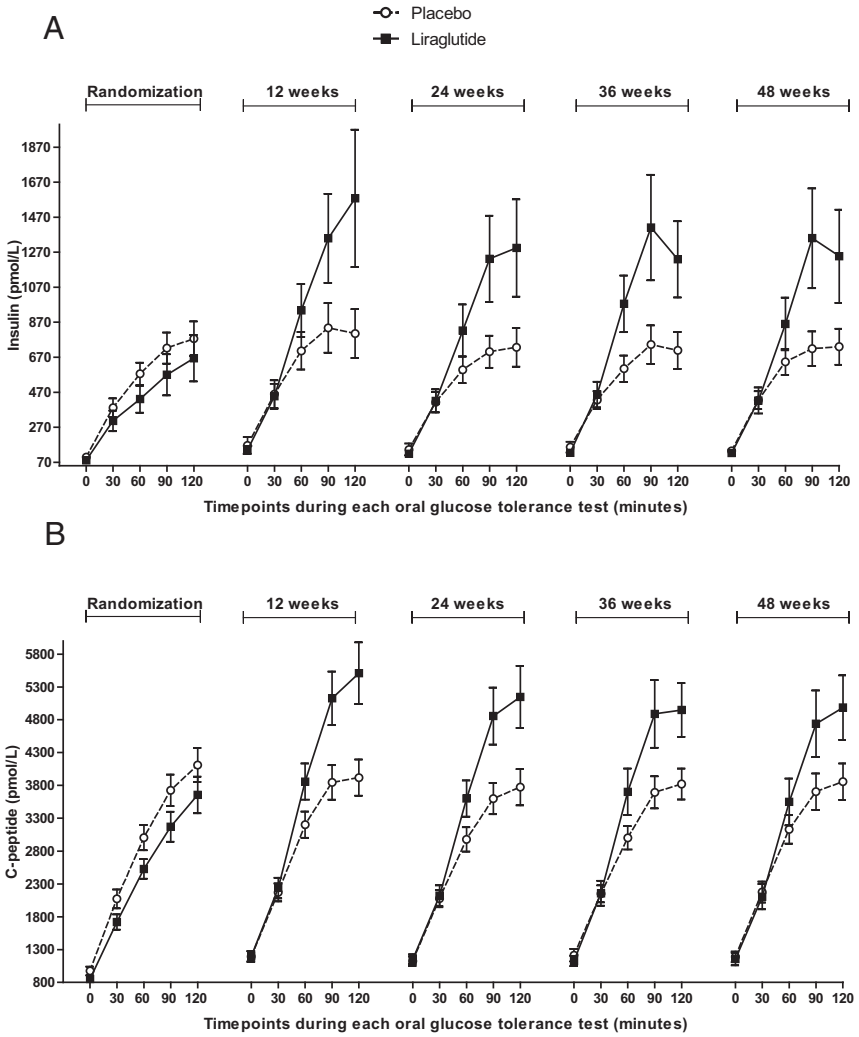

Figure 1. Changes over time in **(Panel A)** insulin response and **(Panel B)** C-peptide response to 75g OGTT at randomization, 12-weeks, 24-weeks, 36-weeks and 48-weeks in the liraglutide (black square) and placebo (open circle) arms. Means ± SEM are shown.

through its effects on hepatic glucose output, hyperglucagonemia may be responsible for up to 50% of the glycemic excursion following an oral glucose challenge in patients with T2DM (4, 5). Glucagon-like peptide-1 (GLP-1) can reduce fasting and postprandial hyperglucagonemia (6) and this acute glucagonostatic effect has been repeatedly observed in response to short-term treatment with GLP-1 agonists, leading to the concept that the reduction of glucagonemia is an important mechanism contributing to the glucose-lowering activity of incretin-based therapies (7–9). However, little is known about the long-term durability of this glucagon effect in the setting of chronic GLP-1 agonist therapy.

The **LI**raglutide and **B**eta-cell **RepA**ir (LIBRA) trial was a randomized placebo-controlled trial that was designed to objectively assess the long-term effect of the GLP-1 agonist liraglutide on the preservation of pancreatic β-cell function in patients who are early in the course of T2DM (10). This trial showed that liraglutide induces robust enhancement of β-cell function that is sustained over 48 weeks of therapy (primary outcome), accompanied by lower A1c and reduction in weight (10). Importantly, this trial also provided an opportunity to assess the longitudinal effect of liraglutide on the glucagon response to an oral glucose challenge through serial assessments that were performed every 12 weeks during the 48-week intervention. Thus, our objective in the current report was to evaluate the impact of chronic liraglutide therapy on glucagonemia in patients with early T2DM in the LIBRA Trial.

