PARTNERS HUMAN RESEARCH COMMITTEE
PROTOCOL SUMMARY

Answer all questions accurately and completely in order to provide the PHRC with the relevant information to assess the risk-benefit ratio for the study. Do not leave sections blank.

PRINCIPAL/OVERALL INVESTIGATOR
Allison B. Goldfine, MD

PROTOCOL TITLE
Metabolic Effects of Betaine Supplementation

FUNDING
American Diabetes Association

VERSION DATE
September 19, 2016

SPECIFIC AIMS

Concisely state the objectives of the study and the hypothesis being tested.

In *Specific Aim 1* we propose to study whether betaine supplementation will improve insulin sensitivity and/or glycemia in overweight insulin resistant persons with abnormal glucose tolerance. In this proof-of-concept study that translates findings from bedside- to- bench, and now back- to- beside, we will administer betaine over 3 months to persons with grade 1-3 obesity and dysglycemia in a single-site randomized, placebo controlled, double masked study. We will assess glycemia during oral glucose tolerance test, insulin action by two- step euglycemic hyperinsulinemic clamp using deuterated glucose to assess hepatic and peripheral insulin sensitivity, and energy expenditure and oxidative and non-oxidative metabolism using indirect calorimetry.

In *Specific Aim 2* will determine if betaine supplementation reduces hepatic fat in obese insulin resistant persons. Hepatic steatosis will be quantified by MRI/MR spectroscopy at baseline and following 3 months of betaine supplementation. As an exploratory aim, we will measure glutathione levels in the liver.

In *Specific Aim 3* we will evaluate whether betaine supplementation reduces oxidative stress and restores endothelium-dependent vasodilation in obese insulin resistant persons. We will use brachial artery flow-mediated dilation and nitroglycerin-mediated, endothelium-independent vasodilation to assess vascular function.

BACKGROUND AND SIGNIFICANCE

Provide a brief paragraph summarizing prior experience important for understanding the proposed study and procedures.

Our group identified low circulating levels of betaine in the blood correlate with insulin resistance. Betaine is important in cellular metabolic pathways. Epidemiologic studies link betaine levels to diabetes and cardiovascular disease. Small human studies suggest benefit for non-alcoholic liver disease. We have shown in rodents that betaine levels fall with high fat feeding and there are metabolic improvements with replacement. In this study we will determine if administration of betaine improves metabolic measures, liver fat and/or endothelial function in humans with glucose intolerance who are overweight.

RESEARCH DESIGN AND METHODS

Briefly describe study design and anticipated enrollment, i.e., number of subjects to be enrolled by researchers study-wide and by Partners researchers. Provide a brief summary of the eligibility criteria (for example, age range, gender, medical condition). Include any local site restrictions, for example, "Enrollment at Partners will be limited to adults although the sponsor's protocol is open to both children and adults."

Patient identification: We will identify patients with prediabetes through a screening 75 gram oral glucose tolerance test. Currently, we diagnose 30% prediabetes in screening persons with one or more risk factor for type 2 diabetes [88]. Thus, we anticipate screening 90-100 persons to identify the 30 subjects for this study. Many participants may come from those screened for other ongoing studies at the Joslin Diabetes Center who have expressed interest in participation in additional studies. Persons screened for this study will meet trial inclusion/exclusion criteria including grade 1-3 obesity and one or more diabetes risk factors including: physical inactivity; high risk ethnic population; first degree relative with T2D; women who delivered an infant >9lbs or with gestational diabetes, or with polycystic ovarian syndrome; hypertension (controlled); low HDL or high triglyceride (but <500 mg/dL); age >45 years; or dysglycemia on prior testing.

Inclusion Criteria: 1) Men and women aged 21-70 years old; 2) Dysglycemia/prediabetes is defined as impaired fasting glucose (≥100 mg/dl), impaired glucose tolerance (2 hour post 75 g oral glucose load 140-200 mg/dl) and/or HbA1c 5.7-6.5%); 3) Overweight to grade 3 obesity (BMI ≥ 25 to 45 kg/m^2).

