HIC#1208010648

YALE UNIVERSITY
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Biomedical Research
100 FR1 (2011-3)

Please refer to the HIC website for application instructions and information required to complete this application. The Instructions are available at http://www.yale.edu/hrpp/forms-templates/biomedical.html Submit the original application and two (2) copies of all materials including relevant sections of the grant which funds this project (if applicable) to the HIC.	**HIC OFFICE USE ONLY**	
	DATE STAMPED-RECEIVED	PROTOCOL NUMBER

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: Impact of Hypoglycemia on Brain Ketone and Neurotransmitter Metabolism in Type 1 DM.			
Principal Investigator: Raimund Herzog, MD		**Yale Academic Appointment:** Associate Professor of Internal Medicine	
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Yale Cancer Center CTO Protocol Correspondent Name & Address (*if applicable*): **N/A**			
Campus Phone:	**Fax:**	**E-mail:**	

Faculty Advisor:(required if PI is a student, resident, fellow or other trainee) ☒ **NA**		**Yale Academic Appointment:**	
Campus Address:			
Campus Phone:	**Fax:**	**Pager:**	**E-mail:**

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Investigator Interests:
Does the principal investigator, co-investigator, or any other responsible research team member, or any of their family members (spouse, child, domestic partner) have an incentive or interest, financial or otherwise, that may be viewed as affecting the protection of the human subjects involved in this project, the scientific objectivity of the research or its integrity? See Disclosures and Management of Personal Interests in Human Research
http://www.yale.edu/hrpp/policies/index.html#COI

o Yes **X No**
If yes, list names of the investigator or responsible person:

The Yale University Principal Investigator and all Yale University and Yale New Haven Hospital individuals who are listed as co-investigators on a protocol with a Yale University Principal Investigator must have a current financial disclosure form on file with the University's Conflict of Interest Office. If this has not been done, the individual(s) should follow this link to the COI Office Website to complete the form: http://www.yale.edu/coi/

NOTE: The requirement for maintaining a current disclosure form on file with the University's Conflict of Interest Office extends primarily to Yale University and Yale-New Haven Hospital personnel. **Whether or not they are required to maintain a disclosure form with the University's Conflict of Interest Office, all investigators and individuals deemed otherwise responsible by the PI who are listed on the protocol are required to disclose to the PI any interests that are specific to this protocol.**

SECTION II: GENERAL INFORMATION

1. **Performing Organizations:** Identify the hospital, in-patient or outpatient facility, school or other agency that will serve as the location of the research. Choose all that apply:

a. Internal Location[s] of the Study:

- ☒ Magnetic Resonance Research Center (MR-TAC)
- ☐ Yale Cancer Center/Clinical Trials Office (CTO)
- ☐ Yale Cancer Center
- ☒ Yale-New Haven Hospital
- ☐ Specify Other Yale Location:
- ☐ PET Center
- ☐ YCCI/Church Street Research Unit (CSRU)
- ☒ YCCI/Hospital Research Unit (HRU)
- ☐ YCCI/Keck Laboratories
- ☐ Cancer Data Repository/Tumor Registry

b. External Location[s]:

- ☐ APT Foundation, Inc.
- ☐ Connecticut Mental Health Center
- ☐ Clinical Neuroscience Research Unit(CNRU)
- ☐ Other Locations, Specify: (Specify location(s)):
- ☐ Haskins Laboratories
- ☐ John B. Pierce Laboratory, Inc.
- ☐ Veterans Affairs Hospital, West Haven
- ☐ International Research Site

c. **Additional Required Documents *(check all that apply)*:** ☐ N/A

- ☐ *YCCI-Scientific and Safety Committee (YCCI-SSC) Approval Date:
- ☐ *Pediatric Protocol Review Committee (PPRC) Approval Date:
- ☐ *YCC Protocol Review Committee (YRC-PRC) Approval Date:
- ☐ *Dept. of Veterans Affairs, West Haven VA HSS Approval Date:
- ☐ *Radioactive Drug Research Committee (RDRC) Approval Date:
- ☐ YNHH-Radiation Safety Committee (YNHH-RSC) Approval Date:

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☒ Magnetic Resonance Research Center PRC (MRRC-PRC) Approval Date: **June 15, 2012**
☐ YSM/YNHH Cancer Data Repository (CaDR) Approval Date:
☐ Dept. of Lab Medicine request for services or specimens form
Approval from these committees is required before final HIC approval is granted. See instructions for documents required for initial submission and approval of the protocol. Allow sufficient time for these requests. Check with the oversight body for their time requirements.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities. 10 years

3. **Research Type/Phase: (Check all that apply)**
a. Study Type
☒ Single Center Study
☐ Multi-Center Study
Does the Yale PI serve as the PI of the multi-site study? Yes ☐ No ☐
☐ Coordinating Center/Data Management
☐ Other:

b. Study Phase ☒ **N/A**
☐ Pilot ☐ Phase I ☐ Phase II ☐ Phase III ☐ Phase IV
☐ Other (*Specify)*

4. **Area of Research: (Check all that apply)** Note that these are overlapping definitions and more than one category may apply to your research protocol. Definitions for the following can be found in the instructions section 4c:
☐ Clinical Research: Patient-Oriented
☐ Clinical Research: Epidemiologic and Behavioral
☒ Translational Research #1 ("Bench-to-Bedside")
☐ Translational Research #2 ("Bedside-to-Community")
☐ Clinical Research: Outcomes and Health Services
☐ Interdisciplinary Research
☐ Community-Based Research

5. Is this study required to be registered in a public database? Yes ☐ No ☒
If yes, where is it registered?
Clinical Trials.gov registry ☐
Other (*Specify*)

6. Will this study have a billable service as defined by the Billable Service Definition?
Yes ☐ No ☒
If you answered "yes", this study will need to be set up in Patient Protocol Manager (PPM)
http://medicine.yale.edu/ymg/systems/ppm/index.aspx

7. Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes____**No _X__***If Yes, please answer questions a through c and note instructions below. If No, proceed to Section III.*

   a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform?

      n/a

   b. Will you be using any new equipment or equipment that you have not used in the past for this procedure?

      n/a

   c. Will a novel approach using existing equipment be applied?

      n/a

If you answered "no" to question 7a, or "yes" to question 7b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

SECTION V: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.

To determine the long-term effect of severe antecedent hypoglycemia in T1DM patients on brain energy substrate metabolism, in particular alternate energy substrate and glucose metabolism. We will measure brain fuel uptake kinetics across the blood brain barrier into the brain, brain glucose levels and neuronal and glial TCA cycle activity. We will further explore the relationships between absolute neurotransmitter and brain metabolite levels in healthy control subjects and Type 1 diabetes subjects and how they vary across different brain regions.

2. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.

2.1.1. Counterregulatory failure in intensely treated diabetic patients.

The long-term impact of hypoglycemia on brain function and development is becoming clearer {Ho, 2008 #1731} and confirms the long held fear of hypoglycemia as the most dreaded immediate complication of T1DM treatment with insulin {DCCT, 1997 #1361;Flykanaka-Gantenbein, 2004

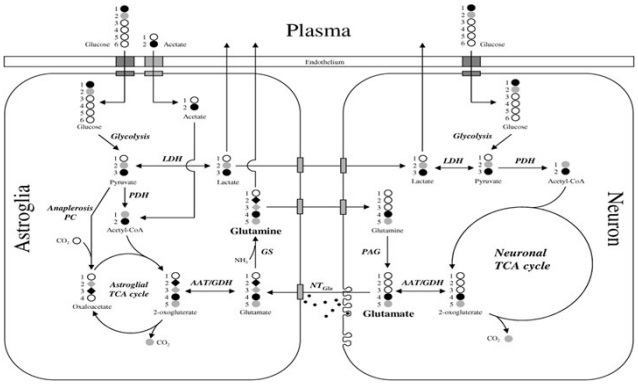

Figure 1: Model of cerebral metabolism in neuronal and astroglial compartments and metabolite ^{13}C labeling patterns following the intravenous infusion of [1-13C]-glucose (black circle), [2-13C]-glucose (gray circle) or [2-13C]-acetate (black circle). Glucose enters both neuronal and astroglial compartments and labels pyruvate and subsequently lactate through

#1391;Kodl, 2008 #1693}. Complications of uncontrolled hyperglycemia in diabetic patients are well established and can be avoided via intensive insulin therapy {DCCT, 1993 #1245;UKPDS, 1998 #1360}. Constrained by these two extremes, measures that allow for intensive glucose control without a negative impact on normal brain function are still needed. The counterregulatory mechanisms to low glucose include responses on several levels: In the pancreas insulin secretion is suppressed; glucagon and epinephrine are increased and conscious measures are taken to reverse this possibly life-threatening state {Cryer, 1994 #1238}. One or several of these are compromised in T1DM patients when compared to non-diabetic individuals mostly by affecting the responsible centers in the brain in their control {Amiel, 1987 #1363;Amiel, 1988 #1364}. **The experiments proposed here are part of the effort to identify ways that would protect the brain from serious hypoglycemic injury.**

2.1.2. Characterization of brain metabolism *in vivo* by NMR spectroscopy.

In vivo biochemical assessment of metabolism by NMR spectroscopy using energy substrates labeled with the stable carbon isotope $^{13}C/^{2}H$ allows real time observation of neurotransmitter cycle activity in the brain (11; 12). Glutamine-glutamate neurotransmitter cycling is stochiometrically coupled to the turns of the TCA cycle, thereby providing the basis for the calculation of TCA cycle flux in astrocytes and neurons in a model of brain metabolism (1)
(Figure 1). This method allows characterization of fuel uptake across the blood brain barrier as well as brain metabolism under different physiological and disease conditions. Infused acetate is predominantly metabolized in glial cells, while labeled lactate, beta-hydroxy-butyrate (BHB) or glucose are preferably metabolized in neurons, where lactate and glucose (but not BHB) enter the TCA cycle by conversion of pyruvate to acetyl-CoA, a reaction catalyzed by the pyruvate dehydrogenase complex (PDH) (13-16). The effects of peripheral changes on plasma metabolite concentrations are very closely monitored and accounted for by frequent plasma sampling and incorporation into the metabolic model (17). The recent observation by members of our group that infused acetate showed enhanced uptake and increased metabolic flux in the cortex of patients with longstanding, but well controlled T1DM (18) led us to perform similar experiments in our animal model of three days of recurrent hypoglycemia (3dRH). **Application of NMR spectroscopy to the study of brain fuel metabolism in our *in vivo* animal model of recurrent hypoglycemia allowed us to measure brain TCA cycle activity in real time. This is particularly relevant to the metabolic adaptations in T1DM patients.**

2.1.3. *In vivo* measurement of brain acetate and lactate metabolism and determination of TCA cycle activity.