Materials and Methods

Study population

The LIBRA trial was a double-blind, randomized, parallel-arm, placebo-controlled trial that was designed to determine whether liraglutide can preserve β-cell function over 48 weeks in early T2DM, following the initial amelioration of glucotoxicity-induced dysfunction using a short course of intensive insulin therapy (IIT) prior to randomization (ClinicalTrials.Gov NCT01270789). The protocol and design of this trial have previously been described in detail (10). In brief, patients with early T2DM underwent 4-weeks of IIT before being randomized to either liraglutide or matching placebo, and then followed for 48-weeks, with serial assessment by oral glucose tolerance test (OGTT) every 12 weeks. Inclusion criteria included duration of diabetes ≤ 7 years, treatment with 0–2 oral antidiabetic medications, and baseline hemoglobin A1c (HbA_{1c}) <9.0% (<75 mmol/mol) if on antidiabetic medications or HbA_{1c} <10.0% (<86 mmol/mol) if not on antidiabetic medication. Exclusion criteria included current insulin therapy, renal/hepatic dysfunction, malignancy, and chronic infection. The study protocol was approved by the Mount Sinai Hospital Research Ethics Board, and all participants provided written informed consent.

Intervention

The protocol for the prerandomization IIT phase has been previously described in detail (10–13). Participants who achieved fasting venous glucose < 7.0 mmol/L one day after stopping IIT (a threshold indicative of the capacity of endogenous insulin secretion to maintain fasting glucose in the nondiabetic range (14, 15)) were considered eligible for 1:1 randomization to either liraglutide or identical placebo. The details of the randomization protocol were described previously (10).

The study medication (liraglutide or placebo) was administered by daily subcutaneous (sc) injection in the morning and titrated weekly over 3-weeks from 0.6 mg daily to 1.2 mg daily to 1.8 mg daily, with the latter dose maintained for the 48-week treatment period. Participants underwent 2-hour 75g OGTT at each of 0-, 12-, 24-, 36-, and 48-weeks. If participants had $HbA_{1c} \geq 8.0\%$ (≥ 64 mmol/mol) at any visit, metformin rescue therapy was initiated at 500 mg twice daily (bid (twice a day)) for the first 2-weeks, before progressing to 1000 mg bid for the duration of the trial. As previously reported (10), six participants required metformin during the trial (five of whom were in the placebo arm).

Laboratory Measurements

Each OGTT was performed in the morning after overnight fast, with the study medication was on the morning of the test, such that the last dose was administered ~24 hours earlier. During each OGTT, venous blood samples were drawn for measurement of insulin, C-peptide, and glucose at fasting and at 10-, 20-, 30-, 60-, 90- and 120-minutes following ingestion of the 75g glucose load. Specific insulin was measured with Roche Elecsys-1010 immunoassay analyzer and electrochemiluminescence immunoassay kit, and C-peptide was measured with Roche Modular system and electrochemiluminescence immunoassay kit (Roche Diagnostics, Laval, Canada). **Serum glucagon was measured from samples at fasting and 30-. 60-, 90- and 120-minutes on each OGTT by manual enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). The samples were collected in chilled tubes with aprotinin and kept on ice before immediate storage at -80C. All samples from a given participant were run in the same assay. The assay has no significant cross-reactivity with gastric inhibitory polypeptide, GLP-1, GLP-2, and glicentin-related polypeptide, and < 12% cross-reactivity with oxyntomodulin. The assay has a detection limit of 14.7 pg/ml and analytical range 31.3–2000 pg/ml. The interassay coefficient of variation (CV) was 11.22% at low concentration (QC1) and 12.61% at high concentration (QC2). The intra-assay CV was 9.76% for QC1 and 9.72% for QC2.**

Whole-body insulin sensitivity was measured by Matsuda index (16) and hepatic insulin resistance was assessed by Homeostasis Model Assessment (HOMA-IR) (17). β-cell function was assessed with the Insulin Secretion-Sensitivity Index-2 (ISSI-2), a validated OGTT-derived measure of β-cell function that is analogous to the disposition index obtained from the intravenous (IV) glucose tolerance test (18, 19). A secondary measure of β-cell function was $\Delta ISR_{0-120}/\Delta gluc_{0-120} \times$ Matsuda index (where ISR is the prehepatic insulin secretion rate determined by C-peptide deconvolution), as previously described (10, 20).

