Protocol Title: Use of a hyperinsulinemic-hypoglycemic clamp to study hypoglycemia: a method development study.

PI Name: David McDougal

Sub-Investigator's Name(s): Daniel Hsia, Leanne Redman, and Eric Ravussin.

Protocol Version Date: November 15th, 2018

Funding: LA CATS/Internal

Objectives

Hypoglycemic complications are a major impediment to the maintenance of healthy glucose levels in persons with diabetes. We recently completed a clinical pilot and feasibility study (GLIMPSE), which identified a novel biomarker that appears to predict the susceptibility to hypoglycemia [**1**]. By providing an assay to predict hypoglycemic events and therefore diabetic complications, the development of this biomarker could significantly improve the treatment of persons with diabetes.

To pursue such line of research requires the induction and maintenance of hypoglycemia in a controlled safe clinical research setting. A hyperinsulinemic-hypoglycemic clamp is an experimental procedure, which allows for hypoglycemia to be studied in a safe and controlled manner [e.g. **2, 3**]. The goal of this study is to establish the hyperinsulinemic-hypoglycemic clamp procedure at Pennington Biomedical Research Center in order to apply the knowledge gained to future studies which will determine the efficacy of our biomarker for predicting susceptibility to hypoglycemia [e.g. aim 2 of the PI's recently funded LA CaTS Roadmap Scholars grant (see appendix A below for project specific aims)]. Additionally, our use of continuous glucose monitoring (CGM) during the clamp procedure will provide novel data regarding the accuracy of CGM during hypoglycemic conditions in a controlled research setting.

Given that the primary purpose of this study is for method development, there is no hypothesis being tested or statistical comparison being made across study participants. We will analyze the time course of the changes in our endpoints in each subject to verify that our procedure produces results that are similar to previously published data from other institutions which have used the hypoglycemic clamp technique. This will allow us to develop an institutional MOP for the hypoglycemic clamp which can be used to safely implement this technique in future studies.

Background

Although Pennington Biomedical has extensive experience with euglycemic clamp procedures since its introduction by Dr. E Ravussin [e.g. **4, 5**], experience performing the hypoglycemic clamp procedure is lacking. The procedural differences between the euglycemic clamp and hypoglycemic clamp are minimal. For example, both procedures involve the paired infusion of insulin and glucose, with the rate of glucose infusion being adjusted to achieve a targeted steady-state blood glucose level. In most instances, the

rate of insulin infusion is identical between the two procedures, whereas the rate of glucose infusion differs due to the difference in the targeted blood glucose concentration, which is approximately 100 mg/dL for the euglycemic clamp and 50 mg/dL in the hypoglycemic clamp. Therefore, in order to achieve the lower steady state blood glucose, the initial rate of dextrose infusion during the first 45 minutes of the procedure will be significantly less than the typical rate of dextrose infusion during a euglycemic clamp.

Given our considerable experience with euglycemic clamp and the similarities between the two procedures, we do not anticipate any issues preventing successful implementation of the hypoglycemic clamp procedure. In addition, we have consulted with Dr. Amir Moheet from the University of Minnesota who has directed multiple studies which utilize this procedure [e.g. **2, 6**], to develop the procedure at Pennington (see attached letter of support).

Study Participants
We will enroll up to 8 healthy men or women (goal n=3 completers) in this method development study.

Inclusion and Exclusion Criteria
Inclusion Criteria:

- Healthy male or female
- Ages 18-40 years
- BMI between 20 kg/m^2 and 30 kg/m^2 (±0.5 kg/m^2 will be accepted)
- Medically cleared for participation in the study

Exclusion Criteria:

- History of clinically diagnosed diabetes or a fasting blood glucose >126 mg/dL
- Average screening blood pressure >140/90 mmHg
- History of cardiovascular disease
- Pregnant, planning to become pregnant, or breastfeeding
- Based on the investigative team's clinical judgement, a subject may not be appropriate for participation in the study.

If eligible, individuals will be enrolled and undergo all testing procedures.

