Efficacy of Glucagon In the Prevention of Hypoglycemia During Mild Exercise

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I. Background and Significance

1.a. Background

Hypoglycemia is a persistent and often unpredictable problem for patients with diabetes mellitus treated with insulin and/or oral hypoglycemic medications. Hypoglycemia can arise from simple miscalculations by the patient: ingestion of too few carbohydrates relative to the amount of medication taken, or too much medication taken with food or for correction of hyperglycemia. Perhaps the most unpredictable cause of hypoglycemia is exercise. Physical activity causes insulin-independent glucose uptake into the muscle both during exercise and for a long period thereafter. When physical activity is coupled with too much medication or not enough carbohydrates, hypoglycemia often results. Identifying, treating and preventing hypoglycemia is critical to avoid both acute and chronic complications including slowed cognition, confusion, unconsciousness, seizures, and death. Recurrent hypoglycemia can lead to reduced or even absent symptoms prior to the onset of neuroglycopenia, which further inhibits the patient's ability to identify and treat their hypoglycemia before it reaches dangerous levels. Tragically, some patients experience irreversible brain damage or die as a consequence of a severe hypoglycemic event that was not detected in time. When this occurs overnight during sleep it is described, chillingly, as the "dead in bed" syndrome. Many patients develop a fear of hypoglycemia, and as a consequence, are not able to maintain tight enough glycemic control to avoid hyperglycemic complications later in life. The tools available to patients with diabetes for identification, treatment and prevention of hypoglycemia are inadequate for the risk they face.

The advent and subsequent improvement of continuous glucose monitoring (CGM) technology has provided a new tool for detecting incipient hypoglycemia, but these devices are invasive, expensive and still require the user to take action on the readings. Currently, only 10-15% of the most vulnerable patients, those with type 1 diabetes (T1D), use a CGM. An experimental bionic pancreas (BP), using micro-dose glucagon in response to hypoglycemic CGM readings, would eliminate the need for the user to take action, or even be aware that the hypoglycemia was occurring. Micro-doses of glucagon would also reduce the need for often calorie-dense oral carbohydrates as treatment.

Recently there has been interest in the use of "diabetes alert dogs" that are said to detect hypoglycemia by smell. If this is true, it suggests that there is an odor signature of hypoglycemia that is detected by dogs, and that this odor signature might also be detected by previously developed nose-like nanosensing technology. A hypoglycemia detecting nanosensor or nanosensor array could be built into a compact, non-invasive warning system that could be employed by diabetics to detect incipient hypoglycemia. Such a system might be particularly useful in the form of a bedside device or one that would be installed in an automobile.

I.b. Bionic Pancreas System

We have developed an autonomous, self-learning BP that requires only the subject's weight for initialization, and then autonomously adapts, modestly or dramatically, as needed, to cope with the wide range of insulin requirements of adults, adolescents, and pre-adolescents with T1D, and potentially for patients with insulin dependent type 2 diabetes. The BP obviates the need for the patient to know, or even appreciate, their insulin requirements, and renders obsolete any need for patients or caregivers to know carbohydrate-to-insulin ratios, basal rates, or insulin correction factors.

Our core technology is the insulin controller, which orchestrates all subcutaneous (SC) insulin dosing. At its centerpiece is a model-predictive control (MPC) algorithm, which bases insulin doses on the glucose data and insulin absorption kinetics. We were the first to incorporate insulin pharmacokinetics (PK) into the algorithm, by augmenting it with a mathematical formulation for estimating the concentration of insulin in the blood and predicting its future concentration. It is essential to compensate for the slow absorption rate of SC insulin analogs (peak time in blood of 30--90 min, clearance in 4--8 hr), and to enable the algorithm to refrain from stacking and overdosing insulin. Furthermore, the MPC algorithm automatically adjusts its insulin-dosing aggressiveness continuously and in real-time to different insulin needs between individuals and variable needs within the same

individual. Running in parallel with the MPC algorithm is an algorithm that automatically modulates basal insulin delivery over multiple time scales, and another algorithm that automatically adapts insulin doses in response to optional meal announcements. Unlike current insulin pumps, and all of the insulin-only control algorithms of which we are aware, the adaptive basal insulin algorithm obviates the need for the user to set, or even know, his or her "basal-rate profile". Instead, it is capable of automatically adapting to, and compensating for, changes in an individual's basal insulin need, such as might occur over a period of hours, days, or weeks (e.g. circadian hormonal fluctuations, intercurrent illness, physical activity, or emotional state) or as might occur over a period of months or years due to developmental changes (e.g. hormonal changes that occur during puberty or menopause). Our adaptive meal dose controller obviates the need for the user to set, or even know, his or her "carbohydrate-to-insulin ratios", as it makes automatic adjustments based on dosing history for similar meal announcements made on previous days, and customizes the dose for each individual and for time of day. Our BP also includes a proportional-derivative algorithm governing SC micro-doses of glucagon to help prevent impending hypoglycemia. Glucagon dosing is based on the glucose level and rate of descent. It could occur preemptively even if glucose is above range and it includes a feedback term to account for the pending effects of recent glucagon doses.

Taken together, these mathematical algorithms provide a universal framework for a glycemic control strategy that requires no quantitative input from, or participation by, the user (besides entering body weight to initialize the system), but which automatically adapts insulin and glucagon dosing to meet the individual needs of each user. Another challenge we have met is enabling the technology to remain completely autonomous in managing insulin and glucagon delivery even when the Dexcom CGM is offline. Specifically, when the Dexcom CGM is offline, the BP invokes the high-resolution "basal rate profile" that it had recently learned and stored when the Dexcom CGM was online. On the basis of what the system learned and stored about meal announcements when the Dexcom CGM was online, it is able to respond to meal announcements in the same manner when the Dexcom CGM is offline. Finally, it automatically responds to user-entered BG values when the Dexcom CGM is offline by issuing a correction dose of insulin or glucagon based on what it learned about the user's insulin and glucagon needs when the Dexcom CGM was online. Thus, the BP never relies on, or burdens the user with, the determination of subjective dosing decisions, which inevitably vary in quality and reliability among different users. The BP provides a turnkey solution for people with diabetes that comprehensively manages glycemia across a broad range of individual needs and a across a large spectrum of circumstances and challenges to glycemic control.

I.c. Preliminary Studies

Our BP hardware platform has evolved over the years from a laptop-driven system, which we used in all of our inpatient studies (between 2008--2012), to the first truly mobile wearable iPhone-driven platform, which we have used in all of our outpatient studies thus far (between 2013--2017). Using the iPhone-driven BP system, we have conducted >160 outpatient experiments of 4--11 days in duration in each subject (> 800 patient days or > 2 patient years of data), and across subjects ranging in age between 6 and 78 years old and in body mass between 21 and 133 kg. The robust adaptation capabilities of the BP is evident in the fact that the average total daily dose of insulin among these subjects varied by over 13-fold (from 11 to 145 units/day). We have tested the BP in bi-hormonal (insulin and glucagon), insulin only and glucagon only configurations. In all of our studies and all configurations, the BP has shown significant reductions in hypoglycemia compared to usual care. An outstanding challenge for the BP is to prove in a head-to-head direct comparison of the insulin only BP with the bi-hormonal BP, demonstrating the benefit of closed loop microdose glucagon in preventing and treating hypoglycemia in a controlled setting. We have unpublished data showing this in the outpatient setting with outcomes based on CGM data, but the Center for Drug Evaluation and Research does not recognize CGM-based outcomes and has requested data showing the incremental benefit of glucagon using outcomes based on plasma glucose measurements made directly on blood. Therefore, we need to perform a study in the in-clinic setting in which frequent reference-quality plasma glucose measurements can be made.

In preliminary studies we have sampled the breath, sweat, and blood of study participants with diabetes wearing the bionic pancreas while they exercised to see if it is possible to identify a volatile organic compounds (VOC) marker for hypoglycemia. These participants wore the bionic pancreas for 4 days as an outpatient run-in to their fasted exercise visit. Some of the subjects were tested multiple times during up to three rounds of testing. This provided sets of clinical samples from 79 exercise sessions. The breath samples have been analyzed by a research team at the MITRE Corporation (a non-profit research corporation) to identify and measure the VOCs present, as well as how their concentrations vary relative to the blood glucose of the participants. The variations in the concentrations of several VOCs in the breath of diabetics appeared to correlate with variations in the blood glucose concentration. However, in experiments performed thus far only two participants developed hypoglycemia due to the efficacy of the BP and the circumstances of the exercise testing. Therefore, more data needs to be collected to allow demonstration of a statistically significant correlation between a VOC breath signature and hypoglycemia. Further clinical testing is necessary to examine the relationship between breath and sweat VOCs and hypoglycemia.