Outcomes

The primary outcome of the trial (baseline-adjusted ISSI-2 at 48-weeks) has been previously reported, along with the secondary outcomes (including HbA_{1c}) and the sample size/power considerations (10). The current analysis focuses on additional outcomes pertaining to the glucagon response. Specifically, the serum glucagon response to the OGTT at randomization (baseline), 12-, 24-, 36- and 48-weeks was evaluated by calculating the incremental area-underthe-glucagon-curve ($iAUC_{glucagon}$) using the trapezoidal rule, thereby enabling the determination of baseline-adjusted $iAUC_{glucagon}$ at each quarterly OGTT. The outcome of primary interest was the baseline-adjusted $iAUC_{glucagon}$ at 48-weeks. To assess the glucagon response in relation to the change in glycemia, we calculated the ratio of the maximal incremental increase in glucagon to the maximal incremental increase in blood glucose during each OGTT, as the relationship between glucagon and glucose can provide an indicator of relative α-cell dysfunction (21). **In addition, we evaluated the insulin to glucagon ratio in response to the OGTT, as this measure reflects a determinant of hepatic gluconeogenesis (22).**

Statistical Analyses

All analyses were conducted using SPSS 18.0 (Chicago, IL). Continuous variables were tested for normality of distribution, and natural log transformations of skewed variables were used, where necessary. The characteristics of the study groups were compared by Student *t* test (continuous variables) or either χ^2 or Fisher exact test (categorical variables)(Table 1). At each study visit, baseline-adjusted fasting glucagon and baseline-adjusted $iAUC_{glucagon}$ on the OGTT were compared between the study arms by analysis of covariance (ANCOVA) (Table 2). The glucagon analyses were performed with no imputation for missing glucagon measurements at any of the 5 timepoints during each OGTT (sample sizes for the glucagon analyses in the placebo and liraglutide groups at 12-, 24-, 36-, and 48-weeks were 24 and 26, 23 and 25, 21 and 25, and 23 and 24, respectively)(Figure 1 and 2, Table 2). To further evaluate the impact of chronic liraglutide therapy on the relationship between the glucagon and glycemic responses to the OGTT, the longitudinal changes over time in the ratio of the incremental increase in glucagon to the incremental increase in glucose in response to the OGTT were assessed with Generalized Estimating Equation (GEE) models, with interaction between treatment group and time evaluated to identify differential change over time between the groups (P for linear trend)(Figure 3). **Finally, at each study visit, we determined the insulin to glucagon ratio in response to the OGTT in both groups (Figure 4) and compared the baseline-adjusted incremental area-underthe-curve for this measure ($iAUC_{insulin/glucagon}$) between the groups by ANCOVA (Supplemental Table 1).**

Results

The study profile and primary results of the LIBRA Trial have been previously reported (10). Fifty-one participants met criteria for randomization to either liraglutide (n = 26) or placebo (n = 25). As shown in Table 1, the groups did not differ in age, gender, ethnicity, pretrial antidiabetic therapy, BMI or waist circumference at randomization. Duration of diabetes was slightly longer in the liraglutide group as compared to placebo (median 3.0 vs 1.5 years, $P = .028$). However, there were no differences between the groups in glycemic control, insulin sensitivity/resistance, β-cell function, or glucagon profile at randomization.