Exclusion Criteria: 1) cystathionine beta-synthase (CBS deficiency) (based on medical history and reported family history); 2) Presence of liver disease other than NAFLD; 3) Use of medications causing steatosis 4) Known alcohol consumption ≥ 2 drink per day; 5) Use of medications known to cause insulin resistance; 6) Use of weight loss drugs (or program) within 3 months of screening; 7) Treatment with any experimental drug within the past 6 months; 8) Subjects must be willing to abstain from use of phosphodiesterase type 5 (PDE-5) inhibitors; 9) Pregnancy or lactation, and women of child bearing potential must use adequate contraception; 10) Surgery within 30 days of screening; 11) Heart disease defined as New York Heart Association Class III or IV cardiac status or hospitalization for congestive heart failure, unstable angina, myocardial infarction, cerebrovascular accident, transient ischemic attack or any revascularization within 6 months; 12) Uncontrolled hypertension; 13) eGFR <60; 14) History of acquired immune deficiency syndrome; 15) History of malignancy within 5 years; 16) Hemoglobin <12 g/dL (males), <10 g/dL (females); 17) Triglycerides (TG) >500 mg/dL; 18) Poor mental function or any other reason to expect patient difficulty in complying with study requirements; 19) Metal clips or implants that preclude magnetic resonance imaging.

Briefly describe study procedures. Include any local site restrictions, for example, "Subjects enrolled at Partners will not participate in the pharmacokinetic portion of the study." Describe study endpoints.

We propose a randomized (1:1) double-masked proof-of concept study of betaine administration over 3 months to 30 patients with prediabetes (defined below) and Overweight to grade 3 obesity (BMI ≥ 25 to 45 kg/m^2). Betaine will be dosed at 333/0 mg/ml oral suspension, 2 teaspoons (3300 mg: 10 ml) twice daily for 10 days, then 1 tablespoon (4950 mg: 15 ml per dose, 10 g/d total) twice daily for the remainder of the 3 months, the dose used to treat patients with homocysteinuria and tolerated with evidence of benefit in pilot studies in humans with NASH. Participants will have oral glucose tolerance test, euglycemic hyperinsulinemic clamp, MRI of

the liver, and brachial artery flow mediated dilation studies performed at baseline and after 12 weeks of randomized assignment to betaine or placebo.

For studies involving treatment or diagnosis, provide information about standard of care at Partners (e.g., BWH, MGH) and indicate how the study procedures differ from standard care. Provide information on available alternative treatments, procedures, or methods of diagnosis.

Patients with pre-diabetes may undergo lifestyle approaches to reduce risk of progression to overt diabetes. Multiple pharmaceutical agents have been demonstrated to reduce risk of progression to overt diabetes, but are not FDA approved for this indication at this time. Consideration of use of metformin is recommended by the American Diabetes Association.

Describe how risks to subjects are minimized, for example, by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk or by using procedures already being performed on the subject for diagnostic or treatment purposes.

Potential Risks and Protection from Potential Risks

Betaine The most serious potential risk is cerebral edema in patients with hypermethioninemia. Patients with cystathionine beta-synthase (CBS deficiency): Homocystinuria due to CBS deficiency is inherited in an autosomal recessive pattern, and patients with family history of CBS deficiency will be excluded from participation. The most common adverse reactions are nausea and gastrointestinal distress. (See concise monograph for betaine anhydrous).

Euglycemic hyperinsulinemic clamp Insulin and glucose are substances which are normally found in the body. The major potential adverse effect of insulin is hypoglycemia. During the insulin clamp study the plasma glucose concentration is measured at 5 minute intervals and as glucose will be infused to maintain the plasma glucose concentration constant, hypoglycemia should not occur.

Isotope administration 6-6-D2 glucose is a glucose analogue containing two stable deuterium atoms at position 6 in the glucose ring. There are no known adverse effects of its administration.

Indirect Calorimetry It is possible that subjects may experience slight claustrophobia when the ventilated hood is placed over their head. It is made of clear plastic to prevent this sensation.

MRI/MRS Magnetic Resonance Imaging is considered non-invasive and is not associated with any known adverse effect except for people with metal or magnetic implants (such as metallic clips in the brain or cardiac pacemakers), or possibly to pregnant women. Persons with metal clips or other devices will not be included. Metal objects can heat up or possibly move in the patient's body. Some types of (home-made) tattoos can also heat up and cause discomfort. Metal objects can also become projectile when placed near the magnetic field. This has been reported at a few sites, but it is a very rare occurrence. Protection from magnetic objects can be safeguarded by the usual safety techniques that are practiced in MRI/MRS sessions such has having subjects and researchers take all metal objects off of their person before entering the environment.

The image procedure can be claustrophobic caused by being in the enclosed space of the MRI/MRS scanners. Any subjects who find that they are anxious or uncomfortable during any part of the procedure will be immediately taken out of the scanner and not tested. Noise levels in the magnet can be uncomfortable for subjects and they will be wearing earplugs through all

MRI/MRS procedures. This will be reviewed with each subject as part of the consent process prior to study participation.

The administration of contrast can be contraindicated by renal disease however gadolinium or other contrast agent will not be used so there is no increased risk of gadolinium induced fibrotic syndromes may be seen in patients with reduced renal function.

