Official Title: Testing glial pathways to HAAF in human subjects using 13C magnetic resonance spectroscopy

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Objectives

CNS metabolism is maintained at the cellular level via interactions between neurons, glia, and the cerebral vasculature. Previous work has identified glial metabolism as a potential biological substrate driving hypoglycemia-associated autonomic failure (HAAF). Specifically, shown in an animal model, low glucose availability induces glial signaling in brain regions responsible for glucodetection, while others have detected altered glial metabolism and substrate preference in human subjects with HAAF. The alterations in glial metabolism associated with HAAF are strikingly similar to those induced by prolonged dietary restriction in rodents. This raises the intriguing possibility that HAAF may be driven by glial adaptations, normally induced only by prolonged starvation, which are triggered in diabetic individuals by treatment-induced exposure to severe hypoglycemia.

Aim 1: Using a prospective observational study design, test whether a 72 hour fast will cause acute alterations in human glial metabolism.

Hypothesis: Prolonged fasting will induce changes in glial metabolism similar to those previously measured in individuals with HAAF.

Aim 2: Determine if variations in fasting induced hypoglycemia and hypoleptinemia are correlated with changes in glial adaptation.

Hypothesis: The magnitude of the hypoglycemia and hypoleptinemia experienced during fasting will predict the level of glial adaptation measured in each subject.

Background

Although diabetes is largely regarded as a disease of hyperglycemia, diabetics are particularly vulnerable to treatment induced hypoglycemia. Following the detection of severe hypoglycemia by the central nervous system (CNS), a series of physiological countermeasures return serum glucose to euglycemic levels. This process is rarely recruited in healthy adults, except in times of prolonged starvation or in endurance athletics (1). Yet, due to the pancreatic dysfunction associated with diabetes, diabetic patients are uniquely reliant on this physiological response. Tragically, repeated episodes of hypoglycemia leads to a progressive loss of CNS hypoglycemic counter regulation. This condition, termed hypoglycemia-associated autonomic failure (HAAF), leaves diabetic patients particularly vulnerable to increasingly severe bouts of treatment-induced hypoglycemia (1-4). These hypoglycemic crises are a significant

impediment to the maintenance of healthy plasma glucose levels in both type 1 and type 2 diabetics (2, 3, 5, 6), and are associated with a 3.4 fold increase risk of death (7). One insidious aspect of HAAF is the development of hypoglycemic unawareness, a situation in which the normal cues of hypoglycemia such as sweating, tremor, and anxiety are lost, thereby impeding a diabetic's ability to behaviorally counteract hypoglycemic episodes (2, 5, 6). Results from long-term longitudinal studies suggest that between 6-10% of individuals with type 1 diabetes (T1DM) die as a result of acute hypoglycemia (8-10), undoubtedly a large majority of these deaths are caused by the development of HAAF (11). Although HAAF can often be reversed by prolonged avoidance of hypoglycemia, intensive glycemic therapies aimed at minimizing hyperglycemia are directly linked to increases in hypoglycemic episodes (12-15). This subjects individuals with diabetes to an untenable Faustian bargain, where reductions in long-term complications are acquired at the price of increased risk of acute death (16). The above only underscores the need for a more complete understanding of the underlying dysfunction which leads to HAAF, with the ultimate goal of reducing the acute risk of hypoglycemia associated with interventions utilizing tight glycemic control.

