Protocol

1. Project Title:
Post-Prandial Carbohydrate metabolism in individuals with and without Post-Bariatric Surgery Hypoglycemia (PBH)

2. Study Sites:
This is a multi-site study with locations at the University of Alabama in Birmingham (UAB) and Joslin Diabetes Center at Harvard University. UAB is the primary site for this study.

2.1 UAB Investigator(s):
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3. Purpose of the study
The study will assess post prandial carbohydrate, hormone and hepatic glycogen metabolism comparing individuals with post-bariatric surgery hypoglycemia (PBH+) to individuals post-bariatric surgery without hypoglycemia (PBH-).

4. Background and significance of proposed studies:
Bariatric/metabolic surgery is a potent tool for the treatment of obesity and metabolic disease, yielding weight loss, rapid improvements in glycemic levels, and reduction in medication use, increased longevity, and remission of type 2 diabetes (T2D). However, along with this metabolic success comes an increased incidence of hypoglycemia (termed post-bariatric hypoglycemia or PBH)[1], which can be severe for a subset of patients. Severe hypoglycemia causes not only distressing adrenergic and cholinergic symptoms, but also neuroglycopenia and hypoglycemia unawareness, impairing safety and increasing risk for syncope, arrhythmias, loss of consciousness and seizures. Moreover, inability to perform job-related tasks or safely operate a motor vehicle can result in disability, and impaired cognitive function in PBH. Although initially thought to occur rarely, and only after Roux-en-Y gastric bypass (RYGB), recent estimates indicate PBH occurs in ~10-30% of patients, after both RYGB and sleeve gastrectomy (SG), with similar presentation and spectrum of severity. Although Glucagon Like Peptide 1 Receptor (GLP1R) and GLP1R/ Glucose-dependent insulinotropic peptide receptor (GIPR) agonists may ultimately reduce need for surgical approaches to metabolic disease, the ongoing dual epidemics of obesity and T2D will likely result in continued use of bariatric surgery for severe obesity. Moreover, >2 million individuals have had bariatric surgery in the past 10 years in the US[2], highlighting the need to care more effectively for those who have or will develop PBH.

Individuals with PBH experience exaggerated postprandial glycemic excursions, with early peaks in glucose, excessive insulin secretion, and rapid decreases to hypoglycemic levels within 1-3 hours. In addition, individuals with PBH can also develop hypoglycemia with activity[3] and during overnight (mid-nocturnal) hours[4]. One study demonstrated both symptomatic and asymptomatic hypoglycemia during and after exercise as early as 3 months

post- surgery[5]. Many patients with PBH have hypoglycemia unawareness, potentially linked to hypoglycemia- associated autonomic failure. For most patients, hypoglycemia is recognized after at least 2-3 years postoperatively.

In a double-blind, placebo-controlled, crossover trial[6], an event-based system to predict and detect postprandial hypoglycemia based on CGM data and recommend delivery of mini-doses of glucagon was tested. Predictive hypoglycemia alerts prompted automated drug delivery, with up to 2 doses of study drug (300/150µg glucagon or equal-volume vehicle). Hypoglycemic outcomes were improved with glucagon. Thus, this pilot study demonstrated that a CGM-guided closed-loop glucagon pump can detect imminent hypoglycemia in the post-meal period and reduce severe hypoglycemia. However, optimal implementation for clinical therapy will require an algorithm responsive to both the unique post-meal physiology of PBH and to hypoglycemia occurring with exercise and mid-nocturnal periods, with responses to glucagon and glycogen metabolism post prandially as will be measured during this study.

5. Specific Aim:

One promising approach to prevent and treat post-bariatric hypoglycemia is closed-loop glucagon (CLG) therapy. In order to develop that, information about glucagon response in patients with PBH is needed. Therefore, the specific aims of this study are:

To characterize glucagon and glycogen metabolism in individuals with PBH (PBH+) as compared with individuals without (PBH-), in both the fasting and post prandial states. These data will provide critical information to guide therapeutic algorithm development.

