HRP-503B – BIOMEDICAL RESEARCH PROTOCOL
(2017-1)

Protocol Title: Mechanisms for restoration of hypoglycemia awareness

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SECTION I: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.
   Aim 1: Determine the impact of frequent hypoglycemia and hypoglycemia unawareness on brain responses to visual food cues as well as brain functional connectivity in T1DM patients.
   Aim 2: Assess the impact of antecedent bouts of hypoglycemia on brain glucose transport/metabolism during hyperglycemia in T1DM Unaware, T1DM Aware as well as healthy controls using 1H, 13C magnetic resonance spectroscopy (MRS).
   Aim 3: Determine if reducing hypoglycemia and glycemic variability using a continuous glucose monitor (CGM) reverses altered brain glucose transport/metabolism and functional connectivity in hypoglycemia unaware T1DM patients.
   Optional Sub Study: Assess the impact COVID-19 pandemic and related shutdown has had on the management of diabetes amongst patients with T1DM.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.
   5 years

3. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.
   The DCCT and EDIC trials established the benefits lowering blood glucose to "near normal" levels in type 1 diabetes (T1DM) patients [1-3]. This has led to the widespread use of more intensified insulin therapy to prevent or delay diabetes complications. However, global application of intensive insulin therapy has been limited by a higher rate of severe hypoglycemia, often occurring without warning symptoms and thus reducing the patient's ability to take corrective action [4]. As a result, many patients do not and/or become afraid to achieve target glycemic goals, since their immediate fear of hypoglycemia exceeds their fear of future long-term complications [5,6]. In non-diabetic subjects, hypoglycemia provokes a multi-tiered defense system including: 1) suppression of

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endogenous insulin secretion; 2) secretion of counterregulatory hormones and 3) subjective awareness of hypoglycemia provoking carbohydrate ingestion. A small decline in glucose (low 70 mg/dl range) initially acts to suppress insulin secretion, which is followed by glucagon and catecholamine release (at 65-69mg/dl) that act in concert to restore euglycemia by stimulating hepatic glucose production and diminishing glucose uptake. Other anti-insulin hormones (e.g. GH and cortisol) come into play somewhat later to enhance and help sustain counter regulation. If glucose falls further (<60-64mg/dl) autonomic and neuroglycopenic symptoms appear, triggering food ingestion. Thus, severe hypoglycemia is rare clinically in the absence of diabetes. In T1DM, this defense system is interrupted at every level. The loss of endogenous insulin and reliance on exogenous insulin make suppression of plasma insulin impossible. Beta cell loss is also closely linked to loss of glucagon responses to hypoglycemia as well, a stimulus specific defect that rapidly appears in nearly all T1DM patients [7-9]. Thus, T1DM patients are very vulnerable to reduced catecholamine release; a scenario associated with intensive insulin therapy, long disease duration, and in particular antecedent hypoglycemia events [10-16]. Loss of adrenomedullary responses appears to be in large part a functional disorder linked to iatrogenic hypoglycemia, since hypoglycemia avoidance commonly reverses the defect [17,18].

The effects of recurrent hypoglycemia, intensive insulin therapy and hypoglycemia unawareness on cognitive function in T1DM patients during hypoglycemia are somewhat controversial with studies reporting differing results. Specifically, it remains uncertain whether the effect of intensive insulin therapy to lower the glucose level needed to activate adrenomedullary responses is mirrored identically by changes in the threshold needed to impair cognitive function, many support this view [19-22], but not all studies [23,24]. Thus, while T1DM patients with a history of frequent hypoglycemia often require a greater glucose fall before cognitive impairment develops, there is some response variability. On the other hand, patients with hypoglycemia unawareness appear to show a more profound disturbance in higher cognitive function than T1DM controls who maintain awareness during acute hypoglycemia [25].

It is noteworthy in this regard, that both healthy human subjects rendered mildly hypoglycemic for several days and T1DM patients receiving intensive insulin therapy are able to maintain brain glucose uptake during acute hypoglycemia, whereas healthy controls show a 20-30% decrease [26,27], suggesting that prior hypoglycemia exposure induces the human brain to more efficiently use glucose much like that seen in rodent studies [28,29]. Furthermore, organ balance studies found a larger fraction of glucose is non-oxidatively metabolized in intensively treated T1DM patients, suggesting an increased ability to use alternative fuels, e.g. monocarboxylic acids [30]. In keeping with this possibility it has been reported that brain acetate transport and metabolism are increased 2-fold in intensively treated T1DM patients [31], findings supported by our data showing upregulation of monocarboxylic acid transporter (MCT) gene expression in diabetic rats exposed to recurrent hypoglycemia [32]. Thus, T1DM patients appear to adapt to antecedent hypoglycemia in part by developing both more efficient brain transport and oxidation of monocarboxylic acids. Use of alternative fuels for brain metabolism may be a useful protective hypoglycemia adaptation, but it might also alter proper awareness of a falling blood glucose since ATP generated by mitochondrial monocarboxylic acid oxidation is most effective in supplying energy for basic brain functions, but is less useful when rapid energy is needed to drive the glutamate-glutamine cycle for complex functions. This is better served by the quick energy supplied by anaerobic glycolysis, much like in an exercising muscle.

Recent studies using the hypoglycemic clamp and fMRI scanning in hypoglycemia unaware and aware T1DM patients and healthy controls have showed distinct differences in patterns of brain responses. In particular, T1DM patients who are aware of hypoglycemia (T1DM-Aware) have greater activity in sensory integration brain regions (e.g. parietal lobe and caudate nucleus) in response to hypoglycemia, whereas hypoglycemia unaware T1DM patients (T1DM-Unaware) show no detectable changes in brain reward regions during hypoglycemia. The underlying mechanisms behind these differences remain unclear. Thus, we believe it is important clinically to assess: 1) if these differences are driven purely by recurrent hypoglycemia or by other closely linked factors

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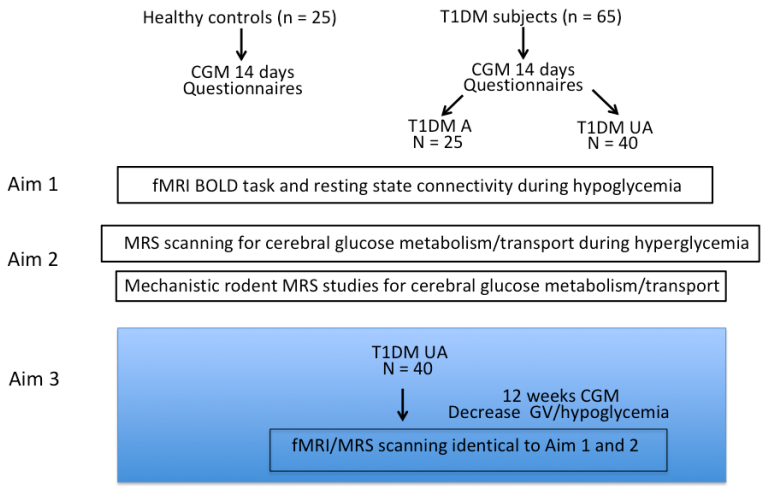
(e.g. glycemic variability); 2) the molecular and metabolic mechanisms by which unawareness leads to the suppression of CNS activity in the context of hypoglycemia; and 3) whether hypoglycemia avoidance using CGM restores CNS activation and metabolism toward normal levels and offers a therapeutic approach to more effectively combat neurocognitive dysfunction associated with intensive treatment of T1DM patients.