Recruitment Methods
Potential participants will be recruited through PBRC via IRB approved recruitment materials (e.g., landing page, listserv, social media). Individuals can either complete the webscreening form directly from the PBRC landing page, call PBRC directly, or e-mail the Recruitment Core. Potentially eligible participants will then undergo a phone screen to answer a series of yes or no questions regarding eligibility. Eligible individuals will be scheduled for a screening visit at PBRC.

Study Timelines
A participant's duration of study participation will be 3 days. The estimated duration to enroll all study subjects is anticipated to be four months. The estimated duration to complete the study is 6 months.

Study Endpoints
Primary endpoints:

- Blood glucose levels

Secondary endpoints:

- Serum epinephrine, norepinephrine, glucagon, and cortisol.
- CGM glucose

The overarching goal of measuring these endpoints is to demonstrate our ability to safely perform hypoglycemic clamps, while simultaneously developing a systematic set of procedures (MOP) which will ensure a high rate of success and reliability in future studies utilizing this procedure.

Procedures
Participants will complete a screening visit, and one study visit conducted in the PBRC Inpatient Unit. See Table 1 for schedule of assessments.

Table 1. Schedule of Clinic Assessments

	Screening Visit	**Run-in Visit** (2-3 days prior to Study Visit)	**Study Visit**
Procedures			
Informed Consent	X		
Metabolic Weight, Height*, BMI	X		X
Vital Signs *(blood pressure, heart rate)*	X		X
Fasting Blood Draw *(CBC and Chem 14)*	X		
Screening Health Questionnaire	X		
Medical History	X		
Physical exam	X		
Run-in meal		X¥	
CGM sensor placement		X	
Pregnancy test (urine)			X
Hyperinsulinemic hypoglycemic clamp†			X
Continuous glucose monitoring			X
Meal (lunch)			X

*Height will only be measured during the Screening Visit
¥Meal will be consumed the night before study visit. Participant will pick up their meal up to 2-3 days prior to Study Visit.
†IV procedure and serial blood collection
CGM: Continuous glucose monitoring

Screening Visit:
Participants will complete a screening visit at the Pennington Biomedical Outpatient Clinic to assess eligibility. Participants will arrive in the morning to the PBRC Outpatient Unit, and after providing written informed consent, the following procedures will be completed: anthropometrics, vital signs, fasting blood draw (CBC and Chem 14), screening heath questionnaire, and medical history and physical examination by one of the medical staff members. The study MI, Dr. Hsia, will conduct a final review of the participants' charts to determine eligibility.

Run-in Visit:
2-3 days prior to the study Visit, participants will be scheduled for a run-in visit to pick up their meal and a continuous glucose monitor (CGM) sensor will be placed on their abdomen.

Study Visit:
Eligible subjects will be scheduled for the Study Visit which will last approximately 8 hours. Participants will be provided a run-in meal (supper) which will be consumed the evening before their visit. Participants will be instructed to 1) eat nothing but the meal provided after 3 PM, 2) eat the entire meal before 9 PM, and 3) drink only water after 3 PM. Participants will be instructed to pick up their meal within 3 days prior to their study visit.

Subjects will arrive at the inpatient unit in the morning after at least a 10 hour overnight fast. Upon arrival to PBRC, participants will be admitted to the inpatient unit. Following measurement of metabolic weight, vitals, and pregnancy test (women only), the 4-hour hypoglycemic clamp procedure (30 minute baseline, 120 minute insulin infusion, and 90 minute recovery) will be performed. Upon completion of the clamp procedure, participants will be provided lunch (Standard American Diet). Following lunch, the CGM sensor will be removed and pending a stable blood glucose level in the normal range, the participant will be discharged from the inpatient unit. This completes the study.

Study Procedure Descriptions

- Anthropometrics: Fasting body metabolic weight will be collected with participants wearing a hospital gown and underwear. Height will be collected once at screening.

- Blood collection (study use): Approximately 7.5 mL of whole blood will be collected during the screening visit. Approximately 120 mL of whole blood will be collected during the hypoglycemic clamp procedure for measurement of glucose, potassium, and counter-regulatory hormones. The total volume of blood that will be collected during the study is approximately 128 mL.

- 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 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 via the blood glucose measurement obtained during the hypoglycemic clamp procedure. The CGM device records interstitial glucose every 5 minutes and will allow a more complete profile of blood glucose changes during the study visit.