6. Research Plan:

6.1 Study population and recruitment

Adult male or female patients with PBH (total n=14; 7 locally at UAB and 7 at Joslin) or without PBH (total n=14; 7 locally at UAB and 7 at Joslin) with at least 2 years following RYGB, will be recruited from clinics at UAB or Joslin, as well as from a database of individuals interested in participation in studies of PBH. Those participants without PBH will serve as controls and will be matched by age, sex, and BMI to the PBH group. The aim will be to obtain at least 7 patients per group with complete data per center. *To account for screen failures, dropouts and incomplete data, we plan to enroll upto 10 individuals with PBH+ and 10 individuals with PBH- at each site.* Study procedures for both study sites will be consistent aside from a MRS scan at UAB vs. subcutaneous glucagon injection at Joslin (detailed in section 6.3 below)

PBH will be defined as: history of symptomatic hypoglycemia, confirmed by plasma glucose value of <54 mg/dL and resolved with carbohydrate ingestion (history of fulfilling Whipple's triad).

Specific inclusion/exclusion criteria:

Inclusion criteria:

1. Age 18-70 years of age, inclusive, at screening.

2. Willingness to provide informed consent and follow all study procedures, including attending all scheduled visits.

3. Males or females at least 2 years following Roux-en-Y gastric bypass (RYGB)

4. For patients recruited to PBH group: diagnosed with ongoing PBH, with documented episodes of hypoglycemia, and history of fulfillment of Whipple's triad.

Exclusion criteria:

1. Documented hypoglycemia occurring only in the fasting state (>12 hours fast);

2. Current diabetes, defined as hemoglobin A1c >6.5% or use of diabetes medications, except for acarbose or miglitol;

3. Chronic kidney disease stage 4 or 5 (including end-stage renal disease);

4. Hepatic disease, including serum ALT or AST greater than 2 times the upper limit of normal; hepatic synthetic insufficiency as defined as serum albumin < 3.0 g/dL; or serum bilirubin > 2.0;

5. Congestive heart failure, NYHA class II, III or IV;

6. History of myocardial infarction, unstable angina or revascularization within the past 6 months.

7. Two or more risk factors for coronary artery disease including diabetes, uncontrolled hypertension, uncontrolled hyperlipidemia, and active tobacco use.

8. History of recurrent syncope (unrelated to hypoglycemia) or active diagnosis of a cardiac arrhythmia;

9. Current administration of β-blocker therapy;

10. History of a cerebrovascular accident;

11. Seizure disorder (other than with suspect or documented hypoglycemia);

12. Active treatment with long-acting (LAR) octreotide or pasireotide;

13. Active malignancy, except basal cell or squamous cell skin cancers;

14. Personal or family history of pheochromocytoma or disorder with increased risk of pheochromocytoma (MEN 2, neurofibromatosis, or Von Hippel-Lindau disease);

15. Known insulinoma;

16. Major surgical operation within 30 days prior to screening;

17. Clinically significant anemia as defined as a hematocrit < 33%;

18. Bleeding disorder, treatment with warfarin, or platelet count <50,000;

19. Blood donation (1 pint of whole blood) within the past 2 months;

20. Active alcohol abuse or substance abuse;

21. Current administration of oral or parenteral corticosteroids;

22. Pregnancy and/ or lactation: For persons of childbearing potential: there is a requirement for a negative urine pregnancy test before any procedures.

23. Not enrolled in another study that uses an investigational drug for this condition.

6.2 Timeline

We plan to recruit all patients in ~18-24 months.

6.3 Study Design and Experimental methods

Visit 1 (Screening)

After signed informed consent, participants will undergo a medical history and physical examination, vital signs, and ECG with emphasis on assessing inclusion/exclusion criteria. Laboratory testing will include hemoglobin A1c, CBC, CMP, urinalysis, urine pregnancy test (if applicable), and an InBody scan.

Participants will be asked to complete a log of foods and drinks consumed (timing/ quantity), timing/severity of symptoms, timing/duration of exercise, and capillary glucose levels (if checked). Surveys assessing hypoglycemia awareness and hypoglycemia symptoms will be completed by participants, either on paper or electronically. Those meeting enrollment criteria will be scheduled for the following visits within 10 days of the screening visit.

In preparation for visit 3, participants will be counseled to follow a low glycemic index diet which is the recommended standard of care for such patients (~90 g CHO per day, consistent with medical nutritional therapy recommendations for PBH) for 3 days and to avoid unaccustomed exercise for 24 hours prior to the study visit. Participants will be instructed to hold hypoglycemia treatments (acarbose. miglitol, if applicable) after the dinner meal on the day prior to the study visit and to fast (nothing by mouth except water) from approximately 8 PM the evening prior to the visit. For individuals taking medication for PBH, medications will be held for at least 5 half-lives, and participants will be asked to monitor finger stick glucose more frequently during this time (1.5-2 hours after meals, bedtime, before driving, and with any symptoms) and to be especially rigorous with dietary recommendations. For individuals who take diazoxide, they will be asked to hold this medication for 5 days prior to visits. For individuals who take octreotide SubQ (short-acting formulation), they will be asked to not take this medication after 6 PM, the day prior to their visits (holding 14 hours prior to the visit, greater than the 9 hours needed for 5 half-lives). Individuals who take pasireotide SubQ (short-acting formulation) will be asked to not take this medication for 2.5 days prior to their visits (holding 50 hours prior to the visit). All recommendations will be provided to participants in writing, reviewed in detail, and questions answered.