Incidental findings from MRI imaging may need additional medical evaluation and lead to unexpected costs or new findings.

Risk Management and Emergency Response: Each subject will complete a checklist that is the same as used by the Brigham and Women's Hospital MRI department to screen for metal implants, pregnancy, and other MR contraindications. Patients who are not able to understand the procedures and risks will not be tested. In addition to completing the MRI screening checklist, subjects will also be asked verbally by the experimenter about metal implants, and surgical procedures. If the answers to these questions are not known, or if the subject is uncertain about any answers, then they will not be tested in the MRI/MRS experiments. All subjects are asked to empty pockets, remove any clothing with possible magnetic materials, and remove all jewelry before the experiments.

A licensed technologist will review screenings in order to comply with MR safety regulations. If there is a possibility of metal in the head and neck area, such as a report of shrapnel in the body somewhere, an x-ray will be done. This x-ray is not routinely done, nor is it done to rule out a cardiac pacemaker. It is only done if there is a question of ferrous metal in the body from a source such as shrapnel or metal from working as a sheet metal worker, or if the subject reports having had metal in the head or neck area, including the eye. In these instances an X-ray is performed. This x-ray will be read immediately and if no metal is found, the MRI/MRS scan will continue. If metal is found, then the MR scan will be cancelled. The x-ray report will become part of the subject's medical record as detailed in the consent form so that it will not be unnecessarily repeated for MRI/MRS exams that the study subject may be required to do in the future.

Noise levels in the MRI/MRS will be reduced, as subjects will be given earplugs. These are used routinely in hospital MRI clinical settings. There is also microphone in the bore of the magnet. Subjects are instructed to talk to the experimenter if they feel uncomfortable for any reason during the testing procedures. If subjects feel uncomfortable they are immediately taken out of the scanner.

Vascular Function Studies The only vasoactive drug used in this study to assess vascular function is nitroglycerin, which is administered sublingually This may cause a fall in blood pressure and symptoms of hypotension. To avoid the latter, nitroglycerin will not be administered to any participant whose systolic blood pressure is ≤100 mmHg. In addition, subjects must be willing to abstain for one week prior to endothelial measures from PDE-5 inhibitors such as sildenafil citrate, (i.e., Viagra, Revatio), tadalafil (Cialis), and vardenafil (Levitra), as use of these drugs could significantly increase risk of hypotension. Ultrasound imaging is non-invasive and has no radiation risk. Blood pressure cuff inflation can cause discomfort and numbness of the hand.

Phlebotomy blood loss and discomfort with required laboratory blood draws and intravenous catheters. Bleeding, bruising or phlebitis at the site of venipucture are also a possibilities. *Blood loss:* The total amount of blood drawn during the protocol is approximately 650 ml. This is approximately equal to 1 ½ units of blood. One unit is the volume typically donated at a single visit to a blood bank, and can be donated every other month. In this study phlebotomy will be distributed over each visit spanning three months. Thus, phlebotomy does not exceed the rate of 1 unit per month and should not present a significant stress to any individual. Volunteers will be requested not to donate blood within 2 months after the study. Any subject who has just donated blood will be asked to postpone entry to the study until 2 months have elapsed.

Screening tests and procedures It is possible that as a result of the screening process a subject may learn that they have a health disorder that they were unaware they had. Every effort will be made to help the participant obtain the care that they need.

Breach of Confidentiality While every effort will be made to protect the confidentiality of participant identifiable information, there is the potential loss of confidentiality by participating in this study.

Inconvenience and Unknown Risks Participants may be inconvenienced by the time commitment involved in participation in the study. There may be other risks from this study not yet identified.

Participants can choose not to participate in the clinical research project and can withdraw consent at any time.

Describe explicitly the methods for ensuring the safety of subjects. Provide objective criteria for removing a subject from the study, for example, objective criteria for worsening disease/lack of improvement and/or unacceptable adverse events. The inclusion of objective drop criteria is especially important in studies designed with placebo control groups.

Participants with be withdrawn from the study for the following reasons:

a. Subject decision to withdraw consent for study participation
b. Evidence of allergy to administered products
c. Acute change in renal function (eGFR decline >50%, validated) or liver function (> 3-fold increase in ALT or AST)
d. New diagnosis of exclusionary medical condition
e. Intolerable adverse event as judged by investigator and participant

FORESEEABLE RISKS AND DISCOMFORTS

Provide a brief description of any foreseeable risks and discomforts to subjects. Include those related to drugs/devices/procedures being studied and/or administered/performed solely for research purposes. In addition, include psychosocial risks, and risks related to privacy and confidentiality. When applicable, describe risks to a developing fetus or nursing infant.

