Use of Functional MRI to Assess Hypothalamic Activation in Response to Diazoxide

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Meredith A. Hawkins, MD, MS

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OVERVIEW:

K_{ATP} channels are potassium channels sensitive to ATP/ADP ratio with their activation resulting in cell hyperpolarization. K_{ATP} channels have been identified on a subgroup of hypothalamic neurons (1) where they play a part in mediating responses of the brain to nutritional signals, while on pancreatic beta cells they have a critical role in regulation of insulin secretion (2).

Previous studies from our and other groups have demonstrated that central KATP channel activation with diazoxide inhibited hepatic glucose production in healthy control rodents and humans (3, 4). Intriguingly, our subsequent studies in a diabetic rat model and in humans with type 2 diabetes (T2D) failed to show these inhibitory effects of diazoxide on glucose production (5). *In vivo* studies in rodents have shown that activation of mitochondrial K_{ATP} channels in cerebral arteries by diazoxide is reduced in diabetes (6), and hypothalamic neurons from obese rats demonstrated defective activation of KATP channels in response to insulin (7). Until now, no measure has been developed for assessment of direct pharmacologic activation of K_{ATP} channels within the hypothalamus and other brain areas (eg. thalamus and/or striatum) *in vivo* in humans, and specifically to examine the difference between diabetic vs. healthy individuals in their ability to respond to diazoxide.

Functional magnetic resonance imaging (fMRI) is a technique for measuring and mapping brain activity that is noninvasive and safe. This technique relies on the fact that cerebral blood flow and neuronal activity are coupled. To assess the effect of diazoxide on activation of hypothalamus and other brain areas in healthy control and T2D patients, fMRI will be performed at baseline and at two hour intervals following administration of diazoxide vs. placebo. Since diazoxide can inhibit insulin secretion and potentially increase blood glucose levels, these will be measured at hourly intervals.

SPECIFIC AIMS:

Aim 1: To determine whether healthy individuals exhibit an activation of K_{ATP} channels in the hypothalamus and other brain areas in response to diazoxide, that can be observed by functional magnetic resonance imaging (fMRI). Based on our previous studies, we hypothesize that diazoxide will activate K_{ATP} channels in healthy participants. Given the connection between K_{ATP} channel activation and neuronal hyperpolarization, as well as the connection between neuronal activity and cerebral blood flow, we predict that K_{ATP} channel activation will be associated with altered activity of hypothalamus and other brain areas recorded by fMRI.

Aim 2: To determine whether hypothalamic responses to diazoxide observed by fMRI are diminished in patients with type 2 diabetes (T2D). We hypothesize that patients with T2D will not respond to diazoxide due to defective activation of K_{ATP} channels resulting from the metabolic defects of chronic diabetes, and that this will be demonstrated by a lack of effect on the activity of hypothalamus and other brain areas recorded by fMRI.

BACKGROUND:

One of the hallmark features of type 2 diabetes (T2D) is impaired regulation of endogenous glucose production (EGP) (8). While EGP is inhibited by both glucose and insulin in non-diabetic animals and humans, T2DM is associated with increased EGP despite elevated plasma glucose and insulin concentrations (9). In fact, we and others have reported direct inhibitory effects of hyperglycemia itself on EGP in nondiabetic subjects, independent of other hormonal or metabolic signals, which are markedly blunted in subjects with T2DM (10).

Animal studies indicate that the brain integrates systemic nutritional signals, such as insulin and glucose, and modulates EGP via vagal efferents as part of an integrated regulatory network for metabolic function. K_{ATP} channels (potassium channels sensitive to intracellular ATP/ADP ratio) have been identified on a subgroup of hypothalamic neurons (1) where they play a part in mediating responses of the brain to nutritional signals, while K_{ATP} channels found on pancreatic beta cells have a critical role in regulation of insulin secretion (2). Activation of hypothalamic K_{ATP} channels results in neuronal hyperpolarization and appears to be an important common mechanism whereby both systemic glucose and insulin suppress EGP. Indeed, activation of these channels may account for almost 50% of EGP suppression by both agents. Furthermore, central administration of diazoxide has been shown to inhibit EGP in rodents via direct pharmacologic activation of K_{ATP} channels in the hypothalamus, while the K_{ATP} channel inhibitor glyburide blocks the activation of these channels (1). We have previously shown that activation of extrapancreatic K_{ATP} channels by orally administered diazoxide under conditions of “pancreatic clamp” in non-diabetic animals and humans inhibits EGP (3). However, this inhibitory effect of diazoxide on EFP was absent in humans and rodents with diabetes (5). Intriguingly, hypothalamic neurons from obese rats demonstrated defective activation of K_{ATP} channels with insulin (7).

Studies with nasally administered insulin in non-diabetic humans have identified the insulin-induced decrease in hypothalamic blood flow as a readout for hypothalamic insulin action, which correlated with the increase in glucose infusion rate from before to after the insulin nasal spray administration (11). Until now, no measure has been developed to assess activation of K_{ATP} channels within the hypothalamus *in vivo* in humans, and more specifically to examine the difference between diabetic vs. healthy individuals. To assess the effect of diazoxide on hypothalamic activation in healthy control and T2D patients, fMRI will be performed at baseline and following administration of diazoxide vs. placebo. Therefore, the current proposal will address the mechanism of central action of diazoxide by elucidating the areas of the brain affected by diazoxide in healthy subjects and those with T2D, with the use of fMRI.

SIGNIFICANCE:

Increased EGP is the major cause of postabsorptive hyperglycemia in T2D which persists despite the presence of hyperglycemia and hyperinsulinemia. Up to one half of regulation of EGP by hyperglycemia is centrally mediated; hypothalamic K_{ATP} channels appear to have a key role in integration of central regulation of EGP by nutrients. While direct pharmacologic activation of central K_{ATP} channels in non-diabetic humans and animals results in significant reduction of EGP, this effect is lost in T2D. Therefore, it is highly plausible that there is an impairment of central regulation of EGP in individuals with T2D. Furthermore, it is possible that the defect of central regulation of EGP in these individuals is at the level of hypothalamic K_{ATP} channels. We are proposing to address this important question by using fMRI: a state-of-the art, non-invasive and safe technique to evaluate neuronal activity in response to K_{ATP} channel agonists in healthy individuals and compare them to patients with T2D.