Figures 1 and 2 shows the pattern of change over time in (Figure 1A) insulin response, (Figure 1B) C-peptide response, (Figure 2A) glucose response, and (Figure 2B) glucagon response to 75g OGTT at each of randomization, 12-weeks, 24-weeks, 36-weeks and 48-weeks in the liraglutide and placebo arms. For each of these analytes, the

Table 1. Baseline characteristics of the study groups at randomization

	Placebo	Liraglutide	P value
	(n = 25)	***(n = 26)***	
Age (years)	57.4 ± 7.4	58.9 ± 8.7	0.50
Gender (% male)	64.0	61.5	0.86
Ethnicity:			0.69
White (%)	68.0	73.1	
Other (%)	32.0	26.9	
Duration of diabetes (years)	1.5 (0.75–3.0)	3.0 (2.0–5.0)	0.028
Diabetes therapy prior to study:			0.69
Diet alone (%)	32.0	26.9	
Metformin alone (%)	64.0	57.7	
Sulphonylurea alone (%)	0	7.7	
Metformin + Sulphonylurea (%)	4.0	7.7	
Body mass index (kg/m^2)	30.4 ± 5.8	30.0 ± 4.3	0.82
Waist circumference (cm)	103.0 ± 13.7	99.8 ± 10.3	0.35
Glycemia:			
Fasting plasma glucose (mmol/liter)	5.7 ± 0.5	5.9 ± 0.7	0.53
HbA_{1c} (%)	6.2 ± 0.4	6.4 ± 0.5	0.07
HbA_{1c} (mmol/mol)	44 ± 4.4	46 ± 5.5	0.07
Insulin sensitivity/resistance:			
Matsuda index	2.2 (1.6–3.8)	3.3 (2.1–5.5)	0.18
HOMA-IR	3.2 (1.8–4.9)	2.5 (1.3–4.1)	0.33
β-cell function:			

(*Continued*)

Table 1. Continued

	Placebo	Liraglutide	P value
ISSI-2	220 (190–289)	193 (146–321)	0.34
$\Delta ISR_{0-120}/\Delta gluc_{0-120} \times$ Matsuda index	0.29 ± 0.06	0.32 ± 0.08	0.77
Glucagon:			
Fasting glucagon (pg/ml)	87.2 ± 26.7	78.6 ± 36.8	0.35
$iAUC_{glucagon}$ on OGTT (pg/ml*2 h)	37.9 ± 87.0	66.1 ± 118.3	0.34

Continuous data are shown are mean ± standard deviation, with the exception of skewed variables (duration of diabetes, Matsuda index, HOMA-IR, ISSI-2), which are shown as median followed by interquartile range in parentheses. Categorical data are presented as proportions.

Table 2. Comparison of placebo and liraglutide groups with respect to baseline-adjusted fasting glucagon (pg/ml) and baseline-adjusted incremental area-under-glucagon-curve ($iAUC_{glucagon}$) (pg/ml*2 h) in response to oral glucose tolerance tests at 12-weeks, 24-weeks, 36-weeks, and 48-weeks

	Placebo	Liraglutide	P value
Fasting glucagon			
12-weeks	97.1 ± 6.5	76.2 ± 6.2	0.03
24-weeks	88.8 ± 5.9	78.8 ± 5.5	0.22
36-weeks	85.4 ± 5.4	78.8 ± 4.9	0.37
48-weeks	111.5 ± 9.9	89.8 ± 9.5	0.12
$iAUC_{glucagon}$			
12-weeks	65.4 ± 36.4	170.2 ± 34.9	0.04
24-weeks	90.7 ± 29.7	122.9 ± 28.4	0.44
36-weeks	55.7 ± 30.4	162.2 ± 27.9	0.01
48-weeks	45.7 ± 27.0	155.5 ± 26.5	0.006

Data shown as mean ± SEM

two groups showed similar responses to the OGTT at randomization, followed by distinct differences that emerged at 12-weeks and were largely maintained for the duration of the 48-week follow-up. Specifically, as expected, liraglutide induced a robust enhancement of the postchallenge insulin (Figure 1A) and C-peptide (Figure 1B) response that was readily apparent at each of 12-, 24-, 36- and 48-weeks, as compared to the placebo group. Consistent with this effect, compared to placebo, the liraglutide group exhibited a concomitant reduction in postchallenge glycemic excursion (Figure 2A) across these visits. Intriguingly, however, liraglutide also induced an unanticipated increase in postchallenge glucagonemia (Figure 2B) that first emerged at 12-weeks and persisted over the course of 48 weeks treatment. Indeed, at each quarterly assessment, the liraglutide arm demonstrated a characteristic pattern of response consisting of the following 3 features: (i) lower or similar fasting glucagon compared to placebo; (ii) an enhanced postchallenge glucagonemic excursion, and (iii) a delayed time to peak serum glucagon concentration (ie, occurring at 60-minutes postchallenge, as compared to the 30-minute peak seen in both groups at baseline and in the placebo group at all 5 visits).

