LONG TITLE: A Randomized, Placebo-Controlled Double-Blind Trial of a Closed-Loop Glucagon System for Post-Bariatric Hypoglycemia

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Patents: Drs. Dassau and Doyle report an issued patent “Health Monitoring System.” Drs. Dassau, Doyle, Laguna-Sanz and Patti report a patent application “Prevention of Post-Bariatric Hypoglycemia Using a Novel Glucose Prediction Algorithm and Mini-Dose Stable Glucagon.” Dr. Patti reports an issued patent PCT/US2017/045061 “Methods and

Compositions for Treating Hypoglycemia." Mr. Cummins, Mr. Newswanger and Dr. Prestrelski acknowledge pending patent PCT/US2019/014815. Dr. Doyle reports equity, licensed IP and is a member of the Scientific Advisory Board of Mode AGC.

ABSTRACT:

Background: Post-bariatric hypoglycemia (PBH) can threaten safety and reduce quality of life. Current therapies are incompletely effective.

Methods: Patients with PBH were enrolled in a double-blind, placebo-controlled, crossover trial to evaluate a closed-loop glucose-responsive automated glucagon delivery system designed to reduce severe hypoglycemia. A hypoglycemia detection and mitigation algorithm was embedded in the Artificial Pancreas System connected to a continuous glucose monitor (CGM, Dexcom) driving a patch infusion pump (Insulet) filled with liquid investigational glucagon (Xeris) or placebo (vehicle). Sensor/plasma glucose responses to mixed meal were assessed during two study visits. The system delivered up to two doses of study drug (300/150 µg glucagon or equal-volume vehicle) if triggered by the algorithm. Rescue dextrose was given for plasma glucose <55 mg/dL or neuroglycopenia.

Findings: Twelve participants (11F/1M, age 52±2, 8±1 years post-surgery, mean±SEM) completed all visits. Predictive hypoglycemia alerts prompted automated drug delivery post-meal, when sensor glucose was 114±7 vs. 121±5 mg/dL (p=0.39). Seven participants required rescue glucose after vehicle but not glucagon (p=0.008). Five participants had severe hypoglycemia (<55 mg/dL) after vehicle but not glucagon (p=0.03). Nadir plasma glucose was higher with glucagon vs. vehicle (67±3 vs. 59±2 mg/dL, p=0.004). Plasma glucagon rose after glucagon delivery (1231±187 vs. 16±1 pg/mL at 30 minutes, p=0.001). No rebound hyperglycemia occurred. Transient infusion site discomfort was reported with both glucagon (n=11/12) and vehicle (n=10/12). No other adverse events were observed.

Interpretation: A CGM-guided closed-loop rescue system can detect imminent hypoglycemia and deliver glucagon, reducing severe hypoglycemia in PBH.

Précis: This trial demonstrates effectiveness of a closed-loop glucagon system to predict and reduce severe hypoglycemia in PBH, an increasingly recognized condition with a paucity of treatment strategies.

INTRODUCTION:

With the rising use of bariatric surgery, one increasingly recognized complication of roux-en-Y gastric bypass (RYGB) is hypoglycemia (1)~~(2)~~. Symptomatic hypoglycemia can also occur after sleeve gastrectomy, but prevalence remains uncertain as the limited number of clinical studies in the post-sleeve population have analyzed glucose response after liquid meals or glucose loads (2,3).This condition, termed post-bariatric hypoglycemia (PBH), is typically recognized between 1 and 3 years postoperatively, and causes not only distressing adrenergic and cholinergic symptoms but also neuroglycopenia and hypoglycemia unawareness, threatening safety. Episodes of hypoglycemia impair cognition and increase the risk for syncope, arrhythmias, seizures, coma, and even death. Moreover, many patients are disabled by their hypoglycemia due to their inability to perform job-related tasks and to safely operate a motor vehicle.

Patients affected by PBH demonstrate distinct postprandial glucose patterns, with very rapid rise in glucose shortly after food intake (mean rise rate 9 ± 1 mg/dL/min in previous study (4)), linked to rapid delivery and intestinal absorption. These high peak glucose levels, together with increased postprandial levels of GLP1 and other intestinally-derived hormones (5-7), stimulate excessive insulin secretion and contribute to the very rapid lowering of glucose to hypoglycemic levels 1-3 hours after eating. Insulin-independent glucose disposal is also increased in PBH. Moreover, counterregulatory hormones, including glucagon (5), catecholamines and cortisol, are reduced during experimentally-induced hypoglycemia in post-RYGB patients (8).

Given the multiple factors promoting hypoglycemia and reduced counterregulatory responses to hypoglycemia, it is not surprising that management of PBH poses distinct challenges for patients and physicians alike. Initial treatment focuses on reducing intake of simple carbohydrates and use of acarbose to minimize the post-meal glucose “spikes.” Strategies aimed at reducing incretin and insulin responses to meals include somatostatin

receptor analogues (e.g. octreotide), diazoxide, and even reversal of the surgical procedure for refractory PBH (9,10). Unfortunately, these therapies are limited by side effects or incomplete efficacy, even in combination. Although partial pancreatectomy was used initially to reduce islet mass (11-13), recurrence of hypoglycemia is observed (14), underscoring the complex metabolic interactions beyond insulin secretion which contribute to hypoglycemia. A major challenge for patients affected by PBH is that glucose levels drop very quickly in the postprandial state (mean fall rate 7 ± 1 mg/dL/min in previous study (4)) making it very difficult to respond to and treat impending hypoglycemia before neuroglycopenia develops; this problem is of even greater magnitude in patients with hypoglycemia unawareness.

With the incomplete efficacy of currently available therapies, there is an urgent need for novel approaches for treatment of severe hypoglycemia to maintain health, improve quality of life, and improve safety. Glucagon can be used successfully to treat acute hypoglycemia in PBH. Currently available glucagon preparations limit utilization due to (1) need to reconstitute powder immediately before use, (2) expense of single-use emergency kits, and (3) side effects such as nausea and rebound hyperglycemia with traditional rescue doses (0.5-1.0 mg)(15). A newly-developed stable liquid formulation of native glucagon (16) can be delivered via infusion pump, allowing lower doses of glucagon to be delivered only when hypoglycemia is imminent.