Risks of Taking Betaine

Betaine occurs naturally in the body. It is a metabolite of choline and is present in small amounts in foods such as beets, spinach, cereals, and seafood. However, taking betaine may cause one or more of the side effects listed below.

Common side effects:

- Nausea (2 out of 100 people reported this side effect)
- Gastrointestinal complaints (2 out of 100 people reported this side effect)

Uncommon side effects:

- A few cases of cerebral edema (brain swelling) have been reported in patients with a rare inherited disorder called cystathionine beta synthase (CBS) deficiency.

There may be other risks of betaine that are currently unknown.

As with any drug, an allergic reaction can occur. Allergic reactions can be mild or more serious, and can even result in death. Common symptoms of an allergic reaction are rash, itching, skin problems, swelling of the face and throat, or trouble breathing.

Betaine is pregnancy class C.

Of note that Cystadene 20g/d (twice the amount proposed in this study) is the upper end of the recommended daily dose range for patients with hyperhomocysteinemia: from Cystadane Product Information (package insert).

Betaine doses of 20 g/d for 12 months have been studied in about 30 patients with liver disease, with this number receiving the active betaine. Safety assessment and findings of these published studies are summarized below.

In the first study, Abdelmalek et al (Am J Gastroenterol. 2001 Sep;96(9):2711-70) surveyed safety with patients contacted by phone by the study coordinator at wk 1, 2, and 4 of treatment, and then monthly for the duration of treatment. At every contact point adverse events, concurrent illnesses, concomitant medications, and compliance with the study medication were assessed. Monitoring laboratory tests including liver biochemistries (ALT, AST, alkaline phosphatase, serum bilirubin, albumin, and prothrombin time), hematology panel, serum creatinine, and glucose were performed at 1, 3, 6, 9, and 12 months during the study. A complete medical history and physical examination were repeated at the end of treatment in each patient. Seven of 10 patients completed 1 yr of treatment with betaine. Two patients moved and were lost to follow-up at 3 and 9 months; and one patient was on treatment for only 6 months as a result of inability to obtain study medication because of a transient shortage in supply by the manufacturer.

Four of 10 patients experienced adverse events during treatment with betaine including nausea, abdominal cramps, loose stools, and body odor. None of these adverse events were dose limiting, and all four patients completed 1 yr of treatment. There were no adverse events noted in hematology or blood chemistry results.

Please note that body odor is not in other reports or the package insert and has not been added to the consent document.

In the second study, Abdelmalek et al (Hepatology. 2009 Dec;50(6):1818-26) followed safety with patients evaluated at the study center every 3 months and phone communication was made with each study participant on a monthly basis to assess compliance with study medication and development of any adverse events. Of those who completed 12 months of therapy (betaine 17, placebo 18), no changes in weight, BMI, or serum aminotransferases or lipid or laboratory safety monitoring parameters were noted. There was no clinically apparent hepatotoxicity (defined as ALT or AST 3x baseline value) rise in total bilirubin value of 2x normal, or severe adverse events reported in either treatment group. The overall incidence of gastrointestinal adverse events (i.e., nausea, vomiting, abdominal bloating, and/or diarrhea) in those treated with betaine was 33% versus 9%, in those treated with placebo, ($P<0.05$). A comparable number of patients in each treatment arm were either noncompliant with study drug and/or voluntarily withdrew their participation. Eighteen subjects (nine in each group) voluntarily withdrew consent for adverse effects, noncompliance, and the “inconvenience”of taking study drug, or inability to continue study participation.

The studies in healthy persons at ≥6g/d note similar side gastrointestinal side effects.

Risks of Phlebotomy and intravenous catheters:
Participants may have a bruise (a black and blue mark) or pain where we take the blood samples. There is also a small risk of infection, lightheadedness, and/or fainting.

The total amount of blood drawn during the entire study is approximately 650 ml. This is about 1 ½ pints of blood. Adults can safely donate 1 pint (2 cups) of blood every two months. In this study blood draws will be distributed over visits spanning three months, with most blood drawn at visits 3-4 and 7-8. Participants should delay enrollment into the study if they have given blood (ie at a hospital or the Red Cross) within 2 months, and should not donate blood during the study or for two months after taking part in this study to minimize risk of anemia (low blood counts).