EXPERIMENTAL APPROACH AND METHODS:

Subject Characteristics:

We will perform a small, non-randomized pilot study in order to optimize fMRI conditions including anatomical localization and software algorithms. The pilot phase of the study will consist of up to 10 individuals (both diabetic and non-diabetic). Once conditions have been optimized, we will progress to the main study and begin randomization. Pilot phase participants may be eligible to subsequently participate in the main study.

The study population for the main phase of the study will consist of 15 normal, healthy, non-diabetic individuals and 15 individuals with moderately uncontrolled T2D (please see below for full inclusion and exclusion criteria).

Screening Procedures:

All subjects will be screened for eligibility prior to enrollment into the study. The purpose, nature, risks and benefits of the study will be explained to all subjects in the Clinical Research Center (CRC) prior to their enrollment in the study, and their voluntary, informed, written consent will be obtained. Screenings will be conducted in the GCRC suite at Albert Einstein College of Medicine. The screening visit will allow for the clinical evaluation of the subjects, including history, physical examination, blood and urine analyses, baseline EKG, and consent procedures. A blood draw (~35 cc/7 tsp) and urine collection will be performed and the following labs will be sent to accessioning for all participants: CBC, LFT, lipid profile, basic metabolic panel, HbA1c, insulin, PT/PTT, urinalysis, and urine drug screen. Additionally, urine microalbumin and urine creatinine will be measured for participants with T2D, and urine pregnancy test will be measured for all for women of childbearing age. If recent documentation of these lab tests is available from the participant's primary care or other physician, the study doctor may decide to use these results, depending on when the previous labs were done in place of those listed above, and if participant consents to providing a copy of these for their study file. The subject will be notified by phone or in writing of his/her eligibility status after his/her chart has undergone review by P.I. or fellow.

Randomization: For the pilot phase of this study, participants will not be randomized, and will receive either diazoxide or placebo as described in the study procedures below.

For the main study, participants will be randomized to receive placebo or diazoxide in a random order. Each subject will receive the experimental agents in random order as determined by study coordinator (using the envelope method). The subject will be blinded as to which agent he/she is receiving.

Drug Storage: All medications received from both Weiler Pharmacy and from external pharmacies will be logged. Each study protocol will have its own drug binder with the medication log. Lot number, expiry date, and date of usage will be documented for all medications and additionally, date received and location of storage will be documented for medication obtained outside of Weiler pharmacy.

STUDY PROCEDURES:

We will perform a small, non-randomized pilot study in order to optimize fMRI conditions including anatomical localization and software algorithms. The pilot phase of the study will consist of up to 10 individuals (both diabetic and non-diabetic). Once conditions have been

optimized, we will progress to the main study and begin randomization. Pilot phase participants may be eligible to subsequently participate in the main study.

Study Visit 1

All subjects will be asked to refrain from strenuous exercise and to fast starting at 10PM (no food or drinks except for water) on the day prior to the study.

Subjects with type 2 diabetes will be admitted to Jack D. Weiler Hospital the evening before the study to acutely normalize plasma glucose levels using variable low dose insulin infusion with a target plasma glucose level of 100 – 120 mg/dL beginning at approximately 1AM. A physician will admit the patient, document the subject's History and Physical, and place the appropriate orders into Epic. An inpatient nurse, familiar with our research studies will take care of the patient overnight, and will be provided with a detailed written protocol for overnight insulin infusion and contact numbers of the fellow and the P.I., in addition to verbal instructions and clarifications by the fellow (*See Appendix 1: diazoxide nursing protocol)*. An IV catheter (18 – 20 gauge) line will be placed in one arm of the subject for infusion of insulin. Another catheter may be placed in the other arm for blood sampling. Subjects' blood glucose will be checked every hour throughout the night, either by drawing blood from an IV catheter or by the finger stick method. The expected volume of blood drawn from these tests will not exceed approximately 10 cc. In order to prevent hypokalemia due to insulin infusion, all subjects receiving insulin will receive an oral dose of potassium chloride (20mEq powder packets dissolved in water) at the time of the initiation of insulin infusion. Most subjects will receive up to 40mEq potassium chloride, not to exceed 80mEq in one day. The infusion will be stopped prior to the first MRI scan.

T2D subjects will be asked to withhold their oral diabetes medication for 2-8 days prior to the study visit (8 days for patients on pioglitazone and 2 days for all other diabetes medications) in order to wash out any medications that could affect study results. In the subjects who use insulin, long acting insulin will be discontinued 24 hours prior to the anticipated start of insulin infusion and intermediate/short acting insulin will be discontinued 12 hours prior to the anticipated start of insulin infusion. The subjects will be instructed to monitor their blood glucose four times daily in this period and to inform the study doctor of any significant episodes of hyperglycemia (blood glucose >300 mg/dl). Any incidents of hyperglycemia (>300 mg/dL) will be evaluated on a case-by-case basis by the P.I., who is an Endocrinologist. The assessment will therefore include a careful history of food intake and activity, timing of blood glucose readings, and symptoms of hyperglycemia. Please note that most patients will be on metformin and/or insulin and hence the entire period of washout will be no more than 2 days, and therefore marked elevations in blood glucose are unlikely to occur. In the unusual case of severe hyperglycemia, the subject will be advised to immediately resume their usual medications and the study will be rescheduled accordingly.

