Pramlintide but not Liraglutide suppresses meal-stimulated glucagon responses in type 1 diabetes

Alfonso Galderisi, Jennifer Sherr, Michelle VanName, Lori Carria, Melinda Zgorski, Eileen Tichy, Kate Weyman, Eda Cengiz, Stuart Weinzimer, William Tamborlane

Pramlintide but not Liraglutide suppresses meal-stimulated glucagon responses in type 1 diabetes

Alfonso Galderisi[1,2]*, Jennifer Sherr[1]*, Michelle VanName[1], Lori Carria[1], Melinda Zgorski[1], Eileen Tichy[1], Kate Weyman[1], Eda Cengiz[1], Stuart Weinzimer[1], William Tamborlane[1]

[1]*Department of Pediatrics, Pediatrics Endocrinology and Diabetes Section, Yale School of Medicine, New Haven, CT, USA*

[2]*Department of Woman and Child's Health, University of Padova, Padova, Italy*

*AG and JS equally contributed to this manuscript

Context. Postprandial hyperglycemia remains a challenge in type 1 diabetes (T1D) due, in part, to dysregulated increases in plasma glucagon levels after meals.

Objective. This study was undertaken to examine whether 3-4 weeks of therapy with pramlintide or liraglutide might help to blunt postprandial hyperglycemia in T1D by suppressing plasma glucagon responses to mixed meal feedings.

Design. Two parallel studies were conducted in which participants underwent mixed meal tolerance tests (MMTTs) without premeal bolus insulin administration before and after 3-4 weeks of treatment with either pramlintide (8 participants aged 20±3yrs, A_{1C} 6.9±0.5%) or liraglutide (10 participants aged 22±3yrs, A_{1C} 7.6±0.9%).

Results. Compared to pre-treatment responses to the MMTT, treatment with pramlintide reduced the peak increment in glucagon from 32 ± 16 to 23 ± 12 pg/mL (p<0.02). In addition, the incremental area under the plasma glucagon curve from 0-120 minutes(Glucagon $iAUC_{0-120\,min}$) dropped from 1988±590 to 737±577 pg/mL*min (p<0.001), which was accompanied by a similar reduction in the meal-stimulated increase in the plasma glucose curve (Glucose $iAUC_{0-120\,min}$) from 11963±1424mg/dL*min pre-treatment vs 2493±1854 mg/dL*min after treatment (p<0.01). In contrast, treatment with liraglutide had no effect on plasma glucagon and glucose responses during the MMTT.

Conclusions: Adjunctive treatment with pramlintide may provide an effective means to blunt post-meal hyperglycemia in T1D by suppressing dysregulated plasma glucagon responses. In contrast, plasma glucose and glucagon responses were unchanged after 3-4 weeks of treatment with liraglutide.

We examined the effect of pramlintide or liraglutide as adjunctive therapy on postprandial glucagon and glucose response to mixed meal feedings in youths with type 1 diabetes.

1. Introduction

Postprandial hyperglycemia remains a challenge in type 1 diabetes (T1D) due to a number of factors that include delays in the absorption and action of pre-meal boluses of insulin from the subcutaneous space and dysregulated glucagon secretion in response to mixed meal feedings.(1-5) In non-diabetic individuals, plasma glucagon levels change very little after eating a mixed meal that includes protein and carbohydrate because the stimulation of glucagon secretion by increases in plasma amino-acids is off-set by the suppression of glucagon secretion by increases

in plasma glucose levels. In contrast, it has been demonstrated that children with type 1 diabetes have higher plasma glucagon responses after a mixed meal feedings compared to healthy peers. (3) Moreover, plasma glucagon responses to mixed meal feeding increase over time, presumably due to the progressive loss of residual β-cell function. (1,6)