Visit 2 (CGM placement -*If not already on CGM per standard of care)

A Continuous Glucose Monitor (CGM) sensor in masked mode (Professional Dexcom G7 or other version available at onset of study) will be placed during visit 2 on the anterior abdomen or arm as specified for the CGM. CGM data, alarms, and timing of symptoms relative to sensor glucose levels will be recorded. CGM data will be analyzed in several ways: (1) percent of time <70 mg/dl (moderate hypoglycemia), (2) percent of time <54 mg/dl (severe hypoglycemia), (3) number of episodes requiring assistance, (4) episodes of severe (<54 mg/dl) or moderate (55-

70 mg/dL) hypoglycemia, and (5) episodes of hyperglycemia (>180 mg/dl). In secondary analysis, data from days and nights (12 AM – 6 AM) will be analyzed separately.

Visit 3 (Glucagon response to Triple Tracer Meal)

This visit will be scheduled within 14 days of screening visit and at least 24 hours after placement of the CGM. If hypoglycemia requiring treatment occurs during the evening (12 hours) prior to study, the visit will be rescheduled. If this visit is delayed beyond the 10 day CGM duration, a new CGM sensor / transmitter will be placed at least 24 hours before the study visit.

Participants will be asked to arrive at the clinical research center (CRC) at ~7:00 AM after an 8-hour fast. Vital signs will be recorded, point of care pregnancy test performed where necessary (and confirmed negative before proceeding) and an intravenous cannula will be inserted into a forearm vein for infusion of stable isotope glucose tracer ([6,6-$^{2}H_2$] glucose). A primed continuous infusion of [6,6-$^{2}H_2$] glucose will be started at ~8 AM (-120 min) and continued until the end of the study. Participants will then be transported to the adjacent radiology suite for a ^{13}C MRS scan to estimate liver glycogen at ~8:30AM (-90 min). Upon return from the radiology suite, another intravenous cannula will be placed in the contralateral distal forearm or hand vein and the forearm/hand will be placed in a heated box (temperature ~55°C (131°F)) to permit arterialization of venous blood. At ~9:30AM (-30 min), an infusion of [^{13}C9, ^{15}N1]-glucagon will be started, and continued until the end of the study. At 10 AM, a triple tracer mixed meal[91] (50% carbohydrates, 15% protein, 30% fat) containing 75 g glucose labeled with [1-^{13}C] glucose (ham, eggs, Jello) will be consumed within 15 minutes. The mixed meal will also contain 1000mg acetaminophen to measure gastric emptying. Simultaneously, [2-^{13}C] glucose will be infused at variable rates to mimic the anticipated systemic rate of appearance of meal glucose; infusion rates of [6,6-$^{2}H_2$] glucose will be varied to mimic anticipated rates of endogenous glucose production (EGP).

As has been our successful long-term practice involving metabolic experiments utilizing isotopic methods, we will analyze data from the first 2-3 participants to determine the tracer-tracee ratios; if necessary, the infusion rates of both glucose and glucagon tracer will be adjusted to minimize fluctuations in tracer-tracee ratios. This is essential to enable accurate measurements of glucose and glucagon turnover. Blood samples will be drawn periodically from the heated hand vein cannula for measurements of glucose, glucose tracer, glucagon tracer, and hormones (insulin, C-peptide, glucagon, GLP-1). Nausea will be evaluated periodically using a visual analogue scale (Fig 2).

Participant will return to radiology suite between 2-5 hours from start of triple tracer meal for a repeat MRS scan to assess post prandial liver glycogen content. After completion of the study ~1PM, all cannulae will be removed as well as CGM. A low-glycemic index snack will be provided following testing, and participants may take acarbose or miglitol if applicable. Following the snack, participants will be monitored for stability with YSI glucose measurements every 15 minutes, and then discharged when glucose is stable or rising (and >80 mg/dL), per study MD clinical judgement. If symptomatic or severe hypoglycemia occurs (neuroglycopenia) during the study visit, blood will be drawn immediately for the above-mentioned analytes, hypoglycemia confirmed with YSI glucose and the Institutional Hypoglycemia treatment protocol will be followed until stable euglycemia is attained.