We thus hypothesized that a novel glucose-responsive glucagon delivery system designed to address the unique pathophysiology of severe PBH, including large glycemic excursions, impaired counterregulation, and hypoglycemia unawareness, would be effective to reduce the occurrence and severity of hypoglycemia. In a pilot study, we demonstrated the feasibility of an event-based hypoglycemia prediction algorithm guided by CGM data to direct manual delivery of small glucagon boluses via a patch infusion pump (4). We now report results of a double-blind, placebo-controlled, crossover trial to determine the efficacy of a closed-loop glucagon delivery system to reduce severe hypoglycemia after a mixed meal.

METHODS

Materials and Methods - Clinical

Study Design

This double-blind, placebo-controlled crossover trial was designed to evaluate the ability of a novel integrated closed-loop glucagon system to reduce meal-provoked hypoglycemia in PBH (**Supplemental Figure 1**) (17).

Participants

Participants with a history of RYGB and PBH with neuroglycopenia, uncontrolled on medical nutrition therapy and medications, were recruited from the Joslin Diabetes Center hypoglycemia clinic and other endocrine clinics in the region. Exclusion criteria included major systemic illness, cardiac arrhythmia, hypertension, active coronary artery disease, fasting hypoglycemia, known insulinoma, pregnancy, substance or alcohol abuse, recent steroid or investigational drug exposure, and use of medications (beyond hypoglycemia treatment) known to affect insulin secretion or action. The Food and Drug Administration approved investigational device exemption (IDE G170159) for the closed-loop glucagon studies. The Joslin Diabetes Center Committee on Human Studies approved the study. Written informed consent was obtained from all participants.

Investigational Glucagon and Vehicle Formulation, Randomization and Masking

Investigational ready-to-use liquid glucagon and matching vehicle (Xeris Pharmaceuticals, Chicago IL) were provided in numbered vials and stored at room temperature. Vehicle was visually indistinguishable from active product. Sets of four vials, one primary and backup vial for each study visit, were grouped in a box for each study participant and labeled with participant number and study visit number. A list of ascending randomization numbers starting with 001 was generated by a Xeris statistician using SAS® version 9.4, Proc Procedure set for a block size of 8. The randomization list was provided to the packager so

that boxes would be packaged with a 1:1 distribution of active and placebo vehicle vials at each visit. After eligibility was confirmed, qualifying subjects were assigned the lowest number kit remaining in inventory at the time of their initial meal challenge. Participants, study personnel, and Xeris study staff were blinded to study drug identity, and identity of vials was unblinded only after data analysis.

At release for use in the study, samples from glucagon vials were analyzed to determine glucagon concentration using ultra-high-performance liquid chromatography (UHPLC, Pyramid Labs, Costa Mesa, CA). Additional samples were placed in an ICH-compliant stability program for concentration analysis during and following completion of the clinical study (UHPLC, MRI Global, Kansas City, MO). The stability results at the timepoint following completion of the clinical trial showed that glucagon concentrations remained well within acceptance criteria.

Initiation of glucagon delivery system and mixed meal tolerance testing

Following screening history, physical exam and laboratory testing, and confirmation of participant eligibility, two blinded continuous glucose monitors (CGMs, Dexcom G4 Professional, San Diego, CA) were inserted on the anterior abdominal wall. Participants were instructed to perform calibrations when prompted and to return 48-72 hours later for the first of two study visits. Medications, including acarbose, short-acting octreotide, and diazoxide, were held for at least 24 hours prior to study visits; long-acting octreotide was withdrawn at least one month prior to study entry.

Participants arrived after an overnight fast. After intravenous catheter placement for blood sampling, an Omnipod pump (Insulet Corporation, Acton MA) filled with either investigational glucagon or vehicle, was placed on the anterior abdominal wall, and the pump catheter was inserted into the subcutaneous space. After calibration, the sensor with glucose values most closely matching the serum glucose was connected to the Windows tablet running the

portable Artificial Pancreas System (pAPS) and the PBH detection algorithm (4,18). After baseline blood sampling, a liquid mixed meal (Ensure Compact, containing 64 g carbohydrate, 18 g protein, 12 g fat, 440 kcal, 236 mL volume) was consumed over 3 to 5 minutes. As previously described(19), this high-carbohydrate meal was chosen as a standardized experimental intervention to increase the likelihood of induction of the postprandial glucose and insulin surge and subsequent hypoglycemia typical of PBH. Use of this protocol allowed testing of the efficacy of glucagon versus vehicle to mitigate hypoglycemia.

Sensor glucose and plasma glucose, insulin, C-peptide and glucagon concentrations were measured at baseline and at predetermined intervals following the mixed meal, at time of hypoglycemia alert, and for at least 2 hours following study drug delivery.

The pAPS system

The portable Artificial Pancreas System (pAPS) is a drug delivery system including a CGM, an Omnipod pump and a tablet computer (Dell Venue™ 10 Pro, Dell Technologies, Round Rock, TX) that centrally manages all the devices (**Figure 1**). The user (study physician or engineer) can interact with the system (e.g. set subject parameters, initiate or terminate the session) through a touch graphical user interface implemented on the tablet. The pAPS is responsible for automated delivery of study drug via the pump; this is accomplished by the hypoglycemia detection and mitigation algorithm that resides on the tablet. More specifically, the CGM sensor transmits a glucose value every 5 minutes to the CGM receiver that is serially connected to the tablet. The transmitted glucose value is used by the algorithm to calculate the dose of study drug, which is then signaled from the tablet to the pump relay via Bluetooth Low Energy (BLE) communication for pump delivery. The pAPS also provides visual and audible alerts in the tablet and sends wireless alerts to the clinical team in the event of impending hypoglycemia.