From the infusion of labeled ^{13}C-acetate into control and recurrently hypoglycemic animals under eu- and hypoglycemia we gained valuable new insights that are now guiding our choice of alternate fuels: Our experiments recapitulated the increase in fuel uptake observed in the human study conducted by our group (18). Due to the ability to infuse larger doses of acetate that allowed us to achieve higher blood concentrations than is ethically permissible in humans, we were able to determine that fluxes contributing to *neuronal* metabolism were particularly affected by antecedent recurrent hypoglycemia

(Figure 2). At euglycemia we observed a striking increase in basal glucose consumption (CMR_{glc}) following pretreatment with three bouts of hypoglycemia in the 3dRH animals when compared to controls, with only a small change in acetate utilization (CMR_{ac}). In contrast under hypoglycemia, whole brain glucose utilization markedly dropped in the 3dRH group, while no such change was observed in controls.

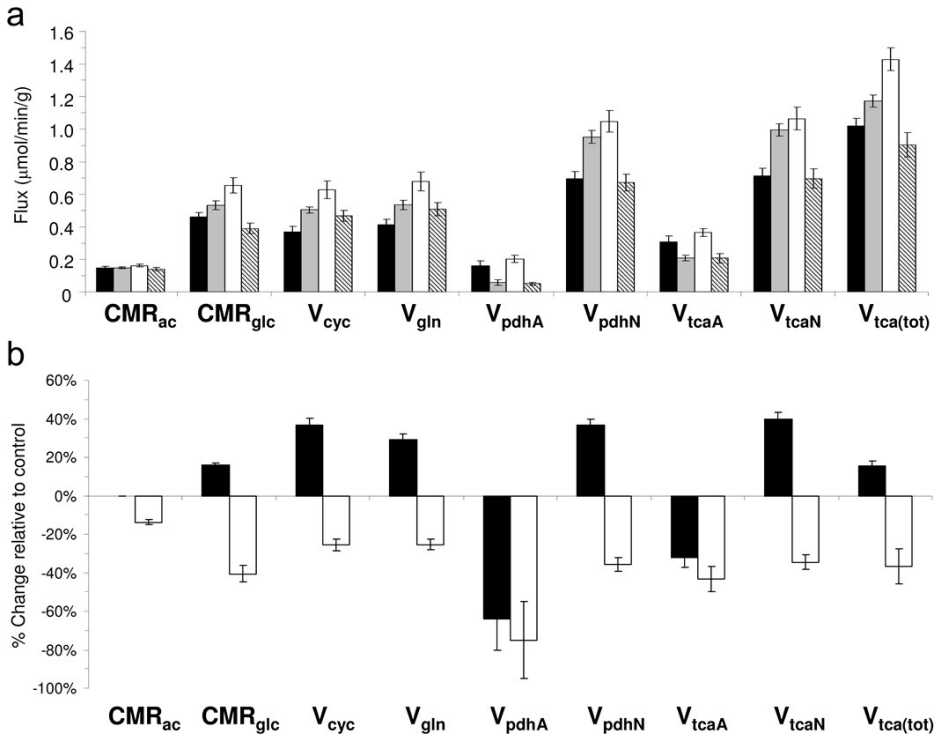

Figure 2: a) Calculated flux rates for key metabolic pathways in the neuronal and astrocytic compartment. (controls euglycemia - black, controls hypoglycemic – grey, 3dRH euglycemia – white, 3dRH hypoglycemia – hatched) b) Percent change in flux between euglycemia and hypoglycemia. (controls – black, 3dRH – white). CMR_{ac}= acetate oxidation rate; CMR_{gl}= glucose oxidation rate; V_{cyc}= glutamine-glutamate cycle flux; V_{gln}= glutamine synthase flux; V_{pdhA}= astrocytic PDH flux; V_{pdhN}= neuronal PDH flux; V_{tcaA}= astrocytic TCA cycle flux; V_{tcaN}= neuronal TCA cycle flux.

We were able to attribute this net change to a neuronal compartment-specific response to hypoglycemia: Animals from both groups decreased their glucose and lactate utilization in astrocytes under hypoglycemia, whereas neuronal pyruvate dehydrogenase flux (V_{pdhN}) increased in control animals, but markedly decreased in 3dRH animals by 50%. This difference translates further into parallel changes of the neuronal TCA cycle rates (V_{tcaN}) between groups (Figure 2), suggesting a subsequent decrease in ATP production and energy deficit. Thus while control animals can increase neuronal TCA cycle activity during hypoglycemia via enhanced substrate influx through pyruvate dehydrogenase, animals pre-exposed to recurrent hypoglycemia do not show such compensation.

These findings suggest that a more *neuron-specific* alternate fuel would be required to prevent neuronal energy deficits under acute hypoglycemia in the clinical setting, since acetate is predominantly supporting astroglial metabolism. Such a candidate fuel that can be safely delivered to humans is the ketone body BHB. It is, like acetate, taken up into the brain via monocarboxylic acid transporters (19). This makes it a particularly attractive candidate molecule, since its brain levels would be enhanced under the very circumstances under which it is supposed to

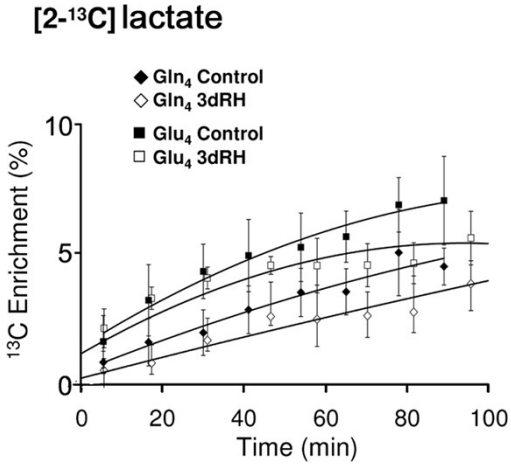

Figure 3: Time courses of ^{13}C label appearance in cortex and subcortex of control and 3dRH pretreated animals; n=5. Single exponential fits. (glu4= glutamate carbon position 4; gln4= fractional enrichment in %).

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support metabolism. Exciting results from a study of infused lactate -another predominantly neuronal fuel - in control and 3dRH animals at euglycemia have revealed a faster label appearance in the metabolites glutamine and glutamate (Figure 3), suggesting enhanced neuronal lactate metabolism after recurrent hypoglycemia and confirming our initial hypothesis.

We have identified changes in fuel transport into the brain and further utilization via neuronal PDH that may underlie the adaptations seen in T1DM patients. BHB may be particularly well suited to compensate for this deficit since it is taken up by the transporters known to be upregulated after exposure to antecedent recurrent hypoglycemia and it particularly supports the neuronal compartment.

2.1.4. Altered Brain Neurotransmitter Homeostasis in Type 1 Diabetes

Following a hypoglycemic period of 2 hours and an infusion of lactate in our animal model, we have observed significant changes in brain metabolite concentrations, when compared to euglycemic controls (Figure 4). Particularly the neurotransmitters aspartate, glutamate and GABA were altered, suggesting changes in the neural excitability of the volume of brain cortex we studied. Whether such a dramatic change in neurotransmitter homeostasis accompanies the physiologic response to hypoglycemia and how it is affected by recurrent hypoglycemia in humans and is not known at this point and will be studied under this proposal. **Proton NMR measurements at high resolution at 7Tesla will allow us to characterize any progressive metabolic disturbance in neurotransmitter level that may occur in the context of recurrent hypoglycemia.**

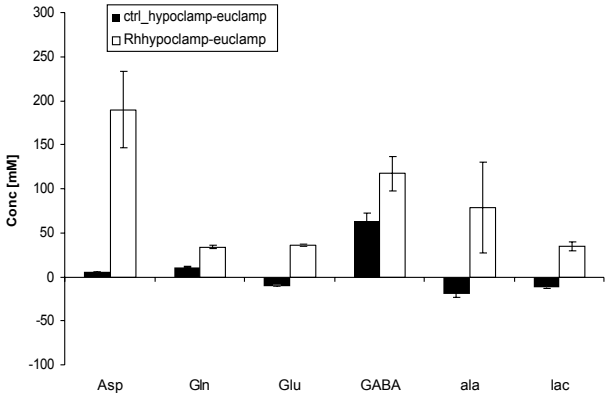

Figure 4: Percent change of brain neurotransmitter levels in control (white bars) and recurrently hypoglycemic (filled bars), comparing clamped euglycemia with clamped hypoglycemia.

2.1.5. Effect of Ketone Supplementation on Cognition under Hypoglycemia.

Cognitive impairment is the most serious adverse consequence of insulin-induced hypoglycemia. It is unknown to what degree hypoglycemia-induced cognitive dysfunction can be alleviated in T1DM subjects by peripheral administration of BHB as an alternate energy source for the brain. In contrast to lactate, our MR spectroscopy measurements of brain metabolism in humans suggest that intensively treated T1DM patients with hypoglycemia unawareness could particularly benefit from oral ketone (e.g. ester) supplementation, which already is safely being used in neurodegenerative diseases many of which are now known to have a neuro-metabolic basis, such as Alzheimer's disease. For this purpose, we will conduct studies designed to examine the potential value of this approach by infusing BHB (or placebo) in T1DM subjects during a hyperinsulinemic-hypoglycemic clamp in conjunction with testing of cognitive function. This will lay the basis for subsequent trials exploring the novel use of therapeutic doses of oral ketone

formulations (such as ketone esters) that are currently under commercial development, which could serve as adjunct therapies to protect the brain from hypoglycemia in T1DM.

