CLOSED-LOOP GLUCAGON ADMINISTRATION FOR THE AUTOMATED TREATMENT OF POST-BARIATRIC HYPOGLYCEMIA

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Version 4, October 5, 2016

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I. Background and Significance

I. a. Historical Background

Bariatric surgery has long been recognized as an effective treatment for obesity associated with complications [1]. It is the most effective method to achieve major, long-term weight loss, and it induces a weight loss of 15-30% of total body weight (30-40 kg), that will sustain for at least 15 years depending on type of operation [2]. In the USA, the number of bariatric operations has increased from approximately 16,000 in the early 1990s to 103,000 in 2003[3].

Bariatric surgery may lead to remission of type 2 diabetes, while improving serious co-morbidities. Among bariatric surgery procedures, Roux-en-Y gastric bypass (RYGB) was shown to account for 41% of all bariatric operations in the USA [4]. However, the long-term risk of RYGB is development of post-bariatric hypoglycemia (PBH) [5]. PBH episodes typically occur 2-3 hours after a meal [6]. The pathophysiology of PBH is not fully understood [7], but postprandial insulin secretion is enhanced, which may be due to increased mass of insulin producing beta-cells or elevated levels of the gut hormones, i.e. glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP). The high GLP-1 secretion after RYGB decreases glucagon levels, which may contribute to the severity of hypoglycemia [8,9].

Fortunately, the frequency of severe hypoglycemia requiring hospitalization after gastric bypass is low (0.2%) [10]. However, mild hypoglycemia with symptoms is much more frequent and is a major burden for the patients [11]. Patients treat mild hypoglycemia with oral intake of carbohydrates. Patients may get trapped in a vicious cycle in which the treatment of hypoglycemia leads to yet another hypoglycemia episode later. These patients may have several episodes per day and unawareness of the hypoglycemia can develop relatively rapidly. Thus, many patients go undiagnosed for extended periods of time, and diagnosis may be delayed until patients lose consciousness or develop seizures, which can result in motor vehicle or other serious accidents.

I. b. The Current Standard of Care

The approach of managing PBH is to avoid eating high-glycemic-index diets and limit portions of any carbohydrates [17]. Unfortunately, post-bariatric hypoglycemia generally responds suboptimally to carbohydrate restriction alone. However, some case reports have reported good results with carbohydrate restriction, with or without the use of pharmacological agents including the α-glucosidase inhibitor acarbose, octreotide, verapamil, and diazoxide [12]. Continuous glucose monitoring can be a useful tool in management and can help ensure timely corrective measures for hypoglycemia prevention [13]. Data evaluating the efficacy of surgery in managing hypoglycemia after gastric bypass are limited to case reports and case series. For instance some patients with severe symptoms have responded well to partial pancreatectomy [14]. In extreme cases, reversal of the bypass may be required [15]. Feeding into the bypassed stomach has been shown to prevent hypoglycemia [16], which suggests the mechanism may be related to lack of signaling from the stomach.

Continuous infusions of glucagon have been successfully used to limit hypoglycemia in patients with congenital hyperinsulinism and in research settings among patients with type 1 diabetes [18]. However, continuous infusions are not well suited for prevention of hypoglycemia in patients with the RYGB. Indeed, continuous infusions may exacerbate post-bariatric hyperglycemic excursions and therefore provoke a stronger endogenous insulin response, thereby worsening late PBH [19]. In contrast, administration of glucagon at the time of impending hypoglycemia could potentially counter the inappropriately elevated insulin concentrations and prevent the post-bariatric hypoglycemia.

I. c. Past Pre-clinical and Clinical Studies

A potentially effective method for achieving consistent blood glucose control in patients with PBH consists of an integrated closed-loop control system, enabled by a continuous glucose monitoring device, pumps, and a control algorithm. This system would actuate a pump to deliver glucagon in response to impending hypoglycemia based on regular glucose measurements by the glucose monitor. Such a system would automate the management of hypoglycemia and result in more consistent glucose regulation.

A device developed by Medtronic and currently available in the US for treatment of type 1 diabetes utilizes a “low glucose suspend” feature on a CGM sensor-augmented pump which stops delivery of insulin when the blood glucose (BG) is below, or is predicted to soon fall below, a threshold [20]. However, this approach is not useful to prevent hypoglycemia caused by high endogenously produced insulin, as in congenital hyperinsulinism [21] and PBH [19].

Since 2008, we have been conducting feasibility studies testing insulin-only and bi-hormonal (insulin and glucagon) configurations of a bionic endocrine pancreas in adult and pediatric subjects with type 1 diabetes at the Massachusetts General Hospital (MGH). Our control strategy uses a bi-hormonal approach by including both insulin and the counter-regulatory hormone glucagon.

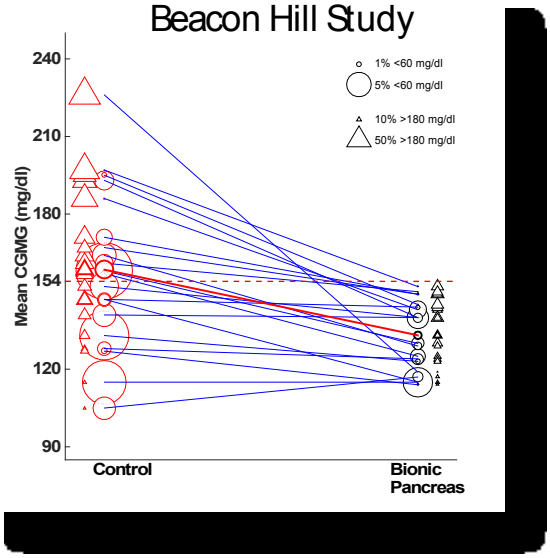
Our first clinical trial, conducted between 2008 and 2009, demonstrated the feasibility of safe and effective bi-hormonal therapy with subcutaneous insulin and glucagon in 27-hour experiments in sedentary adult subjects with type 1 diabetes in the MGH CRC [22,23]. We found that automated subcutaneous microdose glucagon administration by our system was effective at preventing hypoglycemia while maintaining mean plasma glucagon levels near the normal range [10-11]. We also found that our system was able to safely and effectively regulate blood glucose in all of our subjects with essentially no hypoglycemia and without the need for carbohydrate intervention, largely due to the anti-hypoglycemic action of microdose glucagon.

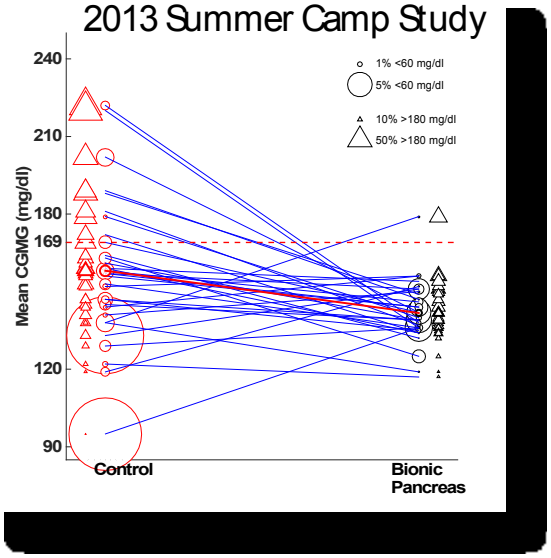
The experiments in our second adult trial and first pediatric trial in subjects with type 1 diabetes, conducted between 2010 and 2011, lasted 51 hours each and included six high-carbohydrate meals and a period of exercise as challenges to glycemic control [24]. We found that automated subcutaneous microdose glucagon administration by our system was effective at preventing hypoglycemia after periods of structured exercise (25-35 minutes at a heart rate of 120-140 beats/minute) and over a control period of 48 continuous hours, longer than any previously reported study. We found no diminution of glucagon efficacy during this control period.