I. d. Rationale and Potential Benefits

We will compare the ability of the bi-hormonal bionic pancreas and the insulin only bionic pancreas to prevent and treat exercise induced hypoglycemia. In this study we have modified the circumstances and type of exercise in ways that we believe will increase our ability to detect differences between the insulin-only and bihormonal bionic pancreas systems. In this study the bionic pancreas will adapt to the individual in a bihormonal configuration at the lowest glucose target we have previously shown to be safe (100 mg/dl) and then either remain in the bihormonal configuration for exercise or be switched to the insulin-only configuration just before exercise at the same glucose target. In the previous study the lowest glucose target used was 110 mg/dl, which likely reduced the incidence of hypoglycemia in the insulin-only configuration. It will be safe to test the insulin-only configuration at this glucose target because it will only be used while participants are under direct monitoring in the Diabetes Research Center (DRC). We will also have participants stay overnight in a hotel near the DRC the night before the exercise visits because we suspect the stress of commuting to the DRC through Boston traffic may have elevated the pre-exercise plasma glucose values in many participants, thereby making it more difficult to observe hypoglycemia during exercise. Finally, we have changed the type of exercise from cycling at a moderate pace with a heart rate of 120-140 beats per minute, to a mild exercise (walking on a treadmill at a comfortable pace) for a longer period of time (up to 1 hour). It is well known that more intense or unaccustomed exercise can often result in elevations rather than reductions in plasma glucose due to release of stress hormones. Therefore, we have chosen a more mild exercise that all participants will be comfortable with and have increased the duration to maximize the chance that hypoglycemia may occur in the insulin-only configuration of the bionic pancreas.

In this setting we will again measure volatile organic compounds in breath and sweat of participants with type 1 diabetes with normoglycemia and exercise induced hypoglycemia to determine if there is a relationship between VOC signature and hypoglycemia. If no hypoglycemia is induced by exercise then after a recovery period we will administer a small insulin dose to increase the likelihood of achieving mild hypoglycemia and thereby increase the number of breath and sweat samples collected under hypoglycemic conditions. This will be done only if hypoglycemia did not occur during exercise or in the recovery period afterwards so this additional intervention will not interfere with interpretation of the results of the exercise study.

II. Hypothesis and Specific Aims

We hypothesize that the bi-hormonal bionic pancreas will reduce the amount of hypoglycemia experienced during and after exercise relative to an insulin-only configuration of the bionic pancreas. We hypothesize that by collecting the breath and sweat of type 1 diabetics while they are in normoglycemic and hypoglycemic states, and comparing the profile of volatile organic compounds in both between these two states, we will be able to determine a relationship between volatile organic compounds in breath and sweat and hypoglycemia.

Aim 1. To evaluate the incremental utility of glucagon in the context of automated insulin delivery by the bionic pancreas in preventing hypoglycemia during mild exercise of extended duration in the fasted state.

Twenty subjects will participate in two experimental periods. Each will include a 24-96 hour outpatient run-in period prior to their exercise visit wearing the bi-hormonal bionic pancreas. This will allow the bionic pancreas to adapt to their diabetes management needs. After the run-in period is complete, the subjects will participate in an exercise visit during which they will arrive fasting and remain fasted until the visit is completed. Subjects will walk on a treadmill for up to 1 hour at a comfortable walking pace. Plasma glucose (PG) measurements will be performed frequently. In one experimental period the bionic pancreas will remain in the bihormonal configuration and will deliver glucagon as needed during the exercise visit. In the other experimental period the glucagon will be replaced with a placebo during the exercise visit. The two experimental periods will be performed in random order. The study will be performed in single-blind fashion in that the participant will not know whether the bionic pancreas glucagon pump is filled with glucagon or placebo during the exercise visits. The outpatient run-in period will always be with the bi-hormonal bionic pancreas delivering glucagon.

Aim 2. To collect samples of breath and sweat during hypoglycemia in 20 adult (≥ 18 years of age) subjects with type 1 diabetes.

Breath and sweat samples will be collected at intervals throughout the exercise visits, with increased frequency during hypoglycemia. Collaborators with the MITRE Corporation will perform analyses on these samples to identify any relationships between volatile organic compounds in breath and sweat and hypoglycemia.

III. Subject Selection

III. a. Inclusion Criteria

- Informed consent by the subject documented prior to any study procedures
- Age ≥ 18 years and have had clinical type 1 diabetes for at least one year
- Diabetes managed using an insulin pump for ≥ 6 months
- Have used a CGM for ≥ 4 weeks over the last 12 months (usage does not need to be consecutive)
- Prescription medication regimen stable for > 1 month (except for medications that will not affect the safety of the study and are not expected to affect any outcome of the study, in the judgment of the principal investigator)
- Live within 120 minute radius of Massachusetts General Hospital
- Willing to remain within a 250 mile radius of the central monitoring location during the outpatient run-in period. No air travel will be allowed, and subjects will still be expected to follow the visit schedule as described.
- Willing to spend the night prior to both exercise visits in a hotel and fast overnight prior to exercise
- Willing and able to avoid deodorant, scented lotions, and scented laundry detergent on your clothes on the days of the exercise visits
- Willing to wear two steel cannula infusion sets (6 mm Contact Detach) and one Dexcom CGM sensor and change sets frequently (a new glucagon infusion set daily and a new insulin infusion set every other day during the outpatient run-in period)
- Have a mobile phone they will have access to at all times during the outpatient run-in period for making contact with study staff

No subjects will be excluded on the basis of gender or race. The requirement that subjects manage their diabetes using subcutaneous insulin infusion pump therapy is imposed because multiple daily injection therapy involves the use of long-acting basal insulin that would require an extended washout period.

III. b. Exclusion Criteria

- Unable to provide informed consent (e.g. impaired cognition or judgment)
- Unable to safely comply with study procedures and reporting requirements (e.g. impairment of vision or dexterity that prevents safe operation of the bionic pancreas, impaired memory, unable to speak and read English)
- Current participation in another diabetes-related clinical trial that, in the judgment of the principal investigator, will compromise the results of this study or the safety of the subject
- Pregnancy (positive urine HCG), breast feeding, plan to become pregnant in the immediate future, or sexually active without use of contraception
    - Subjects must use acceptable contraception for the two weeks prior to the study, throughout the study and for the two weeks following the study.
    - Acceptable contraception methods include:
        - Oral contraceptive pill (OCP)
        - Intrauterine Device (IUD, hormonal or copper)
        - Male condoms
        - Female condoms
        - Diaphragm or cervical cap with spermicide
        - Contraceptive patch (such as OrthoEvra)
        - Contraceptive implant (such as Implanon, Nexplanon)
        - Vaginal ring (such as NuvaRing)
        - Progestin shot (such as Depo-Provera)
        - Male partner with a vasectomy proven to be effective by semen analysis
- Current alcohol abuse (intake averaging > 3 drinks daily in last 30 days) or other substance abuse (use within the last 6 months of controlled substances other than marijuana without a prescription)
- Unwilling or unable or to avoid use of drugs that may dull the sensorium, reduce sensitivity to symptoms of hypoglycemia, or hinder decision making during the period of participation in the study (use of beta blockers will be allowed as long as the dose is stable and the subject does not meet the criteria for hypoglycemia unawareness while taking that stable dose, but use of benzodiazepines or narcotics or other central nervous system depressants, even if by prescription, may be excluded according to the judgment of the principal investigator)
- History of liver disease that is expected to interfere with the anti-hypoglycemia action of glucagon (e.g. liver failure or cirrhosis). Other liver disease (i.e. active hepatitis, steatosis, active biliary disease, any tumor of the liver, hemochromatosis, glycogen storage disease) may exclude the subject if it causes significant compromise to liver function or may do so in an unpredictable fashion.
- Renal failure requiring dialysis
- Personal history of cystic fibrosis, severe pancreatitis, pancreatic tumor, pancreatectomy or any other pancreatic disease leading to diabetes mellitus.
- Any history of insulinoma
- Any known history of coronary artery disease including, but not limited to, history of myocardial infarction, stress test showing ischemia, history of angina, or history of intervention such as coronary artery bypass grafting, percutaneous coronary intervention, or enzymatic lysis of a presumed coronary occlusion)
- Abnormal EKG consistent with coronary artery disease or increased risk of malignant arrhythmia including, but not limited to, evidence of active ischemia, prior myocardial infarction, proximal LAD critical stenosis (Wellen's sign), prolonged QT interval (> 440 ms). Non-specific ST segment and T wave changes are not grounds for exclusion in the absence of symptoms or history of heart disease. A reassuring evaluation by a cardiologist after an abnormal EKG finding may allow participation.
- Congestive heart failure (established history of CHF, lower extremity edema, paroxysmal nocturnal

dyspnea, or orthopnea)