- Hyperinsulinemic-hypoglycemic clamp: An intravenous catheter will be placed in an antecubital vein for infusion of insulin and glucose. A second catheter will be placed retrograde in a dorsal vein of the contra-lateral hand for blood withdrawal. The hand will be placed in a heating box or pad at 70°C for arterialization of venous blood. A primed infusion of regular insulin (120 mU/min/m^2) will be initiated and continued for approximately 2 hours. Beginning 20 minutes prior to the start of the insulin infusion, arterialized venous blood glucose will be measured at 5 minute intervals via a Hemocue analyzer. Following initiation of insulin infusion, blood glucose will be allowed to fall to 50 mg/dL and then maintained at this level using a variable infusion of exogenous dextrose (20% solution). Our goal is to achieve steady-state (blood glucose stabilized at 50 mg/dL) within the first 45 minutes following the start of insulin infusion, thus participants blood glucose will be maintained at this level for approximately 75 minutes.

  In addition to the Hemocue glucose measurements, blood will be collected every 15 minutes starting 15 minutes prior to insulin infusion, for determination of glucose, insulin levels, and counterregulatory hormone levels in circulation (10 blood draws total). Potassium levels will be measured at three of these time points: -15, 60, and 120 minutes (relative to the start of the insulin infusion). See Table 2 for an overview of the timing of blood draws and measurements.

  Following discontinuation of the insulin, blood glucose will be normalized to baseline levels with the 20% dextrose infusion. The participant's blood glucose will continued to be monitored every 15 minutes via Hemocue analyzer for approximately 90 minutes after discontinuation of insulin infusion to ensure baseline levels of blood glucose are achieved and maintained.

  If potassium levels are below 3.8 mmol/l at any of the three measurements (-15, 60, or 120 minutes) a potassium infusion of 10 mEq/hr will be initiated and continued for the remainder of the procedure. In the event that a potassium infusion is initiated, potassium levels may be measured again following discontinuation of insulin infusion, i.e. during the 90 minute recovery period when blood glucose levels will be restored and maintained at baseline levels (120-210 minutes relative to the start of the insulin infusion).

Table 2. Schedule of measurements during clamp								
	Measurement							
	Glucose, insulin, and Potassium				Counterregulatory Hormones			
Time relative to insulin infusion	Glucose (Hemocue)	Glucose (Clinic Chem.)	Insulin	Potassium	Epinephrine	Norepinephrine	Glucagon	Cortisol
-20 minutes	x							
-15 minutes	x	x	x	x	x	x	x	x
-10 minutes	x							
-5 minutes	x							
0 minutes	x	x	x		x	x	x	x
5 minutes	x							
10 minutes	x							
15 minutes	x	x	x		x	x	x	x
20 minutes	x							
25 minutes	x							
30 minutes	x	x	x		x	x	x	x
35 minutes	x							
40 minutes	x							
45 minutes	x	x	x		x	x	x	x
50 minutes	x							
55 minutes	x							
60 minutes	x	x	x	x	x	x	x	x
65 minutes	x							
70 minutes	x							
75 minutes	x	x	x		x	x	x	x
80 minutes	x							
85 minutes	x							
90 minutes	x	x	x		x	x	x	x
95 minutes	x							
100 minutes	x							
105 minutes	x	x	x		x	x	x	x
110 minutes	x							
115 minutes	x							
120 minutes	x	x	x	x	x	x	x	x
135 minutes*	x							
150 minutes*	x							
165 minutes*	x							
180 minutes*	x							
195 minutes*	x							
210 minutes*	x							
Total per Clamp:	**35**	**10**	**10**	**3**	**10**	**10**	**10**	**10**
*Denotes measurements during the recovery period								

- Questionnaire: Participants will complete a Screening Health Questionnaire (to assess general health) at the screening visit.

- 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.

Power analysis
Given that this is a method development study and we are not comparing across groups or individual participants, a power analysis was not performed.