Due to the adverse impact of postprandial hyperglycemia on overall glycemic control and on the risk of complications, (7,8) it has been suggested that treatment with agents approved for use in T2D may be effective in lowering post-prandial glucose peaks in T1D by mechanisms independent of stimulation of insulin secretion (9,10). Specifically, SGLT2 inhibitors reduce plasma glucose by lowering the renal threshold for glucose excretion; (11) whereas, it has been suggested that the glucose-lowering effects of both pramlintide (an analog of amylin) and liraglutide (a GLP1 agonist) are due, in part, to slowing of gastric emptying and suppression of exaggerated post-meal increases in plasma glucagon. (12,13) In two parallel studies, we used a full-closed loop insulin delivery system to control post-meal glucose excursions before and after 3-4 weeks of treatment with pramlintide and liraglutide at maximally recommended doses. Those studies showed durable slowing of gastric emptying with pramlintide but not with liraglutide. (14) In those studies, we also performed mixed meal tolerance tests (MMTTs) in the morning following 24-hours of closed–loop control to examine and compare whether after 3-4 weeks of treatment with pramlintide or liraglutide suppressed meal-stimulated increases in plasma glucagon in T1D. (14) The results of these MMTTs are reported herein.

2. Material and Methods

A. Participants

Participants were eligible to enroll in the pramlintide and liraglutide studies if they had a clinical diagnosis of type 1 diabetes for at least 1 year, A1c ≤9% (≤75mmol/mol) and a normal hematocrit and serum creatinine level. Participants were excluded if they had a history of an eating disorder, celiac disease, gastroparesis, another disorder of intestinal absorption or motility, a history of a hypoglycemic seizure in the past 3 months, another chronic medical condition (except treated hypothyroidism), current use of medications (other than insulin) known to affect blood glucose level or gastrointestinal motility and prior adverse reactions to the drug under study. Female participants could not be pregnant or lactating. The studies were reviewed and approved by the Yale University Human Investigation Committee and written informed consent was obtained by adult participants. Parental consent with participant assent were obtained for participants <18 years.

B. Procedures

Dose Titration Phase:
Participants in the studies underwent two 24 hour periods of closed-loop glucose control before and after 3-4 weeks of treatment with pramlintide or liraglutide. (14) During outpatient treatment, the dose of pramlintide was uptitrated from 30 to 60 ug given 15 minutes prior to each meal and the once daily dosing of liraglutide before breakfast was uptitrated from 0.6 to 1.8 mg/day. In both studies, insulin doses were adjusted, as needed, by frequent telephone contacts with the study participants.

Mixed Meal Tolerance Tests (MMTTs):
In each study, the participants underwent two mixed meal tolerance tests, the first performed before therapy with pramlintide or liraglutide and the second performed after 3-4 weeks of treatment with one of the drugs. All MMTTs were performed at ~8 AM in the morning after an

8-12 hour overnight fast, during which glucose levels were regulated with a Medtronic closed-loop system. (14) The CL system used in both of these studies consisted of four components: a Medtronic Paradigm 715 insulin pump, a Medtronic MiniLink REAL-Time transmitter (MMT-7703) adapted for 1-min transmission, a Medtronic continuous glucose sensor (Sof-sensor in the pramlintide study and Enlite sensor in the liraglutide study), and the Medtronic external Physiological Insulin Delivery (ePID) algorithm modified to include insulin feedback, which was on a laptop computer.

An intravenous catheter was used for frequent blood sampling during the MMTTs. At the start of the 4 hour MMTTs, baseline samples for measurement of plasma glucose and plasma glucagon were obtained, the closed loop system was shut-off and participants were placed back on their usual open loop basal rate settings. Participants then consumed 6ml/kg of Boost High Protein 6cc/kg to a maximum dose of 360mL. Additional blood samples for measurements of plasma glucose and plasma glucagon were obtained every 15-30 min for 240 minutes following ingestion of Boost High Protein; (3) the macro-nutrient content per100 ml of Boost High Protein is protein 6.3g, carbohydrate 13.9g and fat 2.5g. During the second MMTT for each participant, pramlintide (60 mcg) or pramlintide (1.8 mg) was injected just prior to meal ingestion.

The primary outcome of the two parallel studies reported here was the difference in the incremental area under the curve in plasma glucagon levels from baseline to 120 min *(Glucagon iAUC$_{0-120\ min}$).* Secondary outcomes included the *Glucagon iAUC $_{120-240\ min,}$ Glucose iAUC$_{0-120\ min}$ and Glucose iAUC $_{120-240\ min}$*, changes in peak plasma glucagon and peak plasma glucose levels and the time-to-peak for glucagon and glucose over 240 minutes.