- History of TIA or stroke
- Seizure disorder, history of any non-hypoglycemic seizure within the last two years, or ongoing treatment with anticonvulsants
- History of hypoglycemic seizures (grand-mal) or coma in the last year
- History of pheochromocytoma: fractionated metanephrines will be tested in patients with history increasing the risk for a catecholamine secreting tumor:
    - Episodic or treatment refractory (requiring 4 or more medications to achieve normotension) hypertension
    - Paroxysms of tachycardia, pallor, or headache
    - Personal or family history of MEN 2A, MEN 2B, neurofibromatosis, or von Hippel-Lindau disease
    - Adrenal tumor that has not undergone characterization for endocrine function
- Hypertension with systolic BP ≥160 mm Hg or diastolic BP ≥100 despite treatment
- History of any orthopedic conditions rendering exercise unsafe
- Untreated or inadequately treated mental illness (indicators would include symptoms such as psychosis, hallucinations, mania, and any psychiatric hospitalization in the last year), or treatment with anti-psychotic medications that are known to affect glucose regulation.
- Electrically powered implants (e.g. cochlear implants, neurostimulators) that might be susceptible to RF interference
- Unable to completely avoid acetaminophen for duration of study
- History of adverse reaction to glucagon (including allergy) besides nausea and vomiting
- Any known history of hypersensitivity to lactose
- Established history of allergy or severe reaction to adhesive or tape that must be used in the study
- History of eating disorder within the last 2 years, such as anorexia, bulimia, or diabulemia or omission of insulin to manipulate weight
- History of intentional, inappropriate administration of insulin leading to severe hypoglycemia requiring treatment
- Use of oral (e.g. thiazolidinediones, biguanides, sulfonylureas, glitinides, DPP-4 inhibitors, SGLT-2 inhibitors) or non-insulin injectable (GLP-1 agonists, amylin) anti-diabetic medications
- Lives in or frequents areas with poor Verizon wireless network coverage (which would prevent remote monitoring)
- History of poor venous access or inadequate venous access as determined by trial nurse or physician at time of screening?
- Hemoglobin < 12 g/dl for men, < 11 g/dl for women
- Any factors that, in the opinion of the principal investigator would interfere with the safe completion of the study

III. c. Source of Subjects

Volunteers who fit the selection criteria will be considered as candidates for this study. We will contact individuals who have previously inquired about participation in our studies and have asked us to have their contact information kept on file. In addition, advertisements for the study may be posted at the MGH Diabetes Center and other places, and may be distributed in the weekly broadcast email of research studies seeking volunteers. A letter may be sent to adult endocrinologists in the Boston metropolitan as well as selected nearby endocrinologists informing them of the study and asking them to refer any eligible patients who might be interested. We will post information about the trial along with contact information on our website www.bionicpancreas.org and on www.clinicaltrials.gov.

IV. Subject Enrollment

IV. a. Number of Subjects

It is expected that we will have 20 subjects complete the study with a consistent protocol. We expect that the experiments and analysis can be accomplished over a period of 6-12 months. Up to 40 subjects 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. b. Enrollment and Consent Procedures

The study will be approved by the Partners IRB before any subjects are enrolled. Prospective participants will be briefed by a study staff member by phone or e-mail regarding the study procedure and the inclusion and exclusion criteria. Potential subjects will be sent an informed consent document by mail, fax, or e-mail.

Once potential subjects have had time to review the consent document, they will meet with a study provider (MD or NP) that will explain the study, answer any questions, and administer informed consent. In the event that a volunteer is a patient of one of the study MDs or NPs, another staff MD or NP will answer questions and administer consent. A licensed physician investigator will be available to speak with the subjects during the consent process in the event of an NP administering consent.

Study staff will answer any questions that the subjects may have during their participation. They will share any new information in a timely manner that may be relevant to the subject's willingness to continue participating in the trial. The subjects 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
- Date of diabetes diagnosis
- 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
- Duration of insulin pump use
- Type of insulin used in pump
- Average total daily dose of insulin in the last 30 days (from pump history in type 1 diabetes subjects) – for comparison with insulin dosing during the usual care and bionic pancreas arms of the study
- 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)
- Height and weight
- Blood pressure
- EKG (if > 50 years old or duration of diabetes > 20 years)
- Urine HCG (pre-menopausal females)
- Hemoglobin A1c
- Hemoglobin
- Fractionated plasma metanephrines (if testing is indicated by history)

V. b. Drugs

The study involves subcutaneous administration of insulin lispro (Humalog, Lilly) and insulin aspart (Novolog, Novo Nordisk). Both are commercially available by prescription and are indicated for patients with diabetes, but

not for use in a bionic pancreas. Subjects will be provided with and use whichever analog of rapid acting insulin they usually use during all arms of the study. The study also involves subcutaneous administration of glucagon for injection (GlucaGen vials, 10 pack,, Novo Nordisk) which is indicated for the treatment of severe hypoglycemia, but not for use in a bionic pancreas.

The control system can administer bolus doses of each drug up to every five minutes. A single automated bolus of insulin will not exceed 3 units per 5-minute dose [30 µl] and a single meal-priming dose, which is triggered by the user, will not exceed 12 units [120 µl]. A single bolus of glucagon will not exceed 80 µg [80 µl]. The insulin pumps can administer as little as 0.5 µl (0.05 units of U-100 insulin or 0.5 µg of 1 mg/ml glucagon) in single programmable bolus doses.

It is expected that the total daily dose of glucagon will be < 1.0 mg daily as in previous studies. The mean daily glucagon dose in our previous 11 day outpatient study was 0.51 mg/day (range 0.20-0.90 mg/day). The recommended dose of glucagon for adult patients suffering from severe hypoglycemia is 1 mg as a single injection. Mean glucagon levels in our previous inpatient studies have been above the normal fasting range for glucagon only 1% of the time. Therefore, the glucagon exposure of subjects is expected to be modest.

V. c. Devices

Infusion sets: Subjects will wear up to two FDA approved commercially available infusion sets, one for insulin infusion and one for glucagon infusion, when applicable. FDA approved 6 mm steel cannula infusion sets will be provided for use during both arms of the study. If an infusion set falls off or is clinically suspected of failing, it will be replaced with a new one. The insulin infusion set will be changed at least every 48 hours; the glucagon infusion set will be changed every 24 hours.

Continuous glucose monitors: One transcutaneous glucose sensor for the Dexcom G5 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. The whole assembly is held to the skin with an adhesive patch and communicates wirelessly via Bluetooth Low Energy with the G5 application running on a mobile device. If the G5 sensor fails for any reason during the experiment it will be replaced promptly.

Bionic Pancreas Control Unit:. The Beta Bionics mobile application that runs the control algorithm and the Dexcom G5 app are both installed on a stock iPhone 6s running iOS 10. The Betabionics app receives the CGM glucose values that are captured by the Dexcom G5 app.

The control algorithm app has a graphical user interface (GUI) that displays the current Dexcom CGM glucose, a graphical history of the Dexcom CGM glucose, and doses of insulin and glucagon delivered by the control algorithm. The GUI can also be used to input meal announcements, designating the size of the meal as larger than typical, typical in size, smaller than typical, or just a bite, and the type of meal as breakfast, lunch, or dinner. This will trigger a partial meal-priming bolus the size of which will adapt during the course of the trial to meet a target of 75% of the insulin needs for that size and type of meal.

The target glucose level in the bionic pancreas will be programmed by the study staff prior to the start of each experiment. This will be locked for each arm of the study; the subject will be unable to accidentally change or tamper with this setting.

The user will have the option during the outpatient run-in period to trigger the administration of a glucagon dose, intended to be used prior to device disconnection (e.g. for a shower or swimming). The size of the glucagon dose will be automatically determined by the bionic pancreas based on the subject's body mass and will be between 40 and 80 micrograms. This option will provide a means for subjects to raise their BG if they anticipate

they will be at risk for hypoglycemia during a period of disconnection, based on their glucose level and glucose trend at the time.

The GUI can also be used to manage meal boluses as usual, and will administer correction boluses in response to entered BG values, during periods when the Dexcom CGM is offline, such as the period after a sensor is replaced and before the new sensor has been calibrated. During these times the control algorithm will determine and direct the administration of insulin basal rates either based on the subject's weight early in the course of the experiment, or on the average of adaptively determined basal rates for that time of day once sufficient experience has been accumulated (i.e. 24 hours or more) by the control algorithm. The controller will also administer insulin and/or glucagon as appropriate in response to any entered BG values, just as if they were Dexcom CGM values.