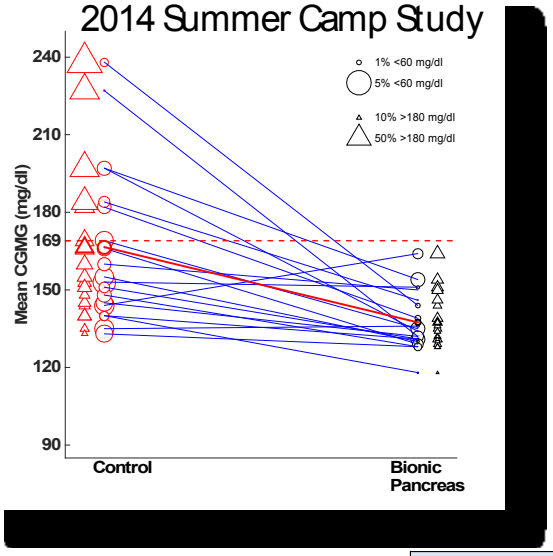
We conducted a third trial testing a control system with an increased ability to adapt online to the insulin needs of individual subjects [24], which demonstrated that the system, initialized only with the subject weight, could adapt to individual insulin and glucagon needs within 18 hours.

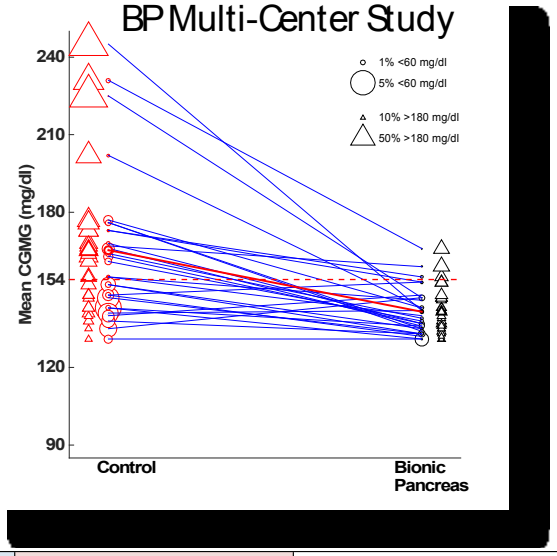
We have now built the first truly mobile platform for testing both bi-hormonal (insulin and glucagon) and uni-hormonal (insulin-only or glucagon-only) bionic endocrine pancreas configurations. It consists of a CGM sensor-transmitter unit, a small custom enclosure housing a smartphone and the CGM receiver, and either one or two infusion pumps. The pump(s) and the smartphone enclosure unit are separable devices that communicate wirelessly and can therefore be conveniently and discretely carried in pockets and pump pouches. In terms of wearability, the system is no more burdensome than the equipment a person on sensor-augmented pump therapy today might routinely carry with them. It is therefore ideally suited for use in outpatient studies. Furthermore, our new platform is highly flexible to allow us to test our system in a variety of configurations (bi-hormonal, glucagon-only, insulin-only).

In November 2012 we obtained FDA approval to conduct our first outpatient study testing our bi-hormonal BP in adults 21 years or older with type 1 diabetes. This study, referred to as the Beacon Hill Study, followed a random-order cross-over design in which 20 adults with type 1 diabetes participated in 5 days on our iPhone-based BP and 5 days of usual care. In the usual care control arm the subjects used conventional insulin pump therapy (and their own CGM if they had one), and they wore a CGM with blinded display and muted alarms. In the BP arm, subjects kept to a three-square-mile geographic area centered around the Beacon Hill neighborhood in Boston. They ate as they chose at local restaurants, and exercised at will with access to two gyms. Analysis was pre-specified to focus on Days 2–5, since glycemic control is more representative of BP performance after most of the adaptation by the BP occurs on Day 1. Results are summarized in the plots and table of Figure 1.









		Bionic Pancreas (BP)			Control			*p*-value (BP versus Control) for:		
Study	**Age** (years)	**Mean CGM glucose level** (mg/dl)	**% of CGM glucose levels <'50'mg/dl** (%)	**70–180'mg/dl** (%)	**Mean CGM glucose level** (mg/dl)	**% of CGM goucose values <'50'mg/dl** (%)	**70–180'mg/dl** (%)	**Mean CGM glucose level**	**% of CGM glucose values <'50'mg/dl**	**70–180'mg/dl**
Beacon Hill (n=20, 5-day experiments)	≥'21	133	1.5	80	159	3.7	59	<'0.001	0.020	<'0.001
2013 Summer Camp (n=32, 5-day experiments)	12–20	142	1.3	76	158	2.2	65	0.004	0.192	<'0.001
2014 Summer Camp (n=19, 5-day experiments)	6–11	137	1.2	81	168	2.8	58	0.004	0.001	<'0.001
BP Multi-Center (n=39, 11-day experiments)	≥'18	141	0.6	78	162	1.9	62	<'0.001	<'0.001	<'0.001

Figure 1. Outpatient results summarizing the distribution of mean CGM glucose levels and hypoglycemia in the bi-hormonal BP and control arms. Mean CGM glucose levels for each subject under usual care (shown as a red circle on the left) is connected with the subject's mean CGM glucose level on the BP (shown as a black circle on the right). For each subject, the circle diameter is proportional to the percentage of CGM glucose values < 60 mg/dl, and the size of the triangle is proportional to the percentage of CGM glucose values > 180 mg/dl. The heavy circles and lines represent the group means. The horizontal red dashed line refers to the glucose level corresponding to the ADA therapy goal for each age group tested, which corresponds to 154 mg/dl (HbA1c <7%) for adults and 169 mg/dl (HbA1c <7.5%) for children. Results are summarized in the table below the plots, where the co-primary outcomes (mean CGM glucose level and percentage of CGM glucose values < 60 mg/dl) for the BP are highlighted in red for each of the four studies.

In April 2013, we obtained FDA approval to conduct our first outpatient study testing our bi-hormonal BP in adolescents 12–20 years old with type 1 diabetes. This study, referred to as the 2013 Summer Camp Study, followed a random-order cross-over design in which 32 adolescents with type 1 diabetes participated in 5 days on our BP and 5 days of supervised camp care in the control arm. In

the control arm the subjects used conventional insulin pump therapy (and their own CGM if they had one), and they wore the bionic pancreas without pumps and with blinded display and muted alarms for remote monitoring. Subjects were monitored remotely according to identical criteria in all arms for proper device functioning and CGM glucose <70 mg/dl lasting more than 15 minutes, which would prompt study staff to call the subject and make sure they were treated. Subjects were fully integrated into normal camp activities without restrictions on diet or exercise. The study used the same iPhone-based BP that was used in our Beacon Hill Study. The mean HbA1c of the entire all 32 subjects at baseline (pre-study) was 8.2%, which corresponds to a mean BG of 189 mg/dl. Results are summarized in the plots and table of Figure 1.

In April 2014 we obtained FDA approval conduct our first outpatient study testing our bi-hormonal BP in pre-adolescents 6–11 years old with T1D. This study, referred to as the 2014 Summer Camp Study, was similar in design to our 2013 Summer Camp Study. Results are summarized in the plots and table of Figure 1.

In April 2014, we obtained FDA approval to conduct our first multi-center study, which was also our first home study, to test our BP in adults 18 years or older with T1D. This study, referred to as the Bionic Pancreas Multi-Center (BPMC) Study, followed a random-order cross-over design in which 39 adults participated in 11 days on our BP and 11 days of usual care. Participants went to work as usual, and lived and slept at home, all without clinical supervision. There were no restrictions placed on diet or exercise. The study included four medical centers (10 subjects per center), which included MGH, the University of Massachusetts Medical School, Stanford University, and the University of North Carolina at Chapel Hill. Results are summarized in the plots and table of Figure 1.

The effectiveness of microdose glucagon in preventing hypoglycemia and the independence of the insulin and glucagon dosing algorithms used in the full-featured bi-hormonal device suggest that closed-loop glucagon delivery is an effective strategy for minimization of hypoglycemia. Closed-loop glucagon delivery could have utility in clinical syndromes of hypoglycemia in which excess insulin is produced endogenously. Unlike the bi-hormonal system, use of automated glucagon delivery poses no additional risk of hypoglycemia beyond the risk that patients with other hypoglycemia syndromes already face on a daily basis. Therefore, the requirements for safety supervision of a study testing the efficacy of automated glucagon delivery for hypoglycemia prevention and mitigation are much lower than for a bi-hormonal bionic pancreas. The development of a mobile bionic pancreas system for bi-hormonal bionic pancreas experiments has also provided the infrastructure for a trial of automated glucagon administration for hypoglycemia prevention. Therefore, we propose to test a closed-loop glucagon delivery system for prevention of hypoglycemia in post-bariatric patients directly in a fully outpatient trial.