Breakfast during closed loop.
Participants in the liraglutide group had their glucagon and glucose response to breakfast assessed during the 24-hr closed loop admissions. Meals were self-selected and were not limited by calorie or carbohydrate content. Samples were obtained every 15-30 min for 180 minutes after the breakfast to assess both glucose and glucagon levels. Closed loop insulin delivery was maintained during the meal test.

C. Laboratory measurements
Plasma glucose was analyzed using the YSI 2300 STAT Plus glucose analyzer (YSI Life Sciences, Yellow Springs, OH). Glucagon was measured by a double antibody radioimmunoassay (EMD Millipore, RIA assay, GL-32K). The lower limit of detection of plasma glucagon was 20 pg/mL and the higher limit of the standard assay curve was 400 pg/mL. The accuracy of the assay was 97±0.8%.

D. Statistical Considerations
Comparisons between the pre- and during treatment measurements were calculated using paired *t* Student test for continuous variables. Fisher exact test was adopted for categorical variables. Changes in plasma glucose and glucagon during the MMTTs were expressed as incremental values from baseline (0 minutes) to the specified timepoints. The incremental areas under the curve (iAUC) and the peak value for both plasma glucose and glucagon were calculated as difference from the baseline measure (0 minutes). Data are expressed as mean±SD. Data were analyzed using GraphPad Prism 7 software (GraphPad Software, Inc., La Jolla, CA).

3. Results

A. Participants

Ten out of 11 participants who enrolled in the CL study (14) with liraglutide agreed to undergo the two MMTTs, as did 8 of 10 participants from the pramlintide study. The clinical characteristics of the 10 liraglutide and 8 pramlintide participants are shown in **Table 1.**

B. MMTT Results

B1. Baseline plasma glucagon and glucose levels

As shown in **Table 2,** in each of the experiments, overnight CL insulin delivery resulted in baseline fasting plasma glucagon and glucose levels that were similar in both groups of subjects both before and during treatment with pramlintide and liraglutide.

B2. Increments in plasma glucagon and glucose before and during treatment with pramlintide

The patterns of incremental changes in plasma glucagon and glucose before and during treatment with pramlintide are shown in **Figure 1A and 1B,** respectively. During the first 2 hours of the MMTTs, treatment with pramlintide markedly reduced the rise in plasma glucagon levels following meal ingestion (**Figure 1A**) and increases in plasma glucose levels were also blunted (**Figure 1B**). Moreover, between 2-4 hours, glucagon levels remained suppressed during treatment with pramlintide (**Figure 1A**), even in the face of a delayed rise in plasma glucose levels (**Figure 1B**). As shown in **Table 3**, adjunctive therapy with pramlintide markedly suppressed the glucagon iAUC $_{0-120 \text{ min}}$ and the peak increment in plasma glucagon, as well as the glucose iAUC $_{0-120 \text{ min}}$, Glucose iAUC $_{120-240 \text{ min}}$ and the peak increment in glucose. Pramlintide treatment also delayed the time to peak glucagon and glucose levels. (**Table 3**).

B3. Increments in plasma glucagon and glucose before and during treatment with liraglutide

The incremental changes in plasma glucagon and glucose before and during treatment with liraglutide are shown in **Figure 1C** and **1D**. As seen in these Figures, after 3-4 weeks of liraglutide treatment, there were no significant differences in the plasma glucagon and glucose responses during the first 120 min and second 120 min following Boost ingestion. As shown in **Table 3**, the peak increment in plasma glucagon, the time to peak glucagon and the iAUC for glucagon were not significantly different before and during treatment with liraglutide. Furthermore, there were no significant changes in the peak increment in plasma glucose, time to peak glucose and iAUC for glucose after treatment with liraglutide.

C. Plasma glucagon and glucose responses during closed loop insulin delivery before and during treatment with liraglutide

To validate that a liquid meal response would be reflective of the physiologic changes in glucagon and glucose following a standard meal under controlled insulin delivery conditions, a self-selected breakfast was provided to participants during both closed loop admissions. The average macronutrient content of the standardized breakfast was 75 ± 49 grams of carbohydrates, 24 ± 16 grams of protein, and 17 ± 14 grams of fat. Corroborating the findings demonstrated during the MMTT, no difference in the glucagon or glucose response was appreciated in the 3-hours following the standardized breakfast meal (**Figure 2** and **Supplemental Table**).