4. **Research Plan:** Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. **Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths**. Describe the setting in which the research will take place.

   Aim 1: Studies will be conducted in non-diabetic controls (N=25) and 2 groups of subjects with T1DM without significant complications: (1) hypoglycemia unaware (T1DM-UA) (N=40) and (2) hypoglycemia aware (T1DM-A) (N=25).

   1. Screening visit: Volunteers will be screened at the Yale New Haven Hospital (YNHH) Hospital Research Unit (HRU) or the Yale Center for Clinical Investigation (YCCI) Church Street Research Unit (CSRU) with a medical history, physical exam, and EKG. Fasting blood work will be collected for A1C, ALT, TSH, electrolytes, renal function, CBC, and C-peptide. A urine pregnancy test will be performed on all women of childbearing potential, and women who are pregnant will be excluded.



   2. Continuous glucose monitoring (CGM) placement: For up to 14 days, all eligible subjects will be asked to wear a blinded continuous glucose monitor (FreeStyle Libre Pro). Given that this data is blinded, patients will be informed that it is not a replacement for self-monitoring of blood glucose. Subjects should continue their normal testing routine while wearing the sensor. CGM placement will take place at the HRU or CSRU. Those not already using CGM in their usual care will be trained in its proper use by experienced study staff. Patients already using CGM will be allowed to use their usual CGM, however, they will also be asked to wear a second blinded study sensor. Subjects will be provided with our contact information in the event that they have any questions, concerns or issues related to the CGM. Intense exercise may cause the sensor to loosen due to sweat or movement of the sensor. If the CGM sensor falls off while the subject is wearing it, we will ask them to return to the HRU or CSRU so that a new sensor can be re-inserted.

      The CGM consists of a sensor that measures glucose levels from the interstitial tissue. The sensor is sterile and comes in an unopened package. It has a plastic wire-like tip that is placed under the skin and continuously measures the glucose levels. A trained study staff member will insert the CGM wire-like tip under the volunteer's skin with the use of the CGM sensor insertion kit. Each sensor is single use only and disposable. The FreeStyle Libre Pro does not require any calibration, as is the case with other CGM devices. This means that no finger sticks are required for its use.

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a. Hypoglycemia questionnaires: All T1DM participants will be asked to complete questionnaires assessing hypoglycemia at the time of the CGM placement. The responses to these questionnaires and the questionnaires during the fMRI scan described below, in addition to analysis of the counterregulatory hormones obtained during the fMRI scan, will be used to categorize T1DM participants as either aware (T1DM-A) or unaware (T1DM-UA).
b. Neurocognitive questionnaires: We will collect metrics of education level and neurocognitive function amongst all participants at the time of the CGM placement to assesses executive function, episodic memory language, processing speed, working memory and attention.

3. CGM removal:
   After 14 days of wearing the CGM, it will be removed by a trained study staff member. To remove the sensor, the adhesive patch will be gently peeled off from the skin, together with the sensor. Removal of the sensor is a painless procedure. The sensor will be discarded after use.
   Note regarding order of visits: CGM placement will occur up to 14 days prior to the subject's first scan (fMRI or MRS). CGM removal will occur on the same day as the subject's first scan (fMRI or MRS). Due to the many complexities of scheduling, although the fMRI is part of Aim 1, the subject may have the MRS scan done first, which is part of Aim 2. The order of scans in no way effects subject safety or research integrity. T1DM subjects will have a second CGM placed after the first scan and will wear this CGM until the second scan, where it will be removed. Therefore, T1DM subjects will wear the CGM two times, once before each scan for up to 14 days prior.
4. fMRI Scan with hyperinsulinemic euglycemia-mild hypoglycemia clamp: All subjects will arrive on the morning of the scan at 7AM following an 8-hr fast. T1DM subjects will be instructed to continue their home insulin regimen until they arrive at the Yale Magnetic Resonance Research Center (MRRC) that is equipped with infusion rooms for IV and line placements. A urine pregnancy test will be performed on all women of childbearing potential, and women who are pregnant will be excluded. Following IV placements and baseline blood collections at ~8 AM subjects will receive an insulin infusion (2 mU/kg/min) together with a variable glucose infusion to keep glucose levels at ~100-110 mg/dl. Once glucose levels are stabilized outside the magnet (~45 min), they will be transferred into the MRI for a 2-step hyperinsulinemic euglycemic (90-100 mg/dl)-mild hypoglycemic (55-60 mg/dl) clamp. Initially, baseline structural scans will be obtained, followed by blood oxygen level dependent (BOLD) contrast acquisitions during visual cues and cognitive tasks. At each clamp step, participants will also be asked to complete questionnaires assessing hypoglycemia. Plasma glucose will be measured every 5-10 min min. Throughout, blood will be obtained for insulin, glucagon, cortisol, catecholamines and other hormonal and metabolic blood tests. A1c will also be measured at this visit.
   a. Food Cues: Picture stimuli for fMRI are high-calorie/high carbohydrate food, and non-food neutral pictures selected from Web sites and from the International Affective Picture System.
   b. Cognitive tasks: include tasks to assess working memory (N-Back), attention (Grad-CPT) and reward (Card guess) that have been adapted for the MRI setting. In order for these tasks to be as accurate as possible, the subjects will need to think that they will get a monetary bonus depending on how they do. In reality, all subjects will "win" and get this bonus.

Aim 2: The same 3 subject groups who participated in Aim 1 will participate in Aim 2. As previously stated, for ease of scheduling, this visit may precede the fMRI visit discussed above.