Glycogen content by C13 MRS: Subjects studied at UAB (n=7/group) will have liver glycogen measured twice using a 3-Tesla whole-body magnetic resonance (MR) imager/spectrometer, with an MRI coil (Pulse Teq) placed over the lateral aspect of each supine subject. A 3-plane

localizer will determine proper coil placement, followed by a higher resolution T1-weighted gradient echo sequence used to determine liver volume. Glycogen 13C spectra will be acquired using a non-localized proton-decoupled 13C pulse-acquire sequence. A vial with a reference solution will be placed at the coil center to calibrate pulse angle and coil loading. Absolute glycogen concentrations will be calculated from the integrated area of the C1-glycogen signal at 100.1 parts per million referenced to glycogen phantom, corrected for receiver gain and reception sensitivity. We will optimize methods using pilot data, and plan to voxel match both visits to obtain the best possible scan data.

Postprandial response to glucagon: Subjects studied at Joslin (n=7/group) will not have MRS. Instead, response to an exogenous subcutaneous glucagon (GCG) injection in the postprandial state (Xeris GCG, up to 300 ug) will be assessed at the nadir glucose level or at 90 minutes (mean nadir time point) after mixed meal (whichever comes first).

Hematocrit will be measured by centrifugation using capillary (microhematocrit) tubes. Blood samples will be processed and stored at -80*C for subsequent analysis of glucose, glucose tracer, hormones (insulin, C-peptide, glucagon, GLP-1), acetaminophen. Plasma glucose will be measured via glucose oxidase (YSI). Plasma enrichments of [6,6-$^{2}H_2$] glucose will be determined using GC/MS via simultaneous quantitation of the C-1 to C-2 and C-3 to C-6 fragments of a methyloxime derivative of glucose. Plasma insulin will be measured using RIA. [^{13}C9, ^{15}N1]-glucagon will be measured by LC-MS/MS with a lower limit of detection of 1.56 pg/mL. GLP-1 (total and intact) will be measured by the Milliplex human metabolic hormone magnetic bead panel-Metabolism Multiplex Assay (Vanderbilt Core). Plasma acetaminophen will be measured by ELISA. Rates of glucose turnover will be calculated as per prior publications using Steele's equation where [6,6-$^{2}H_2$] glucose traces the rate of appearance of total glucose. EGP is determined by subtracting the rate of infusion of [6,6-$^{2}H_2$] glucose from total glucose appearance. Insulin secretion will be calculated by the C-peptide deconvolution model. 7.

7. Statistical Considerations:

Correlations between key variables will be assessed. From previous data[125] we estimate sample size of 14/group (between both study sites) will allow detection of a 50% difference in EGP at 90 minutes after meal testing in PBH+ vs. PBH- (1-β=0.85, α=0.05). Postprandial Ra glucagon has not been previously measured in PBH, but similar sample sizes have been previously employed in the Basu lab[poster, ADA 2022]. We will accept the primary hypotheses if postprandial Ra glucagon and EGP are lower in PBH+ vs. PBH-. Given that postprandial glucagon levels are increased in multiple studies of PBH[9,126,135] , our novel approach to examine glucagon secretion and clearance rates will be critical to unravel these complex interactions.

Primary outcomes of the study will be (1) between-group differences in integrated postprandial response (iAUC) of glucagon secretion (Ra glucagon), and (2) between-group differences in endogenous glucose production (EGP), using mixed model analysis. Secondary outcomes

include (1) comparing differences in glycogen in fasting and postprandial states, (2) Insulin secretion rate between-group differences in response to eating a meal.