4. Discussion

Pramlintide and liraglutide have been widely investigated as adjunctive therapies aimed at limiting post-meal hyperglycemia in T1D (9,12,14-25) due to putative modes of action that include the ability to suppress dysregulated glucagon responses to mixed meal feedings, slowing of gastric motility and earlier satiety. (9,26,27) It should be noted, however, that clinical studies have demonstrated differences between the two drugs on glucose control, glucagon secretion and

gastric emptying (14,16,18,19,22,28-30) with liraglutide surprisingly increasing the glucagon responses to mixed meal feedings after chronic treatment in type 2 diabetes and pramlintide being highly effective in delaying gastric emptying but with conflicting effects on glucagon secretion in T1D. (14,24)

Our parallel studies of the use of pramlintide and liraglutide as adjunctive agents to improve control of post-prandial glucose excursions during closed-loop insulin delivery provided a unique opportunity to examine and compare the effects of these agents on dysregulated increases in plasma glucagon levels after meals. (14) Consequently, the most important findings of the current study are that we were unable to observe any suppressive effects of liraglutide on plasma glucagon responses to mixed meal feedings or any suggestion of a delay in gastric emptying after only 3-4 weeks of treatment. In contrast, marked suppression of 2-hour-plasma glucagon, as well as reduced and delayed increases in plasma glucose levels were sustained after the same duration of treatment with pramlintide.

These findings are consistent with previous studies that supported approval of pramlintide for use as an adjunctive agent in T1D (12,22,23,30,31) and more recent phase 3 studies of liraglutide that indicted little improvement in metabolic control in patients with T1D. (16,17,20) Our results are also consistent with previous reports, indicating that liraglutide is a less effective drug of its class in modulating the gastric motility, with more pronounced action from short-term GLP-1 analogues, like exenatide or lixisenatide. (26,32) As noted in our previous publication in this groups of patients (24) and by others, (19,33) liraglutide may be of benefit to overweight or obese patients with T1D due to its suppression of appetite, which may support weight loss and reductions in insulin doses.(17,27)

A strength of the study, is that the MMTTs were performed after completion of 24 hours of closed-loop insulin delivery, including overnight control just prior to the start of the MMTTs with the last meal being consumed >12 hours earlier. Most MMTT protocols mandate that fasting glucose levels between 70-200 mg/dL be achieved prior to meal ingestion. Our use of the closed loop system ensured that participants had even tighter glycemic control, thus minimizing the potential confounding effects of differences in fasting plasma glucose prior to performance of the procedure. Even plasma glucagon levels were similar prior to the conduct of the pre- and post-treatment MMTTs performed. Use of the closed-loop system also ensured precision in regards to the insulin delivery prior to the start of the MMTTs, eliminating a potential confounding factor of overinsulinization prior to meal ingestion. Thus, in both sets of experiments, the only difference between the two MMTTs in each participant was the injection of the study drug prior to the second MMTT.

Compared to the sharp increases in plasma glucagon and plasma glucose during the pre-treatment MMTT, only a slight increase in plasma glucagon and glucose levels was observed during the first 60 minutes of the MMTT during treatment with pramlintide. (**Figure 1D**) However, it is possible that diminished increases in plasma glucagon and plasma glucose during the first 2 hours of the MMTT were both due to delays in gastric emptying rather than by suppression of glucagon secretion by pramlintide. Arguing against this conclusion is the observation that the relatively flat glucagon response following meal ingestion with pramlintide was present for the full 4-hours of the MMTT, despite delayed absorption of carbohydrate and amino-acids and corresponding increases in plasma glucose after meal ingestion. These data suggest that the ability of pramlintide to mitigate post-meal hyperglycemia is related to both its ability to delay gastric emptying and to suppress α-cell secretion.