Baseline-adjusted fasting glucagon was lower in the liraglutide group than the placebo arm at 12-weeks (mean 76.2 ± 6.2 vs 97.1 ± 6.5 pg/ml, $P = .03$) but did not reach significance at the subsequent visits (Table 2). Consistent with the observed pattern of exacerbated postchallenge glucagonemia, baseline-adjusted $iAUC_{glucagon}$ was significantly higher in the liraglutide group as compared to placebo at 12-weeks (mean 170.2 ± 34.9 vs 65.4 ± 36.4 pg/ml*2h, $P = .04$), 36-weeks (mean 162.2 ± 27.9 vs 55.7 ± 30.4 pg/ml*2h, $P = .01$), and 48-weeks (mean 155.5 ± 26.5 vs. 45.7 ± 27.0 pg/ml*2h, $P = .006$)(Table 2). $iAUC_{glucagon}$ was also higher in the liraglutide arm than placebo at 24-weeks, but this difference did not reach statistical significance (mean 122.9 ± 28.4 vs 90.7 ± 29.7 pg/ml*2h, $P = .44$). These findings were unchanged after further adjustment for duration of diabetes and exclusion of the six participants requiring metformin rescue, respectively (data not shown).

We next sought to evaluate the effect of chronic liraglutide therapy over time on the degree of impairment in glucose-induced suppression of glucagonemia in early T2DM. Figure 3 shows the change over time in each group

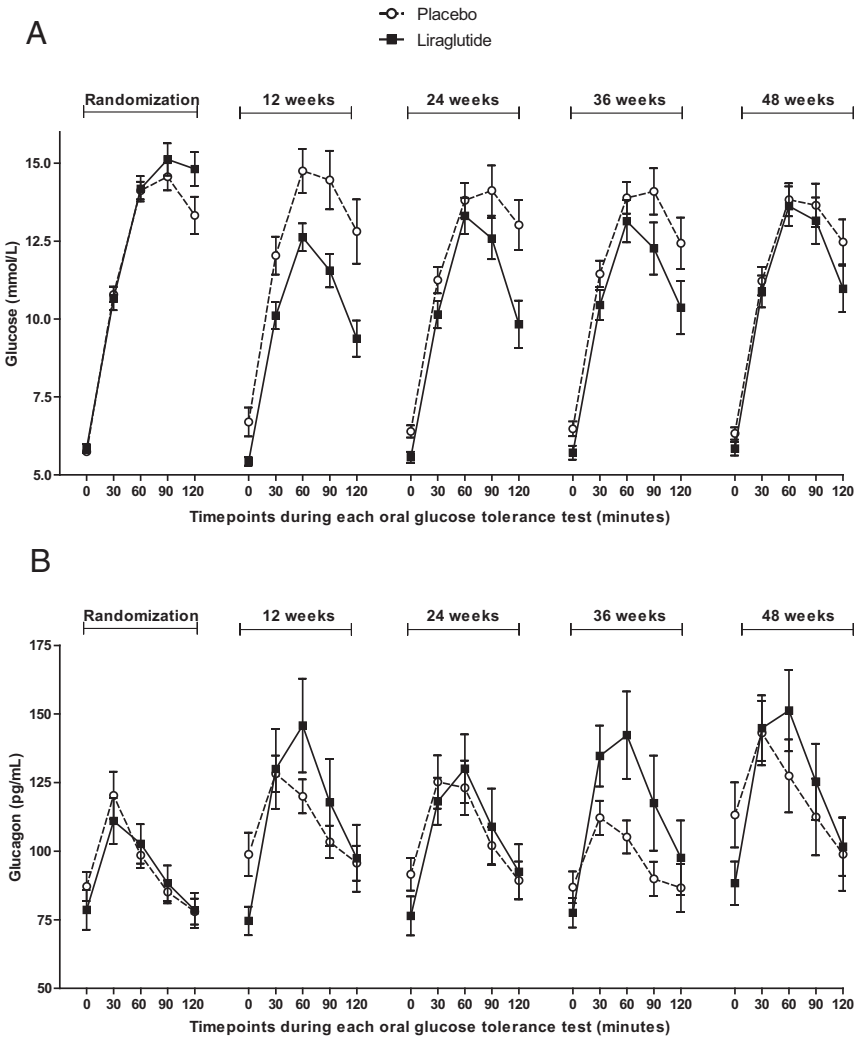

Figure 2. Changes over time in **(Panel A)** glucose response and **(Panel B)** glucagon response to 75g OGTT at randomization, 12-weeks, 24-weeks, 36-weeks and 48-weeks in the liraglutide (black square) and placebo (open circle) arms. Means ± SEM are shown.