2.1.6. Influence of lactate on brain $^{13}C/^{2}H$-glucose metabolism under hypoglycemia in intensively treated T1DM subjects with hypoglycemia unawareness

We have made the surprising observation in our animal model that infused lactate does not contribute to the increased oxidation rate of RH animals during hypoglycemia [20], which has since been supported by studies in T1DM patients [21]. In further follow-up experiments, we used DCA, an inhibitor of brain pyruvate dehydrogenase kinase isoforms and modulator of glucose metabolism, to demonstrate a possible important role for regulation of pyruvate dehydrogenase flux under hypoglycemia in an animal model of RH that recapitulated the effects of lactate. No study to date has directly examined the interaction of glucose and lactate metabolism under hypoglycemia in the brain of intensively treated T1DM subjects. Novel studies conducted under this protocol will yield this critical new information needed to tailor our therapeutic approaches to protect the brain from hypoglycemic injury.

3. References

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21. De Feyter, H.M., et al., *Increased Brain Lactate Concentrations without Increased Lactate Oxidation during Hypoglycemia in Type 1 Diabetic Individuals.* Diabetes, 2013.

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.

Our goal is to measure the uptake kinetics and overall contribution of different neuron-specific energy substrates to brain neurotransmitter metabolism in type 1 diabetic and normal control subjects under hypoglycemia. For that purpose we will compare brain metabolite concentrations and metabolic fluxes by in vivo NMR spectroscopy in healthy controls to that of intensively treated type 1 diabetic patients with a history of severe hypoglycemia and hypoglycemia unawareness during a controlled hypoglycemic state, which we will induce by a hyperinsulinemic-hypoglycemic clamp.

To measure the effect of supplementation with different energy substrates and glucose itself on cognitive function subjects will undergo a 2-step hyperinsulinemic eu- and

hypoglycemic clamp on two separate occasions, i.e. during a concomitant alternate fuel or labeled glucose or saline (control) infusion in random order. During each experimental condition the participants will complete a battery of validated cognitive tests that are sensitive to the cognitive impairment typically caused by hypoglycemia that we used previously in the context of hypoglycemia: Tests of non-memory function included digit symbol substitution, Tests of Everyday Attention, telephone book searching, and map searching in 1 and 2min. Working memory will be assessed by modified versions of the standard Wechsler Memory Scale Digit Span and Letter/Number Sequencing Tests, and map searching.

To define the effect of alternate fuels on brain neurotransmitter metabolism in hypoglycemia unawareness we will compare its ability to modulate the oxidation of infused stable isotope-labeled glucose in healthy adults with that in age- and weight matched subjects with c-peptide-negative T1DM with a history of hypoglycemia unawareness. During clamped hypoglycemia all subjects will undergo an NMR spectroscopy scan that permits detection of the stable isotope used, which will be either carbon13 (^{13}C) or deuterium (^{2}H). Using isotopically labeled-glucose as a tracer together with a co-infusion of 350mM unlabeled lactate, we will measure neuronal glucose metabolite enrichment, visualize them in three dimensions and determine the time courses of enrichment. These will be fitted with our metabolic model to determine whether increased lactate stimulates PDH flux and glucose oxidation under hypoglycemia and to what degree this varies across different regions of the brain.

Subject Selection and Preparation:

Two groups of subjects aged 18-65 years will be studied:

1) *Group 1* will be T1DM subjects (C-peptide negative, HbA1c <7.5%) with a history of severe hypoglycemia and hypoglycemia unawareness as assessed by the Ryan HYPO Score (see below) and as evidenced by interview, questionnaire and glucose log and/or continuous glucose monitoring.

2) *Group 2* will be non-diabetic healthy subjects (fasting plasma glucose < 100 mg/dL, normal HbA1c) who are matched for age and gender to the type 1 diabetic subjects, to serve as controls for the study.

The Ryan HYPO score:

During a 4-week period subjects record blood sugar levels 5 times per day. On the occasion that the glucose recorded is <54 mg/dl subjects are asked to describe the details of the event using a questionnaire recently developed at Edmonton by Ryan et al. to more objectively define hypoglycemia severity. Particular emphasis in the questionnaire is devoted to the type of symptoms and whether help was required to

reverse the hypoglycemic event. From these data a composite HYPO score is generated based on the glucose readings and the patient's reported hypoglycemic events. A composite score in the upper quartile of T1DM control patients will be required for enrollment in this study.

Subjects will be placed into one of following cohorts:

Cohort 1: Subjects from group 1 and group 2 will be scheduled for three study sessions as described below. A screening visit, an MR spectroscopy scan without hyperinsulinemic clamp (euglycemia) and an MR spectroscopy scan during a hyperinsulinemic clamp (hypoglycemia) with a tracer infusion.

Cohort 2: Subjects from group 1 and group 2 will be scheduled for two study sessions. A screening visit as described below. An MR spectroscopy scan that will be conducted during a hyperinsulinemic clamp (combined eu- and hypoglycemia) together with a tracer infusion.

Cohort 3: Subjects from group 1 and group 2 will be scheduled for two study sessions. A screening visit as described below. An MR spectroscopy scan conducted during a hyperinsulinemic clamp (combined eu- and hypoglycemia) without a tracer infusion.

Cohort 4: Healthy subjects from group 2 will be scheduled for one study session. They will undergo an MR spectroscopy scan without a hyperinsulinemic clamp nor any other i.v. infusions (i.e. no i.v. infusions at all).

Cohort 5: Subjects from group 1 and group 2 will be scheduled for two study sessions. A screening visit as described below and a hyperinsulinemic clamp together with a non-labeled ketone or saline infusion without an MR spectroscopy scan.

Session A: Screening visit (YNHH Clinical Research Unit):
At the screening visit, study subjects in both the control and T1DM groups will complete the informed consent form for participation in voluntary clinical research. Demographic and clinical data collected will be reviewed by study personnel to confirm eligibility.

Once consented, all subjects will be further screened with a medical history & physical examination, blood work (electrolytes, hematocrit, creatinine, liver function tests and HbA1c) and a 12-lead electrocardiogram (EKG). Detailed subject inclusion and exclusion criteria are listed below (Human subjects – Subject recruitment). Subjects in the T1DM group only will be instructed to record their glucose readings to determine degree and frequency of hypoglycemia as described above. The subject will perform the preliminary cognitive tests at this point if they are selected for Cohort 5 at this point. The two subsequent MR sessions to complete the study will be scheduled at this point. The subject in the control group will have their two MR sessions scheduled without having to record any glucose readings.

Session B: NMR spectroscopy without tracer infusion (Magnetic Resonance Research Center)

Subjects may be scheduled to arrive on the evening before the study if arriving early will be a difficulty. If such a case occurs, subjects will be admitted to the HRU for an overnight stay to be there for the morning without any delay.

On the morning of study, subjects will arrive directly at the MRRC. A member of the research team will verify that if the subject is from the T1DM group , the diabetic subject (as previously instructed) has taken his/her normal morning dose of insulin (or has continued to wear the insulin pump). This will not apply to subjects that are from the control group of non-diabetics participants. A urine pregnancy test will be completed on all female subjects of child bearing potential. Any female testing positive will be excluded from the study. All subjects will meet the staff, study procedures will be reviewed, and they will be exposed to the instrumentation and then placed in the scanner for NMR spectroscopy.

Imaging Procedures with the 4T/ 7T Bruker Advance NMR spectrometer (Bruker Instruments, Inc, Bellerica, MA) with a 94 cm bore: Proton MRS will be performed using the outer 1H decoupling coils of the ^{13}C probe at 4T. Parameters and pulse sequences for GABA and glutamate acquisition will be the standard ones presently being established for this coil by Drs Mason and Rothman: Subjects will lie supine with the occipital cortex against a 7-cm surface coil tuned to ^{1}H-MRS frequency of 170.4 MHz. T_1-weighted images 30 seconds in duration will be acquired for subject positioning. 1st and 2nd order shimming will be performed automatically using the FASTERMAP procedure. We will then proceed to acquiring proton NMR spectra from a volume adjacent to the NMR coil for a period of 60 minutes. Total and regional power deposition will be below the FDA safety limits for RF power deposition. For 7T studies the pulse sequence will be performed using the multi Tx coil using pulse sequences and parameters established by Dr de Graaf, which will be verified regarding local power deposition using RF simulations. The 7T studies will be exploratory.

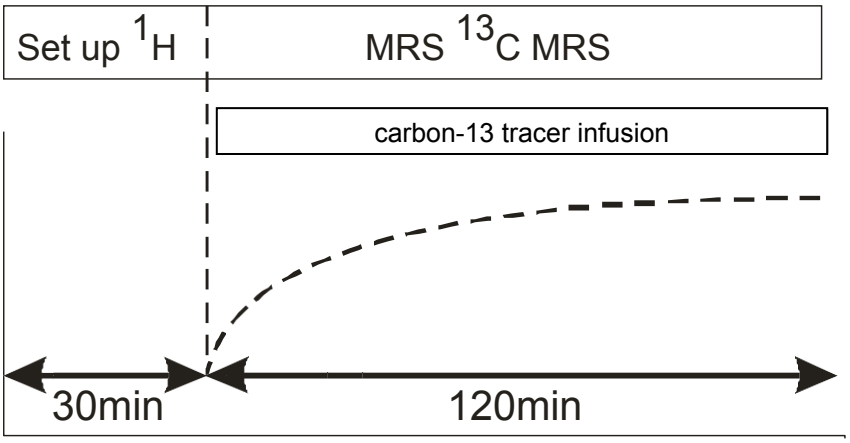
Session C: NMR spectroscopy with ^{13}C/ ^{2}H -tracer infusion (Magnetic Resonance Research Center)

Non-diabetic control subjects will be asked to remain fasting from midnight prior to the study. Upon arrival to the MRRC, their blood glucose will be checked and an IV catheter will be inserted for infusion of insulin and glucose to maintain normal glucose levels and the study will ensue as described below.