A limitation of the present study was that it was not designed to allow for formalized comparison between the two agents. However, comparison of the individual treatments prior to and post treatment in the two separate cohorts studied allows extrapolation of how the two adjunctive therapies may differ. It should also be noted that studies examining physiologic changes induced by pharmacologic agents often have larger sample size. However, the present analysis is a sub-study nested within inpatient closed-loop studies that were designed to examine the feasibility and potential efficacy of adjunctive therapies in conjunction with closed-loop insulin delivery. Although our sample size is relatively small, it was sufficient to show a change in hormonal response during treatment with pramlintide. While we have no direct means of assessing participants' compliance in taking the study drugs during the outpatient phase of the studies, paricipantts were contacted frequently by telephone during this time. These phone calls allowed investigators to encourage compliance and assess for adverse effects of the study medications. Importantly, all participants in both studies tolerated the full therapeutic doses of the drugs during the inpatient studies, which would have been unlikely if they had not been compliant in the outpatient dose titration phase. Finally, as a surrogate marker for compliance, it is notable that during both studies participants on average had a lowering of their total daily insulin dose. (24)

Finally, it is possible that use of a standardized meal instead of a liquid mixed meal would have provided better approximation of how these therapies impact day-to-day life. Although not performed in the pramlintide study, plasma glucagon responses to a standardized breakfast during closed-loop insulin delivery was assessed in the liraglutide study. Furthermore, the standard breakfast meal study conducted during the inpatient closed loop admissions provided the opportunity to see if dynamic insulin delivery impacted the results of meal-stimulated glucagon and glucose responses. As demonstrated in the **Figure 2**, no difference was appreciated with the standard meal; thus, providing justification for assessment of the MMTT in the present analysis. (**Supplemental Table 1**) and confirming the reliability of the use of MMTT, instead of a real meal, to assess the effect of the adjunctive therapy on glucagon and glucose response.

In conclusion, this study has highlighted that liraglutide did not suppress dysregulated increases in plasma glucagon responses to meals even after a relatively short period of treatment. We have also confirmed the effect of pramlintide in limiting the early meal-stimulated increases in plasma glucagon and glucose levels. However, the requirement for subcutaneous injections of pramlintide before each meal has limited the use of this agent in patients with T1D in clinical practice.

5. Acknowledgments

Correspondence. Alfonso Galderisi, Yale School of Medicine, Department of Pediatrics – 333 Cedar Street, LMP3107 – 06520 – New Haven, CT (USA). alfonso.galderisi@yale.edu, Tel +1- 203-785-4646, Fax : +1-203-737-2829, Email: alfonso.galderisi@yale.edu

Author contributions. A.G. researched data and wrote the manuscript. M.A.V., L.C, M.Z., E.T., K.W., E.C. and S.A.W. researched data and contributed to the discussion. J.L.S. and W.V.T researched data, contributed to the discussion and reviewed and edited the manuscript.

Disclosure. Medtronic Diabetes provided the pumps, sensors, infusion sets, reservoirs, and laptop computers for the CL experiments. J.L.S. is a consultant for Medtronic Diabetes and is on advisory boards for Bigfoot Biomedical and Insulet. W.V.T. is a consultant for Eli Lilly, Medtronic Diabetes, NovoNordisk and Sanofi. S.A.W. is a consultant for Medtronic Diabetes and Insulet. E.C. is a speaker for NovoNordisk. No other potential conflicts of interest relevant to this article were reported. No sponsor had any role in the study design, data collection, data analysis, data interpretation, or writing of the report.