Hypoglycemia detection and mitigation algorithm: The pAPS system has reliably supported many inpatient and outpatient AP clinical studies (20-24) using distinct internal algorithms designed for diabetes management. Given the unique post-meal glycemic patterns in PBH, a novel hypoglycemia detection and mitigation algorithm was developed and tested in our previous open-loop pilot study, described in (19). The algorithm has been further optimized to handle missing CGM values in case of lost connectivity or CGM malfunction. The key components of the algorithm, now utilized in a closed-loop mode (requiring no intervention from the clinical team), are detailed in **Supplementary Methods (17)**.

In brief, the algorithm includes two distinct components, designed to permit recognition and response to the unique postprandial glucose metabolism patterns in PBH. For both components, a threshold for drug delivery was chosen which would account for the delay of CGM-derived interstitial glucose values relative to plasma glucose (25), and allow delivery of glucagon prior to the onset of hypoglycemia. The PBH alert, designed to predict postprandial hypoglycemia, is activated if glucose is falling at a rapid rate after a meal has been detected, and hypoglycemia is predicted to occur in <30 minutes; this alert can be activated even if glucose levels are within the normoglycemic range (<150 mg/dL) to allow early detection and response. By contrast, the low glucose prediction (LGP) component can only be activated at a lower glucose, and does not require detection of a meal. If the prespecified conditions were reached, the pAPS system directed the pump to deliver 300 µg of the blinded study drug (glucagon or equivalent volume of vehicle) within 5 minutes. If the system predicted or detected a second hypoglycemic event, 300 µg or 150 µg of blinded study drug were delivered based on filtered glucose levels being below or above 75 mg/dL, respectively. The study team received alerts when a hypoglycemia event was detected or predicted. Though the participants were not directly informed, they could usually feel a sensation of study drug delivery and were aware when the team drew blood samples and began asking about symptoms using the Edinburgh Hypoglycemia Scale.

For all participants, the pump was removed and a standard low-carbohydrate lunch was provided either at 120 minutes following the first hypoglycemia mitigation alert or 30 minutes after a second alert, whichever was later. If at any time during observation the participant developed severe hypoglycemia (serum glucose ≤55 mg/dL or neuroglycopenia), rescue therapy was provided (IV dextrose bolus, subcutaneous glucagon or oral glucose) to achieve resolution of neuroglycopenia. The pump was removed and lunch was provided once glucose was >100 mg/dL. All participants were observed for two additional hours prior to discharge.

Outcomes and Sample Size

The primary endpoint for this study is prevention of meal-provoked hypoglycemia, defined as glucose <65 mg/dL, with the glucagon-containing system but not the vehicle-containing system. Secondary outcomes included prevention of severe hypoglycemia (<60 mg/dL), prevention of rebound hyperglycemia (glucose >180 mg/dL), avoidance of clinically significant hypoglycemia requiring rescue therapy (glucose ≤55 mg/dL and/or severe neuroglycopenia), and improvements in glucose time in goal (65 to 180 mg/dL, reported in minutes). Sample size of 12 subjects was chosen prior to study initiation to provide power >0.9 to detect significant differences between conditions in the primary outcome at $\alpha=0.05$. When 10 participants had completed the trial, a non-comparative (masked) interim analysis was undertaken to determine whether the total number of outcomes thus far provided adequate power to justify stopping enrollment. Discordant responses to treatment between conditions in at least 8 subjects were required to stop enrollment. As 6 subjects had discordant responses, the trial was continued until the planned enrollment target of 12. Enrollment was halted when the pre-specified target sample size was reached.

Additional clinical assessments

The Edinburgh Hypoglycemia Scale was used to assess hypoglycemia symptoms at baseline, at time of hypoglycemia prediction alert, and 15, 30 and 60 minutes after hypoglycemia alert and blinded study drug bolus (26). Scores for the 5 autonomic, 8 neuroglycopenic, and 5 nonspecific symptoms were summed for each time point.

Pain or other symptoms at the infusion site, using a score from 1 (none) to 10 (greatest) were recorded, and the infusion site was examined at 30 and 120 minutes after pump removal using the Draize scale (27).

Hormonal analyses

Plasma glucose was measured by glucose oxidation (YSI 2300 STAT, Yellow Springs, OH). Using solid phase extraction, a validated HPLC method with MS/MS detection was employed to measure plasma glucagon (Algorithme Pharma, Laval, Quebec). C-peptide was determined using a sandwich electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics) using a biotinylated monoclonal C-peptide specific antibody and a monoclonal C-peptide-specific labeled antibody (Medpace, Cincinnati, OH). Insulin was assayed using ECLIA insulin reagent kits and an immuno-analyzer instrument (Roche Diagnostics), which employs a competitive test principle using an anti-insulin monoclonal antibody (Medpace, Cincinnati, OH).

Statistical analysis

Descriptive statistics and figures were produced using GraphPad Prism (GraphPad Software, La Jolla, CA). All other analyses were completed using SAS version 9.4 for Windows (SAS Institute, Cary, NC). All data were tested to confirm that they met the assumptions of the planned analyses. Continuous variables are expressed as mean ± standard deviation or as median with interquartile range. Dichotomous and multinomial outcomes between conditions were compared using McNemar's test, and continuous

outcomes using linear mixed effects models to account for correlation within subjects over time.

RESULTS:

Between October 2017 and August 2018, 23 individuals were screened and 18 enrolled (consort diagram, **Figure 2**). Two participants withdrew prior to the first mixed-meal study visit due to inability to obtain adequate intravenous access. Sixteen participants completed the first mixed meal study visit; twelve participants completed both mixed meal study visits and were included in analysis (**Table 1**). Of the four participants who completed only one mixed meal visit, one developed intractable nausea and emesis after consuming the mixed meal, and a hypoglycemia alert was not triggered in 3 (thus no study drug delivery).