Justification for medication washout: This short-term medication withdrawal is considered standard procedure in the literature and in our studies since 1996, and is carefully explained to all subjects prior to their participation. This is documented in our previous publications. Please

see: Hawkins et al, *Diabetes* 2002; 51:2179-89, in which scientific justification is provided for the following: sulfonylureas and metformin discontinued 48 h before the study, thiazolidinediones (which have longer acting metabolites and prolonged adipose effects) withheld for 8 days, and long-acting insulin was withheld the evening before the study. Specifically, the "wash-out periods" for each medication reflect the amount of time required for drug effects to abate (so that their presence will not affect study results), while avoiding an undue increase in glucose due to a prolonged period off medication. Therefore, this is not considered to put subjects at risk for hyperglycemia significantly above a subject's usual blood glucose range.

Healthy non-diabetic subjects will not require an overnight admission.

All subjects will be drug-tested on arrival to the CRC utilizing an instant result drug panel (*Alere iCup Dx14*). If the test is positive, the subject will be withdrawn from the study and will receive $50 for his/her time (see below under Compensation).

On the day of the experiment, all subjects will be requested to report to the GCRC Study Room at 0800h, after an overnight fast of 10 hours. An 18- or 20- gauge intravenous catheter will be inserted in the subject's arm for hourly blood sampling.

fMRI studies will be conducted in the 3T human MRI scanner in the Gruss Magnetic Resonance Research Center (MRRC) of the Albert Einstein College of Medicine. When placed in the scanner, the subjects will lay supine on the scanner bed, and position within the head coil and advance into the magnetic resonance (MR) scanner until their head is aligned with the magnet isocenter.

Each subject will undergo up to three fMRI sessions over the course of the study day. The first fMRI session will be performed to establish a baseline measurement, while the following two will be performed after administration of either diazoxide or placebo. The number of scans the subject receives will be determined by initial pilot data. The subject will be informed about the number of scans they will receive at the time of scheduling the study. The fMRI sessions will be spaced approximately 2 hours apart and each will last approximately 30 minutes. The subjects will rest in the CRC Study Room between the fMRI sessions. We will be monitoring blood glucose hourly between fMRI scans. If blood glucose exceeds 200 mg/dL, we will resume intravenous insulin infusion using the same insulin infusion protocol during the 2-hr breaks between scans. In the event that the subject's blood glucose level exceeds 300 mg/dL, we will resume insulin infusion and stop the study. The subject will subsequently be monitored until blood glucose reaches the patient's baseline and discharged after with the advice to resume home diabetes medication(s). During the approximately 30 min baseline period, the MR system calibrations, adjustments, and anatomical MRI will be performed as per standard procedures (12). While in the MRI, pseudo-continuous arterial spin labeling (pCASL) fMRI whole brain images will be acquired to localize brain activation.

After the baseline MRI session has been completed, an oral dose of diazoxide (*Proglycem, oral suspension,* at a dose of **4-7 mg/kg**, as explained below, using a syringe for precise measurement of dose) or matched placebo will be administered according to the random assignment. In the pilot phase of our study, participants will receive either diazoxide or placebo, but will not be randomized. In the main phase of the study, the participant will have an equal chance of being assigned to diazoxide or placebo first, and he/she will not be informed as to which medication he/she is receiving first. Since diazoxide can inhibit insulin secretion and potentially increase glucose concentration, hourly blood samples will be collected through the previously placed IV catheter for determinations of blood glucose, insulin, C-peptide, and glucagon. The expected total volume of blood drawn for these tests will not exceed approximately 50 cc. Additionally, as

diazoxide has vasodilatory properties, vital signs (blood pressure and heart rate) will be measured at baseline and then hourly. Of note, in our previous studies we did not note any changes in heart rate, blood pressure or C-peptide levels with diazoxide administration, and therefore do not expect these to change in the current studies. Additionally, the 6 mg/kg dose of diazoxide was safe and well tolerated in our previous studies and we therefore anticipate using this dose in most of the subjects. There is also published data on using diazoxide to restore counterregulation to hypoglycemia that reported safe use of a dose of 7 mg/kg dose (13). We will plan to start with a lower dose and increase as needed to up to 7 mg/kg, if we don't observe the expected response. We will consider using a lower dose (from 4 mg/kg) on a case by case basis, e.g. in subjects with low-normal baseline blood pressure. In the event of symptomatic or significant hypotension and/or tachycardia, the study will be stopped immediately and the subject will be asked to remain supine and will be given an IV saline infusion until his/her baseline blood pressure has been attained and he/she is asymptomatic.

Once the final session of fMRI has been completed, the subject will return to the Study Room, where he/she will receive a meal and final blood draw, if needed. Vital sign measurements and evaluation by research fellow will be done prior to being deemed safe to be discharged home. For participants consenting to take part in the pilot phase of this study, this will be the only visit.

Study Visit 2

There will only be one study visit for participants in the pilot phase of this study. For participants in the main part of the study, study visit 2 will take place after the first study visit, at least 2 weeks apart. The study will be identical to Study visit 1, with the exception that the subject will receive the other study drug.

Analytical Procedures:

Plasma hormone determinations: Measurements of plasma insulin, C-peptide, and glucagon will be performed by the ICTR Biomarker Analytic Research Core Lab (BARC) by human specific RIA and ELISA. Measurement of blood glucose during the study will be performed using an Analox glucose analyzer in the study room. Measurement of blood glucose during the overnight admission for insulin infusion in subject with T2D will be performed using a Precision Xceed Pro glucometer.

fMRI Acquisition Method:

Pseudo-Continuous Arterial Spin Labeling (pCASL) provides high performance brain perfusion imaging at 3.0 Tesla without using contrast agents. Arterial Spin Labeling (ASL; a subtraction technique) is used to generate measures of CBF, an indicator of neuronal activity. In these studies we will employ optimal measurement conditions, including a 3 Tesla magnet and a 32 channel receive only coil, with improved acquisition methodologies that provide for improved measurement of CBF in regions of increased susceptibility, such as the hypothalamus (14).