6. References

1.   Brown RJ, Sinaii N, Rother KI. Too much glucagon, too little insulin: time course of pancreatic islet dysfunction in new-onset type 1 diabetes. Diabetes Care 2008; 31:1403-1404
2.   Dinneen S, Alzaid A, Turk D, Rizza R. Failure of glucagon suppression contributes to postprandial hyperglycaemia in IDDM. Diabetologia 1995; 38:337-343
3.   Sherr J, Tsalikian E, Fox L, Buckingham B, Weinzimer S, Tamborlane WV, White NH, Arbelaez AM, Kollman C, Ruedy KJ, Cheng P, Beck RW, Network DRiC. Evolution of abnormal plasma glucagon responses to mixed-meal feedings in youth with type 1 diabetes during the first 2 years after diagnosis. Diabetes Care 2014; 37:1741-1744
4.   Pörksen S, Nielsen LB, Kaas A, Kocova M, Chiarelli F, Orskov C, Holst JJ, Ploug KB, Hougaard P, Hansen L, Mortensen HB, Diabetes HSGoC. Meal-stimulated glucagon release is associated with postprandial blood glucose level and does not interfere with glycemic control in children and adolescents with new-onset type 1 diabetes. J Clin Endocrinol Metab 2007; 92:2910-2916
5.   Weinzimer SA, Steil GM, Swan KL, Dziura J, Kurtz N, Tamborlane WV. Fully automated closed-loop insulin delivery versus semiautomated hybrid control in pediatric patients with type 1 diabetes using an artificial pancreas. Diabetes Care 2008; 31:934-939
6.   Fredheim S, Andersen ML, Pörksen S, Nielsen LB, Pipper C, Hansen L, Holst JJ, Thomsen J, Johannesen J, Mortensen HB, Svensson J. The influence of glucagon on postprandial hyperglycaemia in children 5 years after onset of type 1 diabetes. Diabetologia 2015; 58:828-834
7.   Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). Diabetes Care 2003; 26:881-885
8.   Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 2006; 295:1681-1687

9.   Bode BW, Garg SK. THE EMERGING ROLE OF ADJUNCTIVE NONINSULIN ANTIHYPERGLYCEMIC THERAPY IN THE MANAGEMENT OF TYPE 1 DIABETES. Endocr Pract 2016; 22:220-230

10.   Ang KH, Sherr JL. Moving beyond subcutaneous insulin: the application of adjunctive therapies to the treatment of type 1 diabetes. Expert Opin Drug Deliv 2017; 14:1113-1131

11.   Wu JH, Foote C, Blomster J, Toyama T, Perkovic V, Sundström J, Neal B. Effects of sodium-glucose cotransporter-2 inhibitors on cardiovascular events, death, and major safety outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. Lancet Diabetes Endocrinol 2016; 4:411-419

12.   Hinshaw L, Schiavon M, Dadlani V, Mallad A, Dalla Man C, Bharucha A, Basu R, Geske JR, Carter RE, Cobelli C, Basu A, Kudva YC. Effect of Pramlintide on Postprandial Glucose Fluxes in Type 1 Diabetes. J Clin Endocrinol Metab 2016; 101:1954-1962

13.   Meier JJ, Rosenstock J, Hincelin-Méry A, Roy-Duval C, Delfolie A, Coester HV, Menge BA, Forst T, Kapitza C. 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

14.   Sherr JL, Patel NS, Michaud CI, Palau-Collazo MM, Van Name MA, Tamborlane WV, Cengiz E, Carria LR, Tichy EM, Weinzimer SA. Mitigating Meal-Related Glycemic Excursions in an Insulin-Sparing Manner During Closed-Loop Insulin Delivery: The Beneficial Effects of Adjunctive Pramlintide and Liraglutide. Diabetes Care 2016; 39:1127-1134

15.   Frandsen CS, Dejgaard TF, Madsbad S. Non-insulin drugs to treat hyperglycaemia in type 1 diabetes mellitus. Lancet Diabetes Endocrinol 2016; 4:766-780

16.   Ahrén B, Hirsch IB, Pieber TR, Mathieu C, Gómez-Peralta F, Hansen TK, Philotheou A, Birch S, Christiansen E, Jensen TJ, Buse JB, Investigators AT. Efficacy and Safety of Liraglutide Added to Capped Insulin Treatment in Subjects With Type 1 Diabetes: The ADJUNCT TWO Randomized Trial. Diabetes Care 2016; 39:1693-1701

17.   Dejgaard TF, Frandsen CS, Hansen TS, Almdal T, Urhammer S, Pedersen-Bjergaard U, Jensen T, Jensen AK, Holst JJ, Tarnow L, Knop FK, Madsbad S, Andersen HU. Efficacy and safety of liraglutide for overweight adult patients with type 1 diabetes and insufficient glycaemic control (Lira-1): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol 2016; 4:221-232

18.   Ilkowitz JT, Katikaneni R, Cantwell M, Ramchandani N, Heptulla RA. Adjuvant Liraglutide and Insulin Versus Insulin Monotherapy in the Closed-Loop System in Type 1 Diabetes: A Randomized Open-Labeled Crossover Design Trial. J Diabetes Sci Technol 2016; 10:1108-1114