The prespecified primary outcome was to test whether the system containing glucagon would be superior to vehicle in preventing hypoglycemia after the mixed meal (moderate hypoglycemia, <65 mg/dL). Avoidance of plasma glucose <65 mg/dL occurred in four of twelve participants in response to glucagon but not vehicle study days (p=0.18). Similarly, avoidance of sensor glucose <65 mg/dL was successful in response to glucagon but not vehicle study day in five of twelve participants (p=0.10) (**Table 2**).

While statistical significance was not achieved for the primary endpoint, several clinically meaningful secondary endpoints demonstrating reduction in severe hypoglycemia were achieved. There was a significant difference in avoidance of severe hypoglycemia or neuroglycopenia; five participants had plasma glucose <55 mg/dL after vehicle, but plasma glucose levels remained above 55 mg/dL after glucagon delivery (p=0.03). No participants reached sensor glucose <55 mg/dL after either treatment. Clinically significant hypoglycemia, defined as plasma glucose <55 mg/dL (n = 5) or neuroglycopenia (n = 2) requiring rescue, occurred after vehicle but not glucagon delivery in seven of twelve participants (p=0.008). One participant required rescue after both glucagon and vehicle; importantly, no participants required rescue after only glucagon treatment. Rebound hyperglycemia (glucose >180 mg/dL) did not occur in any study visits.

Nadir plasma glucose was significantly lower after vehicle versus glucagon (58 vs. 67 mg/dL, p=0.049)(**Table 3**). Plasma glucose remained in target range (70-180 mg/dL) for a greater percentage of time after glucagon versus vehicle (85 vs. 64%, p<0.05). A second hypoglycemia mitigation alert was triggered more often during vehicle than glucagon visits (11 of 12 vs. 8 of 12 visits), reflecting higher glucose levels after the first glucagon delivery vs. vehicle (86 vs. 70 mg/dL, p<0.01) (**Figure 3**). The mean glucose values for participants not requiring rescue glucose was significantly higher at 60 minutes after glucagon compared to vehicle (99 ± 3.5, n=12 vs 72 ± 5.0, n=8, p<0.001).

During both glucagon and vehicle treatment visits, all participants received either 300 µg of glucagon or the equivalent volume of vehicle with the first alarm. During vehicle visits, nine of twelve participants had second alarms due to persistent glucose values below threshold; five received a volume of vehicle equivalent to 300 µg and four received a volume of vehicle equivalent to 150 µg. For the three remaining participants, second alarms were generated for two but study drug was not delivered as the pump had been removed upon rescue glucose treatment for clinically significant hypoglycemia. By contrast, during glucagon treatment visits, six of twelve participants did not have a second alarm, due to higher glucose levels; of the six with a second alarm, five received 150 µg of glucagon, and one received 300 µg of glucagon.

Plasma glucagon levels were similar at baseline, and increased moderately at early time points after the mixed meal, as previously reported (28); post-meal glucagon levels did not differ significantly between study visit days (**Figure 4A, Supplemental Table 1**) (17). At the time of the first hypoglycemia mitigation alert, glucagon levels were not increased from baseline or different between treatment groups. However, 10 minutes after study drug infusion significant differences emerged, with serum glucagon levels 417 ± 91 vs. 14 ± 1 pg/mL vs (glucagon vs. vehicle, p<0.001). Differences between glucagon and vehicle study

days were maximal at 30 minutes after study drug infusion (1231 ± 187 vs. 19 ± 3 pg/mL, p<0.001), representing a 35-fold increase from baseline in the glucagon treatment group (p<0.0001).

Insulin and C-peptide levels were also assessed at baseline and at several intervals following the mixed meal and study drug infusion (**Figure 4B/C, Supplemental Table 2**) (17). Consistent with prior studies, plasma insulin levels rose dramatically after meal ingestion on both glucagon and vehicle study days, peaking at 30 minutes (327 vs. 243 μU/mL, p<0.0001 for both). Insulin levels remained elevated (21- and 36-fold above baseline) at the time of hypoglycemia alert, but did not differ between glucagon and vehicle study days. C-peptide levels demonstrated a similar trend for glucagon vs. vehicle study days.

After study drug delivery, plasma insulin levels continued to fall, with no significant difference between glucagon and vehicle delivery days at any time point. C-peptide levels were significantly higher at 30 minutes after glucagon delivery (12.2 ± 3.1 vs 6.1 ± 0.9 ng/mL, p = 0.03) but did not differ at any other time points.

Hypoglycemia symptoms, assessed with the Edinburgh score, were recorded and analyzed both individually and by category (autonomic, neuroglycopenic or nonspecific symptoms); these did not differ between study days at baseline, at the time of predictive hypoglycemia alert or after drug delivery even in participants who had hypoglycemia.

No serious adverse events were observed during this trial. Nausea was experienced after the mixed meal but before study drug delivery in 12 of 24 study visits in the participants who completed two study visits. Nausea occurred only after glucagon delivery in 2 of 12 glucagon study visits and none of the vehicle study visits. Discomfort at the infusion pump site did not differ between vehicle and glucagon infusions (**Supplemental Figure 3**) (17).

Mild erythema and edema were self-limited and did not differ between glucagon and vehicle infusions (**Supplemental Figure 4**) (17).

DISCUSSION:

This study tested a new closed-loop glucose-responsive automated glucagon delivery system, which demonstrated superior performance as compared with placebo (vehicle) delivery in prevention of significant postprandial hypoglycemia in patients with PBH, without any serious adverse events. Our CGM-informed hypoglycemia mitigation algorithm, designed to detect and respond to the unique rapid and high-magnitude swings in postprandial glucose in PBH, successfully activated the pump to deliver study drug in all participants who developed clinically significant hypoglycemia. Glucagon levels rose in response to pump activation, indicating successful delivery, and post-treatment glucose levels were significantly higher in response to glucagon. Finally, clinically significant hypoglycemia requiring intravenous glucose rescue (neuroglycopenia or plasma glucose <55 mg/dL) occurred only after vehicle infusion, except in the one participant who did not respond to either vehicle or glucagon infusions. Thus, glucagon delivery via the glucose-responsive automated delivery system provided clinical efficacy in mitigating severe postprandial hypoglycemia in PBH.