Arterial water is 'labeled' in the neck using radio frequency signals, after which the labeled water travels to the brain and exchanges with unlabeled water, producing an MRI signal change which can calculate perfusion using modified indicator dilution mathematics (15). During a baseline period, the MR system calibrations, adjustments, and anatomical MRI will be performed as per standard procedures. Optimized conditions will include a 32 channel receive only coil, with improved acquisition methodologies that incorporate multi-coil-sensitivity acceleration, a true 3-dimensional (3D) acquisition method based on hybrid gradient and spin echo based method. This

reduces energy deposition while minimizing T2* based susceptibility artifacts. Background (static) tissue suppression is used to reduce motion artifacts. Collectively, this permits the acquisition of higher signal-to-noise ratios in shorter time frames. *It further provides for improved measurement of CBF in regions of increased susceptibility, such as the hypothalamus.* Blood flow will be calculated using the previously published formula (16). All images and blood flow are processed using software written in MATLAB. Calculated CBF image maps are registered to anatomical 3D data and motion correction using FSL software.

STATISTICAL ANALYSES:

Data collected from each subject will be edited, verified and entered into an Excel data sheet on a password protected computer in the study room. Data quality checking will be performed by computer programs designed to detect errors using accepted ranges, logic checks, and missing values. Outliers will be checked against the original record and correct values will be entered into a final data file. Data from Excel files will be transferred into SPSS for statistical analysis.

- The parameter of interest will be CBF in hypothalamus (if visible) or thalamus
- **Non-paired t-test** will be used to compare CBF between group without diabetes and group with T2D
- **Repeated measures ANOVA (if 3 scans performed) or Paired t-tests (if 2 scans)** will be used to compare CBF in the same subjects under two different experimental conditions (diazoxide vs. placebo) in group without diabetes and group with T2D in Aims 1 and 2.

POWER ANALYSIS:

This pilot study will generate preliminary data to perform a power calculation to define optimal group numbers. The power analysis will be based on an unpaired t-test (CBF response to diazoxide in non-diabetic vs. T2D subjects), using NCSS/Pass 2008 software (Number Cruncher Statistical Systems, Kaysville, Utah) with a *two-tailed of 0.05*. In collaboration with our collaborating statistician, Dr. Kenny Ye, we will calculate the minimal sample size needed to achieve at least 80% power to detect a statistically significant difference in the effect of diazoxide on CBF. In the interest of feasibility, we plan to enroll 15 non-diabetic subjects and 15 type 2 diabetic subjects. It is expected that we may have up to 33% attrition, and will only be able to analyze data from subjects who have completed both studies.

HUMAN SUBJECTS:

1) <u>Subject Population:</u>

All subjects will be screened in the Clinical Research Center (CRC) prior to enrollment in observance of the following inclusion and exclusion criteria:

<u>Non-diabetic subjects (ND):</u>

Inclusion Criteria:

- Age: Between 21 and 70 y.o.

- BMI: <30
- negative family history of diabetes among first-degree relatives
- generally healthy (see below for exclusions)

Subjects with T2D:

Inclusion Criteria:

- Age: Between 21 and 70 y.o.
- BMI: <35
- A1c 8.0-12.0%
- generally healthy (see below for exclusions)
- Not suffering from proliferative retinopathy, significant diabetic renal disease (**urinarymicroalbumin <100 µg/dl)** or severe neuropathy (including cardiovascular and gastrointestinal autonomic neuropathy per history.

Patients will be **excluded** if they have one or more of the following characteristics:

- Age: Under 21 or over 70 y.o.
- BMI: >35 for T2D and >30 for ND
- If BP > 150/90 or <90/60 on more than one occasion
- Severe polydipsia and polyuria (in subjects with T2D) Since polydipsia and polyuria are common symptoms of T2D, the distinction "severe" denotes that the subject indicates a worsening in the symptoms and/or an experience of discomfort related to the symptoms
- Urine microalbumin: >300 mg/g of creatinine (in subjects with T2D)
- Uncontrolled hyperlipidemia defined as TG > 400 mg/dl and/or Total Cholesterol >300 mg/dl
- Clinically significant liver dysfunction: to include including thrombocytopenia (platelets <100,000), anemia (as below), hypoalbuminemia (<3.5), coagulopathy (INR > 1.5), and/or liver enzymes more than 3 times the upper limit of normal
- Clinically significant kidney dysfunction, GFR: <60 mg/dL
- Anemia: HgB <12.5 for men and <11.0 for women
- Clinically significant leukocytosis or leukopenia
- Clinically significant thrombocytopenia or thrombocytosis
- Coagulopathy
- Urine drug screen positive for any of the following: amphetamines, barbiturates, benzodiazepines, cocaine, methadone, opiates, oxycodone, PCP. Amphetamines, oxycodone, opiates, methadone, and benzodiazepines have been shown to affect glucose metabolism (increased glycemia, increased resting insulin levels, delayed insulin response to food ingestion, insulin deficiency) (17, 18, 19). As the drug test available in the CRC is a 7-drug panel, we cannot specifically choose which drugs are screened for. Additionally, in the interest of selecting patients on the basis of their reliability and dependability, we would like to exclude participants using illicit drugs. Occasional use of cannabis (once or twice per week) is not an exclusion.
- Urinalysis: Clinically significant abnormalities
- Clinically significant electrolyte abnormalities
- Smoking >10 cig/day
- Alcohol: Men >14 drinks/wk or > 4drinks/day, Women > 7 drinks/wk or > 3 drinks/day

- History of chronic liver disease, active hepatitis infection, HIV/AIDS, chronic kidney disease (stage 3 or greater), active cancer, cardiovascular disease or other heart disease, systemic rheumatologic conditions, seizures, bleeding disorders, muscle disease
- Surgeries that involve removal of endocrine glands except for thyroidectomy (if euthyroid on thyroid hormone replacement – if such history fT4 and TSH will be checked)
- Pregnant women
- Subject enrolled in another study less than one month prior to their anticipated start date in this study besides those done in our group
- Family history: family history of premature cardiac death
- Allergies to medication administered during study
- Uncontrolled psychiatric disorders
- Any contraindications for MRI: presence of any non-MRI compatible implants including pacemaker, aneurysm clip, cochlear implant, neurostimulator; history of eye injury with metal; history of ever being a metal worker; history of gunshot wounds or any other imbedded metal objects; history of claustrophobia or prior episodes of significant anxiety or discomfort while obtaining an MRI. In addition to our screening, additional comprehensive screening will be performed by MRRC personnel on the day of the study.
- Perimenopausal women who are experiencing/have experienced hot flashes, given the potential that this would affect brain blood flow
- Any condition which in the opinion of the PI makes the subject ill-suited for participation in the study

2) Sources of Material/Use of Data:

All data and records obtained in this study will be used for research purposes only. The protocol will be submitted for review and approval by the Einstein Institutional Review Board (Einstein IRB). The Einstein IRB is responsible to ensure human subject protections in compliance with institutional policies and federal regulations including the HIPAA privacy law.