19.   Kuhadiya ND, Dhindsa S, Ghanim H, Mehta A, Makdissi A, Batra M, Sandhu S, Hejna J, Green K, Bellini N, Yang M, Chaudhuri A, Dandona P. Addition of Liraglutide to Insulin in Patients With Type 1 Diabetes: A Randomized Placebo-Controlled Clinical Trial of 12 Weeks. Diabetes Care 2016; 39:1027-1035

20.   Mathieu C, Zinman B, Hemmingsson JU, Woo V, Colman P, Christiansen E, Linder M, Bode B, Investigators AO. Efficacy and Safety of Liraglutide Added to Insulin Treatment in Type 1 Diabetes: The ADJUNCT ONE Treat-To-Target Randomized Trial. Diabetes Care 2016; 39:1702-1710

8

21.   Chase HP, Lutz K, Pencek R, Zhang B, Porter L. Pramlintide lowered glucose excursions and was well-tolerated in adolescents with type 1 diabetes: results from a randomized, single-blind, placebo-controlled, crossover study. J Pediatr 2009; 155:369-373

22.   Edelman S, Garg S, Frias J, Maggs D, Wang Y, Zhang B, Strobel S, Lutz K, Kolterman O. A double-blind, placebo-controlled trial assessing pramlintide treatment in the setting of intensive insulin therapy in type 1 diabetes. Diabetes Care 2006; 29:2189-2195

23.   Levetan C, Want LL, Weyer C, Strobel SA, Crean J, Wang Y, Maggs DG, Kolterman OG, Chandran M, Mudaliar SR, Henry RR. Impact of pramlintide on glucose fluctuations and postprandial glucose, glucagon, and triglyceride excursions among patients with type 1 diabetes intensively treated with insulin pumps. Diabetes Care 2003; 26:1-8

24.   Weinzimer SA, Sherr JL, Cengiz E, Kim G, Ruiz JL, Carria L, Voskanyan G, Roy A, Tamborlane WV. Effect of pramlintide on prandial glycemic excursions during closed-loop control in adolescents and young adults with type 1 diabetes. Diabetes Care 2012; 35:1994-1999

25.   Whitehouse F, Kruger DF, Fineman M, Shen L, Ruggles JA, Maggs DG, Weyer C, Kolterman OG. A randomized study and open-label extension evaluating the long-term efficacy of pramlintide as an adjunct to insulin therapy in type 1 diabetes. Diabetes Care 2002; 25:724-730

26.   Smits MM, Tonneijck L, Muskiet MH, Kramer MH, Cahen DL, van Raalte DH. Gastrointestinal actions of glucagon-like peptide-1-based therapies: glycaemic control beyond the pancreas. Diabetes Obes Metab 2016; 18:224-235

27.   Anderberg RH, Richard JE, Eerola K, Ferreras LL, Nordbeck EB, Hansson C, Nissbrandt H, Berqquist F, Gribble FM, Reimann F, Wernstedt-Asterholm I, Lamy C, Skibicka KP. Glucagon-Like Peptide-1 and its Analogues Act in the Dorsal Raphe and Modulate Central Serotonin to Reduce Appetite and Body Weight. Diabetes 2017;

28.   Retnakaran R, Kramer CK, Choi H, Swaminathan B, Zinman B. Liraglutide and the preservation of pancreatic β-cell function in early type 2 diabetes: the LIBRA trial. Diabetes Care 2014; 37:3270-3278

29.   Seino Y, Kaneko S, Fukuda S, Osonoi T, Shiraiwa T, Nishijima K, Bosch-Traberg H, Kaku K. Combination therapy with liraglutide and insulin in Japanese patients with type 2 diabetes: A 36-week, randomized, double-blind, parallel-group trial. J Diabetes Investig 2016; 7:565-573

30.   Ratner RE, Dickey R, Fineman M, Maggs DG, Shen L, Strobel SA, Weyer C, Kolterman OG. Amylin replacement with pramlintide as an adjunct to insulin therapy improves long-term glycaemic and weight control in Type 1 diabetes mellitus: a 1-year, randomized controlled trial. Diabet Med 2004; 21:1204-1212