I. d. Rationale and Potential Benefits

Although we used automated glucagon administration as a counter-regulatory agent in the context of bi-hormonal closed-loop control, there is no reason why glucagon could not be used as a free-standing system for hypoglycemia prevention in the absence of insulin administration. Notably, the insulin and glucagon controllers interact only minimally, allowing testing a glucagon-only system for the automated prevention of hypoglycemia without the other component. In this context it has the potential to be used in the absence of any insulin therapy.

Closed-loop glucagon delivery has several important advantages for this application. First, unlike dextrose or other fast-acting sugars, exogenous glucagon effectively mimics a physiologic process in which liver glycogen stores are utilized to raise blood glucose. Subcutaneous glucagon administration is potent in raising glucose [4,5] yet does not introduce exogenous glucose. Second, glucagon is absorbed more rapidly (glucagon mean t_{max} 22 min vs. 70 min for insulin lispro) so that the onset of glucagon action is much faster after dosing than the decay of insulin action after delivery is suspended. In fact, the rise in BG after glucagon administration is faster than can be achieved by delivery of glucose orally. Third, closed-loop glucagon delivery can be effective for hypoglycemia syndromes in which the source of the insulin excess causing hypoglycemia is endogenous and there is no exogenous administration of insulin.

The preparations we have made for transitional and outpatient studies with the bi-hormonal bionic pancreas have poised us to immediately move to outpatient studies if the risk profile of the study allows. Transitional studies are required for bi-hormonal fully automated blood glucose control, but a study of automated glucagon delivery for hypoglycemia prevention has a risk profile that is consistent with moving directly to the fully outpatient setting. Since subjects are not administering any insulin at all, there should be no increased risk of hypoglycemia associated with automated glucagon delivery. The one concern in this regard is that subjects might be less risk-averse or less vigilant regarding the risk of hypoglycemia if they believe there is a backup mechanism to prevent hypoglycemia. To mitigate the hazard that subjects may take more risks, we designed a double blind trial in which subjects refill the reservoir in the closed-loop device daily and are randomized to receive either a placebo or an active glucagon solution each day from coded vials that appear identical. On any given day, subjects will not know if they are receiving glucagon or placebo, and therefore should not take extra risks.

Using this design, we conducted a double-blinded, randomized, placebo-controlled crossover study involving 22 adult subjects with type 1 diabetes who used an insulin pump or multiple daily injections and who have reduced hypoglycemia awareness – the Closed-

loop Glucagon trial. Participants administered their own insulin as usual while receiving either glucagon or placebo (randomized daily) from an automated bionic pancreas. The primary outcome was area over the curve and <60 mg/dl (AOC<60), a measure of total hypoglycemia exposure. On glucagon vs. placebo days AOC<60 mg/dl was reduced by 75% (851±748 vs. 3,414±2,242 mg/dl·min, p<0.0001) and there was a 91% reduction in AOC<60 at night (117±204 vs. 1,309±1,476 mg/dl·min, p<0.0001). There were half as many symptomatic hypoglycemia episodes on glucagon vs. placebo days (0.6±0.4 versus 1.2±0.8 incidents per day; p<0.0001), but no difference in mean CGM glucose (153±28 vs. 152±27 mg/dl, p=0.60). Self-reported nausea was not significantly different on glucagon vs. placebo days (1.1±0.6 vs. 0.4±0.7 cm on a 10 cm visual analog scale, p=0.053) and subjects correctly guessed their daily assignment to glucagon or placebo in a daily survey on only 42% of days (less than chance alone). There was no unexpected or severe adverse events on either glucagon or placebo days. Automated glucagon administration effectively reduced hypoglycemia in patients with type 1 diabetes, and was well tolerated. The AOC results and time less than 60 mg/dl results calculated separately for daytime and nighttime are shown in Figure 2.

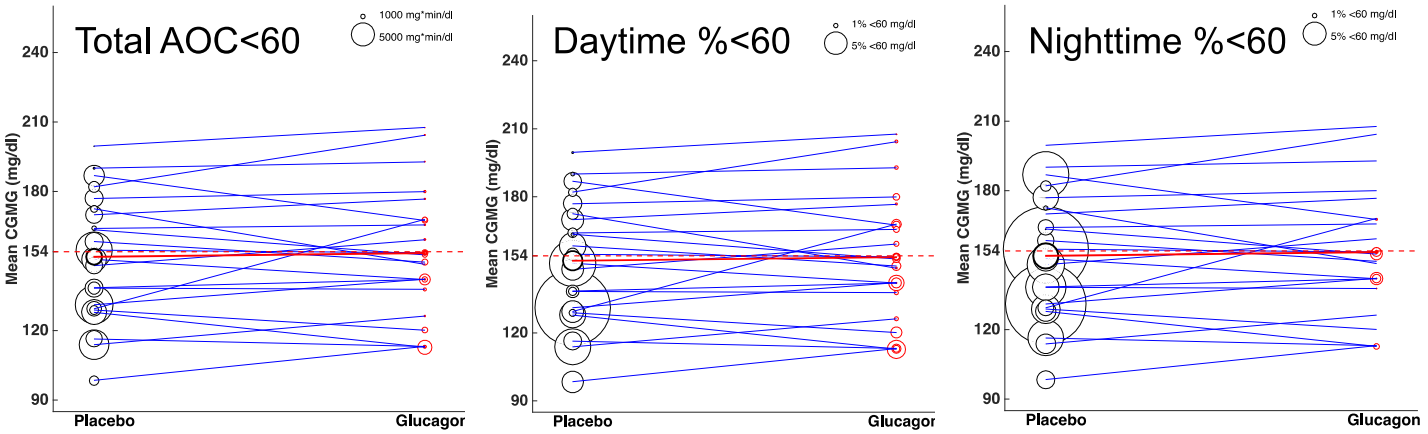


Figure 2. Outpatient results summarizing the distribution of mean CGM glucose levels and hypoglycemia (either AOC<60 or percent time <60 mg/dl) during glucagon and placebo days in the the glucagon-only BP trial. The mean CGM glucose levels for each subject on placebo days (shown as a red circle on the left) is connected with the subject's mean CGM glucose level on glucagon days (shown as a black circle on the right). For each subject, the circle diameter is proportional to either the AOC<60 (left panel) or the percentage of CGM glucose values < 60 mg/dl during the day (middle panel) or night (right panel. The heavy circles and lines represent the group means. The horizontal red dashed line refers to the glucose level corresponding to the ADA therapy goal for each age group tested, which corresponds to 154 mg/dl (HbA1c <7%) for adults.

Based on these results in subjects with type 1 diabetes, we have decided to test the effectiveness of automated glucagon administration to treat PBH. Each subject will serve as their own control by comparing the incidence of hypoglycemia and other adverse events between glucagon to placebo days. During the two-week study period each subject will be treated with glucagon for seven days and placebo for seven days. This multiple crossover design will also have the advantage of allowing paired statistical tests, thereby increasing statistical power to detect differences in both hypoglycemia rates and adverse events.

II. Hypothesis and Specific Aims

We hypothesize that a version of our closed-loop glucose-control system delivering glucagon only can reduce the incidence and severity of hypoglycemia in patients with post-bariatric hypoglycemia (PBH). The specific aim of this study is:

Aim 1: To conduct a randomized, double-blinded, placebo controlled, outpatient feasibilty study testing our automated hypoglycemia prevention and treatment device in 10 adult subjects (≥21 years old) with **post-bariatric hypoglycemia (PBH)**. Subjects will continue to manage any hypoglycemia that occurs according to the current recommendations of their care provider. Subjects will wear a device consisting of a continuous glucose monitor linked to a smartphone running a hypoglycemia prevention algorithm that doses glucagon or placebo from an insulin pump through a subcutaneous infusion set. Each subject will wear the system for two weeks. They will replace the reservoir of the pump daily with a new reservoir they will fill with glucagon or placebo reconstituted from coded vials. The primary outcome will be area over the curve and under 60 mg/dl on glucagon days vs. control days (intention to treat analysis).