1. 1H-[13C] MRS scanning and Hyperglycemic Clamp: Participants will arrive at MRRC at 7AM on the morning of the study after an 8 hour overnight fast. A urine pregnancy test will be performed on all women of childbearing potential, and women who are pregnant will be excluded. Following IV placements and baseline blood collection, at ~8 AM all T1DM subjects will receive a low dose insulin infusion to prevent ketosis during the MRS study and a variable glucose infusion to keep plasma glucose levels at ~100-110 mg/dl. Once glucose levels are stabilized, the subjects will be transferred into the 4T

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scanner (^{13}C ^{1}H for POCE) for the hyperglycemia clamp with labeled glucose. At the start of the study, plasma glucose will be increased to ~180 mg/dl by a primed continuous infusion of 99% 1-13C glucose (Cambridge Isotope Lab) (dextrose 1.1 M) and will be maintained at ~180 mg/dl throughout the study with a variable infusion of 60% 13C glucose (dextrose 1.1 M). Plasma glucose will be measured every 5 min. Throughout, blood will be obtained for insulin, fructose, sorbitol, 13C enrichment and other hormonal and metabolic blood tests. A1c will also be measured at this visit. After tuning, calibration, and acquisition of scout images for anatomical localization, metabolite concentrations will be obtained from 18 ml^3 voxel. Using metabolic modeling we will determine rates of glucose uptake, TCA cycle (VTCA) and glutamate/glutamine cycling (Vcycle) as well as measure absolute glucose and fructose levels and 13C labeling.

Aim 3: T1DM-UA subjects that completed Aims 1 and 2 will participate in Aim 3.

1. Group Assignment: 40 T1DM-UA subjects will be assigned to use a Dexcom G6 continuous monitoring system for 8 to 12 weeks, until they reach stable glycemic variability measured by coefficient of variation (CV) Additional training sessions will be conducted as needed to ensure familiarity and comfort with system operation.
2. Continuous glucose monitoring (CGM): Dexcom G6 continuous glucose monitoring system (DexcomG6 Pro System) is a real time continuous glucose monitoring device indicated for the management of diabetes in persons age 2 years and older. Is factory calibrated so there is no need for calibrations. It replaces fingerstick blood glucose testing for diabetes treatment decisions. The real-time Dexcom G6 System provides glucose trends, several sequential readings over time and glucose excursions facilitating care plan adjustments. This device may also be used as a retrospective glucose-recording device indicated for assessing glycemic variability.

   The CGM consists of a sensor that measures glucose levels from the interstitial tissue. The CGM includes an applicator with a build-in sensor, which is packaged in a sterile unopened package. The sensor has a plastic wire-like tip that is placed under the skin and continuously measures the glucose level. The sensor can be worn for up to 10 days. It also comes with a transmitter, which connects to the sensor wire and sends real-time readings wirelessly to a receiver.
3. Follow-up visits: Subjects will be instructed to share data remotely to the study team through Dexcom Clarity, a continuous glucose monitor management application run by Dexcom. A member of the study team (physician and/or advanced practice register nurse) will review the information on a weekly basis and will make changes to their diabetes management as needed. Study participants will have in-person check-in visits for history, exam, and review of any potential adverse clinical or device-related events, if needed.

   A study team member will communicate directly with the participants and participant's health care providers as they join the study and throughout the study, and will inform them about relevant clinical findings on the CGM including time spent in hyperglycemia and new increase or extended periods of hypoglycemia
4. Repeat brain MRS and fMRI scans: At 12 weeks, all subjects will repeat fMRI and MRS analyses of brain glucose transport/metabolism under hypo- and hyperglycemia exactly as described in Aims 1 and 2. At 12 weeks, the subjects in Group 2 will repeat fMRI and MRS analyses of brain glucose transport/metabolism under hypo- and hyperglycemia exactly as described in Aims 1 and 2. A1c will also be measured at all scan visits. Hypoglycemia questionnaires will also be repeated, in addition to repeat cognitive assessments (using different test versions to reduce practice effects).

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Note regarding order of visits: As is the case in Aims 1 and 2, CGM placement will occur up to 14 days prior to the subject's first scan of Aim 3 (fMRI or MRS). CGM removal will occur on the same day as the subject's first scan of Aim 3 (fMRI or MRS). Due to the many complexities of scheduling, there is no order that the fMRI and MRS must be performed. The order of scans in no way effects subject safety or research integrity.

Optional Sub Study: Subjects from Aim 1 and 2 with T1DM: (1) hypoglycemia unaware (T1DM-UA) (N=40) and (2) hypoglycemia aware (T1DM-A) (N=25) will be given the opportunity to participate in this optional sub study. We will only contact those previously enrolled participants who agreed to be re-contacted for future research by indicating such on the AIM 1 and 2 consent form.

1. Consenting: Subjects will be consented for this optional Sub study through the Qualtrics study link. The consent form will be provided as a study question. Subject will either click yes or no to agreeing to completed issued questionnaires. If subject, clicks yes and provides name and date, questions from survey will be provided. If subject clicks no, subject will not be provided additional questionnaires.
2. Subject Contact: Only subjects with T1DM and already eligible to participate in Aim 1 and Aim 2 will be contacted via telephone and explained the purpose of the sub-study.
3. Diabetes and health status questionnaires: All participants who agree to participate will be asked to complete questionnaires (all standard, well-validated questionnaires) for assessing hypoglycemia, self-evaluate their diabetes healthcare, general stress and anxiety during the COVID-19 pandemic and unprecedented stay-at-home experience. Questionnaires include:

    a. Clarke, Gold, Pedersen : Classifies awareness of hypoglycemia, three different measures

    b. Fear of Hypo : Measures worry about hypo episodes and behavior to avoid them, 2 sub scores available.

    c. Hypo AQ : Hypo Awareness Questionnaire measures awareness, symptom level, and frequency of hypo episodes

    d. Hypo Confidence : measures confidence in managing hypo episodes

    e. DTSQ: Diabetes Treatment Satisfaction Questionnaire, measures satisfaction, hyper frequency, and hypo frequency.

    f. DQOL: Diabetes Quality of Life: four sub scores (satisfaction, impact, worry: diabetes, worry: social)

    g. BDI-II : Beck Depression Inventory: measures depressive symptoms

    h. PSS: Perceived Stress score: measures how "unpredictable, uncontrollable, and overloaded respondents find their lives"

    i. STAI: State Trait Anxiety Inventory: measure of anxiety symptoms as well as generalized anxiety

    j. BIS/BAS: Behavioral avoidance/inhibition scales:

    k. PANAS: Positive Affect, Negative Affect Score: Measures Affect

    l. SF/RAND: short form quality of life questionnaire: designed to be general.