7.1 Calculations:

Glucagon turnover: The rate of glucagon secretion (Ra glucagon) at any time point is - *Ra glucagon* = $\frac{F}{TTR} - \frac{pVC.\frac{dTTR}{dT}}{TTR}$ - *F* where F is the infusion rate of [$^{13}C_9$, $^{15}N_1$] glucagon, TTR is the tracer/tracee ratio of [^{13}C9, ^{15}N1] glucagon to total plasma glucagon, pV is the pool fraction and glucagon volume of distribution (40 ml/kg),[127] C is the concentration of plasma glucagon concentrations, T is the time period in minutes. Glucose Turnover: Fasting and postprandial rates of glucose turnover will be calculated as per prior publications[91] using Steele's equation.[97] Briefly, systemically infused [2-^{13}C] glucose is used to trace the systemic rate of appearance of [1-^{13}C] glucose in the meal, while [6,6-$^{2}H_2$] glucose traces the rate of appearance of endogenously produced glucose. The plasma concentration ratio of [2-^{13}C] glucose to [1-^{13}C] glucose is used to calculate the rate of appearance of ingested [1-^{13}C] glucose; the plasma concentration ratio of [6,6-$^{2}H_2$] glucose to endogenous glucose concentration is used to calculate EGP. The endogenous glucose concentration is calculated by subtracting the concentration of exogenously derived (ingested + infused) glucose (i.e., plasma [1-^{13}C] glucose concentration divided by meal [1-^{13}C] glucose enrichment + plasma [2-^{13}C] glucose concentration + plasma [6,6- ^{2}H2] glucose concentrations) from total plasma glucose.[91,98] Insulin action and secretion. Insulin sensitivity (SI) will be estimated using the oral glucose minimal model.[128] The oral C-peptide and oral insulin minimal models[91,98,129-132] will be used to measure the interactions of plasma glucose, C-peptide, and insulin to derive measures of β-cell responsivity (ϕ) and hepatic insulin extraction (HE). Insulin secretion appropriate for the level of insulin resistance (disposition index, DI) will be derived from the products of SI and corresponding ϕ.[133,134]

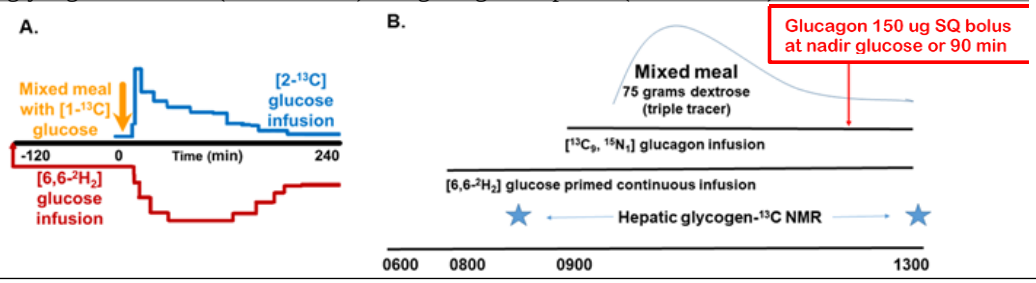
8. Possible Discomforts and Risks:

8.1 Risks to Participants

All risks will be carefully explained by the PI and/or his associates prior to participation and are described in detail in the informed consent.

Glucose Monitoring: The Dexcom G7 Professional continuous glucose monitor is approved for multiple single patient use with appropriate sterilization. The personal Dexcom G7 has not been specifically FDA-approved for use in patients with hypoglycemia, but has been extensively utilized in clinical care of patients with PBH. Glucose sensor placement may cause transient discomfort, bruising, bleeding, or local skin infection.

Fig 1: A. Triple tracer schema. B. Experimental design for Aim 2, with GCG turnover (all), glycogen measures (UAB cohort) and glucagon response (Joslin cohort).



IV catheter placement may cause bleeding, infection, clot formation (all minimal risk), bruising, or discomfort. For all studies, two catheters will be placed, one for phlebotomy and one for infusion.

Adhesive-related skin irritation: Some participants may develop skin irritation or allergic reactions to the adhesives used to secure the CGM. If these reactions occur, different adhesives or “under-taping” (such as with IV 3000, Tegaderm, etc.) will be tried, sites will be rotated frequently, and a mild topical steroid cream or other medication may be required.

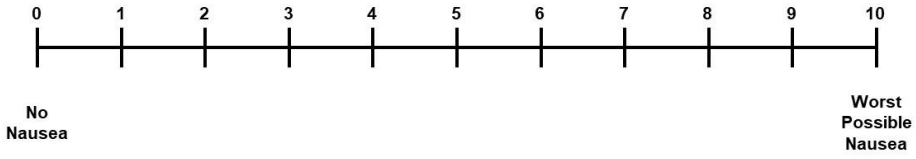
Isotonic saline: A slow infusion of isotonic (normal, or 0.9%) saline is used to keep intravenous lines open. No adverse events are expected.