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: The purpose of this study is to demonstrate proficiency with the hypoglycemic clamp procedure. This will involve demonstrating the ability to achieve and maintain a steady-state blood glucose of approximately 50 mg/dL for approximately 75 minutes and that we are able to measure the known increases in counterregulatory hormone levels associated with exposure to hypoglycemia. This will be indicated by an increase in all four hormones during the steady-state period of the procedure relative to baseline.

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
- Date of onset and date of resolution
- Frequency (single / intermittent)
- Maximum intensity (mild / moderate / severe)
- Outcome (resolved / resolved with sequelae / not resolved)

- Action taken with respect to study drug/intervention (none / dose reduced / temporarily interrupted/ permanently discontinued/ intervention).
- Withdrawal (yes / no)
- Relationship to study drug/intervention
- Whether the AE was “serious” or not (as defined below)

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 possibly 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 involves major risk to study participants but with the possibility of benefitting the population at large. 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, Daniel Hsia, 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.

- 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.

- Metabolic body weight: There is no risk to participants to have body weight measured.

- 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.

- Hyperinsulinemic-hypoglycemic clamp: We expect participants to develop some symptoms of hypoglycemia during the procedure. Symptoms of hypoglycemia include:
  - Sweating,
  - Shakiness
  - Confusion
  - Increased heartbeat
  - feeling “low”

  These can be reversed within minutes by stopping the insulin infusion and by raising the blood sugar via increased rate of dextrose infusion. To be certain that the participant’s blood sugar level does not drop less than 30 mg/dL, blood glucose will be checked every 5 minutes and adjust as needed. If blood sugar drops too low, seizures or abnormalities in heart rhythm can occur. With careful monitoring, it is very unlikely that the participant’s blood glucose will drop to

unsafe levels. In the unlikely event that a participant's blood glucose does drop to less than 30 mg/dL, dextrose will be administered immediately.

The risks of hypoglycemia will be minimized by monitoring the blood glucose every 5 minutes and providing dextrose as needed to achieve the target level of blood glucose. Subjects will also wear a pulse oximeter during the study so we can monitor their heart rate. If we become unable to monitor the glucose during the study or if the participant develops a serious arrhythmia (as defined by the medical staff present), or if blood glucose drops to less than 30 mg/dL, the study will be stopped and dextrose will be given to normalize the blood glucose levels. Medical staff will be present throughout the procedure to monitor the patient. The study will be also be stopped if the medical staff feels that it is no longer safe for the participant to continue. With careful monitoring, it is very unlikely that a participant's blood glucose will drop to less than 30 mg/dL. If blood glucose drops to less than 35 mg/dL, blood samples will be collected every 3 minutes. Additional countermeasures to hypoglycemia will also be utilized as necessary.

- IV Procedure: There is a possibility of pain, bruising, or infection at the site of the needle insertion for the IV line. Trained personnel also minimize this risk.

- Questionnaire: There is a possibility that participants will be uncomfortable answering certain questions. Participants will be told that they may choose to skip any questions.

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

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

Setting
This study will be conducted at Pennington Biomedical Research Center in the Outpatient Unit and Inpatient Unit.

Resources Available
Pennington Biomedical Outpatient and Inpatient staff are highly trained individuals with a breadth of experience in clinical research. All have completed human participants protection training and are highly knowledgeable 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.

Compensation

Participants completing the study visit will receive $200. No compensation will be provided for screening or run-in visit.

Confidentiality
All data and specimens will be obtained solely for research purposes. These will include 1) physical examinations, 2) medical history, 3) blood samples, and 4) adverse events during study participation. All data from individual participants 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 participants protection training. Data will be stored at Pennington Biomedical indefinitely. However, only study investigators, their staff, and collaborators will have access to the data. The study PI is responsible for receipt of the data.

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.

References (bolded references attached with IRB submission).