For type 1 diabetic subjects receiving insulin via an insulin pump , they will be asked to check their blood glucose at home before bed and on awakening to ensure that the glucose level is above 70mg/dl and below 200mg/dl. They will be instructed to call Dr. Herzog with any readings outside of this range. Upon arrival to the MRRC, their blood glucose will be checked as a safeguard, and insulin received via the insulin pump will be held. An IV catheter will be inserted for infusion of insulin and glucose to maintain normal glucose levels and the study will ensue as described below.

For type 1 diabetic subjects using multiple injections of insulin per day will be asked to ensure that the glucose level is above 70mg/dl and below 200mg/dl. As a safeguard, they

will be asked to check their blood glucose at home before bed and on awakening. Upon arrival to the MRRC, their blood glucose will be checked; a nurse will place an intravenous (IV) line to start infusions of glucose and insulin to maintain normal glucose levels.



MRS study design. After a set up period of approximately 30 minutes that includes a 1H MRS scan, subjects will be infused for 120minutes with the isotopic labeled substrate.

At 9:00 AM on the day of this NMR session, a standard IV catheter will be inserted and used to deliver a continuous insulin infusion (2mU/kg/min) and a variable infusion of 20% dextrose. In addition a second IV line will be placed in the opposite arm to receive a normal saline solution and provide access for obtaining blood samples.
Once subjects arrive in the MR center they will be brought to a hypoglycemic state. The plasma glucose level will be gently lowered to 50 ± 3 mg/dL (see blood draw schedule below).

Once hypoglycemia is achieved tracer will be administered (see Figure above). Depending on the cohort assignment this will involve one of the following infusions:

a) A 200mmol/l ^{13}C /^{2}H- alternate substrate infusion, which will be delivered through a port in the already-established IV site at a rate of 22μM/kg/min.

or

b) A 20% [1-^{13}C]/ [^{2}H]-glucose infusion at the same rate as the 20% unlabeled glucose infusion that is part of the ongoing hyperinsulinemic clamp. This 'clamp glucose' infusion will be held for the duration of the ^{13}C/ ^{2}H -glucose tracer infusion. In addition to the ^{13}C/ ^{2}H -glucose infusion subjects will receive a simultaneous infusion of unlabeled 350mM lactate (see adjacent Figure).

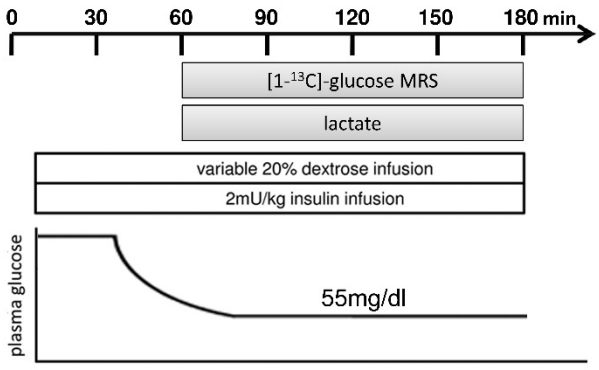


Figure 11: A) Study groups B) Timeline for hyperinsulinemic hypoglycemic clamp and tracer infusions.

Subjects will be moved into the scanner for magnetic resonance spectroscopy (MRS) studies. During the spectroscopy studies, subjects will lie supine in a 4.0 T 94 cm bore magnet. The back of each subject's head

will be superjacent to an 8 cm diameter $^{13}C/^{2}H$-surface coil receiver with a half quadrature coil ^{1}H decoupling channel. Before the $^{13}C/^{2}H$ MRS measurements, an MRI will be obtained for localization purposes. From the image, a 48 cubic centimeter volume in the medical occipital-parietal lobe will be selected to obtain localized $^{13}C/^{2}H$ MRS spectra. The Bo homogeneity in the selected volume will be reduced to less than 0.08 ppm using automated shimming. Following shimming, localized MRS will be performed to the steady state $^{13}C/^{2}H$-concentrations and fractional enrichments of $^{13}C/^{2}H$-tracer, and the 2, 3, and 4 positions of aspartate, glutamate, and glutamine. Throughout the study, samples of plasma will be obtained for measurement of glucose, insulin, ketones, lactate, ^{13}C enrichment metabolites, and counterregulatory hormones (glucagon, epinephrine, and norepinephrine, cortisol). (See sample collection table Appendix A.) In addition, autonomic and neuroglycopenic symptoms will be assessed.
When the study is completed, subjects will receive a bolus and infusion of 10% dextrose to restore normoglycemia; they will then return to the HRU, where they will eat lunch. Once each subject appears clinically well with stable blood glucose levels above 70 mg/dL for at least 60 minutes, the IV cannula can be removed and providing they are considered stable by the study physician, they can be discharged from the HRU.

Session D: Hyperinsulinemic Clamp with Cognitive Testing (Hospital Research Unit)

Non-diabetic control subjects will be asked to remain fasting from midnight prior to the study. Upon arrival to the HRU, their blood glucose will be checked and an IV catheter will be inserted for infusion of insulin and glucose to maintain normal glucose levels and the study will ensue as described below.

For type 1 diabetic subjects receiving insulin via an insulin pump will be asked to decrease their basal insulin rate by ~15% at 9 pm the night prior to the study. As a safeguard, they will be asked to check their blood glucose at home before bed and on awakening to ensure that the glucose level is above 70mg/dl and below 200mg/dl. They will be instructed to call Dr. Herzog with any readings outside of this range. Upon arrival to the HRU, their blood glucose will be checked as a safeguard, and insulin received via the insulin pump will be held. An IV catheter will be inserted for infusion of insulin and glucose to maintain normal glucose levels and the study will ensue as described below.

For type 1 diabetic subjects using multiple injections of insulin per day will be asked to ensure that the glucose level is above 70mg/dl and below 200mg/dl. They will be instructed to call Dr. Herzog with any readings outside of this range, halve their evening dose of long-acting insulin before going to bed and to skip their morning insulin injection. As a safeguard, they will be asked to check their blood glucose at home before bed and on awakening. Upon arrival to the HRU, their blood glucose will be checked; a nurse will place an intravenous (IV) line to start infusions of glucose and insulin to maintain normal glucose levels.

Hyperinsulinemic clamp and cognitive testing. At 9:00 AM on the day of this hyperinsulinemic clamp session, a standard IV catheter will be inserted and used to deliver a continuous insulin infusion (2mU/kg/min) and a variable infusion of 20% dextrose. In addition a second IV line will be placed in the opposite arm to receive a normal saline solution and provide access for obtaining blood samples. During the clamp procedure the plasma glucose level will be gently lowered from the initial level of 95-100mg/dl to the target of 50 ± 3 mg/dL.

Once a stable glycemic state is reached, subjects will receive a bolus-continuous infusion of 40μmol/min/kg unlabeled BHB for 5 min followed by a 12μmol/min/kg of BHB or infusion of a comparable volume of normal saline to control for possible volume effects for the remainder of the study.

Then a cognitive testing battery is administered via questionnaire as done by our group before (HIC#0505000079; PI: R. Sherwin). Tests of non-memory function included digit symbol substitution, Tests of Everyday Attention, telephone book searching, and map searching in 1 and 2min. Tests of immediate and delayed verbal memory and verbal memory recognition are adapted from the Wechsler Memory Scale logical memory tests. Working memory will be assessed by modified versions of the standard Wechsler Memory Scale Digit Span and Letter/Number Sequencing Tests, and map searching.

Throughout, blood is obtained for glucose, BHB, lactate, insulin, glucagon, cortisol, and catecholamines and will be assayed using established RIA, HPLC & ELISA methods in the YCCI Core Lab (see blood draw schedule below).

Blood Draw Schedule

	start hypo clamp	**glucose ~55mg/dl** for ~10-15min	**glucose ~55mg/dl** for ~20-25min							
Timeline	-60 min	-10 min	**$^{13}C/^{2}H$-tracer Baseline** start infusion*	+5	+10	+15	+30	+60	+90	+120
Insulin	X		X			X	X	X		X
Ketones	X		X			X	X	X		X
Lactate	X		X			X	X	X	X	X
$C^{13}/^{2}H$ enrichment (during tracer infusion study)			X	X	X	X	X	X	X	X
Counterreg. hormone	X	X	X				X	X		X
glucose	----------glucose every 5 min during hypo clamp----------									

* blood draws timed to alternate fuel infusion

Counter-regulatory hormones = E, NE, glucagon, cortisol, growth hormone

In the event that scheduling issues preclude the sequencing of scanning sessions as described above, certain modifications can be made to accommodate a subject.

Blood Volumes and Schedule of Events Tables for each group are included at the end of this application.

5. Genetic Testing **N/A** ☒

A. Describe

i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned
ii. the plan for the collection of material or the conditions under which material will be received
iii. the types of information about the donor/individual contributors that will be entered into a database
iv. the methods to uphold confidentiality

B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects?
C. Is widespread sharing of materials planned?
D. When and under what conditions will materials be stripped of all identifiers?
E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials?

i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)?

F. Describe the provisions for protection of participant privacy
G. Describe the methods for the security of storage and sharing of materials

6. **Statistical Considerations:** Describe the statistical analyses that support the study design.