Although the precise mechanisms underlying the development of HAAF are unknown, it is thought to be driven by CNS adaptations, either in the brain cells which detect low glucose availability directly, or in brain regions which contain or regulate these cells (1, 2, 4). Various mechanisms of adaptation have been proposed, which include altered CNS glucose utilization, modified CNS glycogen storage, and alterations in neurotransmission and/or neuromodulation, yet the data supporting each of these mechanisms are largely inconsistent (1, 4). Recent advances in neuroimaging techniques have provided novel insights regarding functional and metabolic changes in the CNS associated with exposure to acute hypoglycemia (17). Most notably, studies utilizing innovative 13C magnetic resonance spectroscopy (MRS) techniques have demonstrated that glial metabolism is altered in humans and animals with type 1 diabetes (18, 19), in diabetic patients with HAAF (20), as well as in animals models of HAAF (21). These findings suggest that the development of HAAF could be directly attributable to alterations in glial metabolism following exposure to hypoglycemia. Therefore, in order to gain a better understanding of the mechanisms underlying the development of HAAF, it is critical to directly link acute reductions in glucose availability to changes in glial metabolism in humans. This is precisely the scientific goal of this research proposal.

Inclusion and Exclusion Criteria

Participants will complete an initial screening (phone or online) and a screening visit at the Pennington Biomedical Outpatient Clinic to assess eligibility.

Inclusion Criteria:

- Male
- BMI 20.0-27.9 kg/m^2
- 18-40 years old
- Willing to reside at Pennington Biomedical for 4 days

Exclusion Criteria:

- Type 1 diabetes mellitus

- Type 2 diabetes mellitus
- Fasting glucose ≥ 110 mg/dL (determined at screening visit)
- Hyperketonuria >15 mg/dL, (determined at screening visit)
- Contraindication to MRI
- History of or current eating disorder
- History of obsessive compulsive disorder
- Unwilling to discontinue use of any medication (including OTC pain medication) during impatient visit.
- Contraindication to prolonged fasting
- Consume >10 alcoholic drinks/week
- Based on the investigative team's clinical judgement, a subject may not be appropriate for participation in the study.

Our eligibility criteria for the study is limited to males in an effort to reduce variability within our small sample size due to the known gender differences in the physiological response to hypoglycemia both acutely (22, 23), as well as during a 72-hr fast (24). Previous studies have demonstrated a reduced release of counterregulatory hormones (e.g. epinephrine and glucagon) in response to hypoglycemia in women. Given that this is a pilot study with a limited number of participants to be studied, we have chosen to minimize the potential variability in our outcome measures caused by these gender differences, by limiting our enrollment to males. This will allow us to minimize our participant numbers.

Number of Subjects
Up to ten subjects will be enrolled in this study.

Recruitment Methods
Standard recruitment methods (email, print, social media advertisements, etc.) will be used to identify potential participants. As this is a study requiring consecutive overnight stays at the Inpatient Unit at Pennington Biomedical, the PI may submit a privacy board request identifying potentially eligible participants who have previously completed extended-stay studies at Pennington Biomedical and have consented to future contact.

Study Timelines
A participant's duration of study participation will be approximately 4 days.
The estimated duration to enroll all study subjects is anticipated to be nine months.
The estimated duration to complete the study (primary analyses) is 1 year (April 2016)

Study Endpoints

Primary endpoints:

- Percent 13C enrichment of cerebral glutamine, glutamate, and bicarbonate, as measured by magnetic resonance spectroscopy (MRS)
- Glial acetate oxidation rate and astroglial TCA cycle rate ascertained through mathematical modeling of MRS data

Secondary endpoints:

- Change in glucose levels
- Change in leptin levels

Procedures Involved

The study design and schedule of assessments are depicted in **Figure 1** and **Table 1**.

Table 1: Schedule of Assessments

Measurement	Screening Visit	Observational Study Visit Day 0	Day 1	Day 2	Day 3
Height	X				
Weight	X	X	X	X	X
Medical History	X				
Physical exam	X				X
Blood collection		X	X	X	X
Blood collection for future use**	X	X	X	X	X
Urine collection	X	X	X	X	X
Blood glucose*	X				X
ECG	X				
CGM		X	X	X	X
Vital signs	X	X	X	X	X
^{13}C - MRS†		X			X
DXA		X			
Adverse events			X	X	X

*via finger-stick.
**Optional
†IV procedure and serial blood collection
CGM: Continuous glucose monitoring

Participants will complete a screening visit, and a 4-day observational inpatient stay.