1. **McDougal, D.H., et al., *Glial acetate metabolism is increased following a 72-h fast in metabolically healthy men and correlates with susceptibility to hypoglycemia.* Acta Diabetol, 2018.**

2. **Moheet, A., et al., *Hypoglycemia-Associated Autonomic Failure in Healthy Humans: Comparison of Two vs Three Periods of Hypoglycemia on Hypoglycemia-Induced Counterregulatory and Symptom Response 5 Days Later.* Journal of Clinical Endocrinology & Metabolism, 2014. 99(2): p. 664-670.**

3. **Seaquist, E.R., et al., *Hypothalamic Glucose Transport in Humans During Experimentally Induced Hypoglycemia-Associated Autonomic Failure.* The Journal of clinical endocrinology and metabolism, 2017. 102(9): p. 3571-3580.**

4. Tam, C.S., et al., *Defining Insulin Resistance From Hyperinsulinemic-Euglycemic Clamps.* Diabetes care, 2012. 35(7): p. 1605-1610.

5. Stull, A.J., et al., *Skeletal Muscle Protein Tyrosine Phosphatase 1B Regulates Insulin Sensitivity in African Americans.* Diabetes, 2012. 61(6): p. 1415-1422.

6. Moheet, A., et al., *Naltrexone for treatment of impaired awareness of hypoglycemia in type 1 diabetes: A randomized clinical trial.* Journal of diabetes and its complications, 2015. 29(8): p. 1277-82.

Appendix A- Project specific Aims of the PI's recently funded LA CaTS Roadmaps Scholars grant.

Specific Aims-

Hypoglycemic complications are the major impediment to the maintenance of healthy plasma glucose levels in persons with diabetes. The applicant recently completed a LA CaTS pilot and feasibility project which identified a novel biomarker, glial acetate metabolism, which predicts susceptibility to hypoglycemia. By providing an assay to predict hypoglycemic complications, the development of this biomarker would revolutionize the treatment of persons with diabetes in a manner analogous to the HbA1c assay. This project will build on this initial study to facilitate the applicant's competitiveness for extramural funding of this innovative line of research.

Previous work established that hypoglycemic complications are associated with alterations in glial metabolism. Glia are the most abundant cell type in the brain and play an integral role in cerebral metabolism. Our prior work and that of others has generated three key observations which link alterations in the metabolic capacity of glial cells with

hypoglycemic exposure and hypoglycemic risk: 1) Exposure to hypoglycemia in both diabetic and non-diabetic subjects is associated with an increase in the ability of glial cells to metabolize acetate. 2) This effect is dose-dependent, i.e., greater exposure to hypoglycemia is associated with higher rates of glial acetate metabolism, and 3) Increases in glial acetate metabolism are correlated with acute susceptibility to hypoglycemia in both diabetic and non-diabetic subjects. Based on these observations, we hypothesize that alterations in glial acetate metabolism could be used as an effective biomarker for assessing the risk of hypoglycemic complications in individuals with diabetes.

Securing extramural funding for the appropriate follow-up studies is presently limited due to a lack of preliminary data, the PI's limited experience with clinical research, and the PI's inexperience with key experimental techniques necessary to measure susceptibility to hypoglycemia. The overarching goal of this project is to overcome these limitations while developing fundamental knowledge regarding the relationship between glial metabolism and hypoglycemia. We will achieve this goal through the following specific aims:

Aim 1: Establish the hyperinsulinemic-hypoglycemic clamp procedure at the Pennington Biomedical Clinical Trials Unit (PBRC CTU). This procedure is the gold standard for determining susceptibility to insulin-induced hypoglycemia and therefore is a necessary technique to extend the applicant's preliminary findings and to compete successfully for extramural research support. Establishing this procedure will not only significantly advance the applicants research efforts, but it will also expand the scope of work available to all research projects at the PBRC CTU. The applicant will work with his mentoring team and existing collaborations with key personnel within the PBRC CTU to accomplish this aim.

Aim 2: Determine if glial acetate metabolism predicts future susceptibility to hypoglycemia. We will measure glial acetate metabolism 7 days prior to exposure to insulin-induced hypoglycemia and determine how well this measurement predicts the magnitude of the counter-regulatory responses to a subsequent bout of hypoglycemia. Hypothesis: Glial acetate metabolism will be inversely proportional to the neuroendocrine response to hypoglycemia, that is, as glial acetate metabolism increases the neuroendocrine response will decrease. This aim is a key test of our principal hypothesis. Establishing a link between glial acetate metabolism and future susceptibility to insulin-induced hypoglycemia will significantly advance our initial findings while providing critical preliminary data to support future grant applications.