Phlebotomy may cause blood loss and discomfort. We have conservatively estimated the total volume of blood sampling is up to a maximum of 400 ml for all visits. This should not pose significant stress to any individual. Screening will verify that hematocrit is over 33 at study entry. Volunteers will be requested not to donate blood within 2 months of the study. Any participant who has just donated blood will be asked to postpone entry into the study until 2 months have elapsed.

Fasting and medication adjustment in preparation for study visits: Participants are asked to fast in preparation for study visits to ensure hormonal profile is at steady-state. This is not likely to induce hypoglycemia, as most hypoglycemia in PBH occurs in response to meals or activity, and glycemic levels are more stable in the fasting state. Withholding of medications which can influence insulin and glucagon secretion is required for accurate assessment of hormonal profiles during study visits. While it is always possible this will induce increase in frequency of hypoglycemia, this participant population with PBH is highly experienced in glucose monitoring and will be asked to monitor glucose more frequently during this time (1.5-2 hours after meals, bedtime, before driving, and with any symptoms) and to be especially rigorous with dietary recommendations. We have safely performed over 120 study visits using this approach to visit preparation. All recommendations will be provided to participants in writing, reviewed in detail, and questions answered. Participants are able to text study staff if questions arise during overnight hours prior to study visit days.

Medical Cost Risks: The participant’s insurance company must pay for any tests or care given beyond what is required in this study. The consent form will indicate that the participant may wish to check with his/her insurance health provider before the study starts to ask what they will cover and if they require the participant to get their permission before enrollment. If the participant is hurt as a result of being in this study, we have no plans to pay the participant for lost wages, disability, or discomfort. If the participant is hurt in the study in a way that is unexpected, the participant’s insurance company may pay for the participant’s treatment.

Fig 2: Visual analogue scale for nausea



Administration of Acetaminophen: This is a medication approved by the Food and Drug Administration (FDA) as a pain reliever; however, measuring the levels of this medication during meal testing allows to measure the rate of stomach emptying. Acetaminophen is generally well tolerated; however, uncommonly can cause nausea, headache, and stomach pain. While high doses can lead to liver injury, the dose of 1000 mg is below the daily limit of 4000 mg and participants are asked to avoid acetaminophen containing medications for 24 hours prior to receiving this dose at visit 2. Rarely acetaminophen can cause skin reddening, a rash, or blisters. As with all medications there is a risk of an allergic reaction. Allergy to Tylenol or acetaminophen, will not be exclusionary to participation in the study. Participants who are allergic to acetaminophen will not receive acetaminophen in the meal test.

Administration of glucagon: Glucagon is a hormone normally found in the body. The dosing regimen used this study may raise plasma glucose levels. Glucagon may cause nausea, vomiting, dizziness, and headache (≈10%), as well as other less common complaints, such as asthenia, palpitations, sweating, thirst, vasodilation (all <10%), abdominal or back pain, confusion, diarrhea, dyspepsia, pain, pharyngitis, pruritus/rash, and syncope (<2%). Glucagon can also cause hypoglycemia in some patients after an initial stimulation of glucose levels. The study physician will be physically present on site in the clinical research unit during testing in order to closely monitor participants and ensure safety. As with any medication, there is risk of allergic reaction. Administered in usual rescue dose of 1 mg intramuscularly, glucagon can cause nausea, vomiting, dizziness, and headache, as well as other less common complaints.

Administration of stable label glucose and glucagon isotopes: [6,6-$^{2}H_{2}$]-Glucose and [2-^{13}C]-Glucose infusion rates will be varied to mimic anticipated rates of EGP. [1-^{13}C] Glucose will be consumed orally. Cold isotopes (isotopes that lack radioactivity) have been increasingly used for research purposes similar to radioactive isotopes—to obtain basic information about drug metabolism or about human physiology, pathophysiology, or biochemistry. When used for these basic research purposes, cold (or stable) isotopes ordinarily present fewer safety concerns than radioactive isotopes. FDA believes there is no need to have more stringent requirements for studies that use cold isotopes than for those that use radioactive isotopes, and historically, FDA has not objected to studies using cold isotopes being conducted without an IND. In exercising its enforcement discretion, FDA does not intend to object to clinical investigations using cold isotopes of unapproved drugs being conducted without an IND, provided the following conditions are met (the conditions are based on the criteria for studies using radiolabeled drugs (see 21 CFR 361.1)):13. Please see link to FDA guidance below.
http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm229175.pdf

- The research is intended to obtain basic information regarding the metabolism (including kinetics, distribution, and localization) of a drug labeled with a cold isotope or regarding human physiology, pathophysiology, or biochemistry.
- The research is not intended for immediate therapeutic, diagnostic, or preventive benefit to the study subject.
- The dose to be administered is known not to cause any clinically detectable pharmacologic effect in humans based on clinical data from published literature or other valid human studies.
- The quality of the cold isotope meets relevant quality standards.
- The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and the requirements for informed consent (21 CFR part 50).