Risks of Oral Glucose Tolerance Test
Nausea or incidental findings

Risks of Use of Insulin
Hypoglycemia

Risks of Use of Glucose
Hyperglycemia

Risks of Use of Nitroglycerin
Headache, dizziness, hypotension, bradycardia

If participants use Viagra (sildenafil), Levitra (vardenafil), or Cialis (tadalafil): The use of these drugs may cause a harmful interaction with the nitroglycerin used in the ultrasound procedure, therefore participants will be advised not to take these medications for one week before participating in the ultrasound procedure. On the day of the procedure, participants will be asked about taking any of these products; and informed an untruthful answer may place them at unnecessary risk.

Risks of Ultrasound Procedure
During the ultrasound a blood pressure cuff will be used to stop the blood in participants arm for short periods of time. This may cause some numbness or discomfort in the arm. This will disappear soon after the cuff is released. Occasionally, bruising occurs in the area where the cuff was inflated. This will disappear within a few days.

Incidental Findings

Risks of Indirect Calorimetry
Slight claustrophobia

Risks of MRI
There is not radioactive exposure from MRI. There are no known or foreseeable risks or side effects associated with conventional MRI procedures except to those people who have electrically, magnetically or mechanically activated implants (such as cardiac pacemakers) or to those who have clips on blood vessels in their brain. If participants have any of the above implants, then they are not eligible for this study. The MRI systems will be operated in a manner accepted by the FDA. System safeguards and screening procedures have been designed and operating guidelines have been provided to minimize any possible risks. Participants comfort will be verified periodically during the study. Participants will be provided with mandatory hearing protection (earplugs), which will prevent discomfort due to scanner noise.

Incidental findings

Loss of Confidentiality

There is the potential loss of confidentiality by participating in this study, but every effort will be made to protect the confidentiality of participant's identifiable information. The study team members are trained in the ethical conduct of clinical research and current privacy protection practices.

Unknown Risks

There may be unforeseen risks associated with participation in this study.

EXPECTED BENEFITS

Describe both the expected benefits to individual subjects participating in the research and the importance of the knowledge that may reasonably be expected to result from the study. Provide a brief, realistic summary of potential benefits to subjects, for example, "It is hoped that the treatment will result in a partial reduction in tumor size in at least 25% of the enrolled subjects." Indicate how the results of the study will benefit future patients with the disease/condition being studied and/or society, e.g., through increased knowledge of human physiology or behavior, improved safety, or technological advances.

The purpose of this study is to further elucidate the pathophysiology of insulin resistance and type 2 diabetes. It is possible that new treatment options to treat or prevent the development of overt disease can be developed. This project is not designed to be of direct benefit to any individual subject, although it is hoped that through the knowledge gained from these studies there will be general benefit to persons with or at risk of development of type 2 diabetes mellitus. Subjects randomized into the trial receive comprehensive medical evaluation and good medical care. As such, risks as such are appropriate in relation to the potential significant benefits for the large number of persons with diabetes and prediabetes. Thus, we feel that the potential benefits to society outweigh the risks to the individual subject.

T2D and prediabetes continues to increase in incidence throughout the USA and worldwide, and remains at epidemic proportions. The long term sequelae of these diseases are substantial and well documented, with substantial individual and public health care cost burden. Data from this grant may lay the groundwork for future studies that could substantially change the understanding of the pathophysiology of diabetes, and/or impact novel therapeutic strategies. This proof-of-concept study may also lays the groundwork for trials evaluating safety and efficacy of betaine supplementation. Significant findings will need to be confirmed in future clinical trials. Despite this limitation, this study potentially could contribute to development of a novel therapeutic intervention to improve outcomes for the millions of Americans affected by type 2 diabetes and/or prediabetes.

Individual participants will not benefit from taking part in this research study. However, they may learn they have diabetes or prediabetes and benefit from improved medical education and care.

Others with obesity, pre-diabetes or diabetes, heart disease or liver disease may benefit in the future from what we learn in this study.

EQUITABLE SELECTION OF SUBJECTS

The risks and benefits of the research must be fairly distributed among the populations that stand to benefit from it. No group of persons, for example, men, women, pregnant women, children,

and minorities, should be categorically excluded from the research without a good scientific or ethical reason to do so. Please provide the basis for concluding that the study population is representative of the population that stands to potentially benefit from this research.

Participants will not benefit from taking part in this research study.

If participants receive betaine, it is possible that their health will improve while taking it.

Others with obesity, pre-diabetes or diabetes, heart disease or liver disease may benefit in the future from what we learn in this study.

We have no reason to believe that risk or benefit will differ based on gender, race, or other categorical differences by group.

When people who do not speak English are excluded from participation in the research, provide the scientific rationale for doing so. Individuals who do not speak English should not be denied participation in research simply because it is inconvenient to translate the consent form in different languages and to have an interpreter present.