Screening:

Participants will complete a screening visit at the Pennington Biomedical Outpatient Clinic to assess eligibility. After providing written informed consent, the following procedures will be completed: anthropometrics, vital signs, 10ml blood draw for future use (optional), fasting glucose by finger stick, urine collection to measure ketonuria, ECG, and medical history and physical examination by a physician or nurse practitioner.

4-Day Observational Inpatient Stay:

Eligible subjects will be scheduled for the observational study visit (OSV) which lasts for approximately 80 continuous hours. The OSV begins (Day -1) with provision of a standard lunch and supper (Standard American Diet) to be consumed the day prior to the fast. Subjects will be instructed to eat only the food provided for lunch and supper, and arrive at the inpatient unit the following morning after at least a 10 hour overnight fast.

On Day 0, subjects will be weighed, vital signs and body composition (DXA) will be measured, and blood (4mL) and urine will be collected for baseline measurements of serum hormones, glucose, and ketones, as well as ketonuria. Blood (10 mL) will also be collected for future use if the participant provides permission. Baseline glial metabolism will be measured via MRS utilizing a simultaneous intravenous infusion of ^{13}C labeled acetate. ^{13}C-acetate will be administered for the first hour of the MRS procedure, at a rate of 6 mg/ kg per min for the first 5 minutes, and 3 mg/kg per min for the remainder.

Following the MRS scan, subjects will be provided a standard breakfast, and then will begin ~3 days and 3 hours of fasting. During the fast, subjects will be allowed to drink water ad libitum and to perform normal ambulatory activity, excluding exercise. A subcutaneous continuous glucose monitoring system will be utilized to track glycemia during the first 72 hours of the fast. After approximately 24, 48, and 72 hours of the initiation of the fast, blood (10mLfor study use and 10mL for future use if the participant provides permission) and urine will be collected, weight and vital signs measured, and adverse events recorded. At 72 hours of the fast, measurement of glial metabolism will repeated via MRS. Subjects will then be provided breakfast (Standard American Diet, overfed by 50%) and discharged from the Inpatient Unit after an end of study evaluation (physical, blood glucose) by the Medical Investigator.

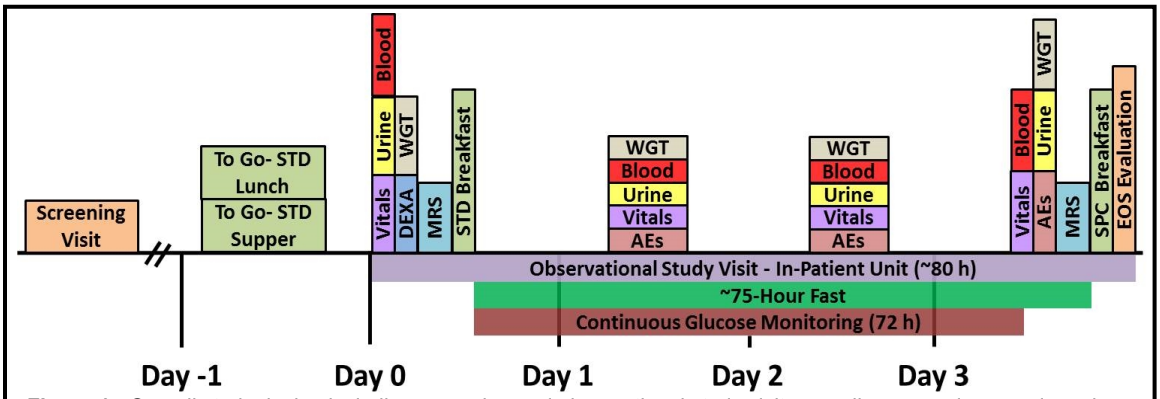

Figure 1 - Overall study design including screening and observational study visit, as well as procedures and meals. Abbreviations: STD (standard), WGT (weight), MRS (magnetic resonance spectroscopy scan), AEs (adverse events), SPC (special), EOS (end of study)