Analysis of glucagon levels during the study revealed several key points. Firstly, glucagon levels did not increase above baseline even as glucose levels approached 55 mg/dL. This indicates that the glucagon counterregulatory response to hypoglycemia is impaired in PBH and parallels findings of Abrahamsson et al. who demonstrated blunted counterregulatory response to hypoglycemia at 6 months post-RYGB, even in individuals without known hypoglycemia (8). Thus, glucagon therapy targets a key element of the dysfunctional hormonal response to hypoglycemia in individuals with PBH.

There was substantial interindividual variation in glycemic responses to mixed meal. Indeed, several participants did not develop adequate hypoglycemic risk to trigger an alert or study drug dosing, despite a history of these events in the outpatient setting. Potential contributors

to this unexpected finding include the study environment, which differs substantially from the ambulatory environment in several respects. Participants consumed a liquid mixed meal high in simple carbohydrates, a meal typically avoided due to propensity to induce dumping syndrome and hypoglycemia (29). Moreover, inactivity (remaining in bed during the study) and the stress of participation may have increased stress-related hormonal responses (e.g. cortisol) and induced relative insulin resistance, reducing the likelihood of hypoglycemia.

While the standard emergency rescue dose of glucagon is 1 mg, we utilized lower doses in this system (300 µg for first dose, 300 or 150 µg for second dose, if needed). Similar submaximal doses have been found to be effective to treat insulin- and exercise-induced hypoglycemia in patients with type 1 diabetes, while minimizing adverse effects such as nausea and rebound hyperglycemia (30-32). For example, in studies by Haymond et al in a pediatric population, age-based micro-dosing of glucagon (20-150 µg) successfully increased glucose by 3.3-5 mmol/L (60-90 mg/dL)(31). Higher doses of glucagon were required following insulin administration, and 52% of participants required 2 or 3 doses to maintain euglycemia. In this study, we utilized a 300 µg dose for the first delivery, based on a more robust rise in glucose with this dose (as compared with 150 µg dose) during the pilot open-loop study (4). We observed significantly higher glucose values at 60 minutes after glucagon dosing as compared with vehicle, with minimal side effects. However, there was substantial interindividual variability in glycemic response, and the magnitude of response to glucagon was generally lower than in studies of T1D. Potential factors contributing to these patterns include the very high insulin levels in the postprandial state in PBH; high-magnitude and prolonged insulin action likely contributed to both ongoing tissue uptake of glucose for several hours after meal ingestion and reduced glucagon responsiveness. Moreover, suboptimal glycogen stores could also contribute to reduced glucagon responsiveness, as observed in patients with type 1 diabetes consuming low carbohydrate diets who have inadequate response to glucagon from insulin-induced hypoglycemia (33). While we recommend a diet which includes complex carbohydrates in controlled portions, some

patients restrict total carbohydrates to reduce hypoglycemia frequency (29), potentially contributing to inadequacy of glycogen stores and reduced glucagon responsiveness. Indeed, one participant did not avoid clinically significant hypoglycemia during either vehicle or glucagon delivery, despite achieving supraphysiologic glucagon levels after glucagon delivery, and similar insulin and C-peptide levels to other participants who did respond to glucagon. We do not understand the precise nature of this variability in response at present. Future studies could include patient-specific dosing optimization.

Given the challenging nature of PBH, additional pharmacologic treatment strategies are being developed. Given the importance of hyperinsulinemia as a contributor to postprandial hypoglycemia in PBH, blocking insulin secretion and/or action may be effective. For example, blockade of the GLP-1 receptor by exendin 9-39 can reduce insulin secretion and hypoglycemia after a glucose tolerance test in short-term studies (34) (35). Inhibition of insulin action using a monoclonal anti-insulin receptor antibody has been shown to reduce insulin action in healthy individuals (36), but results of a clinical trial in individuals with PBH (NCT02772718) have not yet been published. A study testing efficacy of pump delivery of micro-doses of glucagon throughout the day to maintain glycemia has not yet been published (NCT 02966275). Additional studies will be needed to assess the relative efficacy of such approaches, either in isolation or in combination with use of our integrated delivery system to detect and prevent severe hypoglycemia.

We acknowledge limitations of our study. Our study was performed in a single center. Only one male completed both study visits (despite enrolling additional men). This may reflect, in part, the higher number of women undergoing bariatric surgery (37) and the increased risk for PBH in females (38). Future multi-center studies could aim for improved gender parity. We utilized liquid mixed meal testing to reproducibly provoke hypoglycemia in order to test our system, but acknowledge that a liquid meal is not physiologic and may provoke some

features of the dumping syndrome. Frequency of provoked hypoglycemia would likely be lessened with use of a solid meal, as we recommend to our patients in clinical practice. We utilized glycemic patterns detected by CGM to predict hypoglycemia, as a closed-loop glucagon rescue system would be most valuable for patients with hypoglycemia unawareness. While use of CGM in post-bariatric surgery patients is a relatively new strategy, comparison of CGM with plasma glucose-based detection of hypoglycemia has been reported in two studies (39,40). Kefurt et al reported that 75% of post-RYGB patients had glucose <55 mg/dL during blinded CGM, vs. only 29% with plasma glucose <55 mg/dL during meal testing. These differences could reflect high rates of asymptomatic reductions in glucose in this population, erroneous detection of low glucose by CGM, or higher rates of low glucose levels during daily life as compared with post-meal testing (39). Interestingly, Nielsen et al found that minimum interstitial glucose values were an average of 20 mg/dL higher than plasma values measured concurrently during mixed meal, potentially leading to overestimation of glucose values and underestimation of hypoglycemia by CGM (40). Future studies will be required to fully assess the role of CGM and other methods to detect and define hypoglycemia in this population (39,40).