5. Genetic Testing N/A ☒

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6. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.
   25 healthy control subjects, ages ≥ 18 years, BMI ≥ 18.0
   65 subjects with T1DM, ages ≥ 18 years, BMI ≥ 18.0

7. **Subject classification:** Check off all classifications of subjects that will be specifically recruited for enrollment in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☐Children	☐ Healthy	☐Fetal material, placenta, or dead fetus
☐Non-English Speaking	☐ Prisoners	☐Economically disadvantaged persons
☐Decisionally Impaired	☐ Employees	☐Pregnant women and/or fetuses
☐Yale Students	☐ Females of childbearing potential	

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects?
Yes ☐ No ☒

8. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?
   Inclusion Criteria:
   - Ages ≥ 18 years
   - Healthy, non-diabetic control or T1DM
   - BMI ≥ 18.0

   Exclusion Criteria:
   - Creatinine > 1.5 mg/dL
   - Hct < 35% for females, < 39% for males
   - ALT > 2.5 X ULN
   - untreated thyroid disease
   - uncontrolled hypertension
   - neurologic disorders
   - untreated depression or change in antidepressant regimen in last 3 months
   - use of any anxiolytic medications (benzodiazepine) or antipsychotic medications
   - greater than 5% change in weight in last 3 months
   - malignancy
   - current or recent steroid use in last 3 months
   - illicit drug use
   - significant complications related to diabetes (peripheral neuropathy, proliferative retinopathy)
   - inability to enter MRI (per standard MRI safety guidelines)
   - for women: pregnancy or breastfeeding

9. How will **eligibility** be determined, and by whom? Write here
   Eligibility will be determined by a qualified physician or nurse practitioner associated with the research protocol and will be based upon the above inclusion/exclusion criteria. For the sub-study, those previously enrolled subjects who indicated they agreed to future contact will be eligible.

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10. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

Continuous glucose monitoring (CGM) (subjects with T1DM only):

The CGM poses no major risks to the subjects. Participants may feel a mild discomfort (pin prick sensation) during the sensor insertion. Some individuals may experience black and blueness of the skin at the insertion site of the CGM sensor, which resolves by itself in a few days. Redness and discomfort (inflammation) can occur at the sensor insertion site. Some individuals may be sensitive to the adhesive that keeps the sensor attached to the skin. If the subject notices significant skin irritation around or under the sensor, they will be instructed to contact us and it can be removed. Rarely sensors may fracture and a small piece may remain under the skin which will need to be removed by a study clinician. This may cause mild discomfort, bruising, or temporary bleeding. The subjects will be informed that the sensor must be removed before an MRI, a CT scan, or diathermy treatment.

Insulin and glucose infusion:

The infusion of insulin and glucose to achieve hypoglycemia will potentially result in symptoms of varying severity. Some subjects will experience little or no symptoms of sleepiness, hunger, anxiety, palpitations, diaphoresis, difficulty concentrating, mild confusion and/or tremor. There is a small risk that plasma glucose will fall below the predetermined level (3.0-3.5 mM, 55-60 mg/dl) resulting in exaggerated symptoms. All such symptoms would be rapidly reversible with an intravenous dextrose infusion.

The infusion of glucose to achieve hyperglycemia (~180 mg/dl) is not associated with any specific symptoms or significant adverse effects. This modestly high glucose level can be seen in poorly controlled diabetic patients following a meal.

13C glucose:

The infusion of 13C glucose involves no radioactivity, and the glucose is metabolized just as unlabeled glucose. Clinical research studies using 13C glucose have been used at Yale for decades with no adverse effects. The glucose solution is purchased from (Cambridge Isotopes, Inc.) in powder form which has been certified to be sterile and free of pyrogens. The glucose is made into a solution of 20gram/100 ml (D-20) by the Yale New Haven Hospital Investigational Drug Service (IDS). The solutions are tested to be free of pyrogens and to be sterile before being administered. All certifications are kept with the IDS.

MRI/MRS:

Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x- rays, to take pictures and measure chemicals of different parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines.

A member of our research staff will accompany the subject to the MRRC and remain there for the scan. The subject will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens to the subject, they may ask to stop the study at any time and we will take them out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but we will instruct the subject to tell the research staff if they have them.

There are some risks with an MR study for certain people. If the subject has a pacemaker or some metal objects inside their body, they may not be in this study because the strong magnets in the MR scanner might harm them. Another risk is the possibility of metal objects being pulled into the magnet and hitting the subject or personnel. To lower this risk, all people involved with the study must remove all metal from their clothing and all metal objects from their pockets. We also ask all people involved with the study to walk through a detector designed to detect metal objects. It is important to know that no metal can be brought into the magnet room

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at any time. Also, once the subject is in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet.
We instruct the subject to read and answer very carefully the questions on the MR Safety Questionnaire related to their personal safety.
This MR study is for research purposes only and is not in any way a health care examination of the brain. The scans performed in this study are not designed to find abnormalities. The principal investigator, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a health care evaluation of the images. If a worrisome finding is seen on the subject's scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the principal investigator or consulting physician will contact the subject, inform them of the finding, and recommend that the subject seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie only with the subject and their physician. The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that the subject receives based on these findings. The images collected in this study are not a health care MR exam and for that reason, they will not be made available for health care purposes.

Phlebotomy:
Phlebotomy can result in anemia, although the amount of blood taken for these studies should not result in clinically-significant anemia. Subjects are excluded if hemoglobin is less than 10 gms/dL.

Questionnaires:
The questionnaires are generally benign in nature. The major inconvenience is the time taken to complete them and a possible breach of confidentiality.

11. Minimizing Risks: Describe the manner in which the above-mentioned risks will be minimized.
Continuous glucose monitoring (CGM) (subjects with T1DM only):
The CGM catheter will be placed under sterile conditions by experienced staff members. In the case of signs of inflammation or bruising at the sensor insertion site, the sensor will be removed immediately and standard of care will apply. The participants will be given one of the study physician or nurse practitioner's cell phone number to contact for any questions, concerns or problems.

Insulin and glucose infusion:
Intravenous catheters will be placed under sterile conditions by experienced staff members. During glucose infusions, 20% dextrose is used reducing the risk of thrombophlebitis normally associated with the use of excessively hypertonic glucose solutions. All studies will be performed under the direct supervision of a physician or nurse practitioner. All solutions for infusion will be pyrogen free and prepared in a sterile environment. The infusion of insulin and glucose to achieve hypoglycemia will potentially result in symptoms of varying severity. All such symptoms would be rapidly reversible with an intravenous dextrose infusion. To avoid excess hypoglycemia during the hyperinsulinemic clamp, plasma glucose will be checked every 5 minutes using a bedside glucose monitor. Based on these measurements, the plasma glucose will be adjusted via the 20% dextrose infusion.