Study Procedure Descriptions

- Acetate infusion/MRS procedure: Glial metabolism will be measured via MRS utilizing a simultaneous intravenous infusion of 13C labeled acetate. An intravenous catheter will be placed in a vein of each arm, one to infuse 13C-acetate and the other to draw blood samples.
    - Participant alignment and baseline scans: 30 to 40 minutes prior to the start of acetate infusion, participants will be placed in the MRI scanner to conduct both preliminary alignment via MRI as well as baseline MRS scans.
    - Acetate infusion protocol: The infusate, 350 mM [1-13C] acetate (99% enriched; Microbiological/Pyrogen tested), will be prepared by the PBRC Pharmacy. ^{13}C-acetate will be administered for the first hour of the MRS procedure, at a rate of 6 mg/kg per minute for the first 5 minutes, and 3 mg/kg per minute for the remainder.

  - Blood sampling protocol: Venous blood samples (approximately 2 mL each) will be obtained about every 10 minutes, starting 20 minutes before the beginning of the acetate infusion, and will be continued throughout the infusion (~15 total draws).
  - MRS protocol: During IV infusion of [1-13C] acetate, MRS spectra will be collected every 10 minutes for 120 minutes using a 3 Tesla GE SIGNA MRI scanner housed in the PBRC Biomedical Imaging Center.
  - Analysis: The blood collection will be used to measure changes in plasma acetate concentrations and % C13 enrichment of plasma acetate over time, via mass spectrometry. The MRS spectra will be used to calculate changes in cerebral 13C enrichment of glutamine, glutamate, and bicarbonate over time, which reflects glial metabolism and glial amino acid synthesis.
- Anthropometrics: Fasting body weight will be collected with participants wearing a hospital gown and underwear. Height will be collected once at screening.
- Blood collection (study use): Approximately 37.5 mL of whole blood will be drawn during each acetate infusion/MRS assessment on Days 0 and 3 (described above), and approximately 10 mL of additional whole blood will be collected on Days 0, 1, 2, and 3. The total volume of blood that will be collected during the study is approximately 115 mL (37.5 mL + 10 mL + 10 mL + 10 mL + 10 mL + 37.5 mL). Blood will be collected during the MRS assessment to measure plasma acetate concentrations and % C13 enrichment of plasma acetate via mass spectrometry. Blood will be collected on Days 0, 1, 2, and 3 of the observational study to measure metabolic hormones of interest, such as β-hydroxybutyrate, free fatty acids, insulin, leptin, glucagon, glucose, and catecholamine concentrations.
- Blood collection (future use): For participants who provide permission, whole blood will be collected for future research (10mL at screening and 10mL at Days 0, 1, 2, and 3; 50ml total). Samples collected will be stored for future use by PBRC researchers and collaborators.
- Continuous glucose monitoring: Blood glucose will be assessed using continuous glucose monitoring (CGM). Briefly, the abdominal area will be disinfected, and then trained staff from the Inpatient Unit will insert a glucose sensor under the skin in the abdominal area. The sensor has a small needle-like probe that inserts into the subcutaneous fat of the abdomen and that measures blood glucose levels without removing blood from the body. The sensor will then be attached to the recording unit, and the set-up will be secured with adhesive to the participant's body. After an initial period of equilibration with interstitial glucose, the sensor will be calibrated about every 6-12 hours by pricking the participant's finger to measure capillary blood glucose. The CGM device records blood sugar levels every 5 minutes.
- DXA: Dual X-ray absorptiometry (DXA) scans will be performed using a General Electric Lunar iDXA whole-body scanner (General Electric, Milwaukee, WI) during the first day of the Inpatient stay. The protocol requires that subjects lie on a table wearing a hospital gown and no metal containing objects, while the scanner emits low energy X-rays and a detector passes along the body. The scan takes approximately 10 to 15minutes and the radiation dose is less than 1 mrem, equal to about 12 hours of background radiation. In our hands at PBRC, the standard deviations for DXA measurements are 320g (CVs of 0.6%) and 300g (CVs of 1.1%) for FFM and FM, respectively.