While the study was conducted in a clinical research center to ensure participant safety, outpatient “real-world” experience indicates that activity and ambulation may also increase risk of hypoglycemia. Additional studies will be required to test the efficacy of the closed-loop rescue system to prevent hypoglycemia in response to solid meals, activity, and other aspects of outpatient daily life. Our system delivered a maximum of two doses of study drug; future studies will be required to test dose-dependency of the glucagon response and whether additional doses could promote improved efficacy. Future studies could investigate a more personalized dose based on weight or glucagon responsiveness. In summary, we report development and implementation of a novel technology-driven approach to address the unique pathophysiology of PBH, and demonstrate that our closed-loop automated

glucagon system was effective in prevention of severe postprandial hypoglycemia in participants with PBH.

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Patents: Drs. Dassau and Doyle report an issued patent “Health Monitoring System.” Drs. Dassau, Doyle, Laguna-Sanz and Patti report a patent application “Prevention of Post-Bariatric Hypoglycemia Using a Novel Glucose Prediction Algorithm and Mini-Dose Stable Glucagon.” Dr. Patti reports an issued patent PCT/US2017/045061 “Methods and Compositions for Treating Hypoglycemia.” Mr. Cummins, Mr. Newswanger and Dr. Prestrelski acknowledge pending patent PCT/US2019/014815. Dr. Doyle reports equity, licensed IP and is a member of the Scientific Advisory Board of Mode AGC.

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FIGURE AND TABLE LEGENDS

Figure 1. Components of the Closed-Loop Glucagon System: A continuous glucose sensor (A) detects interstitial glucose values, wirelessly transmits data to the CGM receiver (B) which transmits to the pAPS tablet hosting the PBH algorithm (C); when hypoglycemic is predicted, a command for for study drug delivery is sent to an infusion pump containing either study glucagon or vehicle (D).

Figure 2. Consort Diagram: * Exclusion criteria identified during screening included no IV access (n=2), screening lab abnormalities (hypokalemia, n=1), no prior trial of medications for treatment of PBH (n=1), and uncontrolled hypertension (n=1). **Participants were withdrawn from study due to intractable nausea & emesis after MMTT (n=1), no hypoglycemia alert or study drug delivery during observation period (n=3). Abbreviations: IV: intravenous, MMTT: mixed-meal tolerance test.

Figure 3. A. Representative plot of sensor glucose levels for one participant during mixed meal tolerance test study visits during which either vehicle (blue) or glucagon (red) was delivered. During the vehicle delivery visit the participant received two doses of vehicle before reaching clinically significant hypoglycemia, at which time intravenous dextrose was administered and the closed-loop system was discontinued. Sensor glucose values were not plotted after intravenous dextrose administration due to rapid change in glucose leading to signal loss. During the glucagon treatment visit, the participant received two doses of glucagon, and clinically significant hypoglycemia was prevented. **B.** Glucose response in the first 60 minutes after the initial vehicle (blue) or glucagon (red) infusions for all study participants. Black triangles denote administration of rescue glucose and premature discontinuation of the system after clinically significant hypoglycemia (defined by plasma glucose) in 7 of 24 visits; one participant required rescue glucose after glucagon (red line ending in black triangle).

Figure 4. (A) Glucagon, (B) Insulin and (C) C-peptide Levels: Hormone levels measured at baseline, and at specified times after mixed meal ingestion, at the time of 1st and 2nd hypoglycemia mitigation alerts and at specified intervals after alert and study drug infusions. Numbers in grey denote number of participants that had samples drawn at each timepoint. These numbers varied, depending on the timing of alerts; for example, if an alert and study drug delivery occurred before 120 minutes after the meal, additional blood sampling was performed as a post-hypoglycemia alert sample. * $p<0.05$, ** $p<0.001$.

Table 1. Participant Characteristics: Demographic information for 12 enrolled participants who completed two mixed meal study visits and were included in analysis.

Table 2. Dichotomous Outcomes: Glucose values measured during the time defined from the initial hypoglycemia mitigation alert until lunch or delivery of rescue glucose. Rebound hyperglycemia defined as plasma glucose > 180 mg/dL after study drug delivery. Protocol-specified rescue delivery of intravenous glucose was performed if plasma glucose < 55 mg/dL and/or significant neuroglycopenia developed. Abbreviations: G, Glucagon; V, Vehicle

Table 3. Continuous Outcomes: Outcomes are based on glucose values (sensor or capillary, as indicated) and time of key events during study visits. *, Range is 65-180 mg/dL; **Pain Score: 1 (least severe pain) to 10 (most severe)

Table 1.

	Summary	Mean (Median)		SEM (25-75%tile)
Age (years)		52	±	2.4
Gender (M:F)	1:11			
BMI (kg/m^2)		27.6	±	1.5
HbA1c (%)		5.3	±	0.1
Years since surgery		8.4	±	1.5
Years from surgery to neuroglycopenia		(2.2)		(1.4 - 6.1)
On any anti-hypoglycemic medications	10 of 12			
Acarbose	5 of 12			
Diazoxide	4 of 12			
Octreotide	3 of 12			
Pramlintide	2 of 12			

Table 2.

Outcome	Source	Met Criterion on Vehicle Only	Met Criterion on Glucagon Only	p-value [G vs. V]
Glucose < 65 mg/dL	Plasma	4	1	0.180
	Sensor	5	1	0.103
Glucose < 60 mg/dL	Plasma	3	0	0.083
	Sensor	3	1	0.317
Glucose < 55 mg/dL	Plasma	5	0	0.025
	Sensor	0	0	N/A
Rebound hyperglycemia after study drug	Plasma or Sensor	0	0	N/A
Rescue needed		7	0	0.008

Table 3.