3) Recruitment/Consent Procedures:

We will recruit up to 10 participants (both diabetic and non-diabetic) to participate in the pilot phase of this study. We will recruit a total of 15 healthy and 15 volunteers with T2D for the main study. Participants will be recruited using local and online advertising as well as from the clinical research center (CRC) database. We will also be identifying potential subjects for this study by accessing clinical/medical records from local clinics (eg, Montefiore endocrinology clinic, Montefiore Wellness Center). If potentially-eligible subjects are identified, we will contact their physician to ask the patient if they would be interested, and if we may contact them. If they agree, a member of our study team will contact them about the study. These subjects may include subjects who previously participated in our studies.

Consent will be initially obtained during telephone screening performed by the PI, study coordinator or research nurse. Formal consent procedures which adhere to the Clinical Investigations Committee of the Albert Einstein College of Medicine will be followed by the PI, study coordinator or nurse. Specifically, each subject will be verbally informed in layman's language of the purpose, benefits and possible risks of the studies. They will then read the written consent form in the presence of a member of the research team and a physician who will answer any further questions. The subject and the study team member shall be asked to sign the consent

form, one copy of which will be kept in the patient's chart and the other copy will be given to the subject. Each subject's potential participation in related experiments (for example, when each subject is asked to perform repeat studies) will be explicitly stated.

4) Risks and Benefits:

Safety and subject comfort

fMRI is a safe, well validated and generally well tolerated procedure. We will exclude any subjects with any contraindications to MRI, as listed in the "Exclusion criteria" (14). Since subjects can occasionally experience dizziness, they will be accompanied in and out of the MRI by a study team member and advised to rise slowly. To offset the noise of the MRI machine, they will be given earplugs. Between the fMRI sessions, they will be resting in the Study Room. After giving study medication, we will follow every subjects' blood pressure, heart rate and blood glucose levels in hourly intervals. As noted above, in the event of symptomatic or significant hypotension and/or tachycardia, study will be stopped and the subject will be given IV saline infusion.

Potential Risks:

Potential risks to the subjects include the following:

1. Blood withdrawal: The total amount of blood sampled will not exceed approximately 50 cc per study for healthy subjects and approximately 60 cc per study for subjects with T2D.

2. Intravenous Catheter - The intravenous catheter is associated with a small risk of local bruising.

3. Diazoxide (*Proglycem, received from Weiler Pharmacy)* – The risk associated with higher doses of diazoxide therapy is hypotension. Symptoms of low blood pressure are dizziness and light-headedness. During the study, there will be frequent monitoring of blood pressure. Please note that the amount of diazoxide given in this study will be much lower than the doses which cause lowering of blood pressure. Of note, the oral doses the subjects will receive have been used safely in human subjects in the preliminary studies without lowering blood pressure or any other side effects. Diazoxide is approved by the FDA.

4. Insulin (*Novolin R, received from Weiler Pharmacy)*– Intravenous low-dose insulin infusion in T2D subjects performed with simultaneous hourly blood glucose checks should not cause any harm or discomfort to the subject. Transient hypoglycemia may occur during the overnight insulin infusion and will be dealt with as detailed in the overnight nursing protocol. Insulin is approved by the FDA.

5. Potassium chloride oral powder (*Klor-Con 20mEq)*– The most common adverse reactions with oral potassium chloride salts are nausea, vomiting, flatulence, abdominal pain/discomfort, and diarrhea Potassium chloride is approved for use by the FDA.

6. fMRI – fMRI studies may cause some mild discomfort associated with being in a confined space and/or a noisy environment.

7. **Withholding of diabetes medication:** While unlikely, short-term discontinuation of usual diabetes medications may cause short-term hyperglycemia. Specifically, the "wash-out periods"

for each medication reflect the amount of time required for drug effects to abate (so that their presence will not affect study results), while avoiding an undue increase in glucose due to a prolonged period off medication. Therefore, this is not considered to put subjects at risk for hyperglycemia significantly above a subject's usual blood glucose range.

5) **Protection of Subjects:**

As noted above, recruitment involves meticulous exclusion of subjects with any embedded metal objects or medical devices, as well as history of claustrophobia. This is performed at initial screening of subjects as well as a subsequent in-depth questionnaire by MRRC study staff at the time of the fMRI study.