III. Subject Selection

III. a. Inclusion Criteria

The inclusion criteria include:

- Age 21 years or older with a gastric bypass for more than 1 year.
- Post-bariatric hypoglycemia with prior episodes of neuroglycopenia, unresponsive to dietary intervention (low glycemic index, controlled carbohydrate portions) and trial of acarbose therapy at the maximally tolerated dose. Other therapies will not exclude a subject as long as the therapy is continued during the study.
- Otherwise healthy (mild chronic disease such as asthma, hypertension, and depression will be allowed if well controlled).
- Self-reported frequency of documented hypoglycemia (BG < 60 mg/dl verified by capillary blood glucose measurements) of at least 2 times per week.

No volunteers will be excluded on the basis of gender or race. We will exclude children from this study to avoid subjecting this vulnerable population to risk prior to validating the approach in adults.

III. b. Exclusion Criteria

- Unable to provide informed consent.
- Unable to comply with study procedures.
- Current participation in another hypoglycemia related clinical trial other than one that is primarily observational in nature.
- Pregnancy (positive urine HCG), breast feeding, plan to become pregnant in the immediate future, or sexually active without use of contraception.
- Use of insulin and/or insulin secretogues as sulfonylurea,metglitides, and glitazones
- History of cystic fibrosis, type 1 diabetes, or recurrent pancreatitis or episode of pancreatitis within the past 1 year
- End stage renal disease on dialysis (hemodialysis or peritoneal dialysis).
- Any known liver or biliary disease including cirrhosis, alcoholic liver disease, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, any form of viral hepatitis.
- Congestive heart failure (established history of CHF, paroxysmal nocturnal dyspnea, or orthopnea).
- Acute illness or exacerbation of chronic illness at the time of the study.
- Known insulinoma or predominantly fasting pattern of hypoglycemia
- Adrenal insufficiency. Congenital hyperinsulinemia presenting with hypoglycemia during infancy
- History of pheochromocytoma. Fractionated metanephrines will be tested in patients with history increasing the risk for a catecholamine secreting tumor:
    - Paroxysms of tachycardia, pallor, or headache.
    - Personal or family history of MEN 2A, MEN 2B, neurofibromatosis, or von Hippel-Lindau disease
    - Episodic or treatment refractory (requiring 4 or more medications to achieve normotension) hypertension
- Untreated or inadequately treated mental illness (indicators would include symptoms such as psychosis, hallucinations, mania, and/or any psychiatric hospitalization in the last year).
- Current alcohol abuse (intake averaging > 3 drinks daily in last 30 days) or substance abuse (any use within the last 6 months of controlled substances without a prescription).
- Unwilling or unable to refrain from drinking more than two drinks in an hour or more than four drinks in a day during the trial
- Electrically powered implants (e.g. cochlear implants, neurostimulators) that might be susceptible to RF interference.
- History of adverse reaction to glucagon (including allergy) besides nausea and vomiting.
- Unwilling or unable to completely avoid acetaminophen during the study period.
- Any factors that, in the opinion of the principal investigator, would interfere with the safe completion of the study procedures.

III. c. Source of Subjects

Volunteers who fit the selection criteria will be considered as candidates for this study. Patients will be recruited from the patient population of co-investigator Mary-Elizabeth Patti, M.D., who directs the Hypoglycemia Clinic at the Joslin Diabetes Center. Advertisements for the study will be posted at MGH, and will be distributed in the weekly broadcast email of research studies seeking volunteers. A letter may be sent to practitioners in the Boston metropolitan as well as selected endocrinologists in central Massachusetts and nearby population centers (i.e. southern New Hampshire, western Connecticut, Rhode Island), informing them of the study and asking them to refer any eligible patients who might be interested. We will post basic information about the trial along with contact information on our website www.bionicpancreas.org and on www.clinicaltrials.gov. We will also contact individuals who have previously inquired about participation in our studies and have asked us to have their contact information kept on file.

IV. Subject Enrollment

It is expected that we will have 10 volunteers with post-bariatric hypoglycemia complete the study (14 days each). We expect that the

experiments can be accomplished over a period of 6-12 months. Up to 20 volunteers with post-bariatric surgery hypoglycemia will be enrolled. The upper bound is based on the expectation that some volunteers will be excluded after the screening visit and the possibility that some experiments may have to be discontinued before completion (due to, for instance, intercurrent illness or subject withdrawal).

IV. a. Enrollment Procedures

Prospective participants may be briefed by a study staff member by phone regarding the study procedure and the inclusion and exclusion criteria. Potential volunteers may be sent an informed consent document to review by email or post.

IV. b. Consent Procedures

Potential volunteers will talk with a study physician who will explain the study, answer questions, and administer informed consent. In the event that a volunteer is a patient of one of the study MDs, another staff MD will be available to answer questions and administer consent.

The study physician or nurse will also answer any questions that the volunteer may have during their participation. They will share any new information in a timely manner that may be relevant to the volunteer's willingness to continue participating in the trial. The volunteers may choose to discontinue their participation at any time.

V. Study Procedures

V. a. Screening data

- Age
- Sex
- Race and ethnicity
- Date of last menstrual period in female volunteers
- Urine HCG for female volunteers
- Date of bariatric surgery and date of diagnosis with hypoglycemia
- Medical, surgical, and social history, allergies, and review of systems relevant to inclusion and exclusion criteria
- Medications (prescription and non-prescription) and date of last change in medication regimen
- Anti-hypoglycemic regimen
- Hypoglycemia history (number of events per week in the preceding months per patient report, timing of events (fasting or post-bariatric), medical records, meter downloads, and CGM data, as available
- Usage of CGM, if any (type of CGM, days per month worn, usage of data, whether insulin is dosed based on CGM alone, alarm settings)
- Average number of BG tests daily in the last 30 days (from point of care blood glucose meter download)
- Height and weight
- Blood pressure
- Hemoglobin A1c
- Fractionated metanephrines (in patients with history increasing the risk for a catecholamine secreting tumor)

V. b. Drugs

The study involves subcutaneous administration of *recombinant human glucagon* (Lilly), which is commercially available by prescription and is indicated at a dose of 1000 mcg/ dose for treatment of severe hypoglycemia patients with type 1diabetes. Further, glucagon is used by emergency medical services for treatment of hypoglycemia.

The control system can administer bolus doses of glucagon up to every five minutes. A single bolus of glucagon will not exceed 80 µg [80 µl]. The insulin pumps can administer as little as 0.5 µl (0.5 µg of 1 mg/ml glucagon) in single programmable bolus dose.

The total daily dose of glucagon will be less than 2000 µg. The recommended dose of glucagon for a patient suffering from severe hypoglycemia is 1000 µg as a single injection. The mean daily glucagon dose in our previous outpatient studies has been 0.48-0.72 mg/day. We will instruct the subject to fill the reservoir with three vials of glucagon or placebo (3 ml minus some loss to prime the tubing = final volume > 2.5 ml or 2500 µg) each time.

V. c. Devices

Infusion sets. Volunteers will wear an FDA approved commercially available infusion set in the subcutaneous tissue for glucagon infusion. If the infusion set falls off or fails, it will be replaced with a new one by study staff or the subject. The infusion set will be changed every day.

Continuous glucose monitors. One transcutaneous glucose sensor for the Dexcom G4 will be inserted in the subcutaneous tissue and will provide input to the controller. The sensor is powered by the battery within the transmitter that clips to the sensor and the whole assembly is held to the skin with an adhesive patch and communicates wirelessly with the G4 receiver. If the G4 sensor fails for any reason during the experiment it will be replaced promptly. Subjects will be trained to do this and will be provided with a spare sensor.

Bionic Pancreas Control Module. The control module consists of a stock iPhone 4S and a Dexcom G4 AP receiver connected with a custom hardware interface and placed back-to-back in a custom enclosure. The G4 receiver converts the raw wireless signal from the transmitter into an estimated BG signal that is sent via a hardwired connection to the iPhone. The display of the G4 receiver will be blinded for this study so that volunteers will not have access to the CGM data. They will only be able to see whether the device is streaming BG information to the iPhone and they will be able to calibrate the device.