- Ketonuria: Participants will collect a small urine sample in a provided collection cup and ketonuria will be assessed using a Ketostix® (mg/dL).
- Vital Signs: Vital signs will be collected according to PBRC standard operating procedures. Seated vital signs (blood pressure and heart rate) will be measured after a 5 minute rest.

Data and Specimen Banking

Biospecimens banked for future use will be stored indefinitely at Pennington Biomedical in the Clinical Chemistry and McDougal Laboratories. Data will be stored with the participant's de-identified ID number. Specimens will be labeled with the participant's de-identified ID number and date of collection. Data and specimens are accessible by the PI, his staff and designated Clinical Chemistry staff in buildings with restricted access. Requests for release of data and/or specimens will be considered by the PI or a study investigator. Upon agreement to release data and/or specimens, material and/or data transfer agreements will be drafted and approved by respective parties prior to the release of any material.

Power analysis

The primary outcomes of this study are ^{13}C enrichment of brain glutamine and glutamate, measured by MRS. An a priori power analysis was performed by the Biostatistics and Epidemiology Core using means, standard deviations, and effect sizes previously reported in a similar study (20). This analysis estimated that a sample size of 6 would provide adequate statistical power for our primary outcome variables (Table 2). This protocol allows for enrollment of ten subjects to account for withdrawals and allow for complete data on six subjects.

Table 2. Power analysis for % Enrichment of Glutamine C4

Mean Diff	SD	Power	N
4.0	2.3	0.845	8
4.5	2.3	0.860	7
5.0	2.3	0.849	6
5.5	2.3	0.804	5
6.0	2.3	0.863	5

Data and Specimen Management

Study data collected and entered into the Pennington Biomedical Database is handled only by individuals with appropriate HIPAA compliance and Good Clinical Practice training. Participant charts and hard copy data are stored in locked offices with restricted access. Electronic data has exclusive restricted access granted by the Research Computing Group and/or the PI. For quality control, data and charts will be audited for completeness and accuracy (when possible).

Study data and specimens will be stored indefinitely at Pennington Biomedical in the Clinical Chemistry and McDougal Laboratories. Data will be stored with the participant's de-identified ID number. Specimens will be labeled with the participant's de-identified ID number and date of collection. Data and specimens are accessible by the PI, his staff and designated Clinical Chemistry staff in buildings with restricted access.

Data analyses will be conducted as follows:
Acetate infusion/MRS procedure: Using the time courses of 13C enrichment of glutamate, glutamine and bicarbonate (from MRS spectra), as well as the time courses of plasma acetate 13C enrichments (from mass spectroscopy on plasma) we will

mathematically model several parameters of cerebral metabolism, such as brain acetate concentrations ($[acetate]_{BR}$), Glial acetate oxidation rate (V_{gac}), and astroglial TCA cycle rate ($V_{TCA}A$). Each subject will participate in two MRS scans, one immediately prior to, and one immediately following a 72-h fast. Repeated measures ANOVA will be used to compare the mean values of percent enrichment of cerebral glutamine, glutamate, and bicarbonate over time, as well as $[acetate]_{BR}$, V_{gac}, and $V_{TCA}A$ measured before and after the 72-h fast, i.e., differences between first and second MRS assessments. P-values < 0.05 will be considered significance.
Serum hormone and metabolite measurements: Mean values of serum hormone and metabolites (including glucose) measured at each time point will be compared via repeated measures ANOVA. P-values < 0.05 will be considered significance.
Additional analysis: Correlation analysis will also be used to determine if changes in the parameters measured via our acetate infusion/MRS procedure are related to changes in our serum hormone and metabolite measurements.