Outcome	Treatment Condition	Mean	SEM	P-value [G vs. V]
% Time PLASMA Glucose in Range* After Drug Delivery	G	**0.852**	**0.082**	**0.049**
	V	**0.645**	0.074	
% Time SENSOR Glucose in Range* After Drug Delivery	G	**0.987**	0.013	0.056
	V	**0.815**	0.077	
Nadir PLASMA Glucose (mg/dL)	G	**67.4**	2.70	0.004
	V	**58.5**	1.87	
Nadir SENSOR Glucose (mg/dL)	G	**72.7**	2.21	0.059
	V	**65.3**	1.85	
Time to Nadir PLASMA Glucose After Mixed-Meal (Minutes)	G	**138**	11.9	0.195
	V	**125**	7.43	
Time to Nadir SENSOR Glucose After Mixed-Meal (Minutes)	G	**156**	11.6	0.043
	V	**134**	6.04	
Time to Alarm (Minutes)	G	**89.9**	3.90	0.659
	P	**87.7**	4.9975	
Time to Delivery (Minutes)	G	**94**	5.52	0.406
	V	**89.3**	5.33	
Alarm 1 SENSOR Glucose (mg/dL)	G	**134**	5.93	0.611
	V	**139**	4.92	
Alarm 1 Capillary Glucose (mg/dL)	G	**98.1**	7.30	0.183
	V	**109**	5.75	
Alarm 2 SENSOR Glucose (mg/dL)	G	**85.7**	4.96	0.009

	V	**70.1**	2.26	
Alarm 2 Capillary Glucose (mg/dL)	G	**94.2**	1.70	0.789
	V	**91.7**	8.62	
Study Drug Pain Score 1st Dose**	G	**4.00**	0.685	0.865
	V	**3.83**	0.815	
Study Drug Pain Score 2nd Dose**	G	**1.14**	0.615	0.875
	V	**1.31**	0.671	