31.   Nyholm B, Orskov L, Hove KY, Gravholt CH, Møller N, Alberti KG, Moyses C, Kolterman O, Schmitz O. The amylin analog pramlintide improves glycemic control and reduces postprandial glucagon concentrations in patients with type 1 diabetes mellitus. Metabolism 1999; 48:935-941

32.   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

33.   Kuhadiya ND, Malik R, Bellini NJ, Patterson JL, Traina A, Makdissi A, Dandona P. Liraglutide as additional treatment to insulin in obese patients with type 1 diabetes mellitus. Endocr Pract 2013; 19:963-967

Figure 1. Glucose and glucagon profile during mixed meal tolerance test. Glucose profile before and after the treatment with liraglutide (1A) and pramlintide (1B) during mixed meal tolerance test; Glucagon profile before and after the treatment with liraglutide (1C) and pramlintide (1D) during mixed meal tolerance test. Glucose and glucagon are expressed as incremental value from the baseline.

Figure 2. Change in glucose and glucagon levels during full closed loop insulin delivery with a standardized breakfast prior to and 3-4 weeks post treatment with liraglutide.

Table 1 Baseline characteristics

	Liraglutide (n=10)	Pramlintide (n=8)	*p*
Clinical Characteristics			
Sex (F/M)	6/4	5/3	>0.99
Age (y) [age range]	21.9±3.5 [18-27]	19.6±2.8 [16-23]	0.151
BMI (kg/m^2)	23.5±2.9	23.0±1.5	0.665
HbA$_{1c}$ at enrollment % (mmol/mol)	7.5±1.0 (58.0±10.9)	6.9±0.5 (52.0±5.5)	0.142
Diabetes' duration (y)	9.9±6.5y	9.4±4.6y	0.857
Weight (kg)	67.1±9.6	70.7±14.6	0.537
Total daily insulin dose (U/kg/day)	0.8±0.1	0.9±0.3	0.355

Notes: Data are means (SD) unless otherwise indicated.

Table 2. Baseline glucose and glucagon values prior to and post treatment with adjunctive therapy.

Pramlintide	Pre-Treatment	Post-treatment	p-value
Glucose (mg/dL)	121 ± 22	115± 21	0.586
Glucagon (pg/mL)	42 ± 22	45 ± 19	0.775
Liraglutide	Pre-treatment	Post-treatment	p-value
Glucose (mg/dL)	100 ± 16	116 ± 27	0.124
Glucagon (pg/mL)	52 ± 19	47 ± 19	0.563

Notes: Data are means (SD) unless otherwise indicated.

Table 3. Outcomes measures

Glucagon	Liraglutide			Pramlintide		
	Pre-treatment	Post-treatment	*p*	Pre-treatment	Post-treatment	*p*
Glucagon iAUC$_{0-120}$ (pg*min/ml)	1904 ± 651	1801 ± 906	0.774	1988 ± 590	737 ± 577	**0.0007**
Glucagon iAUC$_{120-240}$ (pg*min/ml)	311 ± 564	701 ± 860	0.246	560 ± 807	933 ± 789	0.366
Glucagon incremental peak(pg/ml)	29 ± 16	35 ± 20	0.309	32 ± 16	23 ± 12	**0.026**
Glucagon time-to-peak (min)	47 ± 30	64± 41	0.281	49 ±22	173 ± 66	**0.0097**
Glucose	Pre-treatment	Post-treatment	*p*	Pre-treatment	Post-treatment	*p*
Glucose iAUC$_{0-120}$ (mg*min/dl)	13001 ± 1207	12029 ±1500	0.619	11963 ± 1424	2493 ± 1854	**<0.0001**
Glucose iAUC$_{120-240}$ (mg*min/dl)	20241 ± 1794	18135 ±2580	0.138	17505 ± 2721	13397 ± 2841	0.051
Glucose incremental peak (mg/dl)	200 ±29	171 ±47	0.070	181 ± 46	150 ± 63	**0.011**
Glucose time-to-peak (min)	132 ± 41	135 ± 29	0.85	128 ± 31	221 ± 32	**<0.001**

Notes: Data are means (SD) unless otherwise indicated. p-values in boldface are significant.

10

A Liraglutide — Pre-treatment / Post-treatment

B Liraglutide