Procedures employed to protect subjects from undue risks include the following:

1. Blood withdrawal: Blood withdrawal during any single study will be limited to less than approximately 50 cc per study for healthy subjects and approximately 60 cc per study for subjects with T2D. Subsequent studies will be separated by at least a two-week interval.
2. Intravenous catheter - The infusion and withdrawal catheters may produce infection or local hematoma, but strict aseptic technique will be observed by the experienced physician or registered nurse performing the procedure.
3. Diazoxide – As noted above, vital signs will be carefully monitored throughout the studies. An intravenous line will be established prior to the administration of diazoxide or placebo. In the very unlikely event that the blood pressure drops below 90/60, the patient will be maintained in a supine position and intravenous saline will be administered at a rate sufficient to correct any significant decrease in blood pressure. As noted above, it is highly unlikely that there will be any reduction in blood pressure.
4. Insulin – as noted above, blood glucose will be carefully monitored every hour and insulin infusion rates will be adjusted accordingly. The floor nurse will be given both oral and written instructions regarding the overnight admission procedures. Fellow will be available to the floor nurse by cell phone or pager at all times. The P.I.'s contact information will be available to the floor nurse as well; in the case the floor nurse is unable to reach the fellow for any reason. Careful instructions are provided as to how to manage any episodes of hypoglycemia.
5. Potassium Chloride – Potassium chloride will be given to prevent any significant drop in potassium with insulin infusion. An oral dose of 40 mEq of potassium chloride (oral powder dissolved in water) is in concordance with FDA-recommended dosing for oral powder. The total dose of up to 80 mEq that subjects receive is within the limit approved by the FDA (up to 100 mEq/day). The powder will be given while the subject is sitting up and with a plenty of water. This is to prevent any gastrointestinal distress. Potassium chloride will be given to prevent any significant drop in potassium with insulin infusion.
6. fMRI – in order to minimize discomfort for the subject the duration of the fMRI sessions will be limited to the minimal time sufficient to obtain necessary measures, which should be around 30 min per session. Earplugs will be provided for noise reduction. Between the sessions the subjects will be resting in the Study Room.
7. **Withholding diabetes medication -** The subjects will be instructed to monitor their blood glucose four times daily in this period and to inform the study doctor of any

significant episodes of hyperglycemia (blood glucose > 300 mg/dl). Any incidents of hyperglycemia (>300 mg/dL) will be evaluated on a case-by-case basis by the P.I., who is an Endocrinologist. The assessment will therefore include a careful history of food intake and activity, timing of blood glucose readings, and symptoms of hyperglycemia. Please note that most patients will be on metformin and/or insulin and hence the entire period of washout will be no more than 2 days, and therefore marked elevations in blood glucose are unlikely to occur. In the unusual case of severe hyperglycemia, the subject will be advised to immediately resume their usual medications and the study will be rescheduled accordingly.

Potential benefits:

The study will be of no direct benefit to the subject, although subjects may gain a better understanding of their own metabolic processes and contribute to generalizable knowledge by their participation.

Importance of the knowledge to be gained:

The risks involved are minimal as all procedures are well established. The study may result in a better understanding of the role of the central regulation of glucose production in humans. As described above, a number of mechanisms exist to ensure safety of subjects. All studies will be performed by a physician and nurse in attendance in the GCRC procedure unit with emergency equipment readily accessible. The PI, Co-investigator, and research team have a combined experience of over 20 years in human investigation, and sufficient care in choice of subjects should minimize the risks. The scientific value of these studies is to develop an understanding of the mechanism of an important and potentially-exciting novel therapeutic target for the treatment of diabetes.

RESEARCH INVOLVING CLINICAL TRIALS:

The study does not constitute a phase III clinical trial as defined by the NIH.

DATA SAFETY AND MONITORING PLAN (DSMP):

Though the studies outlined in this application do not constitute a clinical trial, as per the NCRR guidelines for CRC's, all human subject research being conducted at the Einstein CRC must have a DSMP. The guidelines for Data and Safety Monitoring have been established by the CRC Research Subject Advocate (RSA), and approved by the Assistant Dean for Compliance who oversees human research subject safety at the Albert Einstein College of Medicine. http://gcrcweb.aecom.yu.edu/gcrc/dsm.htm

The PI and Co-Investigator will regularly review study progress and any adverse events (see Monitoring Plan below). Reports of any adverse events will be transmitted to the Einstein IRB.

Monitoring Plan: Study progress, including all adverse events, will be reviewed at bi-monthly research group meetings. All abnormal findings from history and physical examination will be documented in the research chart and reviewed by the PI or Co-Investigator. Clinical and laboratory data will be reviewed within 24 hours of receipt and any adverse events will be reported according to Einstein IRB policy. The investigators will periodically assess and review data collection and storage procedures to maintain confidentiality. In addition, annual reports

summarizing study progress (including recruitment progress, interim data analysis and data quality, adverse events and protocol changes) will be prepared and submitted to the Einstein IRB.

Data Safety and Monitoring Board (DSMB): DSMB members will include Dr. Michal L. Melamed, Associate Professor of Medicine and Epidemiology & Population Health and Program Director of the Nephrology Fellowship, a highly experienced patient-oriented investigator who is not involved in this research protocol, but is very familiar with all of the proposed methodologies and the potential risks to human subjects, and Elina Jerschow, who is an experienced diabetes investigator. The DSMB will be chaired by Dr. Nir Barzilai, who has much experience with metabolic research as well as with serving on and chairing DSMBs. The DSMB members will routinely review the safety information related to this project at yearly intervals, and will be contacted immediately should a serious adverse event occur.

Adverse Event Monitoring:

Process: Data and safety monitoring for this study will be performed by the principal investigator on an ongoing basis. All volunteers will be seen by the P.I., Co-Investigator or research staff. Laboratory data will be reviewed by the P.I. within 24 hours of receipt.

Reporting: All adverse events will be compiled, and reported in summary form, on an annual basis to the IRB, and at the conclusion of the study. Unanticipated (non-serious) adverse events will be reported to the IRB within 30 days and serious adverse events will be reported to the IRB within 48 hours by phone, email or fax.

Recruitment Monitoring:

Process: The PI and Co-Investigator will assess the recruitment and retention of study subjects on an ongoing basis. The recruitment goal for this protocol is for 30 subjects to complete ALL of the proposed studies, with a planned enrollment of >45 subjects. We may enroll up to 10 participants for the pilot phase of this study to allow us to optimize the MRI software.

Reporting: Summary statistics regarding recruitment and retention of study subjects will be reported to the IRB on an annual basis, and at the conclusion of the study.

Early Study Termination:

Process: The PI and Co-Investigator will determine if the study is to be terminated prior the scheduled study conclusion. Early study termination will be considered in the event of an unanticipated serious adverse event determined to be possibly, probably or definitely related to the study.