Individuals who do not speak English, will not be denied participation, if there is a hospital interpreter available to translate to the participant's language, and there is an available form in the appropriate language, and there is a translator available to the patient during emergency hours to communicate any potential adverse events to the study team.

- The Principal Investigator (or other member of the study staff with PI-delegated responsibility for obtaining informed consent) will present the PHRC-approved English version of the consent form orally to the subject through a medical interpreter physically present and fluent in English and the language understandable to the subject;
- The subject will be given a written translation of the 'full version' consent document in Spanish to him/her to read; for other languages the "Short Form" Consent will be used.
- The entire consent process will be witnessed by an individual who is fluent in both English and the language understandable to the subject. The interpreter may serve as the witness to the consent process (presentation of the information in the consent form in the language understandable to the subject and the opportunity to ask and receive answers to questions);
- The PHRC-approved English version of the consent form will be signed by the investigator obtaining informed consent and the witness to the consent process;
- The written translation of the 'full version form' will be signed by the subject and the witness to the consent process; and
- The subject will be given signed copies of both the PHRC-approved English version of the consent form and the written translation of the 'full version' consent document.
- The original signed English version of the consent form with the original signed written translation of the 'full version' document attached will be placed in the subject's research record. A copy of both forms will be placed in the subject's medical record, if the information is relevant to their medical care.
- The interpreter will come from the pool of experienced medical interpreters available through Interpreter Services as the PHRC requires that the interpreter.

The PHRC will consider approving an exception to the requirement to use an interpreter from Interpreter Services on a case-by-case basis.

Participants who do not speak English and do not have ready access to an interpreter when the hospital service interpreter is not available will not be enrolled as the study team may not be able to understand emergency related complaints, putting these individuals at increased risk.

For guidance, refer to the following Partners policy:
Obtaining and Documenting Informed Consent of Subjects who do not Speak English
http://healthcare.partners.org/phsirb/nonengco.htm

RECRUITMENT PROCEDURES

Explain in detail the specific methodology that will be used to recruit subjects. Specifically address how, when, where and by whom subjects will be identified and approached about participation. Include any specific recruitment methods used to enhance recruitment of women and minorities.

Subjects will be recruited using web-based advertisement, newspaper and other printed advertisement, use of recruitment brochures, PCP outreach and patient mailings, and will also be identified and recruited through the Partners RPDR and through the identified health information and/or samples from the patients in the Partners HealthCare Biobank. No recruitment materials will be used without first receiving IRB approval.

Provide details of remuneration, when applicable. Even when subjects may derive medical benefit from participation, it is often the case that extra hospital visits, meals at the hospital, parking fees or other inconveniences will result in additional out-of-pocket expenses related to study participation. Investigators may wish to consider providing reimbursement for such expenses when funding is available

Participants will not be compensated for time and effort of participation for screening oral glucose tolerance tests (OGTT). We recognize these take time and cause inconvenience, such as travel, however, only persons with one or more risk factor for diabetes will be screened by OGTT. We will be able to provide them information of personal value that they have normal glucose tolerance, dysglycemia or type 2 diabetes. Persons with type 2 diabetes will be encouraged to share this information with their care provider and receive management for this chronic condition. Persons with dysglycemia will be invited to participate further in the study.

Participants who complete the assessments will receive $400 for their time, effort and inconvenience associated with participation. Participants who complete the baseline assessments and drop out of the study before initiation of study drug, will receive $75 for their time, effort and inconvenience associated with participation.

All participants will receive a light meal or snack following fasted visits.

Parking will be paid by voucher.

For guidance, refer to the following Partners policies:

Recruitment of Research Subjects
http://healthcare.partners.org/phsirb/recruit.htm

Guidelines for Advertisements for Recruiting Subjects
http://healthcare.partners.org/phsirb/advert.htm

Remuneration for Research Subjects
http://healthcare.partners.org/phsirb/remun.htm

CONSENT PROCEDURES

Explain in detail how, when, where, and by whom consent is obtained, and the timing of consent (i.e., how long subjects will be given to consider participation). For most studies involving more than minimal risk and all studies involving investigational drugs/devices, a licensed physician investigator must obtain informed consent. When subjects are to be enrolled from among the investigators' own patients, describe how the potential for coercion will be avoided.

Persons who express interest in the trial will ~~be~~have all procedures, risks and potential benefits described, and will be provided for review IRB-approved consent documents for participation in the trial. All potential participants will have the opportunity to review the consents privately, and to have all of their questions answered prior to signing. Individual appointments for final questions and signing of the consent form with one of the licensed physicians investigators will be scheduled. Subjects will then be scheduled for baseline visits which will be conducted prior to randomization to study drug or placebo.