No adverse events are expected from [6,6-$^{2}H_{2}$], or [2-^{13}C]-Glucose infusion.

[^{13}C,^{15}N]-glucagon will be infused. The labeled glucagon is supplied to the PI (Dr. A. Basu: R01-DK-085516) in powder form. Each lot of the labeled products is carefully analyzed for strict quality control measures and procedures are followed by the Proteomics Core Laboratory as per OSHA guidelines. The labeled glucagon preparations are stored in single dose lyophilized vials. Lyophilized samples aliquoted into single-dose vials are capped and stored in a desiccator at 4°C. On the morning of each study visit, the lyophilized glucagon will be reconstituted in normal saline and infused after appropriate testing to ensure sterility by the bubble test. **This test ensures compliance to the USP 797** procedures for preparing the infusates of the labeled glucagon for the studies proposed. We have conducted IRB approved studies in ~150 healthy and type 1 diabetes participants since 2016 (R01-DK-085516) without any adverse effects related to the labeled glucagon infusions. Appropriate pyrogen and bacteriological testing are performed as per guidelines to ensure sterility. The anticipated risk of allergic reactions to the labeled glucagon per se is extremely low since it is unmodified (except for the labels) human glucagon that is manufactured and infused. Thus far we have not observed any allergic reactions in any of the close to 150 participants studied over the last 5-6 years. Participants experiencing allergies will be excluded from any further participation in studies involving this application.

It is noteworthy that the glucagon isotopes are labeled with non-radioactive, stable isotopes. Hence there is no radiation risk related to these isotopes. Additionally, the amounts of labeled glucagon infused during the study visit will be far less than a clinically relevant dose of glucagon (1 mg) that is normally used to treat hypoglycemia. Hence the risk of hyperglycemia related to the labeled glucagon infusion is minimal.

MRI Scan**:** MRI scanning is a painless procedure that requires participants to lie quietly on a padded table. Because of the magnetic field and radio frequencies, participants with any type of metal in their body will NOT have an MRI. This may include things like pacemakers, aneurysm clips or shrapnel. Therefore, participants with such devices will not be able to participate in the MRI portion of this study. Individuals with clinically significant anxiety related to MRI and/or claustrophobia are also excluded.

Meal Tolerance Testing**:** Eating the meal may cause transient nausea or dumping syndrome in post-bariatric hypoglycemia patients. We have performed over 50 meal tolerance tests in this population without complication. The mixed meal testing protocol often causes hypoglycemia in these patients. However, patients are unlikely to experience severe hypoglycemia because of frequent measurements (every 5 minutes by in-room YSI), clinical observation, and protocols in place to treat hypoglycemia should it develop. The study physician will be physically present on site in the clinical research unit during testing in order to closely monitor participants and ensure safety.

Screening Tests and Procedures: It is possible that as a result of the screening process a subject may learn that they have a health disorder that they were unaware they had. Every effort will be made to help the participant obtain the care that they need.

Breach of Confidentiality: While every effort will be made to protect the confidentiality of participant identifiable information, there is the potential loss of confidentiality by participating in this study.

Inconvenience and Unknown Risks: Participants may be inconvenienced by the time commitment involved in participation in the study. There may be other risks from this study not yet identified.

Participants can choose not to participate in the clinical research project and can withdraw consent at any time.

8.2 Adequacy of Protection from Risks

Participants will be recruited from the Endocrinology Clinic at UAB, the Joslin Hypoglycemia Clinic, and as described above. The study will be explained to the potential subject initially in the clinical setting in a private room, during a phone call, or during a subsequent telephone session. Those interested will be scheduled for a screening visit. The consent process will involve initial discussion of the purpose and scope of the research as well as risks and benefits. Ample opportunity will be provided for participants to read consent, share with family or other health care providers as well as have all questions answered by the Principal Investigator or their designated study staff, prior to obtaining written informed consent using IRB approved documents. The consent form will include a description of risks and benefits, alternative possible procedures, the availability of the investigating physicians throughout the study to discuss any concerns, the availability of the IRB to the patient throughout the study to discuss any concerns, and the fact that the patient can withdraw from the study at any time with no change in his/her standard treatment. If the patient consents to enroll in the study, and the consent form is signed, further evaluation in the form of the medical and screening visit will take place to determine eligibility. One copy of the consent form will be retained by the principal investigator and one copy will be provided to the subject.