13C glucose:
The infusion of 13C glucose involves no radioactivity, and the glucose is metabolized just as unlabeled glucose. Clinical research studies using 13C glucose have been used at Yale for decades with no adverse effects. The glucose solution is purchased from (Cambridge Isotopes, Inc.) in powder form which has been certified to be sterile and free of pyrogens. The glucose is made into a solution of 20gram/100 ml (D-20) by the Yale New

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Haven Hospital Investigational Drug Service (IDS). The solutions are tested to be free of pyrogens and to be sterile before being administered. All certifications are kept with the IDS.

MRI/MRS:
The major risk is the possibility of metal flying into the magnet. All potential study subjects will be screened for MR safety using the standard MR safety protocol developed by the MRRC at the screening visit and again on the day of an MR scan. Subjects and all personnel are also scanned with a metal detector before entering the magnet area and all metal objects are removed. Subjects with contraindications to MR scanning are not eligible for this study. Some subjects feel claustrophobic or experience physical discomfort inside the scanner. Communication is set up so that the subjects can talk to the investigators and may be removed from the scanner if they are uncomfortable. A member of our research staff will accompany the subject to the MRRC and remain there for the scan. A RN will be present inside the scanner at all times with the subject.

Phlebotomy:
Phlebotomy can result in anemia, although the amount of blood taken for these studies should not result in clinically significant anemia. Subjects are excluded if hemoglobin is less than 10 gms/dL. Total blood loss in each subject over a 1-month period will be less than a standard blood donation (470 ml for blood donation). The blood drawn for the screen visit will not exceed 60 ml, for the MRS/hyperclamp visit will not exceed 165 ml and for the fMRI/hypoclamp will not exceed 135 ml. Thus, those that participate in Aim 1 and 2 will have no more than 360 ml total blood drawn. Those that also participate in Aim 3 will have no more than 660 ml total blood drawn over the course of 3 months (360 ml for screen and Aim 1 and 2, and then 3 months later, 300 ml for Aim 3). All subjects having donated blood within 30 days of the study will be asked to postpone study participation (with a repeat blood count prior to future enrollment), and patients will be advised to refrain from blood donation for 30 days after study completion.

Questionnaires:
The major inconvenience of the questionnaires is the time taken to complete them and a possible breach of confidentiality. Study participation is voluntary, and subjects are informed that they are free to drop out at any time without penalty. All data will be kept confidential except in cases of imminent danger to the participants. Such limits to confidentiality will be clearly explained to participants verbally and in the written consent forms. Good clinical and research practice procedures and HIPAA regulations will be followed. No subjects are identified by name in any of the published literature and only by code in major data storage areas.

12. Data and Safety Monitoring Plan: Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.)
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study?
    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? N/A
    c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://your.yale.edu/policies-procedures/forms/420-fr-01-data-and-safety-monitoring-plans-templates for
        i. Minimal risk
        ii. Greater than minimal

    d. For multi-site studies for which the Yale PI serves as the lead investigator: N/A

Greater Than Minimal Risk DSMP

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1. Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency, which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator or the IRB have the authority to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed greater than minimal for the following reasons: (choose those that apply)

1. We do not view the risks associated with the phlebotomy, fMRI/MRS, CGM, , and insulin and glucose infusion as minimal risks.

Although we have assessed the proposed study as one of greater than minimal risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator, Dr. Renata Belfort de Aguiar, according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4. Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

1. Mild adverse event
2. Moderate adverse event
3. Severe

5. Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events:
In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it results in any of the following outcomes:

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1. Death;
2. A life-threatening experience in-patient hospitalization or prolongation of existing hospitalization;
3. A persistent or significant disability or incapacity;
4. A congenital anomaly or birth defect; OR
5. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

6. Plan for reporting UPIRSOs (including Adverse Events) to the IRB

The principal investigator will report the following types of events to the IRB:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND
2. Is related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – *serious, unexpected, and related adverse events* and *unanticipated adverse device effects*. ***Please note*** that adverse events are reportable to the IRB as UPIRSOs only if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the *prompt* reporting requirements described in IRB Policy 710 should be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented. In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

13. Statistical Considerations: Describe the statistical analyses that support the study design.
Data analysis will be conducted in collaboration with James Dziura, PhD at the Yale Center for Analytic Sciences. For Aim 1, we will require 25 T1DM aware and 25 healthy controls to provide 80% power to detect differences of 0.013 between them and the T1DM-Unaware subjects at the Bonferonni corrected 0.017 significance level. This sample size will also provide 80% power at the 0.01 level of significance to detect a difference of 0.22 for average brain glucose for Aim 2. We will enroll 27 aware and 27 healthy controls to accommodate a potential

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5% inadequate scan rate. The T1DM Unaware group sample size (n=40) was first determined to provide sufficient power for Aim 3. T1DM Aware and healthy control sample sizes were then estimated based on power for aims 1 and 2. Our preliminary data shows differences in the co-primary outcome of average brain glucose between those with a low glucose variability index and those with a high glucose variability index of 0.455 (SD=0.26). We've also observed a difference of 0.013 (SD=0.016) in our other co-primary outcome change in brain activity from hypoglycemia to euglycemia in the striatum between T1DM Aware and Unaware. Thus, for Aim 3 a sample size of 20 T1DM Unaware subjects per arm will provide 80% power to detect a difference of 0.22 in brain glucose and 0.013 in change in brain activity. We will enroll 65 Unaware for Aim 3 in order to accommodate a potential 10% dropout rate.

Aim 1 Analysis: Comparison of brain activity between groups will be performed using a repeated measures mixed model. The outcome will be brain activity in our primary region of interest, the caudate region of the striatum. Fixed factors for group (unaware, aware, HC), glucose category (eugly, mild hypo, moderate hypo) and their interaction will be included. Linear contrasts comparing changes in brain activation from euglycemia to moderate hypo between groups will be estimated and evaluated at the 0.017 Bonferroni corrected significance level. Secondary analyses will examine other promising ROIs (hypothalamus and prefrontal cortex). Similar analyses will be done to assess group differences in resting state connectivity and hormones.

Aim 2 Analysis: Comparisons of glucose levels in specific brain regions will be made using a repeated measures mixed model. Fixed factors include group and glycemic state (e.g. acute hyperglycemia) and their interaction. A random effect for subject will be used to accommodate correlation between repeated assessments. Linear contrasts comparing differences in average brain glucose between groups will be estimated. A significance level of 0.01 will be used to accommodate multiple pairwise comparisons of groups. Similar secondary analyses will be conducted to assess differences in V_{TCA} between groups and fructose production during hyperglycemia. For the rodent studies our preliminary data showed a 1.5 standardized difference in fructose and glutathione concentrations between groups, so a sample size of 14-15 rats per group will provide 80% power to detect a large standardized difference in study outcomes at the two-sided 0.05 significance level. Between-group comparisons, ANOVA and t-tests will be used for continuous variables, where applicable.