The GUI also displays local audio and visual alarms if communication is dropped between the Dexcom CGM transmitter and the bionic pancreas control unit or between the control unit and the two insulin pumps. The Dexcom CGM also has its own hard-coded alarm distinct from the bionic pancreas when the CGM glucose crosses below 55 mg/dl. These alarms may be configured so that they require the entry of a code to dismiss.

The iPhone communicates wirelessly via the Bluetooth Low Energy (BTLE) protocol with up to two Tandem t:slim insulin pumps to deliver insulin and glucagon.

The bionic pancreas control unit can be used with two Tandem pumps, one for insulin and the other for glucagon, to make up the bi-hormonal bionic pancreas.

In all configurations, if communication failures between the Dexcom CGM and the bionic pancreas or the bionic pancreas and the cloud are not resolved within 15 minutes they trigger alerts to study staff who will then make contact with the wearer according to study protocol. If communication failure between the bionic pancreas and pumps is not resolved within 20 minutes this triggers an alert to study staff who will make contact with the wearer.

Tandem t:slim Pumps: These pumps are FDA approved insulin pumps with reservoirs capable of holding 300 units (3 ml) of insulin or 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 are slave to the bionic pancreas control unit and are controlled wirelessly via the BTLE protocol by the iPhone6S.

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 in both study arms. This meter will be used to calibrate the Dexcom sensor.

Yellow Springs Instrument (YSI) 2300 STAT PLUS: The YSI model 2300 STAT PLUS Glucose and Lactate Analyzer is a laboratory instrument that is intended for use in clinical care. It provides quick measurements of glucose in whole blood, plasma or serum and will be used to measure plasma glucose during the exercise visit at the end of each arm. This device will be stored at the Diabetes Research Center when not in use, and study staff will follow proper maintenance and quality assurance procedures.

Treadmill: The study will utilize a treadmill for the exercise visit at the end of each study arm. The treadmill will be stored in the Diabetes Research Center when not in use.

V. d. Experimental Procedures and Data Collection

V. d. i. Screening Visit

- All subjects will have a screening visit to confirm eligibility. Informed consent will be obtained and

documented with a signed informed consent document before any trial-related procedures are conducted.

- The subject will be interviewed and the case report form will be completed by study staff to establish whether the subject is eligible to continue with the screening.
- A urine pregnancy test will be performed in female volunteers. If the test is positive the volunteer will be informed of the result and the visit will be ended.
- Height, weight and blood pressure will be measured. An EKG will be performed in subjects who are either ≥50 years of age or who have had diabetes for ≥20 years.
- If the volunteer is not excluded based on historical criteria, blood pressure, EKG or urine pregnancy test, blood will be drawn for hemoglobin and hemoglobin A1c. Plasma fractionated metanephrines may be obtained if indicated by history.
- Once all of the laboratory results have been returned, a study MD or NP will review the case report form to determine subject eligibility. If subjects are not eligible to continue in the study the results of abnormal tests will be reported to the subjects and to a health care provider of their choosing.
- Subjects who have been screened and are eligible can participate without having to be re-screened for a period of one year. The study staff should verbally confirm that there have been no health events that would make them ineligible if the interval between screening and participation is longer than 3 months.

V. d. ii. Randomization of Study Visit Order: Once the subject has been enrolled and eligibility of subjects has been established, subjects will be randomized to one of the possible two visit-orders. In random order, subjects will complete an insulin only exercise visit and a bi-hormonal exercise visit. The outpatient run-in period for both exercise visits will use the bi-hormonal bionic pancreas.

V. d. iii. General Study Policies during the Outpatient Run-In:

- Subjects will remain at all times within a geographic boundary established on the basis of 250 miles from the designated base for study personnel and will avoid any air travel.
- Subjects will keep a charged mobile phone on their person (or at their bedside) at all times and will answer calls from the study staff.
- Subjects will sleep in a hotel nearby the Diabetes Research Center the night prior to both exercise visits. This is meant to avoid increases in counter-regulatory and stress hormones that may occur during a lengthy and/or stressful commute to the study center.
- Study subjects will keep a StatStrip Xpress glucometer easily accessible at all times in case a calibration is needed, and they will do all calibrations with this meter. They will keep a glucometer, fast-acting carbohydrates, and a glucagon emergency kit easily accessible at home for hypoglycemia as needed.
- Subjects should use the study provided StatStrip Xpress glucometer for all BG checks throughout the study. They are encouraged to check their BG at least four times a day, before meals and before bedtime. They will also be encouraged to check before exercise and at intervals during exercise, and for any symptoms of hypoglycemia. There are no restrictions on additional checks and subjects should check as often as they wish to confirm the accuracy of the Dexcom CGM and for safety.
- Subjects will wear 6 mm steel cannula (contact detach) infusion sets during both the outpatient run-in periods and both exercise visits.
- Subjects will not use any recreational drugs or drugs of abuse, other than alcohol. The need to take prescription drugs that dull the sensorium, reduce sensitivity to symptoms of hypoglycemia, or hinder appropriate decision-making may be grounds for exclusion from the trial or discontinuation of participation, a decision that will be made by the principal investigator.
- Subjects may not take acetaminophen during both study arms due to potential interference with

CGM sensing. Acetaminophen is known to interfere with the accuracy of the Dexcom CGM.

- Subjects will not tamper with the bionic pancreas device in any way, including changing any settings. Subjects will not use the bionic pancreas iPhone for anything beyond its intended purpose in this study.
- During the experiment the bionic pancreas will be worn by the subject or kept nearby (such as when sleeping) at all times to ensure good radio-frequency signal reception.
- Subjects will keep their bionic pancreas charged, which will require charging at least once per day.
- The bionic pancreas is not water resistant and therefore must be removed for showering. Subjects are urged to take appropriate precautions when they are disconnected from the bionic pancreas, including frequent BG checks and having carbohydrate readily available. Subjects may give a glucagon bolus prior to disconnecting.
    - The Dexcom CGM transmitter is water resistant and can be left on for bathing and swimming.
    - Subjects may not remove the bionic pancreas for more than 1 hour at a time (e.g. for bathing) and may not remove it for more than 2 hours total in any 24 hour period.
- Any medical advice needed by the subjects during their participation, which is not directly related to BG control during the experiment, should be obtained by them in the usual manner with their primary care physician or endocrinologist.
    - If a subject develops an illness during the experiment, they can seek medical care as usual. As long as the subject is not hospitalized, the study can be continued. 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.
- Subjects may participate in any activities that they wish, as long as they abide by the policies above.
- There are no restrictions of any kind on diet or exercise, although subjects should attempt to maintain similar dietary habits and exercise habits during each arm of the study. The bionic pancreas must be kept dry during exercise.
- Subjects may choose to withdraw from the study at any time. If they withdraw from the study, they should contact a provider immediately. If they are wearing the bionic pancreas, a provider will help them transition to their own insulin regimen safely.

V. d. iv. Remote Monitoring

Local Alarms

- The system will generate the following local alarms to the subject:
    - Low threshold audio alarm of 50 mg/dl Dexcom CGM glucose values on the bionic pancreas
    - The Dexcom CGM app also has a low threshold audio and vibrating alarm of 55 mg/dl
    - Audio and visual alarms on the bionic pancreas for pump disconnections after 10 minutes, and then every 5 minutes following that until the disconnection is resolved
    - Audio and visual alarms on the bionic pancreas for Dexcom G5 disconnections after 10 minutes, and then every hour following that until the disconnection is resolved
- Subjects will be trained in recognizing and responding to all of these alarms.

Remote Monitoring

- A central monitoring station will be staffed 24 hours a day. There will be at least one provider (MD or NP) on call at all times in addition to the staff member monitoring for alarms. Additional study staff members may assist with on-call duties. A study staff member will make contact with subjects as necessary and help them troubleshoot any issues that may arise, leaving the monitor free to focus on identifying alarms and communicating them to the study staff.
- The system generates the following alarms to the monitoring center:
    - If the Dexcom CGM has been disconnected and has not spontaneously reconnected after 20 minutes
    - If the wireless connection between the bionic pancreas control unit and a Tandem pump has

been lost and has not spontaneously reconnected within 20 minutes
    - No internet connectivity (either through cellular or WiFi), and therefore unable to monitor, for 20 minutes
- When an alert comes to the monitoring station, a study staff member will contact the volunteer on any of the provided phone numbers.
- 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 throughout. We may also encourage subjects to connect to public but secure wireless networks if they are having trouble connecting to cellular service.
    - If we are unable to monitor a subject remotely for greater than 20 minutes, a study staff member will contact the subject to check that the bionic pancreas is functioning properly and to resolve problems with network coverage. If there are no indications of device malfunction as the cause for lost connectivity, the glucose level is in safe range, and a subject chooses to remain in an area with poor network coverage, we will instruct the subject to check the bionic pancreas display at least every 30-60 minutes for alert icons. We will call the subject every 2 hours to check on safety and device function until remote monitoring is restored.
- If there is a technical problem with the bionic pancreas that cannot be resolved over the phone, a member of study staff may be dispatched to the location of the subject to provide in-person assistance. The subject may be asked to come to the Diabetes Research Center or study staff may meet them in another public place. If this is not possible or would be too disruptive (i.e. in the middle of the night) the subject will be asked to take over their own glycemic control using their insulin pump until such time as a meeting can be arranged for in-person inspection of the device. This should occur in most cases within 12 hours. Staff will not go into subjects' houses or other non-public places, nor will they go to any place to meet the subject that is not public or where they do not feel safe.