The iPhone runs iOS 6 in "Guided Access" mode, where the only app accessible to the subject is the Beta Bionics app, which runs the control algorithm. Access to other functions on the iPhone (namely, the home screen and the Settings app) is password protected and will be accessible to study staff personnel, but not to the subject. The control algorithm app has a graphical user interface (GUI) that is able to display (in bi-hormonal mode) the current CGM glucose, a graphical history of the CGM glucose, and doses of insulin and glucagon delivered by the control algorithm as well as diagnostic information such as the status of the CGM and the connection with the pumps. However, all aspects of this display except the CGM and pump diagnostic information will be disabled for this study so that the CGM data is not available to the volunteers.

The blinding of the display is necessary because we do not wish data from the CGM to modify the behavior of the subjects. On one hand it could lead them to intervene with carbohydrates based on CGM data to prevent or treat hypoglycemia, thereby reducing our ability to evaluate the effectiveness of glucagon. On the other hand, volunteers who are not familiar with using CGM data might make inappropriate and even dangerous decisions based on the CGM data

The iPhone communicates wirelessly with the Tandem t:slim pump to deliver glucagon. The iPhone also transmits all operational data every five minutes to the cloud over the Verizon 3G wireless network, so all aspects of device operation can be monitored remotely if required. Remote monitoring is only possible when the subject has Verizon network coverage and data can be transmitted to the cloud service. There may be times when a subject enters an area where Verizon coverage is not available. We may provide subjects with WiFi boosters for their homes or WiFi hot spots to carry with them in order to improve data throughput. We may also encourage subjects to connect to public but secure wireless networks if they are having trouble connecting to cellular service.

The connection is secure and password protected, and will be set up so that only viewing of the screen is possible - no input or changes to the controller can be made remotely. For privacy reasons, no audio or video connection will be made to the iPhone.

Tandem t:slim Insulin Pump. This pump is an FDA approved insulin pump with reservoirs capable of holding up to 3 ml of a glucagon solution. The pumps have a mechanical dosing resolution of 1/120 (0.00833) unit and can deliver liquids at a maximal rate of ~ 33 µl per minute (2 ml per hour). They can be controlled wirelessly via Bluetooth Low Energy protocol by the iPhone 4S.

Nova Biomedical StatStrip Xpress Glucose Meter. The StatStrip Xpress glucose meter is an FDA approved glucose meter that is commercially available. Blood glucose measurements for CGM calibration will be obtained via fingerstick with the StatStrip Xpress throughout the study.

Subjects will be provided with a meter and enough strips to obtain blood glucose measurements via fingerstick with the StatStrip Xpress meter to calibrate the CGM device in the morning before breakfast and in the evening before dinner each day. In addition, we will ask subjects to perform all routine BG measurements using the StatStrip Xpress. The meter will be downloaded at the end of the study.

Multiple use equipment will be cleaned between subjects using a sanitizing wipe such as Super Sani Cloth or a 10% bleach solution or equivalent.

V. d. Experimental Procedures and Data Collection

General policies

- Subjects may not take acetaminophen because of potential interference with CGM sensing.

- Subjects will not drink more than two alcoholic drinks in one hour or more than four drinks in one day. This policy is in place because excessive alcohol consumption may dull the sensorium, reduce sensitivity to symptoms of hypoglycemia, and hinder appropriate decision-making. It may also reduce the effectiveness of glucagon in preventing or treating hypoglycemia.
- Subjects are not allowed to tamper with the bionic pancreas device in any way, including changing any settings. They will maintain the bionic pancreas in guided access mode unless instructed otherwise by study staff.
- During the experiment the bionic pancreas must be worn by the subject or kept nearby (such as when sleeping) at all times to ensure good radio-frequency signal reception.
- Subjects must keep their bionic pancreas charged, which will require charging when sleeping and likely during one other period during the day.
- The bionic pancreas is not water resistant and therefore must be removed for showering and swimming. Subjects are urged to take appropriate precautions when they are disconnected from the bionic pancreas, including frequent BG checks and having carbohydrates readily available.
- We will request that subjects not remove the bionic pancreas for more than 1 hour at a time (e.g. for swimming or bathing) and or for more than 2 hours total in any 24 hour period.
- Study subjects must keep a StatStrip Xpress glucometer easily accessible at all times in case a calibration or a BG test is needed. They will use the StatStrip Xpress glucometer for all BG tests. Study subjects will keep fast-acting carbohydrates easily accessible for treatment of hypoglycemia as needed.
- Any medical advice needed by the subjects during their participation which is not directly related to the experiment, should be obtained in the usual manner with their primary care physician or endocrinologist.
- If subject develops an illness during the experiment, they can seek medical care as they usually would. As long as the subject is not hospitalized, the study may continue. If the subject is unable to eat for a period exceeding one day, they must notify study staff so that the medical staff can assess the safety of continuing in the study.
- We will ask that other than the BG measurements for calibrations, subjects continue whatever BG monitoring pattern is typical for them, including checking a blood glucose with symptoms of hypoglycemia. Subjects will be asked to enter all fingerstick BG measurements into the Bionic Pancreas.
- Subjects who normally wear a CGM are encouraged to do so during the study.
- Subjects may participate in any activities they wish, as long as they abide by the policies above. There are no restrictions on any kind of diet or exercise, although subjects should not modify their dietary and exercise habits based on their suspicion of whether they are on a placebo or glucagon day. This should not be a problem if blinding is effective, but if blinding is not effective because of glucagon action, we will ask that they not "take advantage" of suspected glucagon administration to be less careful with food intake or BG monitoring.
- Subjects may choose to withdraw from the study at any time. If they withdraw from the study, they should contact a provider immediately.

Screening and Testing Visit

- All volunteers will have a screening visit to confirm eligibility.
- The volunteer will be interviewed and the case report form will be completed by a study nurse or study physician to establish whether the volunteer is eligible to continue with the screening.
- A urine pregnancy test will be performed in female volunteers pre-menopause.
- A blood sample will be drawn for hemoglobin A1c testing.
- A study physician will review the case report form to determine volunteer eligibility.

Training Visit:

- A mandatory training visit will take place within approximately one week of the scheduled start of the study. Study staff will verify that the subjects have understood the material and are competent to participate safely in the study.
- Volunteers will be trained on study procedures including the use of the StatStrip Xpress BG meter, bionic pancreas device, CGM calibrations, loading of pump reservoirs, priming of tubing, insertion of infusion sets and CGM sensors, and basic troubleshooting.
- Volunteers will be trained in reconstituting the study drug or placebo (including a protocol for gentle mixing of the vials after the addition of the diluent).

Closed-loop Initiation Visit (Day 1)

- On arrival to the Diabetes Research Center, a Dexcom G4 CGM sensor will be placed by the subject and study staff will confirm they are doing it properly.
- The body weight of the subject will be documented.
- The date of the last menstrual period will be documented, along with usual cycle length, for female subjects.
- Female volunteers pre-menopause will have urine tested for HCG. If the test is positive, the volunteer will be informed of the result and the visit will be ended.

- The CGM and bionic pancreas displays will be blinded to the volunteers and the high and low glucose alarms and rate of change alarms will be turned off. This will ensure that volunteers will not use this data to modify their BG control strategy.
- The volunteers will be provided with a one week supply of coded vials (21 vials, three vials with identical contents per day to provide 3 ml of glucagon or placebo) with identical opaque covering with a space for documenting the date and time of reconstitution. The 21 vials for each volunteer will be numbered 1A, 1B, and 1C through 7A,7B, and 7C. On each study day A, B, and C vials for the day number will be reconstituted and loaded in to the pump. The order of glucagon and placebo days will be randomized in blocks of two. This means that there can be no more than two glucagon or placebo days in a row.
- Study staff will provide other supplies (such as the bionic pancreas control unit and pump, extra G4 sensors, StatStrip Xpress glucometer and strips, infusion sets), and go over the study procedures again. Volunteers will be asked to report reservoir changes, thoughts about reservoir contents, symptoms of hypoglycemia, carbohydrate interventions for hypoglycemia, exercise, any adverse events, including any nausea or vomiting, number and timing of any unscheduled calibrations, and other information through a daily email survey.
- Volunteers will be instructed to keep all coded vials and document on each used vial the day and time the vial was loaded into the bionic pancreas. This will allow us to determine if there were any mistakes made in the order of vial use. This will also deter and detect any attempts to unblind the study by opening the vial or removing their opaque coverings.
- They will load the pump with coded vials, prime the tubing, and insert an infusion set under observation by study staff. The pump will be connected prior to calibration of the CGM, but will not be infusing until the closed loop is started.
- Approximately two hours after placement of the sensor, the volunteers will perform the first Dexcom G4 calibration under observation by study staff.
- The staff will start the bionic pancreas as close as possible to a minute divisible by 5 minutes (i.e. on a 5-minute mark).