Plan for Missing Data: Prevention is the obvious way to control for bias and loss of power from missing data (71). We will follow the intent to treat principle, requiring follow-up of all subjects regardless of treatment [34]. Postcard and telephone visit reminders will be sent and alternative contacts identified to minimize loss to follow-up. Timely data entry and weekly missing data reports will trigger protocols for obtaining missing data items. Despite these efforts it is reasonable to assume missing data will occur most likely at random [35]. We will evaluate the plausibility of this assumption using logistic regression to identify factors linked to dropout.

SECTION II: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

If this section (or one of its parts, A or B) is not applicable, check off N/A and delete the rest of the section.

A. RADIOTRACERS ☒N/A

B. DRUGS/BIOLOGICS ☒N/A

1. Use of Placebo: ☒Not applicable to this research project

2. Continuation of Drug Therapy After Study Closure ☒Not applicable to this project

B. DEVICES ☐N/A

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1. Are there any investigational devices used or investigational procedures performed at Yale-New Haven Hospital (YNHH) (e.g., in the YNHH Operating Room or YNHH Heart and Vascular Center)? ☒Yes ☐No

If Yes, please be aware of the following requirements:

A YNHH New Product/Trial Request Form must be completed via EPIC: Pull down the Tools tab in the EPIC Banner, Click on Lawson, Click on "Add new" under the New Technology Request Summary and fill out the forms requested including the "Initial Request Form," "Clinical Evidence Summary", and attach any other pertinent documents. Then select "save and submit" to submit your request; AND

Your request must be reviewed and approved in writing by the appropriate YNHH committee before patients/subjects may be scheduled to receive the investigational device or investigational procedure.

2. Background Information: Provide a description of previous human use, known risks, and any other factors that might influence risks. If this is the first time this device is being used in humans, include relevant data on animal models.
   The FreeStyle Libre Glucose Monitoring System is a continuous glucose monitoring (CGM) device indicated for the management of diabetes in persons age 18 and older. It is designed to replace blood glucose testing for diabetes treatment decisions. The System detects trends and tracks patterns aiding in the detection of episodes of hyperglycemia and hypoglycemia, facilitating both acute and long-term therapy adjustments. Interpretation of the System readings should be based on the glucose trends and several sequential readings over time. The System is intended for single patient use and requires a prescription.
   The CGM poses no major risks to the subjects. Participants may feel a mild discomfort (pin prick sensation) during the sensor insertion. Some individuals may experience black and blueness of the skin at the insertion site of the CGM sensor, which resolves by itself in a few days. Redness and discomfort (inflammation) can occur at the sensor insertion site. Rarely sensors may fracture and a small piece may remain under the skin which will need to be removed by a study clinician. This may cause mild discomfort, bruising, or temporary bleeding.

3. Source:
   a) Identify the source of the device to be used. Abbott
   b) Is the device provided free of charge to subjects? ☒Yes ☐No

4. Investigational device accountability: State how the PI, or named designee, ensures that an investigational device is used only in accordance with the research protocol approved by the HIC, and maintains control of the investigational device as follows:

   a) Maintains appropriate records, including receipt of shipment, inventory at the site, dispensation or use by each participant, and final disposition and/or the return of the investigational device (or other disposal if applicable): Electronic records (shipment receipts, inventory, participant use, return of device) will be stored in the study folder on the secured server on a password protected computer or laptop, which is kept in the PI's locked office. Any paper records will be scanned and stored in the file as well.

   b) Documents pertinent information assigned to the investigational device (e.g., date, quantity, batch or serial number, expiration date if applicable, and unique code number): Pertinent information assigned to the investigational device will be stored in an electronic file in the study folder on the secured server on a password protected computer or laptop, which is kept in the PI's locked office.

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c) Stores the investigational device according to the manufacturer's recommendations with respect to temperature, humidity, lighting, and other environmental considerations: Per the manufacturer's recommendations, the sensor kit will be stored in a location between 39-77 degrees Fahrenheit and between 10-90% non-condensing humidity. There are no other environmental considerations.

d) Ensures that the device is stored in a secure area with limited access in accordance with applicable regulatory requirements: The sensor kit will be stored in the PI's locked office in a locked cabinet.

e) Distributes the investigational device to subjects enrolled in the IRB-approved protocol: The device will be provided to subjects enrolled in this IRB-approved protocol after consent has been obtained and a study clinician has explained how to use the device and has determined that the subject understands how to use the device. Sufficient time will be provided in order for the subject to ask any questions they may have about the device and feel confident in using the device. The participants will be given one of the study physician or nurse practitioner's cell phone number to contact for any questions, concerns or problems.

SECTION III: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. **Targeted Enrollment: Give the number of subjects:**
   a. Targeted for enrollment at Yale for this protocol: 150
   b. If this is a multi-site study, give the total number of subjects targeted across all sites: N/A

2. **Indicate recruitment methods below.** Attach copies of any recruitment materials that will be used.

☐ Flyers	☐ Internet/web postings	☐ Radio
☐ Posters	☒ Mass email solicitation	☒ Telephone
☒ Letter	☒ Departmental/Center website	☐ Television
☒ Medical record review*	☒ Departmental/Center research boards	☐ Newspaper
☒ Departmental/Center newsletters	☐ Web-based clinical trial registries	☒ Clinicaltrails.gov
☒ YCCI Recruitment database	☐ Social Media (Twitter/Facebook):	
☐ Other:		

* Requests for medical records should be made through JDAT as described at http://medicine.yale.edu/ycci/oncore/availableservices/datarequests/datarequests.aspx

3. **Recruitment Procedures:**

a. Describe how potential subjects will be identified.

Potential subjects will be identified as indicated below using the methods identified in #2 above:

- Self-Identify
- Provider Referrals
- Study Team Members
- Existing registries, studies, and websites where participants have previously provided informed consent or a request to be contacted for research utilizing their preferred method of contact when known.
- Joint Data Analytics Team (JDAT)

How these options will be utilized is further described in section "b" below.

b. Describe how potential subjects are contacted.

- Self-Identify and Provider Referrals: Participants will self-identify using IRB approved recruitment materials that will be posted locally. Recruitment materials will display the contact information of the study team in addition to a description of the study. Potential participants may also be approached by their medical

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provider. Additionally, participants may be approached by study personnel at community events, or at various locations where potentially eligible patients are seen when facilitated through the participant's treatment team, including at Yale SOM, YNHH, YMG, and VA Connecticut Healthcare System. These sites include the Adult Endocrine Clinic/Yale Diabetes Center (YDC), community clinics and Yale University Health.