Provisions to Monitor the Data to Ensure the Safety of Subjects
We will use the definitions of *Adverse Events*, *Serious Adverse Events*, and *Unanticipated Problems Involving Risks to Subjects or Others* below. Events will be recorded from the participant during their inpatient stay by experienced staff trained in the ascertainment of adverse events from research participants. For each sign, symptom or adverse event, the following information will be recorded:

- A brief descriptor of the adverse event
- Start and stop dates
- Intensity (mild / moderate / severe)
- Whether the AE was “serious” or not (as defined below)
- Causal association with the intervention assigned (none / doubtful / possibly / probably / very likely)
- Outcome (resolved / resolved with sequelae / improving / still present and unchanged / death)
- Action taken with respect to the intervention (none / intervention temporarily discontinued / medical therapy required / intervention permanently discontinued / other).

The Pennington Biomedical Research Center’s Human Research Protections Program’s definitions for adverse event, serious adverse event, and unanticipated problem involving risks to subjects or others (Policy 8) will be applied in this study and are as follows:

- An **adverse event** is any untoward physical or psychological occurrence in a human subject participating in research, including any abnormal sign (e.g., abnormal physical exam or laboratory finding, symptoms or disease associated with the research or the use of a medical investigational test article), symptom, or disease, temporally associated with the subject’s participation in the research. An adverse event does not necessarily have to have a causal relationship with the research, or any risk associated with the research or the research intervention, or the assessment.

- A **serious adverse event** is defined as an adverse event that is fatal or life-threatening, permanently disabling, requires or prolongs hospitalization or results in significant disability, congenital anomaly or birth defect.
- An **unanticipated problem involving risks to subjects or others** is defined as any incident, experience, outcome or new information where all three elements exist:
    - Is unexpected;
    - Is related or possible related to participation in the research, and
    - Indicates that subjects or others are at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

While federal guidelines do not require the reporting of adverse events to the IRB, unanticipated problems involving risks to subjects or others will be reported to the IRB within 10 working days of ascertainment of the event per HRPP guidelines.

Upon completion of each participant, participant's anthropometric and adverse event data will be reviewed by the Medical Investigator with the Principal Investigator to evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe. When incidental findings on imaging studies or out of range values on lab tests are obtained by study personnel, the participant will be notified and a copy of the report sent to his physician. For lab tests, this pertains only to those tests for which results are obtained in real-time.

Withdrawal of Subjects

Participants may be withdrawn from the research without their consent if they fail to comply with the prolonged fasting protocol or leave campus during their Inpatient stay. Participants that withdraw from the research will not be followed long-term as this protocol employs an acute intervention.

Risks to Subjects

This study does not involve major risk to study participants. To minimize the potential risks of the assessment methods and outcome variables, investigators will frequently monitor the study to assure that no volunteer suffers any adverse effects from participating in the research. Risks of complications will be reduced by carefully selecting only healthy participants to enroll in the study. The inclusion and exclusion criteria were created to ensure participants would have minimal risk for completing the study protocol. The Medical Investigator, Kishore Gadde, MD, will monitor the study closely and review all non-serious and serious adverse events. Potential risks associated with the study procedures include (listed alphabetically):

- Blood draw. There is the possibility of pain and bruising at the vein on the arm where the needle is inserted. Aseptic (sterile) technique and trained nursing staff minimizes these risks. Each acetate infusion/MRS assessment will collect approximately 30 mL of whole blood. The maximum amount of whole blood collected throughout the 4 day evaluation period (including for future use) is about 165 mL.