Week 1 (Days 1-7)

- Over the next week, volunteers will manage their BG according to their usual practice. They will perform BG measurements with the StatStrip Xpress meter at least two times daily for calibrations. We will ask that other than the BG measurements for calibrations, they continue whatever BG monitoring pattern is typical for them, but to enter any BG values into the bionic pancreas.
- Volunteers will calibrate the Dexcom G4 before breakfast and dinner each day, approximately 12 hours apart. Calibrations will only be performed with the StatStrip Xpress meter. Volunteers will load the t:slim pump with the next numerical coded vial, prime the tubing, and insert a new infusion set each day, 24 hours after the last pump refill.
- Volunteers will continue to complete a survey daily by email, noting if they believe that the vials loaded contained glucagon or placebo (this information will be used later to assess effectiveness of blinding).
- The volunteers will be reminded to complete the survey every day by email.

Mid-study Visit (Day 7)

- After the end of the first week volunteers will return to the Diabetes Research Center to drop off all used coded vials for the first week, pick up a second week of coded vials (8A, 8B, and 8C through 14A, 14B, and 14C), pick up any additional needed supplies (such as StatStrip Xpress test strips). The bionic pancreas data and the StatStrip Xpress meter will be downloaded.
- Study staff will remind subjects to replace their Dexcom G4 sensor today, and assist with the insertion as needed.

Week 2 (Day 8-14)

- This week will be the same as week one.

Final Visit

- After the end of the second week volunteers will return to the Diabetes Research Center to drop off all used coded vials, and answer a brief series of questions.
- The sensor will be removed and the volunteers will return all of the study materials. The bionic pancreas data and the StatStrip Xpress meter will be downloaded.
- The body weight of the subject will be documented.
- The date of the last menstrual period will be documented, along with usual cycle length, for female subjects.

If the Dexcom G4 sensor stops working or comes off, it will be replaced promptly. The subjects will be trained to do this and will be provided with a spare sensor.

V. e. Quality Assurance for Blood Glucose Values

The StatStrip Xpress will undergo quality control testing prior to use. The QC testing will be done by study staff prior to the first week visit and at the second week study visit. Volunteers will calibrate the Dexcom G4 CGM twice a day using the StatStrip Xpress, which is a highly accurate meter with a mean error less than half that of other consumer BG meters.

V. f. Quality Assurance for Study Drug Administration

The used vial will be returned to the study staff who will confirm that they have not been unblinded and that the dates for reconstitution marked on the vials match the time when those vials should have been reconstituted.

V. g. Response to Hypoglycemia

- Volunteers will be asked to investigate symptoms of hypoglycemia with a StatStrip Xpress glucose measurement
- Volunteers will be instructed to treat hypoglycemia according to their usual practice throughout the study.
- If the Dexcom G4 CGM overestimates BG then glucagon or placebo may not be administered even though the BG is actually trending towards hypoglycemia or is actually in the hypoglycemic range. If the CGM values were not blinded, then the error of the CGM might be noted and corrected through calibration. In this study, this will not be possible. This is a limitation that is inherent in the design of the study, which blinds the CGM to the volunteers. However, due to the blinded nature of the study the volunteers will not be counting on glucagon to prevent hypoglycemia, so they will not be at increased risk of hypoglycemia compared to their risk when not participating in the trial.
- If a subject experiences a seizure or unconsciousness associated with hypoglycemia, his or her participation in the study will be discontinued.

V. h. Response to Hyperglycemia

- Volunteers will be asked to respond to hyperglycemia according to their usual practice throughout the study.
- A potential risk of the study is that the Dexcom G4 CGM could be reading below the true BG. In this circumstance the bionic pancreas could administer glucagon unnecessarily. In the case of a large under-reading of BG, this could lead to hyperglycemia. If the volunteer believes that their BG is inappropriately high for the circumstances they will be instructed to perform a calibration using a StatStrip Xpress BG value. If calibrating the CGM does not resolve the issue, subjects will be instructed to replace the CGM sensor.
- Subject may call study staff with any concerns, and staff may look at actions of the bionic pancreas remotely in order to decide on the correct advice to the subject. If the CGM is reading much lower than the subject's BG, then a calibration will be advised.

V. i. Response to Nausea/Vomiting and other medical needs

- Minimal nausea was noted in clinical trials of the closed-loop system to date. In a previous blinded study with similar design, there was no difference in nausea between glucagon and placebo day. However, nausea is a potential side effect of glucagon.
- If a subject has nausea or vomiting, this could be due to excessive glucagon dosing related to the CGM reading low. Therefore, subjects will be encouraged to calibrate the sensor if nausea persists more than 30 minutes.
- If the subject develops vomiting they will be instructed to contact study staff who will be able to monitor the dosing behavior of the bionic pancreas remotely. Based on the data available to the study staff, who will be able to see the amount of material dosed although not whether it is glucagon or placebo, the subject may be instructed to disconnect the bionic pancreas infusion set to avoid additional glucagon or placebo delivery while troubleshooting is going on. They may be instructed to calibrate the bionic pancreas if the CGM is reading significantly below their BG. Regardless of the intervention, they will be instructed to monitor their BG closely and to treat any hypoglycemia or hyperglycemia according to their usual practice as they should throughout the study.
- If nausea and/or vomiting persists for more than three hours after infusion set disconnection or after the last dose of glucagon (after which time no significant glucagon should be present in the bloodstream, making the symptoms unlikely to be related to glucagon) then the volunteer will be advised to use sick-day management strategies and possibly to call their physician's office. The subject will be given the option of reconnecting and continuing with the study once the acute illness resolves.
- If the nausea/or vomiting resolves after disconnection of the bionic pancreas, the subject will be given the option of reconnecting and continuing with the study. If they chose to do so and they experience a second episode of nausea and/or vomiting that also persists after appropriate calibration of the system and then resolves after disconnection, then their participation in the trial will be discontinued.
- If the subject is unable to eat for a period exceeding one day, they must notify study staff so that the medical staff can assess the safety of continuing in the study. If the anorexia is thought to be due to an unrelated illness rather than use of the device, their participation may be suspended until they are able to eat again. However, if their anorexia resolves after suspension of device usage and recurs with resumption of device usage, their participation in the study will be discontinued.
- If the volunteer experiences any non-emergent medical concerns outside the scope of the study, he or she will be asked to call their PCP. If the volunteer experiences urgent or emergent medical concerns the volunteer will be advised to call 911.

V. j. Monitoring of Closed Loop Device Performance

An engineer will be readily available by phone for consultation at all times during the course of each experiment. They will have the capability of viewing the controller (iPhone) display and diagnostic information remotely during the experiment, in order to monitor and assist in any needed troubleshooting. The connection will be secure and password protected, and will be set up so that only viewing of the screen is possible - no input or changes to the controller can be made remotely. For privacy reasons, no audio or video connection will be made to the iPhone.

V. k. Supervision by Study Staff

A study physician will be available by telephone and/or page 24 hours a day during the course of all experiments. The clinicians will also have the capability of viewing the controller display and evaluating diagnostic information remotely during the experiment to facilitate implementation of the protocols for dealing with hyperglycemia and nausea or vomiting. Study staff will perform 4 checks per day at regularly scheduled intervals of the controller display to identify device connectivity issues and may reach out to the subjects to fix the problem if it persists.

V. l. Modifications to the Control Algorithm

Certain parameters of the control algorithm may be adjusted between experiments, as approved in the Investigational Device Exception, but no parameters may be adjusted during an experiment. A cohort of 10 experiments will be completed with an algorithm that is locked so that all experiments are comparable.