Sample size calculations were performed using PASS 2002 (Hintze J. 2001. NCSS and PASS Number Cruncher Statistical Systems. Kaysville, Utah.). In our preliminary magnetic resonance spectroscopy experiments (see preliminary studies), under hypoglycemic conditions, we observed significantly greater fractions of glial oxidative metabolism supplied by acetate in T1DM subjects compared to non-diabetic controls (0.19 ± 0.066 vs 0.10 ± 0.020; a 90% increase in diabetics compared to controls). We will expect differences between severe hypoglycemia unaware and control subjects in this study to be on a similar scale. With a pooled standard deviation of 0.033, our proposed sample sizes (17 diabetic subjects and 17 non-diabetic controls) will provide 80% power at the two-sided 0.05 significance level to detect differences of 0.033 between diabetic subjects and non-diabetic controls representing a 33% increase from non-diabetic subjects. In short, we should easily have sufficient numbers of to address our primary endpoint of enhanced ketone body uptake and metabolism following recurrent hypoglycemia. Using this data, our proposed sample sizes of 17 subjects per group will provide >80% power to detect a 20% difference in the severe hypoglycemia unaware compared to each control group. We anticipate the changes seen will be much greater than that. Because small effects cannot be detected with sufficient power, conclusions based on failure to reject the null hypothesis will be avoided.

The same group size considerations apply to the cohorts studied with glucose/lactate co-infusions and the cognitive performance analysis under ketone infusion outside of the MRI scanners.

Data analysis will be performed using SAS statistical software version 8.2. Prior to the fundamental analysis, all data will be appropriately screened for accuracy, missing data, outliers and agreement with assumptions for the analyses. Univariate descriptive statistics and plots will first be employed to determine out-of-range values as well as plausible means and standard deviations. Non-normal variables will be identified through the inspection of normal probability plots and with the Kolmogorov-Smirnov test. If required, appropriate transformations or non-parametric tests will be applied to variables in violation of the model assumptions. James Dziura, Ph.D., the GCRC biostatistician, is available for advice in data analysis.

To test the hypothesis that patients with hypoglycemia unawareness exhibit enhanced CNS ketone body metabolism we will assess brain beta-hydroxybutyrate transport and its absolute levels and the ratio of brain beta-hydroxybutyrate contribution to the neuronal and astroglial TCA cycle. In addition, we will determine the absolute concentration of brain neurotransmitter levels using proton NMR. Group comparisons will be assessed by analysis of variance (ANOVA) for repeated measures. Data will be tested for multisample sphericity using Mauchly's and Box tests. If the assumption of homogenous covariance matrices is violated a covariance pattern model will be employed to allow for separate sets of covariance parameters for type 1 diabetic vs. nondiabetic controls. Evaluation of a significant diagnosis by clamp condition interaction will be determined by paired or unpaired t-tests (for within and between subjects factors respectively), using the interaction variance term from the ANOVA with a Holm correction for multiple testing.

7. **Subject classification:** Check off all classifications of subjects that will be targeted 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 (If yes, see Instructions section VII #4 for further requirements)

8. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?

Inclusion criteria for subjects:

1. Type 1 diabetes mellitus of > 5 years (C-peptide negative age 18-60 years, on intensive insulin therapy (HbA1c <7.5%)
2. Non-diabetic volunteers, age 18-65 years that do not meet any of the exclusion criteria.

The following criteria will exclude subjects from study:

1. Pregnancy
2. Significant baseline anemia (hemoglobin <11.0 or hematocrit < 33%)
3. A history of liver cirrhosis or porto-caval shunt surgery.
4. Any contraindications for MRI scanning.
5. Subjects that follow a vegetarian diet
6. Subjects that exercise heavily on a regular basis (i.e. marathon runners)
7. Subjects with a history of anxiety/ panic attacks

9. How will **eligibility** be determined, and by whom?

Eligibility to participate in the study will be determined by Raimund Herzog, MD. Eligibility will be determined based on information collected at the screening visit after consent is obtained. This will include findings from physical examination, medical history, EKG, and laboratory testing in accordance with the inclusion and exclusion criteria listed above.

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

10.1. Intravenous catheters. The placement of intravenous catheters and/or the use of intravenous infusions can result in local hematoma formation or thrombophlebitis. Transient vasovagal symptoms including nausea, sweating, and lightheadedness may also occur during intravenous catheter placement.

10.2. Blood sampling. Same as above and frequent blood sampling may result in a drop in hematocrit.

10.3. Hyperinsulinemic hypoglycemic clamp technique. The hyperinsulinemic hypoglycemic clamp technique can result in hypoglycemia symptoms of varying severity. Subjects may experience autonomic symptoms including hunger, anxiety, tremor, palpitations, and/or diaphoresis. Others may experience fatigue, concentration difficulties, and/or mild confusion. The risk of plasma glucose levels falling below the target value of 50 ± 3 mg/dL is small but present; affected patients may experience exaggerated symptoms, as well as the development of dizziness, blurred vision, and/or confusion. All such symptoms would be rapidly reversible with an intravenous dextrose infusion.

10.4. Infusion of lactate. In some subjects with severe panic disorder lactate infusions at a lever higher than proposed here for MR purposes can precipitate a panic attack. In healthy subjects without panic attacks lactate infusion is not known to induce such attacks. For this reason we are screening potential study subjects for a past medical history of panic attacks and they will be excluded from participation in our study.

10.5. Magnetic resonance imaging/spectroscopy. Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various 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.

The Subject will be watched closely throughout the MR study. Physiological monitoring will be done, including O_2 saturation, pulse rate and blood pressure. Some subjects may feel uncomfortable or anxious. If this happens to the subject, the subject may ask the study nurse that is present in the scanner room to stop the study at any time and the MR staff will take the subject out of the MR scanner. On rare occasions, some subjects might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly however the subject will be instructed to please tell the research staff if they experience them. Dr. Herzog, who has admitting privileges at YNHH will be available by cell phone at all times surrounding a study.

There are some risks with an MR study for certain people. If the subject has a pacemaker or some metal objects inside their body, the subject may not be in this study because the strong magnets in the MR scanner might harm them. Another risk is a metallic object flying through the air toward the magnet and hitting the subject. To reduce this risk the MR staff requires that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. Nothing metal can be brought into the magnet room at any time. Subjects will walk through a ferromagnetic detector. 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.

The MR staff want the subject to read and answer very carefully the questions on the MR Safety Questionnaire related to the subject's personal safety. The subject will be asked to take a moment, to be sure that they have read the MR Safety Questionnaire and be sure to tell the MR staff any information they think might be important.

This MR study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. The primary 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 diagnostic 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 primary investigator or consulting physician will contact the subject, inform the subject of the finding, and recommend that the subject seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie solely with the subject and the subject's 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 receive based on these findings. The images collected in this study are not a clinical MR exam and for that reason, they will not be made available for diagnostic purposes.

10.6 Stable isotope infusions: $^{13}C/^{2}H$ –labeled alternate energy substrates or $^{13}C/^{2}H$-glucose. Carbon-13 and ^{2}H (deuterium) are stable isotopes of naturally occurring substances (carbon12 and hydrogen, respectively). ^{2}H has been used as metabolic tracer in the early days of in vivo MRS, but was never as popular as ^{13}C approaches. Recent technical developments have motivated us to explore the usefulness of ^{2}H in metabolic imaging. There are no known side effects are associated with the administration of the labeled substrates acetate, BHB and lactate or glucose, all of which have been used by our group and colleagues at MRRC for many years. The MRRC has extensive experience with stable isotope infusions including acetate, BHB, glucose and lactate (HIC # 10605, Rothman PI)) and to date there have been no adverse events due to the infusion.

11. **11. Minimizing Risks:** Describe the manner in which the above-mentioned risks will be minimized.

The primary risks to the subject are impact by magnetic objects accidentally brought into the magnet room, anxiousness, irritation due to the IV blood sampling, and hypoglycemia after the glucose infusion. The investigators in this protocol pioneered human infusion studies of stable isotope labeled metabolic substrates in MR systems and have been routinely performing these studies since 1988. Studies of brain metabolism using stable isotope infusion in the MRC have been performed since 1990. To date there have been no patient injuries during these studies and only minor adverse incidences (anxiety, IV irritation).

To confront the specific risks listed above:

11.1 Intravenous catheters will be placed under sterile conditions by experienced HRU staff members. All infused solutions will be prepared in sterile form by the YNHH pharmacy and will be tested for pyrogenicity and sterility prior to use. During dextrose infusions, 20% dextrose will be used to reduce the risk of thrombophlebitis normally associated with the use of excessively hypertonic glucose solutions.

11.2 No subject with a hematocrit less than 37% for males and less than 33% for females will be studied. 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 each study completion. Total blood loss will not exceed half the amount of a regular blood donation for each study group. All blood losses will be replaced quantitatively with normal saline.

11.3 All study procedures, including the hyperinsulinemic clamp, will be performed under the direct supervision of a qualified, licensed practitioner.

11.4 No subject with a history of panic attacks will be enrolled in our study. To identify these subjects specific screening questions are administered during the screening visit. Licensed physicians and study nurses are present throughout the study to immediately recognize any distress subjects may experience. Studies would be immediately terminated should despite careful screening such an event still occur.

11.5 To avoid excess hypoglycemia during the hyperinsulinemic clamp, plasma glucose will be checked every five minutes using a bedside glucose monitor. Based on these measurements, the plasma glucose will be adjusted via the D20 infusion.

11.6 All potential study subjects will also be screened for MR safety using the standard MR safety protocol developed by Yale New Haven Hospital's Diagnostic Imaging department. Subjects with claustrophobia or with contraindications to MR scanning will be excluded from the study. The MR safety screening process will be performed both during the screening interview and on the day of an MR study.

11.7 To reduce MRI risks we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. Nothing metal can be brought into the magnet room at any time. Subjects will also walk through a ferromagnetic detector. Also, once subjects are in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet. In addition, patients can stop study at any time if anxiety is a significant issue.

11.8 To reduce the risk of injury in the MRI scanner, subjects will go through a ferromagnetic metal detector, to prevent any metal objects being taken into the scanner.

11.9 Stable isotope infusions will be prepared in sterile form by the YNHH pharmacy and will be tested for pyrogenicity and sterility prior to use.