- Blood pressure testing. Participants may experience discomfort during blood pressure recordings due to the pressure of the cuff inflating on their arm. This discomfort is only temporary and individuals performing this assessment are experienced in clinical research studies.
- Body composition assessment: Dual energy x-ray absorptiometry (DXA) scans are determined to be minimal risk for study participants. The radiation exposure from a DXA scan with our equipment (the iDXA), which is 4 to 6 minutes in duration, is less than the equivalent of one day of environmental sun exposure.
- Body weight: There is no risk to participants to have body weight measured.
- ^{13}C-MRS: There are no known biological risks associated with magnetic resonance scanning. It has been used routinely for over 20 years. It produces side effects in very few situations. Those situations include:
    - *Metal*: Because the magnetic resonance machine uses a magnetic field, it can move any metallic objects that are inside the body. *This disruption of metal inside the body is extremely dangerous and may even be life threatening.* If the participant thinks he/she may have a cardiac stent, metallic implant, metallic piercings, shrapnel, or any other metallic material in the body, it is of utmost importance that the pariticpant alert the study coordinator or MR technician. If the participant has metallic materials in the body that cannot be removed, we will exclude the participant from this study.
    - *Electronics*: Magnetic resonance imaging involves the use of radio frequency energy that can disrupt the functioning of electronic devices. If the participant possesses a pacemaker or any other electronic medical device inside the body, the participant will be excluded.
    - *Tattoos and cosmetics*: Some tattoos and cosmetics contain metallic materials that can heat up during scanning, especially if they are located on the part of the body being scanned. If the metallic material heats up enough, the participant may feel an uncomfortable burning sensation, and a skin burn may develop. In some cases, the amount of metallic material in the area being scanned is so excessive that the scan cannot proceed without risk of a burn developing. In other cases, a cold compress placed over the metallic material can be used to prevent burning.
    - *Confinement*: During the MR scan, the participant will be lying down on a table inside of a metal tube. The metal tube is a confined place. This might produce a feeling of claustrophobia, which can be distressing. A participant who has experienced claustrophobia in the past might become too distressed to complete the scan. In this case, the scan will be halted.
    - *Noise***:** The MRI machine creates a loud, rhythmic noise that sounds like grinding or churning. This can be distressing to those who are sensitive to loud noises. The participant will be provided with earplugs to reduce the noise. But, if the participant finds the machine noises distressing, the MR technician can halt the scan.
    - *Peripheral nerve stimulation***:** During the MR scan, the magnetic field around the body goes through rapid changes. These changes are all within safety limits set by the Food and Drug Administration. But, some people experience twitching in the nerves of their arms or legs as a result

of these magnetic field changes. This twitching is generally not painful, and it stops at the end of the MR scan. But the feeling of inadvertent muscle twitching may make individuals feel disoriented or uncomfortable. Any participant who experiences this and wishes to stop the scan as a result will be allowed to do so.

- Continuous Glucose Monitoring (CGM): Because CGM involves the placement of an implantable device below the skin, there is the possibility of discomfort, pain, and bruising at the site where the device is inserted. There is also a small risk of bleeding and a very small risk of infection at the site of the blood draw. Aseptic (sterile) technique and trained personnel minimize these risks. Finally, the adhesive may cause redness or irritation of the skin.
- Prolonged fasting (72 hours): The risks associated with prolonged fasting are minimal in healthy adults, and is routinely used in clinical research studies (e.g. 22, 23), as well as for clinical diagnoses of insulinoma (e.g. 23, 24, 25). Participants are likely to experience symptoms of hypoglycemia including cold seats, palpitations, shakes, unsteadiness, drowsiness. The participants will be continually supervised by the nursing staff in the Inpatient Unit to ensure safety, and any adverse events will be recorded.
- Intravenous ^{13}C Acetate infusion: There is the possibility of pain and bruising at the vein where the needle associated with the IV line is inserted. Aseptic (sterile) technique and trained nursing staff minimizes these risks. We have significant experience in preparing and administering sterile solutions intravenously. All solution will be prepared by a licensed pharmacist. ^{13}C is a naturally occurring stable isotope of carbon which is commonly use in clinical research studies. There are no reported risks associated with administration of ^{13}C labeled compounds. The amount of acetate infused relative to blood volume is very low and therefore plasma pH is not anticipated to be affected.
- Urine collection: There is no risk to participants in collecting their urine.