in the glucagon response to glycemia, as measured by the incremental increase in glucagon per incremental increase in blood glucose in response to the OGTT at randomization, 12-,24-,36-, and 48-weeks. This analysis demonstrated that there was a significant treatment effect from randomization to 48-weeks ($P = .01$) with worsening of the paradoxical incremental increase in glucagon in relation to glucose in the liraglutide group as compared to placebo (Figure 3), **suggestive of α-cell insensitivity to the glucagon-suppressive effects of glucose.**

Lastly, we evaluated the effect over time of chronic liraglutide therapy on the insulin to glucagon ratio in response to the OGTT at each study visit. As shown in Figure 4, there was an enhanced postprandial insulin to glucagon ratio in response to the oral challenge in the liraglutide arm as compared to placebo at each of 12-, 24-, 36- and 48-weeks. Accordingly, baseline-adjusted $iAUC_{insulin/glucagon}$ was higher in the liraglutide arm at each of these visits (all $P < .002$) (Supplemental Table 1).

Discussion

In this randomized placebo-controlled trial, we demonstrate that, despite having sustained beneficial effects on insulin secretion and glycemic control (**10**), chronic liraglutide therapy is associated with a paradoxical enhancement of postchallenge hyperglucagonemia that emerges by 12-weeks and persists over 48-weeks of treatment in early T2DM. Specifically, compared to placebo, liraglutide induced a characteristic glucagon response profile that was consistently observed at each quarterly OGTT, consisting of (i) lower/similar fasting glucagon, (ii) an enhanced postchallenge glucagonemic excursion, and (iii) a delayed time to peak glucagon concentration. These data thus highlight the need for further understanding of the impact of chronic GLP-1 agonist therapy on α-cell and islet physiology.

Several previous studies have demonstrated the acute glucagon-lowering effect of GLP-1 and its agonists in patients with and without T2DM (7–9, 23–25). However, there has been little study of the durability of this effect with chronic GLP-1 agonist therapy. In the open-label DURATION-1 study, long-acting exenatide administered once weekly reduced fasting serum glucagon over 30-weeks treatment, as compared to twice daily exenatide (26). Similarly, in the Get-Goal-M (27) and Get-Goal-S (28) studies, lixisenatide reduced 2-hour postprandial glucagon in response to a meal test after 24-weeks treatment in which it was added to metformin and sulfonylurea +/- metformin, respectively (likely through its effects on gastric emptying). In contrast, Gudipaty et al reported that indices of the glucagon response to arginine stimulation were unchanged after 6 months of treatment with exenatide (n = 14) or sitagliptin (n = 12), though they were increased by the sulfonylurea glimepiride (n = 14), which served as an active comparator (29).

Against this background, the current study extends the literature though the serial measurement of 5-point glucagon profiles (0-,30-,60-,90- and 120-minutes) in response to an oral glucose challenge on 5 occasions across

48-weeks of treatment with either liraglutide or placebo. Indeed, the design of this study offers several advantages for elucidating the impact of chronic GLP-1 agonist therapy on glucagon regulation. First, it provided randomized blinded comparison of the impact of liraglutide vs placebo in the absence of background antidiabetic therapies. Second, the 5-point glucagon profiles offer greater characterization than isolated fasting or 2-hour measurements, as evidenced by the observed differences in postchallenge excursion and time to peak concentration that would not be detectable with the latter measurements alone. Third, although differences exist between commercial glucagon assays, this study used an established assay that demonstrated divergent effects of liraglutide on fasting and postchallenge glucagon that were consistently observed on 4 postrandomization tests across 48 weeks. Fourth, the serial assessments at randomization, 12-, 24-, 36- and 48-weeks provide insight into the temporal course and durability of effects on glucagon regulation, coupled with a longer duration of therapy than earlier studies. With these design features, the current study revealed a very consistent effect of chronic liraglutide treatment on postchallenge glucagonemic excursion and time to peak thereof across all 4 postrandomization assessments (with the only exception being that the higher $iAUC_{glucagon}$ in the liraglutide arm compared to placebo at 24-weeks did not reach statistical significance). This longitudinal consistency over repeated tests strongly supports the robustness of these data.