NOTE: When subjects are unable to give consent due to age (minors) or impaired decision-making capacity, complete the forms for Research Involving Children as Subjects of Research and/or Research Involving Individuals with Impaired Decision-making Capacity, available on the New Submissions page on the PHRC website:
http://healthcare.partners.org/phsirb/newapp.htm#Newapp

For guidance, refer to the following Partners policy:

Informed Consent of Research Subjects
http://healthcare.partners.org/phsirb/infcons.htm

DATA AND SAFETY MONITORING

Describe the plan for monitoring the data to ensure the safety of subjects. The plan should include a brief description of (1) the safety and/or efficacy data that will be reviewed; (2) the planned frequency of review; and (3) who will be responsible for this review and for determining whether the research should be altered or stopped. Include a brief description of any stopping rules for the study, when appropriate. Depending upon the risk, size and complexity of the study, the investigator, an expert group, an independent Data and Safety Monitoring Board (DSMB) or others might be assigned primary responsibility for this monitoring activity.

NOTE: Regardless of data and safety monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for protecting the rights, safety, and welfare of subjects under his/her care.

In brief, the Principal Investigator, Dr. Goldfine, will be responsible for ensuring that the study is conducted in accordance with the IRB approved protocol and will review all adverse events, serious adverse events. The study nurse coordinator will also perform a periodic review on all data. The members of the study team will conduct regularly scheduled meeting approximately weekly as well as on an add needed basis to review status of the trial inclusive of enrollment, oversight of informed consent, general conduct of the trial as well as reporting of any complications as needed to the NIH or IRB as stated below.

All adverse events will be reported to the IRB.

- Serious adverse events including death, life-threatening experience, event resulting in hospitalization, event resulting in an intervention, and event resulting in congenital anomaly/birth defect will be reported within 24 hours followed by a full written report within one week by the principal investigator.
- Adverse events and unanticipated problems will be reported per PHRC reporting guidelines.
- Mild to moderate adverse events that are unexpected but not related to the study will be reported to IRB in the periodic review.
- Expected adverse events that are not serious will be reported to the IRB in the periodic review.
- A written periodic review will be submitted to the IRB for continuing review annually.
- All adverse events at all performance sites will be reviewed by the principal investigator.

Safety reviews. The Principal Investigator (Dr. Allison Goldfine, MD) will review the safety and progress of this study on a bi-monthly basis. She will report to the Institutional Review Boards, Safety Officer and Patient Advocates, any adverse event of moderate or greater severity, and a Data Safety Monitor Board if appointed by the NIH, and FDA. She will review the study and potential new literature on an ongoing basis to ensure no new information is present to cause early study termination.

In time safety– in addition all information including medical history, physical examination and laboratory assessment will be assessed by a study investigator as collected or completed by the laboratory. Summary data will be reviewed bi-monthly by the Principal Investigator (Dr. Goldfine).

Annual reviews. The Principal Investigator (Dr. Allison Goldfine, MD) will review this protocol on a continuing basis for subject safety and provide written summary of results annually for review in the annual progress reports submitted to the IRB's,and safety officer, FDA and American Diabetes Association (Sponsor).

Annual reports. The annual report will include a list of adverse events. These will be provided to the institutional review boards. Annual reports will be generated both site specific and for the overall conduct of the trial. *Content of annual reports:* The annual report will address: (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely.

Laboratory review: Laboratory assessment will be performed at scheduled and predetermined intervals. The study investigators will review all laboratory reports within 24 hours of completion by the laboratory for participant safety.

Rules for stopping the trial in the event that expected or unexpected adverse events arise, particularly, but not exclusively, as explained in the Risks and Discomforts section of Protocol and its Informed Consent.

Study therapy should be discontinued for any of the following reasons:

a. Subject decision to withdraw consent for study participation
b. New diagnosis of exclusionary medical condition
c. Evidence of allergy to administered products
d. Acute change in renal function (eGFR decline >50%, validated) or liver function (> 3-fold increase in ALT or AST)
e. Intolerable adverse event as judged by investigator and participant
f. Pregnacy or unwillingness to use appropriate contraception.

The trial may be stopped by the regulatory oversight committees including, but not limited to the IRB, American Diabetes Association, or FDA.

Describe the plan to be followed by the Principal Investigator/study staff for review of adverse events experienced by subjects under his/her care, and when applicable, for review of sponsor safety reports and DSMB reports. Describe the plan for reporting adverse events to the sponsor and the Partners' IRB and, when applicable, for submitting sponsor safety reports and DSMB reports to the Partners' IRBs. When the investigator is also the sponsor of the IND/IDE, include the plan for reporting of adverse events to the FDA and, when applicable, to investigators at other sites.