V. d. v. Visit Procedures

Visit 1:

- On arrival to the first study visit, the body weight of the subject will be documented.
- A urine pregnancy test will be performed in female volunteers at the start of the first arm. If the test is positive the volunteer will be informed of the result and the visit will be ended. The date of the last menstrual period will also be documented, along with usual cycle length, for female subjects.
- The subjects will place a Dexcom G5 sensor and study staff will confirm they are doing it properly.
- Study staff will provide supplies and review the study procedures again. Study staff will supervise the setup of the insulin and glucagon pumps and infusion sets.
- The control algorithm will be initialized only with the subject's weight. Diagnostics will be performed to ensure that the Dexcom CGM device is appropriately calibrated and that all of the components of the bionic pancreas are in good communication with each other.
- The subject's own insulin infusion pump will be stopped and disconnected, and its infusion set will be removed.
- 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). Study staff will verify that data streaming is working prior to the subject leaving the Diabetes Research Center.

Outpatient Run-In:

- The subjects will calibrate the Dexcom CGM twice daily, preferably before breakfast and supper, using the StatStrip Xpress.

- Subjects will be advised to delay calibration if there is a steep rise or fall in the blood glucose (>2 mg/dl/min), or if they suspect a steep rise or fall while in blinded mode, there has been carbohydrate intake in the last 30 minutes, or there has been a glucagon dose in the last 15 minutes. In the immediate aftermath of carbohydrate intake or glucagon dosing it is possible for the BG to be rising without a change in interstitial fluid glucose. If a calibration is delayed for any of these reasons, it will be performed at the next opportunity.
- Subjects may perform additional calibrations if the Dexcom CGM is inaccurate relative to a BG measurement as long as they do not calibrate within 30 minutes of food intake or 15 minutes of glucagon dosing. Subjects will be discouraged from performing extra calibrations if the Dexcom CGM is within 15 mg/dl when the BG is ≤ 75 mg/dl and within 20% if the BG is >75 mg/dl at times when the rate of change is low. They will also be trained to understand that the apparent error can be higher than this when the BG is changing rapidly, and that it is typical for the Dexcom CGM to underestimate BG when the trend is upward and to overestimate BG when the trend is downward as a result of physiologic lag. Errors in these directions should typically not prompt extra calibrations unless they are very large (≥ 50%).

- Subjects will be contacted if Dexcom CGM streaming is interrupted for more than 15 minutes. If the sensor has been lost, it will be replaced promptly. If there is a technical fault with the bionic pancreas, study staff will troubleshoot this with the subject. If necessary, a staff member will meet the subject to assist with troubleshooting. This meeting may be delayed until morning if the problem occurs overnight - in this case, the subject will use their own pump until a meeting is possible. If necessary, the bionic pancreas control unit may be replaced.
  - When meeting subjects in an off-site location, the principal investigator will always be notified. A member of the clinical team (MD, NP or RN) will be dispatched if the problem is clinical in nature. If the principal investigator determines the problem to be purely technical, a trained engineer may be dispatched to assist the subject with troubleshooting their device.
  - If there is a complete failure of bionic pancreas operation and it is anticipated that restarting it will take more than an hour, subjects may take over their own BG control using their own insulin pump or with insulin injections until the bionic pancreas can be brought back online with the help of study staff. During the day, this should be rare. If the failure occurs at night, every effort should be made to correct the problem as soon as possible, which should almost always be possible within 12 hours.
  - If a Dexcom CGM sensor fails during the course of an experiment the system will provide basal insulin based on past requirements and will allow announcement of meals and entry of fingerstick BG measurements, which will be treated as Dexcom CGM data and may result in administration of insulin and/or glucagon. The Dexcom CGM sensor will be replaced as soon as possible and normal bionic pancreas control will resume when the new sensor is calibrated.
- Alarms will sound and a visual alert will appear on the iPhone screen of the bionic pancreas control unit if the Dexcom CGM glucose is less than 50 mg/dl. Subjects will test their BG and enter the results into the bionic pancreas in response to such an alarm.
- Subjects will be trained on troubleshooting for various scenarios that could lead to a low threshold alarms. For instance, a threshold alarm could be due to true hypoglycemia, poor Dexcom CGM calibration, or a compression artifact at the site of the sensor.
  - The first step for all low glucose-related alarms will be to perform a finger stick BG measurement.
  - If the BG measurement is not consistent with the fact that a threshold alarm has occurred: the subject will assess the possibility of a compression artifact (they will be trained in the causes and recognition of these events). If a compression artifact is suspected, they will take steps to relieve the pressure on the transmitter. If compression is not suspected, they will calibrate the Dexcom CGM as long as there has been no food or carbohydrate intake in the last 30 minutes. If a calibration is delayed for this reason, it will be performed at the next opportunity.
  - If the BG measurement is consistent with a low threshold alarm: the subject will treat

hypoglycemia with carbohydrate ingestion according to their usual practice. When investigating suspected or persistent hypoglycemia, subjects will also be trained to investigate the glucagon infusion set and consider replacing it.

- Subjects will be asked to change their insulin infusion set and reservoir at least every other day during both arms in the study.
- Subjects will be trained on troubleshooting for various scenarios that could lead to hyperglycemia. For instance, hyperglycemia could be due to true hyperglycemia or poor Dexcom CGM calibration.
    - The first step in responding to hyperglycemia according to the CGM will be to perform a finger stick BG measurement.
    - If the BG measurement is not consistent with the CGMG: the subject will calibrate the Dexcom CGM as long as there has been no carbohydrate intake in the last 30 minutes and there is no steep rise or fall in glucose (>2 mg/dl/min). If a calibration is delayed for this reason, it will be performed at the next opportunity.
    - If the BG measurement is consistent with the CGMG: the subject will investigate their insulin infusion site and consider replacing it.
- Subjects will be asked to announce the three major meals of the day, but not snacks, to the bionic pancreas. The meal announcement will consist of choosing the type of meal (breakfast, lunch, dinner) and the size of the meal relative to typical meals for that subject (snack, smaller than typical, typical, larger than typical).
- The glucagon reservoir will be replaced every day during the outpatient run-in period. Each reservoir will be filled with two vials of freshly reconstituted GlucaGen vials (Novo Nordisk). The glucagon infusion set will be changed daily with the reservoir change. We have received an IND exemption from the FDA for use of glucagon in this application for up to 27 hours. The insulin reservoir will be changed every other day during the outpatient run-in period.
- On days when both the insulin and glucagon reservoirs will be changed, subjects will be asked to change them at different times in the day, separated by at least one hour. They will label the infusion sets and tubing with supplied labels to avoid confusion or cross connection.

Exercise Visits:

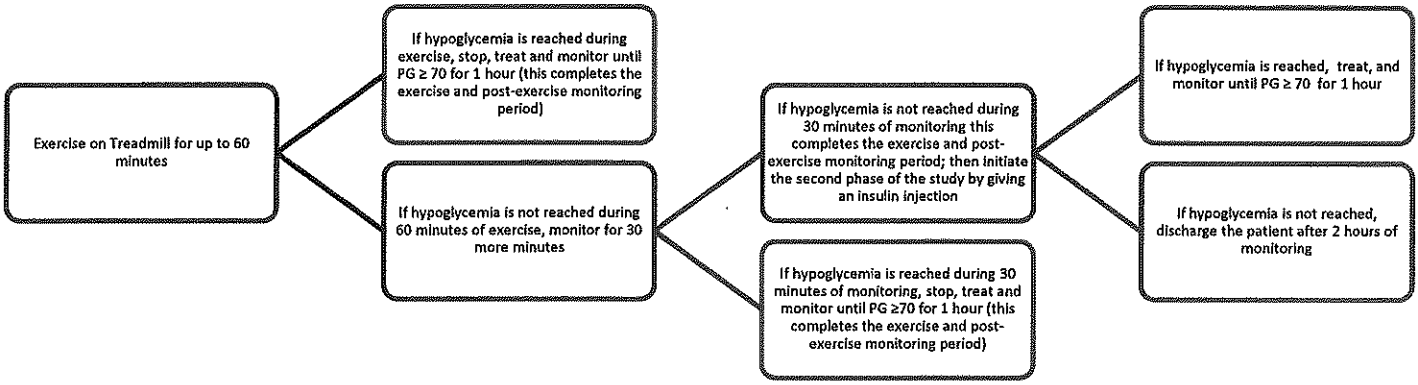
- Subjects will have the option of completing the exercise visit at least 24 hours and up to 96 hours after completing Visit 1
- Subjects will arrive to the exercise visit having fasted since 12:00 AM the night before (treatment with simple carbohydrates of up to 30 grams for a low blood sugar is allowed). If the patient is not fasted, the visit will be rescheduled. Subjects will sleep in a local hotel the night before the exercise visit to eliminate the effects of their morning routine and the commute to the Diabetes Research Center on their glycemic control.
- A plasma glucose will be checked via fingerstick using the Statstrip Xpress upon arrival. If PG is ≥150 mg/dL, the subject will either wait for the PG to fall below 150 mg/dL or the visit will be rescheduled. If PG is <50 mg/dL, the patient will be treated with simple carbohydrates according to the 15/15 rule (15 grams of carbohydrates, repeated in 15 minutes if necessary).
- We will ask subjects to avoid certain skin care products that will interfere with sweat sample collection on the day of the visit.
- Study staff will fill the tandem t:slim glucagon pump with either glucagon or placebo (normal saline) according to the subject's randomization order. The subject will not be aware of their randomization. New infusion sets for both the glucagon/placebo and insulin pumps will be placed using an FDA approved steel cannula infusion set.
- The body weight of the subject will be documented.
- A 20 gauge or smaller peripheral I.V. will be placed.
- Subjects will walk on a treadmill which is intended to induce hypoglycemia. Subjects will not be allowed to move faster than a comfortable walking pace. Subjects will be reminded that the goal is not to raise

their heart rate or sweat, but to keep moving and keep their leg and core muscles activated without excessive exertion.

- Subjects will continue walking until the hypoglycemia threshold has been reached, or up to 1 hour. Exercise will be terminated when:
  - Subjects reach a PG of 50 mg/dl or lower
  - Subjects reach a PG of < 60 mg/dl but > 50 mg/dl for three consecutive measurements taken every 5 minutes and thereafter choose to treat with oral carbohydrates (subjects may elect not to treat hypoglycemia with carbohydrates if the PG is >50 mg/dl)
  - They have completed 1 hour of walking with no treatment for hypoglycemia
- During exercise, BG measurements using the YSI will be obtained according to the following protocol:
  - If PG is ≥ 80 mg/dl, BG measurements will be obtained off of the IV line every 10 minutes
  - If the PG is < 80 mg/dl, BG measurements will be obtained off of the IV line every 5 minutes.
  - If PG is <60 mg/dL for more than three consecutive measurements but is still > 50 mg/dl, subjects will have the option to treat with carbohydrates, and if subjects choose to treat exercise will be terminated.
  - If PG is < 50 mg/dL, exercise will be terminated and subjects will be required to treat with carbohydrates.
    - Carbohydrates will be given according to the following protocol: Dextrose (g) = BSA (m^2)/[1.7 m^2 (women) or 1.9 m^2 (men)] *15g
    - Repeat treatments will be given at 15 minute intervals as long as hypoglycemia continues
  - If the YSI fails, the Nova StatStrip Xpress meter will be used for glucose measurements.
- Study staff will collect samples of sweat from the subject's underarm and will ask subjects to exhale into a breath collection device at the beginning of, at intervals during, and at the completion of exercise, with increased frequency during any episodes of hypoglycemia. These samples will be collected, de-identified and shipped out to collaborators at the MITRE Corporation for analysis of the relationship between volatile organic compounds in breath and sweat and hypoglycemia.
- PG monitoring off of the IV line will continue after the exercise is completed or stopped according to the following protocol:
  - If the PG is ≥ 120 mg/dl, BG measurements will be obtained off of the IV line every 20 minutes.
  - If PG is 80-119 mg/dl, BG measurements will be obtained off of the IV line every 10 minutes
  - If PG is < 80 mg/dl, BG measurements will be obtained off of the IV line every 5 minutes
- If the subject reached the hypoglycemia threshold during exercise, their PG will continue to be monitored after exercise is stopped. They will be discharged from the DRC when they have maintained a PG ≥ 70 mg/dl for 1 hour. In this scenario the end of this hour will be the end of the post-exercise monitoring period. They will be allowed to treat with oral carbohydrates at their discretion during this time to maintain their blood glucose.
- If the subject did not reach the hypoglycemia threshold during exercise, they will be monitored for an additional 30 minutes after exercise is stopped.
  - If they reach the hypoglycemia threshold during this additional 30 minutes, they will continue to be monitored until they maintain a PG ≥ 70 mg/dl for 1 hour. In this scenario the end of this hour will be the end of the exercise and post-exercise monitoring period. They will be allowed to treat with oral carbohydrates at their discretion during this time to maintain their blood glucose.
  - If they do not reach the hypoglycemia threshold during this additional 30 minutes, this will complete the post-exercise monitoring period.
  - After the post-exercise monitoring period is completed, a correction bolus of insulin will be given via syringe.
    - This dose of insulin will be calculated using their own correction factor, correcting them down to a PG of 50 mg/dl using their current PG.
    - The PG will continue to be monitored after the insulin injection is given and hypoglycemia will be treated per protocol.

- If the subject reaches the hypoglycemia threshold following the insulin injection, they will be monitored until they maintain a PG ≥ 70 mg/dl for 1 hour
- If the subject does not reach the hypoglycemia threshold, after two hours, they will be discharged

- Once the post-exercise monitoring is complete and the subject's plasma glucose is stable, they will be discharged off of the bionic pancreas.
  - Their glucose meters and bionic pancreas will be downloaded.
  - Subjects will have the option to begin the next arm of the study on the same day they complete the first arm. If subjects choose this option, they will start over with Visit 1 procedures.
  - If the subject has chosen to complete their second study arm at a later date or has completed both study arms, they will resume their usual care on their own insulin pump, and study staff will review the last several hours of insulin and/or glucagon dosing with them.



V. d. vi. Response to Hypoglycemia

- While wearing the bionic pancreas at home, subjects are encouraged to check their BG for any symptoms of hypoglycemia.
- Subjects are encouraged to treat hypoglycemia according their usual practice or according to the "rule of 15s": take 15 grams of rapid acting carbohydrate and recheck in 15 minutes, then repeat as needed.
- Subjects will be instructed to check their glucagon infusion site and their bionic pancreas for normal operation any time hypoglycemia occurs. If there is any suspicion of glucagon infusion set malfunction, the site should be replaced.
- If a subject experiences a seizure or unconsciousness associated with hypoglycemia in the study, his or her participation in the study will be discontinued.

V. d. vii. Response to Hyperglycemia

- Subjects will be instructed to check their insulin infusion site and their pump or bionic pancreas for normal operation any time BG is greater than 300 mg/dl. If there is any suspicion of insulin infusion set malfunction, the site should be replaced.
- Subjects may contact a study provider (MD or NP) for advice at any time, and may contact the troubleshooting support team, as they wish. During both study arms, subjects will be assisted in checking the bionic pancreas for any malfunction and correcting any problems that are found.
- If no correctable fault is found, but there is doubt regarding the correct function of the bionic pancreas system, an entirely new backup bionic pancreas system may be brought to the subject's location by study staff.
- If a subject experiences diabetic ketoacidosis requiring hospitalization during the study, his or her participation in the study will be discontinued.

V. d. viii. Response to Nausea/Vomiting

If significant nausea, nausea that prevents the subject from eating normally, or any vomiting occurs during either arm of the study, subjects will be encouraged to contact a study provider (MD or NP). They will document the report of nausea or vomiting. Study providers may assist the subject in troubleshooting, such as checking the BG and the calibration of the Dexcom CGM (excessive glucagon dosing may occur if the Dexcom CGM is reading lower than the true BG). If a subject experiences persistent nausea and vomiting thought to be related to glucagon dosing, his or her participation in the study will be discontinued.

V. d ix. Response to Other Medical Needs

If the subject experiences any non-emergent medical concerns outside the scope of diabetes care, he or she will see their personal physician. If the subject experiences urgent or emergent medical concerns outside the scope of diabetes care and their primary care physicians, they should visit a walk-in clinic or emergency room, or if necessary call 911.