Potential Benefits to Subjects
There is no direct benefit to participants.

Sharing of Results with Subjects
Results will not be shared with subjects.

Setting
This study will be conducted at Pennington Biomedical Research Center in the Outpatient Unit, Inpatient Unit and Bioimaging facility. Measurement of 1-13C acetate levels and 12C acetate levels will be completed by the Biological and Small Molecule Mass Spectrometry Core of the Department of Chemistry at the University of Tennessee-Knoxville through a contractual agreement.

Resources Available
Pennington Biomedical Outpatient and Inpatient staff are highly trained individuals with a breadth of experience in clinical research. All have completed human subjects protection training and highly knowledge of local study site and culture. A physician is

on-call 24 hours a day and is available for consultation or evaluations if necessary. The study regulatory documents will be provided to all staff involved and a startup meeting will be held to ensure all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions. Measurement of 1-13C acetate levels and 12C acetate levels will be completed by the Biological and Small Molecule Mass Spectrometry Core of the Department of Chemistry at the University of Tennessee-Knoxville through a contractual agreement. Specimens will be collected, prepared, processed, labeled, stored, and shipped by the PBRC Clinical Chemistry Lab.

Compensation
Participants will be paid up to $900 for their participation in the study. If for some reason participants are unable to complete all overnight stays and test days, they will receive payment for the procedures they have completed. They will receive up to $200 for completing Days 0, 1 & 2 each (including the overnight stays) and an additional $300 for completing through Day 3.

Confidentiality
All data and specimens will be obtained solely for research purposes. These will include 1) physical examinations, 2) medical history, 3) blood and urine samples, 4) body composition measures by DXA, 5) glial metabolism by MRS, and 6) adverse events during study participation. All data from individual subjects will be maintained for confidentiality and names and identities will not be disclosed in any published document.

Data will be stored in hard copy in participant charts, which are kept in a locked, secure location only accessible to individuals with human subjects protection training. Biospecimens will be stored within the Clinical Chemistry Laboratory and Dr. McDougal's Laboratory, both located in a building at Pennington Biomedical with restricted access. Data and biospecimens will be stored at Pennington Biomedical indefinitely however only study investigators, their staff and collaborators will have access to the data/biospecimens. The study PI is responsible for receipt of the data and biospecimens.

All specimen samples sent to the contracted Lab for analysis will be coded with unique identifiers. No personal information or personal identifiers will be sent.

Provisions to Protect the Privacy Interests of Subjects
Provisions to protect privacy interests will be untaken during this study to ensure participants feel at ease with the research situation. Participants will be continuously reminded they ask any questions or discuss any concerns in private at any time. Examinations, interviews and study procedures will be conducted in private rooms whenever possible.

Compensation for Research-Related Injury
No form of compensation for medical treatment or for other damages (i.e., lost wages, time lost from work, etc.) is available from the Pennington Biomedical Research Center

for this study. In the event of injury or medical illness resulting from the research procedures, participants will be referred to a treatment facility. Medical treatment may be provided at participant's expense or at the expense of the participant's health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.) which may or may not provide coverage. The Pennington Biomedical Research Center is a research facility and provides medical treatment only as part of research protocols.

Economic Burden to Subjects
The participants enrolled in this study are not anticipated to incur any costs during their participation.

Consent Process
Informed consent will be obtained from each study screener and participant prior to the initiation of any study procedures. The informed consent process with take place in a private room at Pennington Biomedical Research Center and participants will be allowed ample time to read and review the informed consent documents. The informed consent process will be ongoing as Pennington Biomedical staff with continue to discuss the study, its procedures and the participants' options throughout study participation.

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