Reporting: The P.I. will report the decision to terminate the study to the IRB within 48 hours of this determination. The P.I. will submit a narrative description of the reasons for early termination of the study within 10 days.

DATA SHARING:

We respect that the rights and privacy of people who participate in NIH-sponsored research must be protected at all times. Thus, data intended for broader use will be free of identifiers that would permit linkages to individual research participants. We and our collaborators will make the data and associated documentation available to users only if they provide: (1) a commitment to using the data only for research purposes and not to identify any individual participant; (2) a commitment

to securing the data using appropriate computer technology; and (3) a commitment to destroying or returning the data after analyses are completed. We expect that the results of this study will be presented at national scientific meetings and published in peer reviewed journals.

COST AND COMPENSATION TO SUBJECTS:

There will be no costs to the participants in the study. Subjects will receive payment by Clincard Greenphire Payment for time and inconvenience associated with the study. Subjects will be asked to provide their social security numbers to receive monetary compensation, which will be loaded within 24-48 business hours from the day of their study. It will be kept confidential. For completion of the pilot phase of this study, subjects will receive $50 per fMRI session study visit for a total of up to $150. Pilot phase subjects with T2D will receive an additional $100 for the overnight admission. For completion of the main study, subjects will receive $50 per fMRI session study visit for a total of up to $150. Subjects with T2D will receive an additional $100 for the overnight admission. If subjects choose to withdraw or cannot complete the study, the compensation will be prorated based on the time spent. All subjects will be drug-tested on arrival to the CRC utilizing an instant result drug panel (*Alere iCup Dx14*). If the test is positive, the subject will be withdrawn from the study based on our exclusion criteria.

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Appendix 1: DZX Nursing Protocol

DR. HAWKINS NURSING PROTOCOL
TYPE 2 DIABETES INPATIENTS
STUDY (Diazoxide) Overnight Admission

Purpose:

These subjects (with Type 2 diabetes) will be participating in experimental studies to assess glucose metabolism. The Prinicipal Investigator is Dr. Meredith Hawkins.

Admission Day 1:

Subjects will arrive in Room 2S-42 (GCRC Study suite) on the evening of admission to Weiler Hospital. They will arrive on 11 South before dinner. Routine admission procedure will be followed including vital signs and nursing assessments. Orders will be placed by the research team.

1. Diet and Activity:

The orders will be written by the research fellow. The diet is **diabetic consistent carb test diet**. The patient should be ambulatory. Vitals signs per shift. Dinner should be provided at approx. 6:00 pm. Pre-bedtime snack will be given at approx. 9:30 pm. The patient should have nothing to eat after 10:00 P.M except drinking free water is allowed *ad lib* and encouraged. Please record the time snack and dinner are consumed on the blood glucose monitoring sheet in the space provided.

2. Medications:

The subjects will have washed out from their usual diabetes medication, medications for other indications such as hypertension will be taken as usual.

3. I.V Access:

An 18 to 20 gauge catheter must be inserted in one arm for insulin infusion. Should there be any difficulty in placing line (i.e. more than 2 attempts on one side) please call the research fellow on call. Another 18-20 gauge IV catheter may be placed in the other arm for blood sampling.

4. Insulin:

Insulin infusion: will be started at approximately 01 am and continued through the morning of Day 2 until the patient is ready to be transferred to the Room 2S-42 (GCRC Study suite) for the study around 7.45 am on Day 2. **Please do not stop the insulin pump until the patient is ready to be transferred immediately to the GCRC Study suite as holding insulin will result in hyperglycemia.**

The insulin infusion will be prepared per the standard hospital pharmacy protocol for Insulin regular (e.g. Humulin R) 100 units in 100cc of 0.9% NaCl (1unit/cc). Connect the bag to the IV tubing, flush and discard approx. 20ml to avoid changes in insulin concentration due to adherence to the tubing. The insulin infusion should be administered using a usual insulin pump on the floor.

On admission and then hourly at the beginning and during the insulin infusion, blood glucose should be checked on a glucose meter using a drop of blood from the fingerstick. The results of blood glucose should be logged in the EPIC and the provided blood glucose flowsheet. The following formula should be used to adjust the insulin infusion rate.

INSULIN INFUSION PROTOCOL

Blood Glucose **mg/dL**	**Intravenous Infusion Rate** **(mL/hr)**	**Insulin Infusion Rate** **(U/hr)**
>250	6	6
201-250	5	5
171-200	4	4
141-170	3	3
121-140	2	2
101-120	1	1
80-100	**0.5**	**0.5**
<80	only normal saline	only normal saline

Check glucose levels every 30 minutes if the previous value was <80 and insulin infusion was stopped. If you don't run any insulin infusion, please change that line to saline to keep vein open.

5. Treating hypoglycemia:

If at any time the blood glucose is less than 70mg/dl or patient experiences signs/symptoms of hypoglycemia the patient should be given D10% glucose at the rate of 100cc/hr and recheck the blood glucose within 30 minutes as described above. If there are any problems with persistent hypo or hyperglycemia, or if intravenous catheter fails to work properly, **please call Research Fellow (the name and number provided on the form). Please do not omit hourly blood sugar determinations at any point because blood glucose may fall quickly.**

6. Potassium supplementation:

Patient should receive 40 mEq of KCl PO once at approximately 01 am prior to starting insulin infusion to prevent hypokalemia.

Admission Day 2.

Last glucose value will be checked at approximately 7:30 am and insulin continued based on the algorithm unless below 80 mg/dl.

At around 7:45 am, the subject should void and then be transferred to the bed in room 2S-42 (ext. 2190). **Please continue insulin infusion when the subject is brought to 2S-42. IT IS VITALLY IMPORTANT THAT THE INSULIN NOT BE STOPPED!!!**

The subject will then undergo the research procedure. The experimental protocol will end between 4 and 5 pm. The subject will be discharged after a period of observation at approximately 6pm. For any questions, call **Dr. Sandra Aleksic 718-664-3408, pager 917-956-6638** or Dr. Meredith Hawkins 646-708-2721 THANK YOU!