V. d. x. Monitoring of Bionic Pancreas Performance

Collaborators (and bionic pancreas inventors and developers) Edward Damiano, Firas El-Khatib and/or an engineer trained by them will be readily available by phone for consultation at all times during the course of each experiment. They will have the capability of viewing diagnostic information regarding the connection of the Dexcom CGM with the bionic pancreas, the functioning of the bionic pancreas, and the connection of the bionic pancreas with the insulin and glucagon pumps 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. d. xi. Supervision by Study Staff

A study provider (MD or NP) will be on call at all times during the course of each study arm. All trained staff will have the capability of remotely viewing diagnostic information to facilitate phone troubleshooting with subjects and decide about whether additional assistance is needed.

VI. Biostatistical Analysis

VI. a. Data Collected

VI. a. 1. Prior to start of experiment:

- Age
- Sex
- Race and ethnicity
- Date of last menstrual period in female subjects
- Date of diabetes diagnosis
- 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
- Duration of insulin pump use
- Type of insulin used in pump
- Insulin regimen (basal rate, sensitivity factor, and carbohydrate ratio)
- Average total daily dose of insulin in the last 30 days as available
- Usage of CGM(type of CGM, days per month worn, usage of data, whether insulin is dosed based on CGM alone, alarm settings)

- Height and weight
- Blood pressure
- EKG if applicable
- Hemoglobin A1c
- Hemoglobin
- Urine HCG (pre-menopausal females)
- Fractionated plasma metanephrines (if indicated by history)

VI. a. 2. During the Outpatient Period of Both Study Arms:

- CGMG (CGM glucose) every five minutes from the Dexcom CGM
- All fingerstick BG measurements taken by the subject (meter download)
- Insulin total daily dose
- Glucagon total daily dose
- Timing of meal announcements and size of meals announced
- Timing and doses of glucagon boluses
- Time subjects were not under bionic pancreas control
- List of technical faults associated with the bionic pancreas including cause and resolution

VI. a. 3. During the Exercise Visit:

- Plasma BG measurements every 10 minutes, every 5 minutes when BG < 80 mg/dl, or every 20 minutes when exercise is complete and BG > 120 mg/dl
- Time from start of exercise to first glucose measurement < 60 mg/dl
- Grams of oral carbohydrates given to the subject to treat hypoglycemia
- Timing of exercise and duration

VI. b. Study Endpoints

VI. b. 1. Primary endpoint analysis (considers only the exercise and post-exercise monitoring period)

Number of subjects discordant between insulin-only and bionic pancreas visits for reaching a PG <60 mg/dl (using the YSI) for >2 consecutive measurements during the exercise and post-exercise monitoring period; only the first such event will be counted for this analysis

VI. b. 2. Secondary endpoint analyses (during the exercise and post-exercise monitoring period)

- Duration of plasma glucose <60 mg/dl
- Nadir plasma glucose
- Area over the plasma glucose curve and < 60 mg/dl
- Time from the start of exercise to first PG < 60 mg/dl, if found

VI. b. 3. Other endpoint analyses

Exercise and post-exercise monitoring period

- Plasma glucose at the start of exercise
- Mean slope of the CGM glucose curve in the 30 minutes prior to the start of exercise
- Area over the plasma glucose curve and <60 mg/dl accumulated at the time the participant requests carbohydrate treatment or treatment is required by protocol during exercise visit
- Duration with CGM glucose <60 mg/dl during exercise visit
- Nadir CGM glucose during exercise visit
- Area over the plasma glucose curve and <60 mg/dl during exercise visit, calculated by CGM
- Time from the start of exercise to first CGM reading < 60 mg/dl

- Total glucagon dosing by bi-hormonal bionic pancreas from the start of exercise until the end of the exercise and post-exercise monitoring period
- Grams of oral carbohydrates given to the subject to treat hypoglycemia during the exercise and post-exercise monitoring period
- Total glucagon dosing by bi-hormonal bionic pancreas from the start of exercise until the end of the visit
- **VOC outcomes**
  - Identification of volatile and organic compound or compounds in breath and sweat that correlate(s) with hypoglycemia
  - Identification of volatile organic compounds in breath and sweat that correlate with normoglycemia and/or hyperglycemia

Outpatient period

- **CGM-based outcomes**
  - Mean CGM glucose – 24-hours, daytime (7:00 AM – 11:00 PM), and nighttime (11:00 PM – 7:00 AM)
  - Time in the following ranges – 24-hours, daytime (7:00 AM – 11:00 PM), and nighttime (11:00 PM – 7:00 AM):
    - <70 mg/dl
    - <60 mg/dl
    - <54 mg/dl
    - <50 mg/dl
    - 70-180 mg/dl
    - 70-120 mg/dl
    - 70-140 mg/dl
    - >180 mg/dl
    - >250 mg/dl
  - Percentage of participants achieving a mean CGM glucose of ≤154 mg/dl
  - Correlation between mean Dexcom CGMG and mean number of meal announcements per day
  - CGM MARD from Dexcom G5 CGM versus time-stamped BG values from meter downloads (any other BG values will not be considered)
  - CGM Reliability index, calculated as percent of possible values actually recorded by CGM
- **PG-based outcomes**
  - All of following metrics will be generated from any fingerstick data available (downloaded from the subjects meter) during the outpatient bionic pancreas and usual care periods.
  - Mean number of daily BG measurements

Analysis of the Effect of Glucagon During the In-clinic Experiment

We will assess the incremental effect of glucagon in the context of automated insulin delivery by the bionic pancreas in preventing hypoglycemia during exercise in the fasted state. The primary outcome will be derived from the plasma glucose values collected throughout the exercise visits. Mean glucose, time-in-range, and time in hypoglycemia measures will be separately calculated using both plasma glucose values and CGM glucose values for the in-clinic exercise experiments and both will be reported. For the primary outcome we will compare the proportions of patients having at least one hypoglycemia event between the bi-hormonal and insulin-only bionic pancreas arms. A hypoglycemia event is defined as an event with PG <60 mg/dl for more than two consecutive measurements.

In the crossover design, each subject will serve as their own control and the hypoglycemia event is considered to be correlated within the same patient. We will apply a two-sided exact McNemar's test to compare

hypoglycemia rates between the bi-hormonal and insulin-only bionic pancreas arms. We use a conservative threshold for p-value <0.025 as indication of statistical significance.

In the case of a subject who withdraws from the study after participating in only one of the in-clinic experiments we will use multiple imputations to replace the missing data and perform a sensitivity analysis comparing the result using multiple imputations to the result obtained including only subjects that have completed both of the in-clinic visits.

For the time from the start of exercise to first PG <60 mg/dl endpoint we will perform survival analysis using the Kaplan-Meir method and will compare arms with a Cox Proportional Hazards model accounting for within patient correlations.

Analysis of Outpatient Outcomes

For the outpatient run-in period only outcomes based on CGM glucose values will be reported. The primary analysis of the designated endpoints will be calculated on an intention-to-treat basis, including data from periods when the bionic pancreas was not in use, if available (Dexcom CGM data may not be available in some failure modes). In cases where an arm was not completed we will use the available data from that arm in the data analysis. We will calculate percentages, means standard deviations (and medians and interquartile ranges as appropriate), and ranges in descriptive analyses. We will use the Shapiro-Wilk test to determine normality of the variables. We will use paired t-test for comparison of means for normally distributed outcomes and the Wilcoxon Signed Rank test for comparisons on non-normally distributed outcomes. In a secondary analysis we will look for any period effect in normally distributed outcomes 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 may, in exploratory analyses, also stratify subjects for secondary analyses of the pre-specified endpoints by the following characteristics: sex, age, usual care insulin total daily dose, body mass index, baseline A1c, and use of CGM in usual care.

VI. c. Power Analysis

Power Calculation for Aim 1

The sample size for this study is being based on the power calculations for Aim 1. From our clinical experience, we expect the hypoglycemia rate to be around 25% in the bi-hormonal arm and around 75% in the insulin-only arm, corresponding to a difference of 50 percentage points.

The table below provides power calculations of a two-sided exact McNemar's test with 2.5% type I error under varying degrees of within-subject correlations.

Based on our clinical experience with bi-hormonal bionic pancreas, patients who develop hypoglycemia using bi-hormonal bionic pancreas are highly likely to also develop hypoglycemia when not given glucagon. We expect 5% of patients who develop hypoglycemia with glucagon will have no hypoglycemia when glucagon is not given. If our expectation is correct, our planned sample size of 20 patients will provide ≥90% statistical power to detect a difference in hypoglycemia rates between the two arms. If this proportion is three times as big as we expect (15%), we will still have 82% power to detect the difference.

Statistical power of two-sided exact McNemar's test with 2.5% type 1 error, assuming a difference of 50% (25% vs. 75%) in hypoglycemia rate between two arms. Crossover design, N=20.