11.10 Study subjects will be closely followed by an experienced clinical research team, including an experienced nurse with ACLS training who is with a patient at all times, a licensed MD or practitioner, and an experienced MRS technologist. All personnel involved in the study will be trained in MR safety procedures. The MD or licensed practitioner and technologist who are outside of the room have direct visual contact with the subject and nurse through a window, a TV monitor, and audio contact through a speaker system. Subjects who become claustrophobic in the magnet will be removed immediately from the magnet room. Patients who experience distress in the magnet room for any reason including seizures, loss of consciousness, or pain will be removed from the magnet room in accordance with well-established procedures developed for the Magnetic Resonance Research Center. Should an emergency arise, the MR technologist will call 911 and have an ambulance with personnel trained in resuscitation sent to the MR Center. The other members will remove the patient via the detachable patient bed from the magnet room If immediate resuscitation is needed the physician present during the study will perform this procedure, using a fully equipped crash cart which will be in the console room during the study.

All subjects will be given a telephone number to enable immediate communication with the P.I. or one of the co-investigators, to report any delayed adverse effects and to receive recommendations for treatment of any adverse effects.

HIC#1208010648

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.) For more information, see the Instructions, page 24.

    a. What is the investigator's assessment of the overall risk level for subjects participating in this study?

    Moderate

    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. Copy, paste, and then tailor an appropriate Data and Safety Monitoring Plan from http://www.yale.edu/hrpp/forms-templates/biomedical.html for
        i. Minimal risk
        ii. Greater than minimal/moderate risk
        iii. High risk

The risks associated with the current study are deemed moderate for the following reasons:
We do not view the risks associated with the combined use of insulin and dextrose as minimal. Given our experience with the combined co-administration of insulin and dextrose, we do not view the proposed studies as high risk.

Although we have assessed the proposed study as one of moderate 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:

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 Herzog according to the following categories:

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

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

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:

1. is life-threatening
2. results in in-patient hospitalization or prolongation of existing hospitalization
3. results in persistent or significant disability or incapacity
4. results in a congenital anomaly or birth defect OR
5. results in death
6. 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, or
7. adversely affects the risk/benefit ratio of the study

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 HIC or HSC is necessary.

Plan for reporting serious AND unanticipated AND related adverse events, anticipated adverse events occurring at a greater frequency than expected, and other unanticipated problems involving risks to subjects or others to the HIC or HSC.

13. The investigator will report the following types of adverse events to the HIC or HSC:
    a) serious AND unanticipated AND possibly, probably or definitely related events;
    b) anticipated adverse events occurring with a greater frequency than expected; and
    c) other unanticipated problems involving risks to subjects or others.

These adverse events or unanticipated problems involving risks to subjects or others will be reported to the HIC or HSC within 48 hours of it becoming known to the investigator, using the appropriate forms found on the website.

Plan for reporting adverse events to co-investigators on the study, as appropriate the protocol's research monitor(s), e.g., industrial sponsor, Yale Center for Clinical Investigation Research Subject Advocates (RSAs), Cancer Center's Quality Assurance, Compliance and Safety Committee (QUACS) Protocol Review Committee (PRC), DSMBs, study sponsors, funding and regulatory agencies, and regulatory and decision-making bodies.

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

- All Co-Investigators listed on the protocol.
- Yale Center for Clinical Investigation Research Subject Advocates (RSAs)

The principal investigator Dr Herzog will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

d. For multi-site studies for which the Yale PI serves as the lead investigator:
   i. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed?
   ii. What provisions are in place for management of interim results?
   iii. What will the multi-site process be for protocol modifications?

N/A

12. **Statistical Considerations:** Describe the statistical analyses that support the study design.

Sample size calculations were performed using PASS 2002 (Hintze J. 2001. NCSS and PASS Number Cruncher Statistical Systems. Kaysville, Utah.). In our preliminary magnetic resonance spectroscopy experiments (see preliminary studies), under hypoglycemic conditions, we observed significantly greater fractions of glial oxidative metabolism supplied by acetate in T1DM subjects compared to nondiabetic controls (0.19 $\pm$ 0.066 vs 0.10 $\pm$ 0.020; a 90% increase in diabetics compared to controls). We will expect differences between severe hypoglycemia unaware and control subjects in this study to be on a similar scale. With a pooled standard deviation of 0.033, our proposed sample sizes (17 diabetic subjects and 17 nondiabetic controls) will provide 80% power at the two-sided 0.05 significance level to detect differences of 0.033 between diabetic subjects and nondiabetic controls representing a 33% increase from nondiabetic subjects. In short, we should easily have sufficient numbers of to address our primary endpoint of enhanced ketone body uptake and metabolism following recurrent hypoglycemia. Using this data, our proposed sample sizes of 17 subjects per group will provide >80% power to detect a 20% difference in the severe hypoglycemia unaware compared to each control group. We anticipate the changes seen will be much greater than that. Because small effects cannot be detected with sufficient power, conclusions based on failure to reject the null hypothesis will be avoided.
Data analysis will be performed using SAS statistical software version 8.2. Prior to the fundamental analysis, all data will be appropriately screened for accuracy, missing data, outliers and agreement with assumptions for the analyses. Univariate descriptive statistics and plots will first be employed to determine out-of-range values as well as plausible means and standard deviations. Non-normal variables will be identified through the inspection of normal probability plots and with the Kolmogorov-Smirnov test. If required, appropriate transformations or non-parametric tests will be applied to variables in violation of the model assumptions. James Dziura, Ph.D., the GCRC biostatistician, is available for advice in data analysis.

To test the hypothesis that patients with hypoglycemia unawareness exhibit enhanced CNS alternate fuel metabolism we will assess brain beta-hydroxybutyrate transport and its absolute levels and the ratio of brain alternate fuel contribution to the neuronal and astroglial TCA cycle. In addition, we will determine the absolute concentration of brain

neurotransmitter lebels using proton NMR. Group comparisons will be assessed by analysis of variance (ANOVA) for repeated measures. Data will be tested for multisample sphericity using Mauchly's and Box tests. If the assumption of homogenous covariance matrices is violated a covariance pattern model will be employed to allow for separate sets of covariance parameters for type 1 diabetic vs. nondiabetic controls. Evaluation of a significant diagnosis by clamp condition interaction will be determined by paired or unpaired t-tests (for within and between subjects factors respectively), using the interaction variance term from the ANOVA with a Holm correction for multiple testing.

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

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

A. DRUGS and BIOLOGICS

1. **Identification of Drug or Biologic:** What is (are) the **name(s)** of the drug(s) or biologic(s) being used? Identify whether FDA approval has been granted and for what indication(s).

[^{13}C] / ^{2}H -labeled BHB
[^{13}C] / ^{2}H -labeled acetate
[^{13}C] / ^{2}H -labeled lactate
[^{13}C] / ^{2}H -labeled glucose
Unlabeled BHB
Unlabeled acetate
Unlabeled lactate
Unlabeled glucose

All protocols which utilize a drug or biologic **not** approved by, but regulated by, the FDA must provide the following information:

a. What is the Investigational New Drug (IND) **number** assigned by the FDA?

b. Who holds the IND?

Alternatively, an **exemption from IND filing requirements** may be sought for a clinical investigation of a drug product that is lawfully marketed in the United States. If there is no IND and an exemption is being sought, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Exempt Category 1
The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:

i. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug. ☐ Yes ☐ No
ii. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and

the intention of the investigation is NOT to support a significant change in the advertising for the product. ☐ Yes ☐ No

iii. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. ☐ Yes ☐ No

iv. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). ☐ Yes ☐ No

v. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. ☐ Yes ☐ No

Exempt Category 2 (all items i, ii, and iii must be checked to grant a category 2 exemption)

☐ i. The clinical investigation is for an *in vitro* diagnostic biological product that involves one or more of the following (check all that apply):

☐ Blood grouping serum
☐ Reagent red blood cells
☐ Anti-human globulin

☐ ii. The diagnostic test is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure; and

☐ iii. The diagnostic test is shipped in compliance with 21 CFR §312.160.

Exempt Category 3

☐ The drug is intended solely for tests in vitro or in laboratory research animals if shipped in accordance with 21 CFR 312.60

Exempt Category 4

☐ A clinical investigation involving use of a placebo if the investigation does not otherwise require submission of an IND.

2. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

^{13}C-labeled acetate, ^{13}C-labeled BHB, ^{13}C-labeled lactate and ^{13}C-glucose have been administered by the PI and his mentors Drs Sherwin and Rothman in previous studies (HIC # 10605) without any side effects:
Alternate Substrate infusion rates consist of a bolus of 16.7ml/min for 20 minutes followed by a continuous rate of 0.77ml/min for an additional 100 minutes. Glucose will be infused at the same rate as required to maintain a subject's plasma glucose level at the target hypoglycemic level of 55mg/dl. Infusion of unlabeled lactate (350 mmol/L) will occur at a priming dose of 300mol/kg given over 5 min followed by a continuous infusion of 20mol/kg/min for 120min.
Of note, carbon-13 and deuterium are natural isotopes of carbon. They are non-radioactive and have no side effects.

3. **Source:** a) Identify the source of the drug or biologic to be used.

The tracer-labeled energy substrates are purchased in powder form (from Sigma-Aldrich or Cambridge Isotopes, Inc.) and have 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.

Clinical grade labeled acetate, BHB and lactate are purchased in powder form (also from Sigma-Aldrich or Cambridge Isotopes, Inc.) which has been certified to be sterile and free of pyrogens. The acetate is made into a solution of 350mmol/liter by the Yale New Haven Hospital Investigational Drug Service (IDS). The solutions are tested to be free of pyrogens and to be sterile, and they have a neutral pH balance before being administered. All certifications are kept with the IDS.

b) Is the drug provided free of charge to subjects? ☐ Yes ☐ No If yes, by whom?

N/A

4. **Preparation and Use:** Describe the method of preparation, storage, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.

Check applicable Investigational Drug Service utilized:

☒ **YNHH IDS**	☐ **Yale Cancer Center**
☐ **CMHC Pharmacy**	☐ **West Haven VA**
☐ **Other:**	☐ **None**

Note: If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.