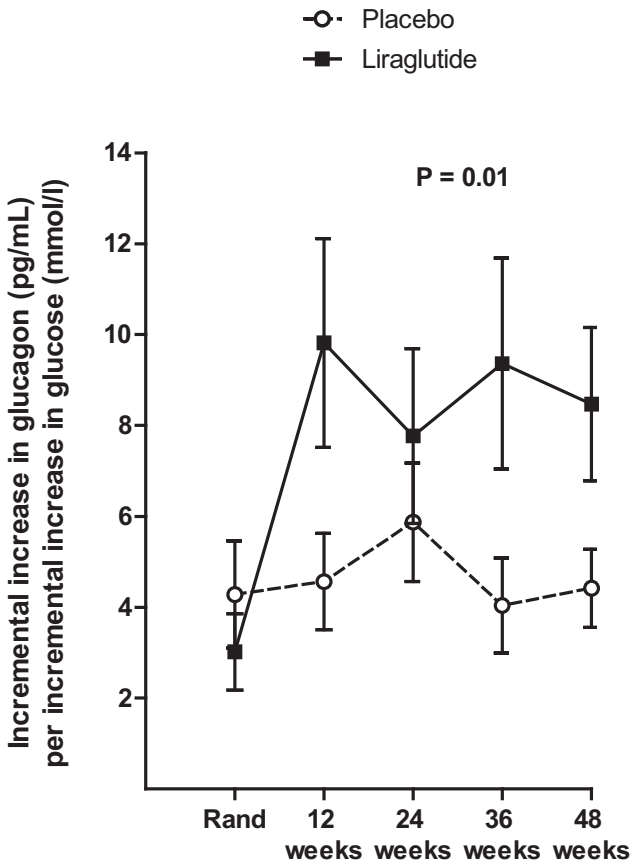

Figure 3. Changes over time in glucagon response to glycemia as measured by the incremental increase in glucagon per incremental increase in blood glucose in response to 75g OGTT at randomization, 12-weeks, 24-weeks, 36-weeks and 48-weeks in the liraglutide (black square) and placebo (open circle) arms. Means ± SEM are shown. P for linear trend

At each OGTT, the higher postchallenge glucagonemia in the liraglutide arm occurred in the presence of robust enhancement of the insulin/C-peptide responses and lower glycemic excursion. These findings are suggestive of a dissociation of the expected inverse relationship between serum glucagon and insulin/C-peptide responses. Interestingly, the similarly unexpected coupling of higher glucagonemia with better glycemic control has been observed recently with SGLT-2 inhibitors, although only with short-term treatment to date (30, 31). It is also noteworthy that, following gastric bypass surgery, patients exhibit elevated postprandial serum concentrations of both GLP-1 and glucagon (32), features which potentially parallel those arising with long-term liraglutide in the current study. Overall, our findings raise the possibility that the sustained glucose-lowering effects of chronic liraglutide therapy might not be attributable to the improved postprandial glucagon suppression that has been observed with short-term treatment.

The mechanism by which chronic liraglutide may induce postchallenge hyperglucagonemia remains unclear. However, preclinical studies of chronic inhibition of glucagon signaling may provide relevant insight in this regard. Specifically, in mouse models, both long-term inhibition of the glucagon receptor by monoclonal antibody (33) and knockout of the receptor (34) have caused α-cell hyperplasia and hyperglucagonemia, coupled with improved glycemic control. An inactivating mutation of the glucagon receptor has also been described in humans, again resulting in α-cell hyperplasia and hyperglucagonemia (35). Accordingly, these data raise the possibility that, over the course of long-term treatment, the glucagon-lowering activity of liraglutide may lead to similar α-cell compensation that produces an exaggerated hyperglucagonemic response to a carbohydrate challenge. While this mechanism remains speculative at this time, the current data nevertheless highlight two key points. First, the effect of chronic GLP-1 agonist therapy on postprandial glucagonemia may be very different from that of short-term treatment. Second, there are likely differences in the determinants of fasting and postchallenge glucagonemia in T2DM, given that the glucagon-lowering effect of liraglutide at fasting was generally preserved during the study whereas the anticipated acute postprandial glucagonos-