Figure 1

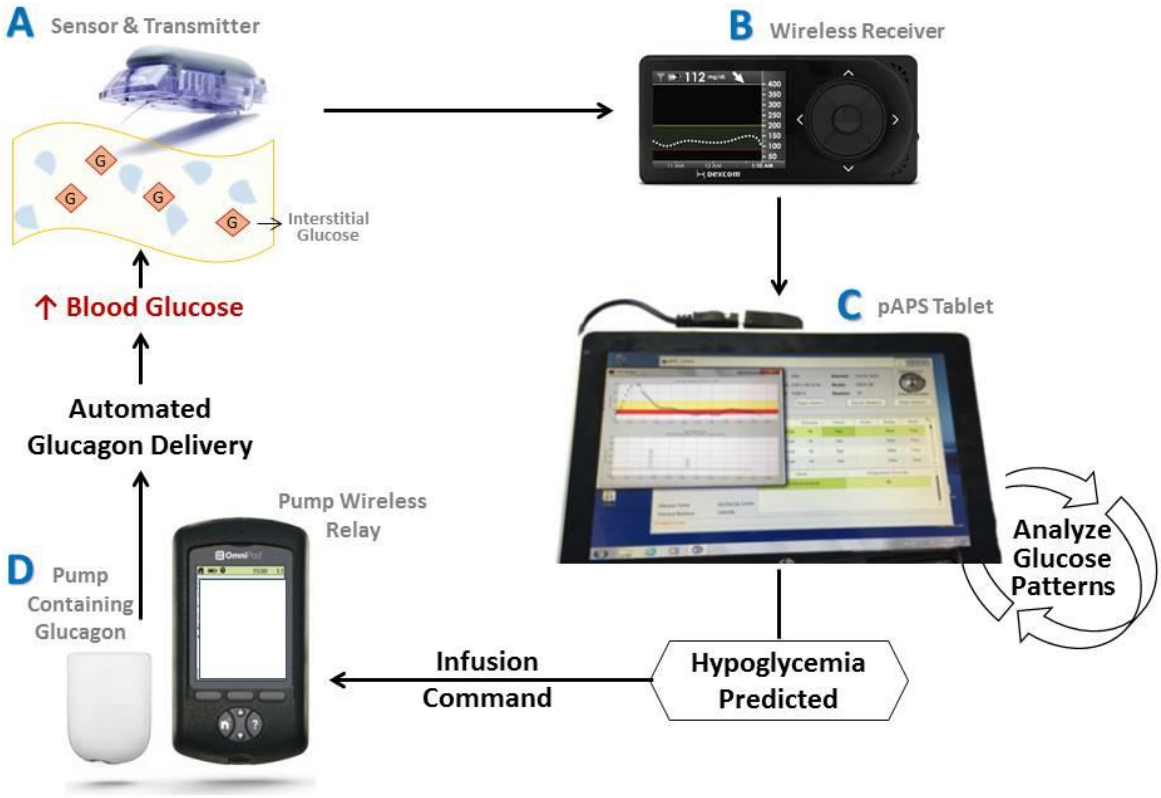

Figure 1

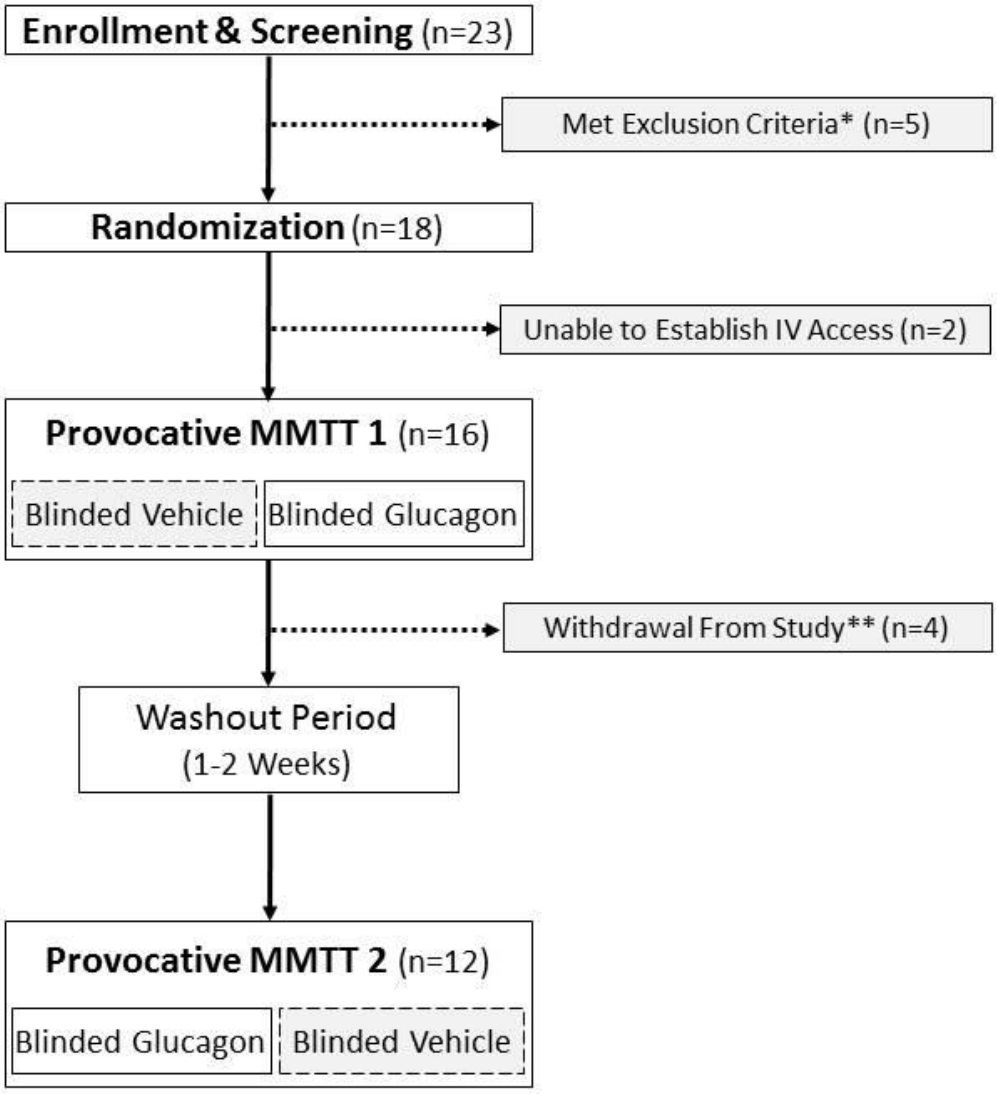

Figure 2

Figure 3

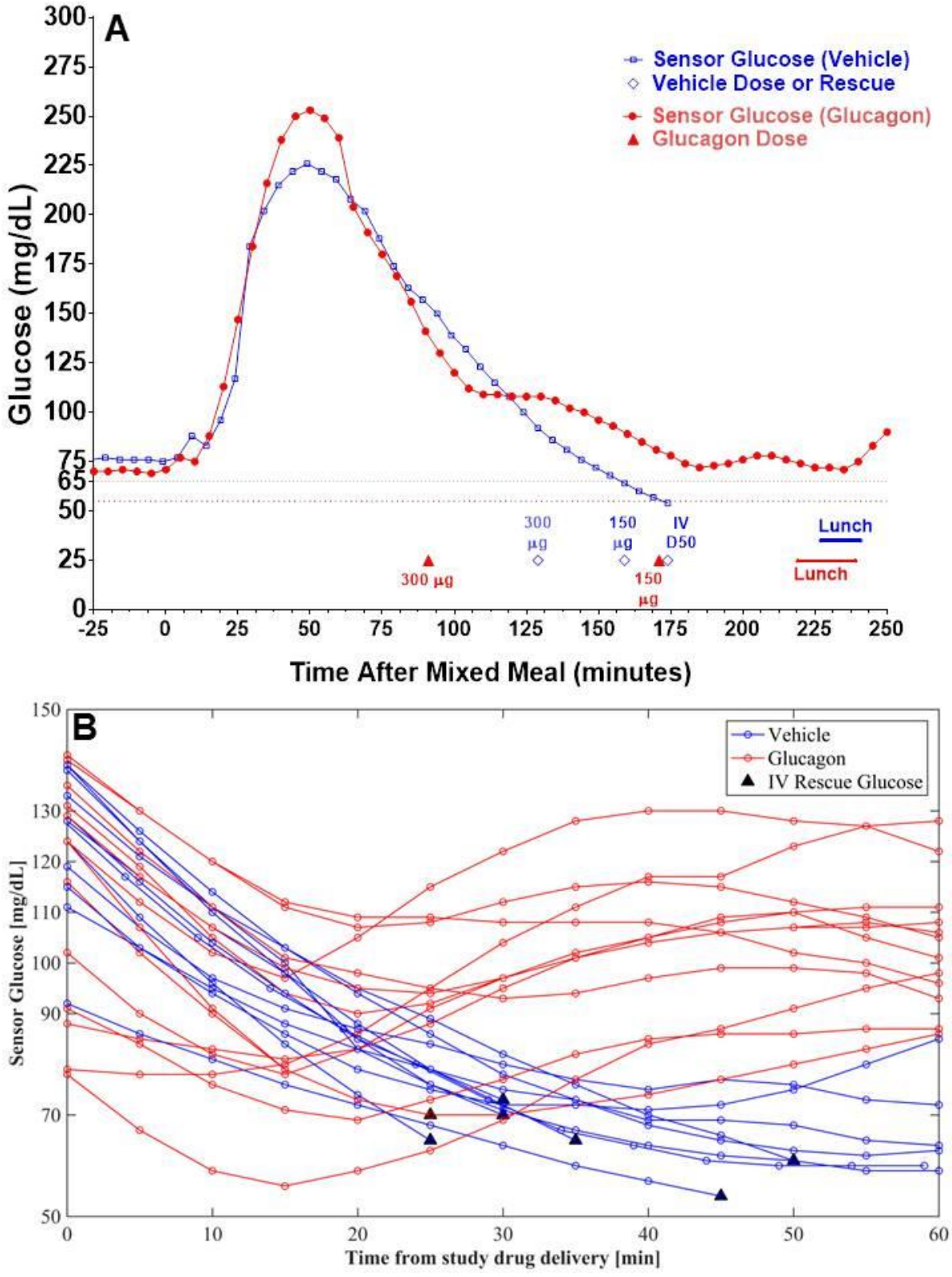

Figure 4