The compounds will be sterility tested and certified to be free of pyrogens by the company. All certifications are kept in YNHH-IDS. YNHH-IDS prepares the study infusion the day before the study and the study infusate is kept at 4°C.

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

If use of a placebo is planned, provide a justification which addresses the following:

a. Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.
b. State the maximum total length of time a participant may receive placebo while on the study.
c. Address the greatest potential harm that may come to a participant as a result of receiving placebo.
d. Describe the procedures that are in place to safeguard participants receiving placebo.

6. **Use of Controlled Substances:**

Will this research project involve the use of controlled substances in human subjects?
☐ Yes ☒ No *See HIC Application Instructions to view controlled substance listings*.

If yes, is the use of the controlled substance considered:
☐ Therapeutic: The use of the controlled substance, within the context of the research, has the potential to benefit the research participant.
☐ Non-Therapeutic: *Note, the use of a controlled substance in a non-therapeutic research study involving human subjects may require that the investigator obtain a Laboratory Research License. Examples include*

controlled substances used for basic imaging, observation or biochemical studies or other non-therapeutic purposes. See Instructions for further information.

7. Continuation of Drug Therapy After Study Closure ☒ **Not applicable to this project**
Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended?
☐ Yes If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access.

☐ No If no, explain why this is acceptable.

B. DEVICES

1. Are there any investigational devices used or investigational procedures performed at YNHH, e.g., YNHH Operating Room or YNHH Heart and Vascular Center? Yes ☐ No ☒

SECTION VII: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. Targeted Enrollment: Give the number of subjects:

1.1. targeted for enrollment at Yale for this protocol 100_
1.2. 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 E-mail Solicitation	☐ Telephone
☒ Letter	☒ Departmental/Center Website	☐ Television
☐ Medical Record Review	☒ Departmental/Center Research Boards	☐ Newspaper
☐ Departmental/Center Newsletters	☐ Web-Based Clinical Trial Registries	

☒ Other (describe): Yalestudies.org and yalestudies social media Facebook and Twitter
☐ Clinicaltrials.gov Registry (do not send materials to HIC)

3. Recruitment Procedures:
3.1. Describe how potential subjects will be identified.

Some subjects will be identified through their previous participation HIC #1009007394: "Effect of a long-term Medium Chain Triglyceride diet on cognitive function and brain activation." and HIC #0505000079: "Effect of Medium Chain Fatty Acids in cognitive function during acute hypoglycemia in patients with type 1 diabetes.".
Possible study participants will be approached by their caregivers or study staff at the different locations where Type 1 Diabetic patients are seen at Yale SOM, YNHH and YMG. These sites include the Adult Endocrine clinic (Yale Diabetes Center, YNHH), the Yale Pediatric Endocrine Clinic, Veterans Affairs Hospital, West Haven, community clinics and Yale University Health. We will also accept referrals from outpatient clinics in the community. In addition, the diabetes registry will be used to identify eligible patients and contact them by phone or letter, since this category of patients have already given their consent to be contacted for potential trials research. Flyers will be put up on notice boards at and around Yale University and Yale New Haven Hospital. Study volunteer listings will be requested from the YCCI volunteer database (HIC#0805003779) to assist with recruitment of subjects. Also, the Diabetes Registry (HIC#0911005973) will be used to contact Type 1 Diabetic subjects to discuss enrollment into the study. Subjects will be contacted through email and telephone. Brief screenings will take place either over the telephone or email and subjects will then be invited to a full screening at the HRU.

3.2. Describe how potential subjects are contacted.
3.3. Who is recruiting potential subjects?

Flyers will be put up on notice boards at and around Yale University and Yale New Haven Hospital. Study volunteer listings will be requested from the YCCI volunteer database (0805003779) to assist with recruitment of subjects. Also, the Diabetes Registry (HIC# 0911005973) will be used to contact Type 1 Diabetic subjects to discuss enrollment into the study. Subjects will be contacted through email and telephone. Brief screenings will take place either over the telephone or email and subjects will then be invited to a full screening at the HRU.

The text to appear on yalestudies.org is as follows:
Purpose (healthcare version): The goal of this study is to characterize the adaptations of brain metabolism in T1DM subjects exposed to frequent hypoglycemic episodes.
We will determine the contribution of alternate energy substrates to brain neurotransmitter metabolism under experimentally controlled low blood sugar levels. For purposes of this study we are looking to enroll healthy controls and T1DM subjects.
Purpose (subject friendly version): We are trying to understand how brain metabolism in T1DM subjects changes in order to develop therapies that can protect the brain from injury during events of extreme low blood sugar.
Inclusion Criteria: Type 1 Diabetes Mellitus >5 years duration
HbA1c <7.5%
A person in good health

Exclusion Criteria:
1. Significant Systemic Disease
2. Pregnancy
3. Significant Anemia
4. Excessive Exercise
5. Vegetarian Diet
6. Excessive Alcohol Use
   Substance Abuse

Compensation offered: **Up to $350**

4. Screening Procedures
4.1. Will email or telephone correspondence be used to screen potential subjects for eligibility prior to the potential subject coming to the research office? ☒ Yes ☐ No

Through telephone correspondence, potential subjects will be asked the following information: Name, age, gender, date of birth, place of birth, race/ethnicity, address, phone number, height, weight, history of diabetes including treatment and complications, most recent HbA1c level, medications, history of other medical problems, history of claustrophobia, vegetarian, right/left handed, and previous studies at Yale. This information will be protected according to HIPPA policies and will be destroyed if the individual is

determined to not be eligible for the study during this telephone correspondence. The purpose and potential complications of the study will be explained to each subject in detail.
The actual screening visit cannot occur until the participant has signed the informed consent. During the screening visit, the informed consent form and study details are reviewed in detail by research staff and the subject will be asked to read the informed consent form (approved by the Yale Human Investigations committee). The subject will be given time to ask questions and only after that will the subject be asked to give written informed consent to participate.
At the screening visit, the participant will undergo the following assessments: physical examination with height and weight, medical history, blood draw for hematocrit, HbA1c levels, potassium, bicarbonate, creatinine, ALT/AST, lipid profile, ketones, and c-peptide. If female and of childbearing potential, a pregnancy test will be performed. Inclusion will be based on the above information and determined by the PI.

4.2. If yes, identify below all health information to be collected as part of screening and check off any of the following HIPAA identifiers to be collected and retained by the research team during this screening process.

Prior to the potential subject traveling to Yale for the screening visits, a collection of information concerning their eligibility will be done to help determine if they are eligible to be screened for inclusion. This will be done only to prevent unnecessary screening and travel for those who can be determined to be ineligible based on recognizable exclusion criteria. Through telephone correspondence, potential subjects will be asked the following information: Name, age, gender, date of birth, place of birth, race/ethnicity, address, phone number, height, weight, history of diabetes including treatment and complications, most recent HbA1c level, medications, history of other medical problems, history of claustrophobia, vegetarian, right/left handed, and previous studies at Yale. This information will be protected according to HIPPA policies and will be destroyed if the individual is determined to be not be eligible for the study during this telephone correspondence.

HEALTH INFORMATION TO BE COLLECTED:

HIPAA identifiers:
☒ Names
☒ All geographic subdivisions smaller than a State, including: street address, city, county, precinct, zip codes and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly-available data from the Bureau of the Census: (1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people, and (2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.
☒ Telephone numbers
☐ Fax numbers
☒ E-mail addresses
☐ Social Security numbers
☐ Medical record numbers
☐ Health plan beneficiary numbers
☐ Account numbers
☒ All elements of dates (except year) for dates related to an individual, including: birth date, admission date, discharge date, date of death, all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older
☐ Certificate/license numbers
☐ Vehicle identifiers and serial numbers, including license plate numbers
☐ Device identifiers and serial numbers
☐ Web Universal Resource Locators (URLs)
☐ Internet Protocol (IP) address numbers
☐ Biometric identifiers, including finger and voice prints

☐ Full face photographic images and any comparable images
☐ Any other unique identifying numbers, characteristics, or codes

5. 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.

Subjects with type 1 diabetes will be recruited from the surrounding area. Therefore it is possible that some patients may be under the care of the principle investigator, Dr Herzog, in the setting of the Yale Diabetes Center.

6. 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 purposes only: Yes___X___

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data;

We are requesting a waiver of HIPPA Authorization in order to utilize the YNHH admission lists that would indicate patients we may not be able to contact prior to their discharge from the hospital. Such cases may be admissions that are over the weekends and holidays that may meet our criteria for inclusion. We would like to be able to identify Type 1 diabetic potential subjects that may be interested in participating, but were discharged prior to being able to discuss the study in person. We will contact the clinician that the patient is scheduled to see for follow up care and ask that they assist with referring the potential subject to contact us, or will allow us to speak to the subject at their next visit directly if the subject agrees when asked by their clinician.

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; We will obtain signed consent for the entire study if the subject agrees to participate.

By signing this protocol application, 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.

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.

7. **Required HIPAA Authorization:** If the research involves the creation, use or disclosure of protected health information (PHI), separate subject authorization is required under the HIPAA Privacy Rule. Indicate which of the following forms are being provided:

   ☒ Compound Consent and Authorization form
   ☐ HIPAA Research Authorization Form

   Consent Personnel: List the names of all members of the research team who will be obtaining consent/assent.

   Raimund Herzog, M.D. ; Katrin Endrikat
   Yvette Strong; Domenico Trico, M.D.

8. **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.

The study will be described to the subject in detail, including the purpose and potential risks associated with the study. This will be explained by the physician, including the intervention and process for the study day. The subject will be required to read and sign the consent form approved by the HIC, and the patient will retain a copy of this consent to review. Additionally, the patient will sufficient time to ask questions during the screening and will be encouraged to contact investigators with further questions or concerns.

9. E**valuation 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.

In this study, we are not using vulnerable subjects. Subjects will be asked open-ended questions in order to evaluate their understanding of the research protocol and the risks involved.

10. **Documentation of Consent/Assent:** Specify the documents that will be used during the consent/assent process. Copies of all documents should be appended to the protocol, in the same format that they will be given to subjects.