- Recruitment through Existing Registries and Studies: In addition, the YDC diabetes registry will be used to identify eligible patients and contact them with IRB approved materials by phone, email or traditional mail (letter), since this category of patients has already given their consent to be contacted for potential trials. Furthermore, participants will be recruited through the YCCI website/registry/Help Us Discover and ClinicalTrials.gov website. Participants may also be identified through their previous participation in HIC #0108012609, 2000021046, 2000020059, 2000020041 and 1408014461.
  Study personnel will use MyChart, traditional mail, telephone or email of patients recruited through this method. If the EPIC registration module provides a preferred method of contact, that method will be utilized. If the participant is not registered in EPIC but has expressed interest in a research study by calling, emailing, filling out an online questionnaire, or speaking with study personnel face-to-face, the patient will be contacted by their means of preference, if stated.
- Recruitment through JDAT: The Joint Data Analytics Team (JDAT) will also be used to query eligible patients in EPIC based on the eligibility criteria outlined above (#8). JDAT will also be used to identify potential subjects with the various permutations of diagnosis codes related to type 1 diabetes. JDAT will identify and send a study communication to potential participants based on these criterion that fall within the following time period: the last 2 years. Potential participants who do not use MyChart will receive a paper mailing including information about the study.
  JDAT will not identify the potential participants to the researchers, and therefore the researchers will receive no identifiable information from JDAT.
- Messaging to Study Participants:
  The following wording will be used in the letter or MyChart message patients receive via JDAT describing the study and inviting them to participate:
    - "You are receiving this notification because you may qualify and be interested in a study looking at mechanisms for restoration of hypoglycemia awareness. The Yale New Haven Health electronic health record system has searched medical conditions to find people who may be good matches for research studies. No one has looked at your record and no information has been shared with any research doctor or research team member. Just because you received this message does not mean that you are in a research study or that you have to decide to be in this or any study.
      You may be interested and eligible to participate in a research study conducted by Yale University investigators to better understand diabetes.
      To opt-out of research, including opting out of receiving future messages about research studies, please email optout@yale.edu or call 1-877-978-8348 and select option #3.
      Title of study: Mechanisms for restoration of hypoglycemia awareness
      Principal Investigator: Renata Belfort de Aguiar, MD
      Study Coordinator: Mari-Lynet Knight
      Phone # 203-737-6067

  The message/mailing to potential study participants will also include the following information, regardless of who sends it:
    - "You are receiving this notification because you may qualify and be interested in a study looking at mechanisms for restoration of hypoglycemia awareness. Approximately 65 subjects will be enrolled in this study.
      Description of Study:

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Aim 1: Determine the impact of frequent hypoglycemia and hypoglycemia unawareness on brain responses to visual food cues as well as brain functional connectivity in T1DM patients.

Aim 2: Assess the Impact of antecedent bouts of hypoglycemia on brain glucose transport/metabolism during hyperglycemia in T1DM Unaware, T1DM Aware as well as healthy controls using 1H, 13C magnetic resonance spectroscopy (MRS).

Aim 3: Determine if reducing hypoglycemia and glycemic variability using CGM reverses altered brain glucose transport/metabolism and functional connectivity in hypoglycemia unaware T1DM patients.

Confidentiality and Privacy: Any personal health, financial data, and other information gathered in the study will remain confidential and will be stored on a password-protected computer, only accessed by study personnel. When the results of the research are published or discussed, no information will be included that would reveal your identity. We understand that information about you obtained in connection with your health is personal, and we are committed to protecting the privacy of that information. If you would like to learn more about participating in this study, please contact the study coordinator at 203-737-4777.

Possible Benefits: This research may not benefit you directly. However, knowledge gained from the results may help us to better understand diabetes.

Participation in this study is completely voluntary. You are free to decline to participate, to end participation at any time for any reason, or to refuse to answer any individual question at any time. Refusing to participate will involve no penalty or loss of benefits to which you are otherwise entitled (such as your health care outside the study, the payment for your health care, and your health care benefits).

Questions: If you have any further questions about this study, you may contact the investigator, Dr. Renata Belfort de Aguiarat 203-737-6067. If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688."

- Screening and Informed Consent Discussion: Research team members will contact interested potential participants to assess eligibility and provide the participant with additional information about the study, including the study procedures, purpose and potential complications. The brief screen via email or phone will ask for the following information to confirm study eligibility:
    - Name
    - Age/Date of Birth
    - Place of Birth
    - Phone Number/Email
    - Address
    - Marital Status
    - Race/Ethnicity
    - Height/Weight
    - Medical/Surgical History
    - Medications
    - Drug/Alcohol Use

  It is necessary to collect this information prior to the subject traveling to Yale for an in-person screening visit, in order to determine their eligibility and avoid unnecessary screening and traveling on the part of the participant. This information will be protected according to HIPAA policies and will be destroyed if the individual is determined to not be eligible for the study during this correspondence, or is otherwise not interested in participating in the study, unless the subject consents for his/her screening information to be stored for future studies that he/she may be eligible for.

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During the in-person screening session, the informed consent form and study details are reviewed in detail by one of the project investigators or key study personnel and the subject will be asked to read the informed consent form (approved by the Yale Human Investigation Committee). The subject will be given time to ask questions and only after that will the subject be asked to give informed consent to participate. The informed consent form and study details will again be reviewed with the subject on each study day prior to beginning the study.
The Principal Investigator in this study or key study personnel will be responsible for obtaining consent.

c. Who is recruiting potential subjects?
All research team members as well as the individuals listed above (JDAT, referring providers, etc.).

4. Assessment of Current Health Provider Relationship for HIPAA Consideration:
Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?
☐Yes, all subjects
☒Yes, some of the subjects
☐No

If yes, describe the nature of this relationship. An investigator may be the treating clinician for subjects recruited from the YNHH Endocrine Clinic/YNHH Diabetes Center.

5. Request for waiver of HIPAA authorization: (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)
Choose one:
☐ For entire study
☒ For recruitment/screening purposes only
☐ For inclusion of non-English speaking subject if short form is being used and there is no translated HIPAA research authorization form available on the University's HIPAA website at hipaa.yale.edu.