Proportion discordant (higher value corresponds to lower correlation between two arms)	Proportion of those having hypoglycemia with glucagon	Statistical power

	that do not have hypoglycemia when glucagon is withdrawn	
0.625†	25%	75%
0.6	20%	78%
0.575	15%	82%
0.55	10%	86%
0.525	5%	90%
0.505	1%	93%

†corresponding to independence between two arms

Power Calculation for Aim 2

No formal power analysis has been completed for the determination of a relationship between VOCs and hypoglycemia. Combining hypoglycemia that occurs during exercise and hypoglycemia that occurs after a correction bolus with a glucose target of 50 mg/dl, we expect to capture breath and sweat in at least 30 visits during which hypoglycemia (BG < 60 mg/dl) occurs. During visits in which hypoglycemia occurs, we will capture at least one set, and likely 2 or more sets, of samples of breath and sweat during hypoglycemia, and at least 4 during normoglycemia in the same participants on the same day. This is anticipated to provide samples from 30-40 hypoglycemic episodes and paired samples during normoglycemia (both before and after hypoglycemia) from the same participants. If there is a robust correlation between a VOC marker and hypoglycemia, this should provide sufficient poswer to identify VOC markers of hypoglycemia.

VII. Risks and Discomforts

There is a risk of hypoglycemia. In the outpatient run-in period, this risk is expected to be less than the risk during the subjects' lives outside the trial based on data from earlier trials. All of our previous studies have shown that hypoglycemia is significantly reduced in all configurations of the bionic pancreas when compared with usual care. Based on our experience, we believe that the risk of hypoglycemia in the outpatient run-in period will be less than or equal to the risks that they are exposed to on a daily basis while living with type 1 diabetes outside of the trial. The exercise visit protocol is designed to cause hypoglycemia. Given frequent PG monitoring, protocols for treating hypoglycemia, and direct supervision by an RN or MD at all times, the risk of a hypoglycemic episode leading to significant harm to volunteers is expected to be very low. A small meal or snack will be made available to subjects after completion of the study.

Subjects may experience discomfort with insertion of the peripheral intravenous line, infusion sets, and Dexcom sensor into the subcutaneous tissues. The risk of discomfort due to insertion of infusion sets and Dexcom sensors

may be greater than in their lives outside the trial because more infusion sets and sensors will be inserted than they may be used to.

There is a risk of risk of dizziness or lightheadedness from blood loss. However, typical blood loss will be limited to no more than 216 ml. Exclusion of subjects with anemia will help mitigate this risk.

There is a risk of headache, nausea, or vomiting in subjects due to the administration of exogenous glucagon. There is a possible risk of skin rash due to administration of exogenous glucagon. There may be risks of daily, low-level glucagon administration that have not become apparent during trials lasting up to 11 days. One possible risk is weight loss although no changes in weight have been observed in trials lasting up to 11 days. Others may include changes in blood chemistries or blood counts. The magnitude of the other possible risks due to daily administration of small amounts of glucagon are unknown, but are not expected to be high because mean glucagon levels have been in the normal fasted range in previous trials and there have be no other adverse events in previous bionic pancreas trials lasting up to 11 days. Of note, the incidence of nausea or vomiting has been low in prior studies and in one randomized double-blinded placebo controlled study it was not different from placebo.

VIII. Potential Benefits

Based on evidence from previous trials of the bionic pancreas and the design of this trial, subjects enrolled in the study may benefit from a reduction in risk of hypoglycemia and hyperglycemia and a better mean glucose during the outpatient run-in period.

Additionally, this study will help us to identify the utility of microdose glucagon in a bionic pancreas to prevent and treat hypoglycemia in the context of exercise, and FDA CDER has indicated that PG data demonstrating this will be required by in order to approve a bionic pancreas delivering glucagon on a chronic basis.

The data derived from this study will allow us to evaluate whether or not there is a relationship between volatile organic compounds in the breath and sweat and hypoglycemia. This information could be useful in developing noninvasive technologies to identify impending and actual hypoglycemia.

Subjects will be financially compensated for participating in the study.

IX. Data and Safety Monitoring

IX. a. Monitoring of Source Data

During the experiment, Dexcom CGM data will be collected in various ways. Dexcom CGM data, calibration data, insulin dosing data, and glucagon dosing data will be automatically stored in the bionic pancreas device (from which it will be downloaded at intervals) and wirelessly streamed to the cloud where it will be stored to provide redundancy in data storage and mitigate the risk of data loss. All of the data 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.

Study staff will be encouraged to raise any concerns they may have or problems they have identified at any time. The PI, in consultation with the co-investigators, will decide a course of corrective action, and resolution or progress will be assessed no later than the next meeting.

An audit of procedures, regulatory documentation, and a sample of subject files will be performed by a member of the Diabetes Research Center at least biannually. The audit will be conducted by a 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 subject files, including a review of consents, case report forms, and other data from study visits.

A numeric code will be substituted for the subjects personal identifying information in the study database, which will be password protected. The key linking the medical record number of the subject 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 at Boston University and at the MITRE Corporation (a non-profit research corporation), but only in a form in which all personally identifiable information has been removed (e.g. combined database including BG values, record of insulin and glucagon delivered by the device, and blood insulin and glucagon levels). Shared data will be in the form of a database in which only a number identifies subjects.

Subjects may not withdraw their data, as it will be stored in non-personally identifiable form.

IX. b. Safety Monitoring

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. Additionally, the DSMB will be informed in the event of any severe 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.

The participation of individual subjects will be discontinued if they experience:

- Diabetic ketoacidosis requiring hospitalization
- Seizure or unconsciousness associated with hypoglycemia
- Persistent nausea and vomiting thought to be related to glucagon dosing

If more than 2 subjects must be withdrawn from the study for these reasons, the study will stop and a vote of the DSMB will be required to restart it. All serious and unexpected events will be reported to the DSMB within 72 hours.

Note that subjects may discontinue participation at any time and subjects may be removed from the trial for other reasons, for instance failure to comply with study procedures or concurrent illness that is unrelated to the bionic pancreas but that precludes safe participation. Discontinuation of participation for these reasons will not contribute to a decision to discontinue the trial.

IX. c. Adverse Event Reporting Guidelines

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered/using product and which does not necessarily have to have a causal relationship with this treatment. An Adverse Event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether considered related to the product or not.

A serious adverse event (SAE) is an experience that at any dose results in any of the following: (death, life-threatening experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity or is a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require Hospitalization may be considered a serious adverse event when, based upon appropriate medical judgement, they may jeopardize the patient or subject or require medical or surgical intervention to prevent one of the outcomes listed in this definition. Suspicion of transmission of infectious agents must always be considered an SAE.

Serious adverse reaction (SAR) is an adverse event that fulfils both the criteria for a serious adverse event and the criteria for an adverse reaction.

Whether adverse events are expected or unexpected will be base on product labeling for the insulins and glucagon used in the study, the expected functioning of the bionic pancreas, and the potential risks described in the consent document.

The causality of each AE should be assessed by the Investigator per the following classification:

- Probable: Good reason and sufficient documentation to assume a causal relationship
- Possible: A causal relationship is conceivable and cannot be dismissed
- Unlikely: The event is most likely related to etiology other than the trial product

The PI and co-investigators will review any adverse events after each experiment. Any serious or unexpected but possibly related adverse events will be communicated to the PI as soon as possible and within 48 hours of the time they are detected. Adverse events will be reported promptly to the Partner's IRB and to the BU IRB. Collaborator Ed Damiano is the sponsor of the Investigational Device Exception (IDE) for the bionic pancreas to be used in this trial. Reports of adverse events will be made to the FDA in compliance with the terms of IDE.
The Investigator will forward all serious adverse events (SARs) and reports on pregnancy during the use of Novo Nordisk product to Novo Nordisk. These safety reports must be sent to Novo Nordisk within 15 calendar days from the investigator's first knowledge of the event. In addition to SARs, any other events that have been submitted to the IRB must be sent to Novo Nordisk at the time of submission.

AE information should include the following:

- Study name
- Patient identification (i.e. subject number, initials, sex, age)
- Event (diagnosis)
- Trial drug
- Reporter
- Causality
- Outcome

X. Subject Compensation

Financial compensation will be provided to all subjects who complete the screening visit. Subjects will be paid $25 for completing the screening visit whether or not they are eligible to participate in the study. Study participants will be compensated $50 for completing each study visit. Thus the total compensation for a subject who completed the study could be up to $225. Parking expenses will be paid for up to $30 per subject. Subjects who are unable to complete the study or chose to stop participation will receive prorated compensation for the portion of the study visits that they complete.

XI. Publication Plans

The data derived from this study will be published in a peer-reviewed journal. Candidate journals include: Diabetes Care; Diabetes; Diabetologia; Diabetes, Obesity and Metabolism; and Journal of Clinical Endocrinology and Metabolism.

XI. References

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