Hypoglycemia safety and mitigation plan: Firstly, our inclusion/exclusion criteria are designed to exclude individuals at high risk for complications related to hypoglycemia, such as ischemic cardiovascular disease. Secondly, our protocol provides close monitoring of participants to ensure safety at all times. Plasma glucose will be measured at 10 minute intervals, and every 5 minutes when glucose levels are <90 mg/dL, using an in-room instrument calibrated daily. A second instrument is available in the same room, serving as backup in case of instrument malfunction. A medically-trained member of the study team (study nurse, physician, nurse practitioner, or all) remains with the patient at all times during the study.

If at any time during the study visit the glucose level falls below 54 mg/dl, or if the patient develops neuroglycopenia, or if the participant develops severe discomfort with signs and symptoms of hypoglycemia, then treatment for clinically significant hypoglycemia will be initiated as clinically indicated. Options will include (1) intravenous glucose (one ampule of D50 [25 grams in 50 mL, 50% glucose solution]) administered over 2-5 minutes via the intravenous line. (2) commercial glucagon 1.0 mg, administered subcutaneously. For both approaches, glucose will be re-measured every 5 minutes and treatment repeated as indicated, based on achieving glucose >54 mg/dL and resolution of neuroglycopenia, at the discretion of the study physician. If 2 doses of intravenous glucose are required, an infusion of 20% dextrose will be initiated at a rate of 100 mL/hour and glucose levels will be monitored every 10 minutes, and rate adjustments will be made according to glucose results. Once glucose levels are above 100 mg/dL, intravenous infusion will be tapered with continued monitoring as indicated clinically by the study physician.

If after treatment for clinically significant hypoglycemia the glucose remains <70 mg/dL, but >54 mg/dL, neuroglycopenia is resolved, and if the patient is able to swallow, then the team will administer oral glucose 15-30 grams (glucose tablets or gel). If the patient is unable to tolerate oral intake (e.g. due to nausea), the team will administer ½ ampule of D50 (12.5 grams in 25 mL) over 2-5 minutes intravenously. Once hypoglycemia has resolved (glucose >70 mg/dL), neuroglycopenia has resolved, and patient is able to able to swallow, then the team will provide

a standard lunch meal containing complex, low-GI carbohydrates, fat and protein to maintain euglycemia.

Safety monitoring will include frequent blood glucose monitoring during study visits by study staff. A study physician will be physically present in the study rooms, or on site, at all times to evaluate clinical status and glycemic levels. All labs will be reviewed by a designated member of the study team within 24 hours. Abnormal labs are communicated by alerts to the principal investigator or other medical research staff directly from the laboratory and will be communicated to the participant and treating physician if clinically actionable. Adverse events will be reported to the IRB, as appropriate, to ensure the safety of participants.

All records will be kept in a locked office and computer files secured by a password. The identifying information will be kept separate from study data. Plasma, serum, and urine samples will be labeled only with a numeric and acrostic code. Participants will be given the opportunity to decide whether or not the clinical information gained from the study should be shared with their health care providers. All personal identifying information, such as address, and date of birth, will be maintained separately from study data and will not be shared with collaborating laboratories, repositories or other investigators. Participants will be made aware that, despite these measures, confidentiality cannot be totally ensured.

8. Possible Benefits:

This project is not designed to be of direct benefit to any individual subject, although it is hoped that through the knowledge gained from this study, there will be general benefit to persons with or at risk of development of PBH. Participants enrolled in this study receive comprehensive medical evaluation. As such, risks are appropriate in relation to the potential significant benefits for the significant number of persons with or at risk for hypoglycemia after bariatric surgery. Thus, the potential benefits to society are likely to outweigh the risks to the individual subject.

The ultimate purpose of this study is develop a single-hormone glucagon-only closed loop system for treatment and prevention of hypoglycemia. Given the epidemic of obesity in the US, and the increasing use of bariatric surgery to treat obesity, coupled with the prevalence of up to 30% of post-bariatric patients who develop PBH (likely a chronic/life-long condition), and that there are no currently FDA approved treatments for this indication, understanding the causes underlying this condition is important in order to develop new approaches to identify high risk patients before bariatric surgery, to identify markers of risk, and to develop new, more effective treatment strategies

9. Conflict of Interest: none.

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