V. m. Stopping Rules

- If a subject experiences a seizure or unconsciousness associated with hypoglycemia, his or her participation in the study will be discontinued.
- If a subject experiences recurrent nausea and vomiting that does not resolve with calibration of the system but does resolve with disconnection of the system (and is therefore thought to be due to appropriate glucagon dosing), his or her participation in the study will be discontinued.
- If the subject is unable to eat for a period exceeding one day, they must notify study staff so that the medical staff can assess the safety of continuing in the study. If the anorexia is thought to be due to an unrelated illness rather than use of the device, their participation may be suspended until they are able to eat again. However, if their anorexia resolves after suspension of device usage and recurs with resumption of device usage, their participation in the study will be discontinued.
- If the participation of five subjects is discontinued according to these criteria then the trial will be stopped and may only be restarted if the DSMB determines that its continuation does not pose an unreasonable risk to subjects. This decision will be based on a review of the circumstances of each discontinuation using unblinded data on glucagon vs. placebo administration to determine if they were, in fact, due to the use of the experimental device. In the case of recurrent nausea and vomiting, the decision will be largely based on whether the subjects were randomized to delivery of placebo on the days when the episodes occurred.

VI. Biostatistical Analysis

VI. a. Data Collected

At the time of enrollment:

- Age
- Sex
- Race and ethnicity
- Urine HCG for female volunteers pre-menopause
- Date of last menstrual period in female volunteers
- Date of bariatric surgery and date of diagnosis with hypoglycemia (post-bariatric surgery subjects)
- Medical, surgical, and social history, allergies, and review of systems relevant to inclusion and exclusion criteria
- Anti-hypoglycemic regimen
- Medications (prescription and non-prescription) and date of last change in medication regimen
- Usage of CGM, if any (type of CGM, days per month worn, usage of data, whether insulin is dosed based on CGM alone, alarm settings)
- Hypoglycemia diagnostic workup and current treatment regimen, including medical nutrition therapy and medications
- Average number of BG tests daily in the last 30 days (from meter download or other volunteer documentation if available – if more than one meter is used all will be downloaded)

- Hypoglycemia history (number of events per week in the preceding months per patient report, fasting or post-bariatric timing of episodes, degree of unawareness, history of neuroglycopenia, use of emergency glucagon, medical records, meter downloads, and CGM data, as available
- Height and weight
- Blood Pressure
- Hemoglobin A1c
- Fractionated metanephrines (in patients with history increasing the risk for a catecholamine secreting tumor)

During the study period:

- CGMG every five minutes from the Dexcom G4 CGM
- StatStrip Xpress capillary BG values from meter download
- Timing of exercise (from daily email survey)
- Number of symptomatic hypoglycemic events (from daily email survey), number of neuroglycopenic events noted by others, number requiring assistance of others
- Number of carbohydrate interventions for hypoglycemia (from daily email survey)
- Number of BG checks per day from meter download
- Glucagon doses administered by the control system
- Bionic pancreas downtime – number, timing, and duration of periods offline, reasons for being offline (CGM sensor loss, system crash, communication problem between CGM and bionic pancreas, communication problem between bionic pancreas and pumps, pump malfunction, tubing occlusion, infusion set failure/pulled out)
- Log of calls for hyperglycemia, hypoglycemia and device troubleshooting, and actions taken
- Adverse events noted by the volunteer specifically including any nausea, any local reactions at the glucagon infusion set or rash, any episodes of unexplained hyperglycemia, or any other symptoms noted by the volunteers.
- Date of last menstrual period and usual cycle length in pre-menopausal female volunteers

VI. b. Study Endpoints

Primary endpoint:

- CGM glucose area over the curve and less than 60 mg/dl (intention to treat analysis)

Secondary endpoints – CGM (all intention to treat analysis):

- Mean CGMG
- Time with CGM glucose<60 mg/dl
- Area over the curve and less than 60 mg/dl overnight (11:00 PM – 7:00 AM)
- Area over the curve and less than 60 mg/dl during the daytime (7:00 AM – 11:00 PM)
- Time with CGM glucose less than 60 mg/dl overnight (11:00 PM – 7:00 AM)
- Time with CGM glucose less than 60 mg/dl during the daytime (7:00 AM – 11:00 PM)
- Fraction of time spent within each of the following glucose ranges:
    - 70-120 mg/dl
    - 70-180 mg/dl
    - >180 mg/dl
    - >250 mg/dl
- Mean absolute relative deviation (MARD) vs. all StatStrip Xpress BG measurements
- Number of carbohydrate interventions for hypoglycemia from daily email survey
- Total number of grams of carbohydrate taken for hypoglycemia from daily email survey
- Total glucagon dosing (mcg/kg/24 hours)
- Number of symptomatic hypoglycemias from daily email survey
- Fraction of days on which volunteers correctly stated which study drug (glucagon or placebo) they had received during the preceding 24 hours (could be greater than chance even with effective vial blinding based on amount of hypoglycemia or adverse events).
- Number and severity of episodes of nausea from daily email survey

Other/exploratory endpoints – CGM (all intention to treat analysis):

- Additional accuracy measures using all StatStrip Xperss measurements Statistics will include:
    - Mean absolute relative difference (MARD) between CGM and BG in hypoglycemic (< 70 mg/dl), normoglycemic (70-120 mg/dl), post-prandial target (70-180 mg/dl), hyperglycemic (> 180 mg/dl and > 250 mg/dl) ranges.
    - Reliability index calculated as percent of possible values actually recorded by CGM.
- Number of unscheduled infusion set replacements
- Area over the curve and less than 50 mg/dl

- Area over the curve and less than 70 mg/dl
- Time with CGM glucose<50 mg/dl
- Time with CGM glucose<70 mg/dl
- Other adverse events from patient logs, specifically including any local reactions at the glucagon infusion set or rash, any episodes of unexplained hyperglycemia, or any other symptoms noted by the volunteers.

Other/exloratory endpoints – BG (all intention to treat analysis):

- Number of hypoglycemic events as determined from StatStrip Xpress measurements (episodes separated by < 15 minutes are considered the same episode):
    - < 70 mg/dl
    - < 60 mg/dl
    - < 50 mg/dl
- Mean number of daily BG measurements

Other endpoints– Non-glycemic (all intention to treat analysis):

- Timing and duration of exercise from daily email survey
- Exercise exposure (duration X intensity)
- Relative risk of hypoglycemia in the nights following bouts of exercise vs. nights without exercise in the preceding day.
- Correlation between exercise exposure and risk of hypoglycemia
- Amount of alcohol ingested by subjects each day and daily average intake per subject
- Relative risk of hypoglycemia on nights following days with alcohol intake vs. nights following days without alcohol intake
- Correlation between mean alcohol intake and risk of hypoglycemia per subject
- Fraction of days that CGM was used by participants as part of their usual care
- Fraction of time bionic pancreas disconnected by the subject for bathing or swimming (self-report on daily questionnaire)
- Number of unscheduled CGM sensor changes
- Time without CGM monitoring data
- List of technical faults associated with the bionic pancreas including cause and resolution

Other endpoints – Monitoring for Adverse Events (all intention to treat analysis):

- List of technical faults associated with the bionic pancreas including cause and resolution
- Number of episodes of nausea/vomiting that resulted in a calibration
- Patient calls from volunteer to study staff for hyperglycemia
- Number of calls for hyperglycemia that resulted in a calibration
- Number of calls for hyperglycemia that resulted in a calibration and were associated with CGM underestimating BG so that the error could have resulted in more glucagon dosing and fraction on glucagon vs. placebo days
- Number of episodes of nausea/vomiting that resulted in a calibration
- Patient calls from volunteer to study staff for nausea/vomiting
- Number of episodes of nausea/vomiting that resulted in a calibration and were associated with CGM underestimating BG so that the error could have resulted in more glucagon dosing and fraction on glucagon vs. placebo days
- Episodes of nausea and nausea index (sum of number of episodes times severity from VAS) on days 1-14
- Change in body weight from day 1 to day 14 of each study arm
- Any skin rash, either local to infusion sites, or more generalized with subject reported severity and timing
- Episodes of reported diarrhea with subject reported severity and timing

We will calculate means, median, percentages, standard deviations, standard errors, inter-quartile ranges, and 95% confidence intervals in descriptive analyses. We will use the Shapiro-Wilk test to determine if differences between glucagon and placebo days are normally distributed. For normally distributed data we will use the paired Student's t-test for comparison of means. For non-normally distributed data we will use the Wilcoxon Signed Rank test for comparison of medians. In a secondary analysis we will look for any period effect and any interaction between treatment and period, although no such interaction is predicted and there is probably insufficient power to identify a small interaction. We will use multivariate regression models with repeated measurements to compare means and percentages while adjusting for patient demographics characteristics (age, gender, body mass, insulin pump vs. MDI, total daily insulin dose)

VI. c. Power Analysis

The sample size of 10 subjects is meant to provide preliminary data from a feasibility trial and will provide data that will be used to power a definitive trial.