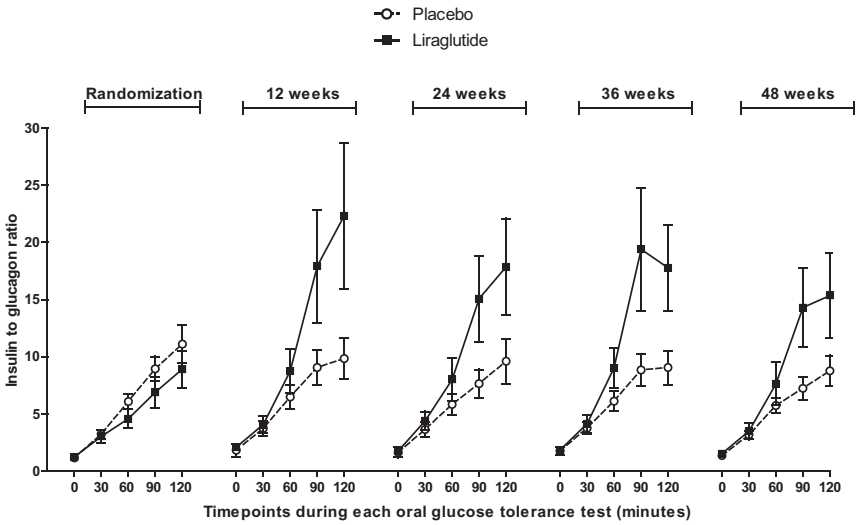

Figure 4. Changes over time in the insulin to glucagon ratio in response to 75g OGTT at randomization, 12-weeks, 24-weeks, 36-weeks and 48-weeks in the liraglutide (black square) and placebo (open circle) arms. Means ± SEM are shown.

tatic effect may have been lost within 12 weeks of chronic treatment. Further study of the long-term impact of GLP-1 agonist therapy on α-cell physiology is needed.

In the LIBRA Trial, the study medication was held on the morning of each OGTT, such that the most recent dose of liraglutide or placebo would have been administered ~24 hours earlier. A limitation of the study is that we did not directly measure liraglutide concentrations at each OGTT. However, it should be noted that Degn et al have reported that the half-life of liraglutide in steady state is 17.9 hours and have demonstrated its sustained activity 24 hours after its last subcutaneous administration (8), suggesting that a rebound effect in response to the withdrawal of liraglutide is probably not responsible for the observed glucagonemia. There is also ongoing debate regarding endogenous GLP-1 levels in T2DM (36, 37), although the effect of such an underlying factor on the observed results is likely be mitigated through the randomization process in the setting of a trial. Another limitation is that this study does not provide insight on the response to a mixed meal or to repeated meal challenge, as would occur in daily living. Finally, it remains to be determined whether the current findings would extend to long-term treatment with other GLP-1 agonists with shorter half-lives or if they specifically reflect a tachyphylactic response to chronic liraglutide therapy.

In summary, chronic treatment with liraglutide induces a paradoxical enhancement of postchallenge hyperglucagonemia in early T2DM, despite sustained beneficial effects on insulin secretion and glycemic control. Specifically, by 12-weeks of treatment, a characteristic glucagon response profile emerges that persists over 48-weeks and consists of lower/similar fasting glucagon, enhanced postchallenge glucagonemic excursion, and a delayed time to peak glucagon concentration. It thus appears that the acute suppressive effect of liraglutide on postchallenge glucagonemia may be lost with longer duration of therapy, highlighting the need for further study to elucidate the α-cell and islet response to chronic GLP-1 agonist treatment.

AUTHOR CONTRIBUTIONS

RR and BZ designed the study and protocol. CKK, HC, BZ and RR implemented the study and acquired the data. CK performed the statistical analyses and wrote the manuscript. PWC contributed to interpretation of biochemical analyses. All authors contributed to interpretation of the data and critical revision of the manuscript. All authors approved the manuscript. The protocol is available from RR on request. RR had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgments

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