NOTE: In addition to the adverse event reporting requirements of the sponsor, the principal investigator must follow the Partners Human Research Committee guidelines for Adverse Event Reporting

See above

MONITORING AND QUALITY ASSURANCE

Describe the plan to be followed by the principal investigator/study staff to monitor and assure the validity and integrity of the data and adherence to the IRB-approved protocol. Specify who will be responsible for monitoring, and the planned frequency of monitoring. For example, specify who will review the accuracy and completeness of case report form entries, source documents, and informed consent.

NOTE: Regardless of monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for ensuring that the study is conducted at his/her investigative site in accordance with the IRB-approved protocol, and applicable regulations and requirements of the IRB.

See above

For guidance, refer to the following Partners policies:

Data and Safety Monitoring Plans and Quality Assurance
http://healthcare.partners.org/phsirb/guidance.htm#13

Reporting Unanticipated Problems (including Adverse Events)
http://healthcare.partners.org/phsirb/guidance.htm#7

PRIVACY AND CONFIDENTIALITY

Describe methods used to protect the privacy of subjects and maintain confidentiality of data collected. This typically includes such practices as substituting codes for names and/or medical record numbers; removing face sheets or other identifiers from completed surveys/questionnaires; proper disposal of printed computer data; limited access to study data; use of password-protected computer databases; training for research staff on the importance of confidentiality of data, and storing research records in a secure location.

NOTE: Additional measures, such as obtaining a Certificate of Confidentiality, should be considered and are strongly encouraged when the research involves the collection of sensitive data, such as sexual, criminal or illegal behaviors.

Research data will be obtained in the form of medical history and review of selected medical record information including personal and family history including personal and family history, anthropometric measurements, laboratory tests, questionnaires. Data will be collected by study staff and stored in computerized databases. Only those involved directly with the study and patient care will have access to any data collected. Computer files with subject information will be kept locked with restricted access. No information about a subject will be shared with other parties unless the subject has given written or witnessed consent to do so.

SENDING SPECIMENS/DATA TO RESEARCH COLLABORATORS OUTSIDE PARTNERS

Specimens or data collected by Partners investigators will be sent to research collaborators outside Partners, indicate to whom specimens/data will be sent, what information will be sent, and whether the specimens/data will contain identifiers that could be used by the outside collaborators to link the specimens/data to individual subjects.

Specimens collected for this protocol will be procured and processed at laboratories including those at BWH, the Joslin and Quest Diagnostic, the Catalyst Core laboratory and LabCorp. Health data will be shared by the investigative teams at the Joslin Diabetes Center and Brigham and Women's Hospital. All investigators and study staff will be trained in the ethical conduct of clinical research and privacy protection practices and credentialed by their institutions for the level of their staff positions. Biospecimens obtained at the time of the study visits will be stored at the Joslin Diabetes Center for batch analysis. Data may be analyzed at a later date for cost effectiveness as described in the protocol. Clinical research specimens and research level data will be labeled with a unique identifier. However, as clinical care is coordinated at two institutions and in a certified clinical laboratory (Quest and/or LabCorp. A study nickname and numeric code will be used, but Date of Birth must be provided for age relevant normative interpretation. It is hard to identify an individual in the New England Area based solely on DOB.

Some specimens will be saved for batch analysis in core laboratories. Samples will be stored at the Joslin Diabetes Center. The Joslin Diabetes Center has ceded review to Partners. Application ID: 1291 - Metabolic Effects of Betaine Supplementation, August 16th, 2013.

Specifically address whether specimens/data will be stored at collaborating sites outside Partners for future use not described in the protocol. Include whether subjects can withdraw their specimens/data, and how they would do so. When appropriate, submit documentation of IRB approval from the recipient institution.

RECEIVING SPECIMENS/DATA FROM RESEARCH COLLABORATORS OUTSIDE PARTNERS

When specimens or data collected by research collaborators outside Partners will be sent to Partners investigators, indicate from where the specimens/data will be obtained and whether the specimens/data will contain identifiers that could be used by Partners investigators to link the specimens/data to individual subjects. When appropriate, submit documentation of IRB approval and a copy of the IRB-approved consent form from the institution where the specimens/data were collected.

Specimens obtained during the study of "Metabolic Effects of Betaine Supplementation" will be labeled with unique alpha numeric identifier. Samples may be assayed in clinical or core laboratories at Partner's Healthcare, Joslin, or Quest or LabCorp laboratories. Only CLIA certified labs will be used for safety measures.