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data: It is necessary to collect this information prior to the subject traveling to Yale for an in-person screening visit, in order to determine their eligibility and avoid unnecessary screening and traveling on the part of the participant. This information will be protected according to HIPAA policies and will be destroyed if the individual is determined to not be eligible for the study during this correspondence, or is otherwise not interested in participating in the study, unless the subject consents for his/her screening information to be stored for future studies that he/she may be eligible for. In addition, we will need the participant's full name, address, date of birth, place of birth, phone numbers, marital status, height and weight in order to schedule a screening visit at the HRU or CSRU.

ii. If requesting a waiver of signed authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data: *Write here*

The investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

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Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

6. Process of Consent/Assent: Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.
   During the in-person screening session at the YNHH Hospital Research Unit (HRU) or YCCI Church Street Research Unit (CSRU), the informed consent form and study details are reviewed in detail by one of the study investigators or key study personnel. The subject will be asked to read the informed consent form (approved by the Yale Human Investigation Committee). The subject will retain a copy of this consent to review. The subject will be given time to ask questions and only after that will the subject be asked to sign to give informed consent to participate. The informed consent form and study details will again be reviewed with the subject on each study day prior to beginning the study. Any subject who appears incapable of providing informed consent (e.g., due to apparent cognitive impairment) will be excluded.

7. Evaluation of Subject(s) Capacity to Provide Informed Consent/Assent: Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.
   We do not plan to recruit subjects with limited decision-making capacity. Potential subjects will undergo a face-to-face interview. At that time, a study clinician will meet the subject, review the informed consent form, explain the purpose of the study and risks associated with participation, and will be available for questions. To ensure that the study subject understands the study, the subject will be asked questions about the study procedures and the risks associated with participation. If any concern arises that the study subject did not fully understand the study, the study clinician may decide that the subject is not suitable for participation. If the subject is still interested after all questions have been answered, a study physician will ask the subject to sign the informed consent form.

8. Non-English Speaking Subjects: Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.
   N/A, we do not plan to recruit non-English speaking subjects.

As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly encounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment? YES ☐ NO ☒

Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

Several translated short form templates are available on the HRPP website (yale.edu/hrpp) and translated HIPAA Research Authorization Forms are available on the HIPAA website (hipaa.yale.edu). If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via modification prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

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If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

9. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☐Not Requesting any consent waivers

☒Requesting a waiver of signed consent:

☒ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only) and for the Optional substudy.*

☐ Entire Study (Note that an information sheet may be required.)

For a waiver of signed consent, address the following:

- Would the signed consent form be the only record linking the subject and the research? YES ☒ NO ☐
- Does a breach of confidentiality constitute the principal risk to subjects? YES ☒ NO ☐

OR

- Does the research pose greater than minimal risk? YES ☐ NO☒ (optional substudy)
- Does the research include any activities that would require signed consent in a non-research context? YES ☐ NO ☒ (optional substudy)

☐ Requesting a waiver of consent:

☐ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ Entire Study

For a full waiver of consent, please address all of the following:

- Does the research pose greater than minimal risk to subjects?
  ☐ Yes *If you answered yes, stop. A waiver cannot be granted.*
  ☐ No
- Will the waiver adversely affect subjects' rights and welfare? YES ☐ NO☐
- Why would the research be impracticable to conduct without the waiver? *Write here*
- Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date? *Write here*

SECTION IV: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

1. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?
   - Medical record number

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- Name
- Address
- Phone number/email
- Age/date of birth
- Race/ethnicity
- Medical and surgical history, allergies, medications taken
- Blood test results
- CGM reports

2. How will the research data be collected, recorded and stored?
   Research data will be collected directly from the subject as well as via the electronic medical record. Subjects will be assigned a study number. The principal investigator will create a computer worksheet where the name of the subject's medical information is linked to the coded information. This will be housed on a password protected computer on a secured network. Only the investigators will have access to the computer records, which include the subject's identity, in order to evaluate the information generated by the study. No further public disclosure of this information will be made. One copy of the consent form will be kept in a secured and locked cabinet in the PI's office (which is also locked). The subject's name will be kept separate from the results.

3. How will the digital data be stored? ☐CD ☐DVD ☐Flash Drive ☐Portable Hard Drive ☒Secured Server ☐Laptop Computer ☐Desktop Computer ☐Other

4. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?
   All identifiable subject information that is collected during recruitment, screening, and participation will appear only on the initial paper forms, which will be kept under lock and key in the academic office of one of the study investigators. All digital files that could link a code number to an individual study subject will be password protected and stored on a shared drive in a password protected secure server, on a laptop or computer which will be kept locked in the PI's office.

All portable devices must contain encryption software, per University Policy 5100. If there is a technical reason a device cannot be encrypted please submit an exception request to the Information Security, Policy and Compliance Office by clicking on url http://its.yale.edu/egrc or email it.compliance@yale.edu

5. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.
   Procedures to ensure confidentiality follow the regulations and policies of the Yale University School of Medicine. The security mechanisms specified above will continue to be in place to protect study data.

6. If appropriate, has a Certificate of Confidentiality been obtained? N/A

SECTION V: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)

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This research may not benefit the subject directly. However, knowledge gained from the results may help us to better understand diabetes.

SECTION VI: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. Alternatives: What other alternatives are available to the study subjects outside of the research?
   The alternative is to decline to participate in the study.

2. Payments for Participation (Economic Considerations): Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.
   Aim 1 and 2:
   $25 for screening visit
   $50 to wear CGM and complete questionnaires (with > 80% compliance/time points collected)
   $200 for fMRI scan/hypoclamp #1
   $200 for MRS scan/hyperclamp #1

   Aim 3:

   $275 for fMRI/hypoclamp (+ an additional $25 bonus depending on how you do at the tasks/questionairre during the scan)
   $300 for MRS scan/hypercplamp visits

   Optional Sub-study:
   $25 for completion of questionnaires

3. Costs for Participation (Economic Considerations): Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.
   All study interventions and procedures will be provided at no cost to study participants.

4. In Case of Injury: This section is required for any research involving more than minimal risk, and for minimal risk research that presents the potential for physical harm (e.g., research involving blood draws).

   a. Will medical treatment be available if research-related injury occurs? Yes
   b. Where and from whom may treatment be obtained? YNHH
   c. Are there any limits to the treatment being provided? No
   d. Who will pay for this treatment? Insurance/patient
   e. How will the medical treatment be accessed by subjects? Medical treatment will be provided by study staff with referral to appropriate care at YNHH as needed

IMPORTANT REMINDERS

Will this study have a billable service? Yes ☒ No☐

A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by

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professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects.

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ☒ No ☐

If Yes, please answer questions a through c and note instructions below.

a. Does your YNHH privilege delineation currently include the specific procedure that you will perform? Yes ☒ No ☐

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? Yes ☐ No ☒

c. Will a novel approach using existing equipment be applied? Yes ☐ No ☒

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