Overnight Insulin Record
Protocol (Diazoxide)

Patient MR#_____ GCRC#_____

Date_____

Research fellow name & number: _____

Time	Blood Glucose (mg/dL)	Insulin Infusion Rate (units/hour)	Saline Infusion Rate/Comments
1.00 am			
2.00 am			
3.00am			
4.00am			
5.00am			
6.00am			
7.00am			
7.30am			

Time dinner consumed (use 24 hour clock): _____

Time snack consumed (use 24 hour clock): _____

Meredith A. Hawkins, MD, MS

_____	_____	_____
Print name and title	Signature	Date

DR. HAWKINS STUDY ROOM PROTOCOL
TYPE 2 DIABETES PARTICIPANTS 9040-2018 MRI STUDY DAY(Diazoxide)

Purpose:

These subjects (with Type 2 diabetes) will be participating in experimental studies to assess glucose metabolism. The Prinicipal Investigator is Dr. Meredith Hawkins.

MRI Study Day/Study Medication:

Subjects will be discharged from the inpatient floor and checked in to the GCRC Study Room at approximately 8:00 AM. Weight, vital signs, and IV placement (as needed) will be done at this time. Participant blood glucose will be monitored according to flowsheet below. Participant will be escorted to the Gruss Magnetic Resonance Research Center of the Albert Einstein College of Medicine for their first MRI scan at approximately 9 AM. The first MRI scan will last approximately 30 minutes. After the scan, the participant is escorted back to the GCRC Study Room by the study coordinator. They are given study medication (diazoxide or placebo according to randomization) at the beginning of their 2 hour rest period before their second scan. Participants blood glucose and vital signs are monitored during this rest period as described below. The second MRI scan is at approximately 12 noon, and the third MRI scan (if done) is at approximately 3 PM with a 2-hour rest period between scans as described above.

1. Diet and Activity:

Participant is fasting for entire period of study except for water and diet soda. Participant is encouraged to remain resting during the break periods between scans. They are given a meal upon the completion of the last MRI scan.

2. Medications:

Participants will have washed out from their usual diabetes medication, medications for other indications such as hypertension will be taken as usual.

3. I.V Access:

Participant will have an 18 to 20 gauge catheter placed in the arm during overnight admission. This catheter should be used for blood draws between the MRI scans, if functional, and if not needed for ongoing insulin infusion (as below). In the case that insulin infusion is needed or if the catheter is not functioning, another 18-20 gauge IV catheter will be placed in the other arm for blood sampling.

4. Insulin:

Insulin infusion: If blood glucose exceeds 200 mg/dL, we will begin intravenous insulin infusion using the insulin infusion protocol below during the 2-hr breaks between scans. In the event that the subject's blood glucose level exceeds 300 mg/dL, we will resume insulin infusion and stop the study. At the end of the MRI scans, the subject will subsequently be monitored until blood glucose is below 200 mg/dL and discharged with the advice to resume home diabetes medication(s).

The insulin infusion will be prepared per the standard hospital pharmacy protocol for Insulin regular (Humulin R) 100 units in 100cc (or 50 units in 50 cc, depending on availability) of 0.9% NaCl (1unit/cc). Connect the bag to the IV tubing, flush and discard approx. 20ml to avoid changes in insulin concentration due to adherence to the tubing. The insulin infusion should be administered using a hospital pump, if still available from overninght admission, or a usual insulin pump in the GCRC.

Glucose: On arrival to GCRC Study Room, and then hourly at the beginning and during the insulin infusion, blood glucose should be checked on a glucose meter using a drop of blood from a fingerstick or from IV draw using Analox machine. The results of blood glucose should be logged in the provided blood glucose flowsheet. The following protocol should be used to adjust the insulin infusion rate:

INSULIN INFUSION PROTOCOL

Blood Glucose **mg/dL**	**Intravenous Infusion Rate** **(mL/hr)**	**Insulin Infusion Rate** **(U/hr)**
>250	6	6
201-250	5	5
171-200	4	4
141-170	3	3
121-140	2	2
101-120	1	1
80-100	**0.5**	**0.5**
<80	only normal saline	only normal saline

Check glucose levels every 30 minutes if the previous value was <80 and insulin infusion was stopped. If you don't run any insulin infusion, please change that line to saline to keep vein open.

5. Treating hypoglycemia:

If at any time the blood glucose is less than 70mg/dl or patient experiences signs/symptoms of hypoglycemia the patient should be given D20% glucose at the rate of 100cc/hr and blood glucose should be rechecked within 30 minutes as described above.

Discharge:

After finishing the last MRI scan, participants will be given a meal and will have their blood glucose monitored. Once blood glucose levels are <200 mg/dl, participants will have vital signs and a final blood glucose check done. Participant is monitored for general comfort during this time. The participant will be discharged after a period of observation after research fellow clears participant to go home. At the end of the study visit, participant will be escorted to transportation by the research coordinator.

For any questions, call **Dr. Shiksha Sharma 201-683-1500, Dr. Sandra Aleksic 718-664-3408** or Dr. Meredith Hawkins 646-708-2721

THANK YOU!!

Study Day Insulin Record (if given)
Protocol (9040-2018 MRI STUDY Diazoxide)

Patient MR#_____ GCRC#_____ Date_____

Research fellow name: _____

Time	Blood Glucose (mg/dL)	Insulin Infusion Rate (units/hour)	Saline Infusion Rate	Additional Comments and Measurements
8:00 AM				
9:00 AM				
10:00 AM (If done, participant may be in MRI scan)				
11:00 AM				
12:00 PM (If done, participant may be in MRI scan)				
1:00 PM				
2:00 PM				
3:00 PM (If done, participant may be in MRI scan)				
4:00 PM				
5:00 PM				
6:00 PM (if needed)				

_____ _____ _____

Print name and title Signature Date