A compound authorization and consent form will be used.

11. **Non-English Speaking Subjects:** Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. Translated copies of all consent materials must be submitted for approval prior to use.

N/A

12. **Consent Waiver: In certain circumstances, the HIC may grant a full or partial 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 a Consent Waiver**
    ☒ **Requesting a waiver of signed consent**
    ☐ **Requesting a full waiver of consent**

A. Waiver of **signed** consent: (Verbal consent from subjects will be obtained. **If PHI is collected, information in this section must match Section VII, Question 6**)

☒ **Requesting a waiver of signed consent for recruitment/screening only**

☐ **Requesting a waiver of signed consent for the entire study (**Note that an information sheet may be required.)

If requesting a waiver of signed consent, please address the following:

a. Would the signed consent form be the only record linking the subject and the research?
☐ Yes ☐ No

b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☐ No

OR

c. Does the research pose greater than minimal risk? ☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** ☐ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

B. **Full waiver** of consent: (No consent from subjects will be obtained.)

☐ **Requesting a waiver of consent for recruitment/screening only**

☐ **Requesting a full waiver of consent for the study (Note: If PHI is collected, information here must match Section VII, question 6.)**

If requesting a full waiver of consent, please address the following:

a. Does the research pose greater than minimal risk to subjects? ☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** ☐ No

b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No

c. Why would the research be impracticable to conduct without the waiver?

d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

SECTION VIII: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

a. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?

Protected health information will include all blood work, urine pregnancy in women, medical history and physical examination results.

b. How will the research data be collected, recorded and stored?

To comply with HIPAA standards, patient identification will be kept confidential. Subjects will be identified by code number, not by name during the data analysis. This data will be stored in computer files by code number, and their names will appear only on the initial form. Data that links the subject's number to the subject will be kept in password

protected documents and kept on a secured server. Any data that contains patient's identifying information will be released only with written consent from the patient.

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

d. 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?

The documents will be password protected and held on a secured server which will only be available to the principle investigator and the co-investigators.

Do all portable devices contain encryption software? ☒ Yes ☐ No
If no, see http://hipaa.yale.edu/guidance/policy.html

e. 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.

Individual's names will appear only on the initial forms and these will be kept under on a secured server. All data files that could link a code number to an individual study subject will be password protected. Once the study is completed zeroing will be used to remove any identifiable subject information.

f. Who will have access to the protected health information (such as the research sponsor, the investigator, the research staff, all research monitors, FDA, QUACS, SSC, etc.)? (please distinguish between PHI and de-identified data)

Principle investigators and co-investigators only, no external agencies.

g. If appropriate, has a Certificate of Confidentiality been obtained?

N/A

h. Are any of the study procedures likely to yield information subject to mandatory reporting requirements? (e.g. HIV testing – reporting of communicable diseases; parent interview -incidents of child abuse, elderly abuse, etc.). Please verify to whom such instances will need to be reported.

N/A

SECTION IX: 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.)

Study subjects will derive no direct medical benefits from the study. However, the potential benefits of this study to society at large, and to the T1DM population in particular, are substantial in that the knowledge gained from this work should provide important information concerning the effects of recurrent, antecedent hypoglycemia on CNS responses to hypoglycemia. This information could contribute significantly to clinical indications and patient selection for the islet transplantation procedure.

SECTION X: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?

No alternative treatments are available.

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.

Subjects assigned to Cohort 1 will receive a total of $350 for participating in the study ($50 for the completion of the first scan and $300 for completion of the second scan). Subjects assigned to Cohort 2,3 and 5 will receive $300 for completing the study. Subjects assigned to Cohort 4 will be paid $60 for their participation. Payments will be given to the subject via a Bank of America pre-paid debit card using the Yale electronic payment service.

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.

Subjects will not be responsible for any costs associated with the study or the screening portion prior to the study, including blood work, history and physical examination, or urine pregnancy test. We believe that given the length and number of procedures performed, the remuneration is fair and is not an excessive inducement for people to volunteer for the study.

4. **In Case of Injury:** This section is required for any research involving more than minimal risk.
   a. Will medical treatment be available if research-related injury occurs?
   b. Where and from whom may treatment be obtained?
   c. Are there any limits to the treatment being provided?
   d. Who will pay for this treatment?
   e. How will the medical treatment be accessed by subjects?

If injury were to occur, we will provide any care of treatment required. Our study has no provision for additional compensation. While participating in the study procedures the overseeing physician, Dr. Herzog or one of his designated co-investigators will be responsible for providing treatment. If necessary and the treatment requirement exceeds what can be provided at the study site, the participant will be transferred to the YNHH Emergency Department for further care. The participant's insurance will be billed for the

cost of this treatment. Should problems arise after completion of the study the subjects will be provided with a telephone number to reach the PI's office and additional numbers

that will allow 24hr access to the study investigators.

TIMELINE and Blood Drawing Schedule for Hypoglycemic clamp study and recovery

Time	Real Time	YSI glucose q5 min	Insulin	Glucagon	Catachols	GCMS / other analytes	Lactate & Ketones	Cortisol and GH	Notes
A.1.___	Baseline	X	X	X	X		X	X	
B.1._____	Start insulin/ start clock	Glucose is checked every 5 minutes	X	X	X				Enter scanner
B.2._____	B.1 +30	X	X	X	X				
B.3._____	B.1 +60	X	X	X	X				
B.4._____	B.1 +90	X	X	X	X				
C.1._____ BHB Baseline	**Start BHB infusion**	Glucose is checked q5 min	X	X	X	X	X	X	Target glucose ~55mg/dl
C.2._____	C.1 +5	↓				X			↓
C.3._____	C.1 +10	↓				X			↓
C.4._____	C.1 +15	↓				X			↓
C.5._____	C.1 +30	↓	X	X	X	X	X		↓
C.6._____	C.1 +60	↓	X	X	X	X	X	X	↓
C.7._____	C.1 +90	↓	X	X	X	X	X		↓
C.8._____	C.1 +120	Glucose check	X	X	X	X	X	X	End Clamp

COHORT 1:

Schedule of Events:	Screening Visit **Session A**	Proton-NMR Spectroscopy **Session B**	Carbon 13-NMR Spectroscopy **Session C**	Total Blood Collected
Informed Consent	X			
Medical History	X			
Physical Exam	X			
EKG	X			
Hematocrit (3 mLs)	X (3mLs)			
Creatinine (2 mLs)	X (2mLs)			
Liver Function Tests (3mLs)	X (3mLs)			
HbA1c (3mLs)	X (3mLs)			
Inclusion/Exclusion Check list	X			
Record Blood Sugar Levels 5x daily *	X			
Complete questionnaire for glucose readings <54mg/dl *	X			
Schedule two MR sessions	X			
Urine Pregnancy (females)	X	X		
IV Placement			X	
Hypoglycemic Clamp			X	
Blood Volume per visit	11 ml	none	< 250ml	< 250ml

*: For diabetic subjects only

COHORT 2:

Schedule of Events:	Screening Visit **Session A**	Carbon 13-NMR Spectroscopy **Session C**	Total Blood Collected
Informed Consent	X		
Medical History	X		
Physical Exam	X		
EKG	X		
Hematocrit (3 mLs)	X (3mLs)		
Creatinine (2 mLs)	X (2mLs)		
Liver Function Tests (3mLs)	X (3mLs)		
HbA1c (3mLs)	X (3mLs)		
Inclusion/Exclusion Check list	X		
Record Blood Sugar Levels 5x daily *	X		
Complete questionnaire for glucose readings <54mg/dl *	X		
Schedule MR sessions	X		
Urine Pregnancy (females)	X	X	
IV Placement		X	
Hypoglycemic Clamp		X	
Blood Volume per visit	11 ml	< 250ml	< 250 ml

*: For diabetic subjects only

COHORT 3:

Schedule of Events:	Screening Visit **Session A**	Proton-NMR Spectroscopy **Session B**	Total Blood Collected
Informed Consent	X		
Medical History	X		
Physical Exam	X		
EKG	X		
Hematocrit (3 mLs)	X (3mLs)		
Creatinine (2 mLs)	X (2mLs)		
Liver Function Tests (3mLs)	X (3mLs)		
HbA1c (3mLs)	X (3mLs)		
Inclusion/Exclusion Check list	X		
Record Blood Sugar Levels 5x daily *	X		
Complete questionnaire for glucose readings <54mg/dl *	X		
Schedule MR sessions	X		
Urine Pregnancy (females)	X	X	
IV Placement		X	
Hypoglycemic Clamp		X	
Blood Volume per visit	11 ml	< 250ml	< 250 ml

*: For diabetic subjects only

COHORT 4:

Schedule of Events:	Screening Visit **Session A**	Proton-NMR Spectroscopy **Session B**
Informed Consent	X	
Inclusion/Exclusion Check list	X	
Urine Pregnancy (females)	X	X
Blood Volume per visit	none	none

COHORT 5:

Schedule of Events:	Screening Visit **Session A**	**Cognitive Testing Session D**	Total Blood Collected
Informed Consent	X		
Medical History	X		
Physical Exam	X		
EKG	X		
Hematocrit (3 mLs)	X (3mLs)		
Creatinine (2 mLs)	X (2mLs)		
Liver Function Tests (3mLs)	X (3mLs)		
HbA1c (3mLs)	X (3mLs)		
Inclusion/Exclusion Check list	X		
Record Blood Sugar Levels 5x daily *	X		
Complete questionnaire for glucose readings <54mg/dl *	X		
Schedule cognitive testing session	X		
Urine Pregnancy (females)	X	X	
IV Placement		X	
Hypoglycemic Clamp		X	
Alternate Fuel (BHB) infusion		X	
Cognitive Testing	X	X	
Blood Volume per visit	11 ml	< 250ml	< 250 ml

*: For diabetic subjects only

Any unused serum collected from all cohorts may be kept for additional metabolic studies or for confirmation of findings at a future time for both subject groups (non-diabetic and diabetic).