VI. d. Criteria for Success of the Study

We will consider the study to be a success if there is a statistically significant difference in the primary outcome, CGM glucose area over the curve and less than 60 mg/dl, between the glucagon and placebo days.

VII. Risks and Discomforts

Volunteers may experience mild discomfort associated with the insertion of the infusion sets and Dexcom CGM sensor into the SC tissue. Once the infusion sets and sensors are in place, there should be no significant discomfort. The risk for developing inflammation in the SC tissue at the insertion sites is expected to be extremely low. There have been no instances of inflammation or infection in our experiments using similar sensors and infusion sets in our previous studies including over 100 volunteers.

A potential risk of the study is that the Dexcom G4 CGM could be reading below the true BG. In this circumstance the bionic pancreas could administer glucagon unnecessarily. In the case of a large under-reading of BG this could lead to hyperglycemia. The mechanism in the protocol to check the accuracy of the CGM if hyperglycemia occurs should mitigate this risk. Alternatively, rapid post-bariatric drops in glucose values could result in CGM readings above the true BG. In this situation, the bionic pancreas could fail to administer glucagon, even though dosing would be indicated. If hypoglycemia develops, subjects would be still be able to use their usual hypoglycemia treatment approach, as guided by symptoms or capillary glucose monitoring.

There is a potential risk of nausea or vomiting in volunteers due to the administration of exogenous glucagon. These experiments, however, involve *small and infrequent* SC glucagon doses. The largest single dose to be administered in our study is 80 mcg. In experiments testing our bi-hormonal bionic pancreas the mean total daily dose of glucagon is 0.5 mg and very rarely greater than 1.5 mg. The recommended dose of glucagon for treatment of an adult diabetic with hypoglycemia is 1 mg, given as a single subcutaneous injection. In practice, a smaller dose of 0.5 mg is sometimes used initially to reduce the risk of nausea and vomiting. This risk could be increased if the Dexcom G4 CGM is reading below the true BG and therefore administering more glucagon. The mechanism in the protocol to check the accuracy of the CGM if hyperglycemia occurs should mitigate this risk. Nausea was rare in our previous closed-loop studies, and in a previous study with a very similar randomized blinded design to this one, there was no difference in nausea between the glucagon and placebo days.

In individuals with post-bariatric hypoglycemia, administration of glucagon, even in small doses, has the potential for increasing glucose values above normal, triggering release of insulin and a subsequent "roller-coaster" effect with recurrent hypoglycemia. Since the doses of glucagon to be infused are low, and the glucagon delivery algorithm attempts to avoid overcorrecting glucose above the normal range, we do not anticipate a post-treatment surge in glucose sufficient to trigger this problem.

There is a risk of hypoglycemia for everyone with post-bariatric surgery hypoglycemia. However, we do not believe that the study intervention will increase this risk. On the contrary, our hypothesis is that this risk will be reduced on glucagon days. The double-blind nature of the study was chosen, in part, so that volunteers would not change their behavior in ways that might put them at greater risk of hypoglycemia, such as taking more insulin or skipping snacks they might have otherwise had. Since they will not know if they are getting glucagon we hope they will behave in the same way they would ordinarily do, so that their risk is not increased on placebo days and may be reduced on glucagon days.

VIII. Potential Benefits

Subjects enrolled in the study may have a reduced incidence or reduced severity of hypoglycemia on glucagon days.

The data derived from this study will allow us measure the effectiveness of glucagon in preventing and minimizing hypoglycemia. This information will be helpful in assessing the feasibility of using automated glucagon delivery in the treatment of post-bariatric surgery hypoglycemia. It may also lead to the use of closed-loop glucagon delivery to prevent hypoglycemia in other populations at risk for hypoglycemia, such as patients with insulinoma or congenital hyperinsulinism.

IX. Data and Safety Monitoring

IX. a. Monitoring of Source Data

The principal investigator ora study clinical research fellow (physician) will review the eligibility of each volunteer based on the case report form from the screening visit and review the laboratory data. CGM data, calibration data, and glucagon dosing data will be stored in the bionic pancreas device and wirelessly streamed to the cloud where it will also be stored electronically to provide

redundancy in data storage and mitigate the risk of data loss. All of the data for each sub-study will be combined in a single database that will be compared against the primary data files for integrity. The computer database will be backed up at least monthly and the backup media stored in a secure location.

The Study will be conducted by the staff of the MGH Diabetes Research Center (the Center). The PI will conduct meetings with Study staff at least twice a month to review Study progress, discuss any issues in Study conduct, and review procedures. Study staff will be encouraged to raise any concerns they may have or problems they have identified at these meetings. The PI, in consultation with the Nursing Director of the Center as appropriate, will decide a course of corrective action, and resolution or progress will be assessed no later than the next bimonthly meeting. An audit of procedures, regulatory documentation, and a sample of volunteer files will be performed by a member of the Center at least biannually. The audit will be conducted by a Center staff member who is not directly involved in the conduct of the Study. This audit will include a review of regulatory documentation, such as IRB and FDA correspondence, and a review of volunteer files, including a review of consents, case report forms, and other data from Study visits.

A numeric code will be substituted for the volunteers personal identifying information in the study database, which will be password protected. The key linking the medical record number of the volunteer with the numeric code, along with case report forms, and all information that is personally identifiable, will be kept in a locked filing cabinet in an investigator's locked office. All electronic records will be kept in a password protected computer database. All printed computer data will be disposed of confidentially when no longer needed. Only the study staff will have access to the study database. Subjects may not withdraw from the de-identified database, but they may elect to have the key linking their medical record to the de-identified database destroyed.

The study data may be shared with collaborators outside of Partners, but only in a form in which all personally identifiable information has been removed (e.g. combined database including patient diary data, CGM data, BG values, and glucagon delivered by the device). Shared data will be in the form of a database in which only a number identifies volunteers.

A de-identified dataset as described above will be stored for possible future analysis in the laboratory of the Boston University by an engineer. Subjects may not withdraw their data, as it will be stored in non-personally identifiable form.

IX. b. Safety Monitoring

Once participants are enrolled in the study, a study physician or nurse will monitor the initial insertion of the infusion sets and CGM sensor, ensuring that proper procedures are followed.

This study is considered moderate risk. An external Data and Safety Monitoring Board will oversee the conduct of the study and review its results on a regular basis. The DSMB will have unblinded access to the drug assignments to assess whether adverse events are associated with glucagon administration. They may also perform interim analyses on the primary outcome to determine whether the study should be stopped early due to futility or adverse events that are excessive as compared to the benefits.

Additionally, the DSMB will be informed of any serious or unexpected adverse events. The DSMB will be informed if there are any changes to the study protocol that could significantly impact the safety or scientific validity of the study. A final DSMB meeting will convene after the completion of the study. Safety and efficacy data will also be reported to the FDA in compliance with applicable regulations.

It should be noted that there were no serious adverse events in the earlier phases of our closed loop studies including over 100 volunteers.

IX. c. Adverse Event Reporting Guidelines

The Principal Investigator and Co-investigators will review any adverse events after each experiment. Adverse events will be reported promptly to the Partner's IRB and to the Joslin IRB according to their respective adverse event reporting guidelines. Edward Damiano, PhD of Boston University is the sponsor of an IDE for this trial. Reports of adverse events will be made to the FDA in compliance with the terms of the IDE.

X. Subject Compensation

Financial compensation ($50) will be provided to all volunteers who complete the Screening Visit.

Subjects will be compensated $350 for satisfactorily completing the study. Volunteers will receive a bonus of $100 for complete documentation (completion of the email survey every day) and return of all blinded vials promptly at the end of each study week. Thus, the total compensation for a volunteer with PBH will be up to $500. We will also cover parking expenses for participants.

Compensation will be pro-rated at a rate of $25 per completed day if volunteers chose to stop participation prior to the completion of the study.

XI. References

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