Protocol for: Lipid Biomarkers for Diabetic Heart Disease

BACKGROUND

The cardiovascular complications of type 2 diabetes (T2DM) present a formidable challenge because of the high prevalence of T2DM and the adverse effects of cardiovascular disease on quality of life and survival. Despite generally accepted treatment goals for glucose control, diabetic cardiomyopathy remains a significant cause of morbidity and mortality. Moreover, recent data from the ACCORD trial suggest that focus on glucose lowering alone is insufficient.

Evidence is emerging that hyperlipidemia plays a central role in the pathogenesis of heart failure in diabetic patients, independent of coronary atherosclerosis (CAD). Elevated serum triglycerides (TGs) and free fatty acids (FFAs) cause increased FFA import into cardiac myocytes, in which excess lipid accumulation leads to cell dysfunction and cell death. We propose that this lipotoxicity is a primary cause of diabetic cardiomyopathy, which manifests early as isolated diastolic dysfunction, and later as both systolic and diastolic heart failure.

There are presently no available non-invasive tests for predicting those diabetics at highest risk for developing diabetic cardiomyopathy and no proven therapies for preventing this complication. Currently available plasma FFA and TG measures vary substantially with diet, exercise and sympathetic nervous system activity and are so weakly associated with cardiac dysfunction (e.g., $R^2 = 0.1$) that they are ineffective clinical tools for this outcome. Positron emission tomography (PET) and magnetic resonance spectroscopy are expensive and available only in specialized academic centers. Linking changes in cardiac function to lipid metabolites in cardiac tissue is also not practical, given that endomyocardial biopsy, an invasive test, is not clinically indicated. There is a pressing need to identify new DCM disease markers to facilitate early detection and intervention.

OBJECTIVES

This study will test the overall hypothesis that excessive myocardial FFA delivery contributes to cardiomyopathy in patients with T2DM. Moreover, systemic alterations in lipid metabolism will be manifested by changes in blood lipids, and these measures can be exploited to develop novel biomarkers for early detection and management of diabetic cardiomyopathy. Our aims are to:

1. Test whether pharmacological approaches to lower delivery of excess lipid to the heart in human subjects with T2DM improves cardiac function.

2. Determine whether specific lipid molecular species in plasma, quantified by highly sensitive mass spectrometry based lipidomic profiling, can serve as biomarkers for diabetic cardiomyopathy.

This study will provide novel insights into the relationship between lipid metabolic abnormalities in diabetes and cardiac function. This study may result in new diagnostic and therapeutic approaches to diabetic cardiomyopathy.

INCLUSION/EXCLUSION CRITERIA

Subjects will be recruited from clinics, practices, and laboratories within the Barnes-Jewish Hospital consortium, from the Volunteers for Health program and the Diabetes Research Connections recruitment program at Washington University School of Medicine, and through the use of local advertisements, posters, emails and flyers. Proposed dates of enrollment are from 4/1/12 through 2/28/18. One of the principal investigators or their proxy will address all questions of potential participants before written informed consent is obtained and prior to all studies. Consent procedures and forms will be approved by the Human Research

Protection Office (HRPO). Should a subject choose to withdraw from the study at any time, blood and tissue samples will be destroyed.

Because diabetes and diabetic cardiomyopathy commonly occurs in women, it is important that women are adequately represented among study participants. Our goal is to achieve ~50% enrollment of women in this study. Diabetes and diabetic cardiomyopathy commonly occurs in minorities. Thus, it is equally important that adequate numbers of subjects from these groups are represented among study participants. Our goal will be to recruit a total of 130 subjects of different ethnic and racial backgrounds in proportion to their representation in the greater St. Louis population.

Potential subjects who have T2DM and who have systolic cardiac function in the low normal or mildly abnormal range (20% < fractional shortening <27%), and who are not taking fibrates will be recruited. We will enroll subjects with normal or elevated triglycerides. However, our initial focus will be on subjects whose triglycerides are >100mg/dL because these individuals are likely to have the greatest benefit from treatment with fenofibrate. The diagnosis of diabetes will be based on a history of or treatment for type 2 diabetes. Subjects with poor diabetic control (HbA1c > 10%) will be excluded. Subjects on lipid-lowering therapies other than fibrates will be included as long as their doses of these medications are stable. Subjects who are hypothyroid, have HIV, or who are taking steroid medications will be excluded because these can affect plasma lipid levels.

Obese subjects who weigh more than 300 lbs will be excluded to ensure that subjects will be able to undergo the dual energy X-ray absorptiometry (DXA) and ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS).

In order to minimize possible confounding effects on cardiac function, subjects will be excluded if they are < 30 or > 65 years of age; are smokers; or have moderate or severe hypertension (blood pressure $\geq$ 140/90 mm Hg), atrial fibrillation, heart failure symptoms, moderate or severe valvular heart disease, paradoxical septal motion, or infiltrative myocardial diseases. Individuals with significant obstructive coronary artery disease based on symptoms or stress testing or cardiac catheterization will be excluded. Individuals with evidence of any of the above-listed cardiovascular diseases by history or during stress echocardiography will be excluded. Subjects with obstructive coronary disease who have been revascularized and have no evidence of current significant obstruction by stress testing may be included, as long as there is no evidence of prior myocardial infarction. We will also exclude subjects with significant systolic dysfunction, because our study is focused on the early phase of diabetic cardiomyopathy and this will limit potentially confounding effects of conditions such as idiopathic dilated cardiomyopathy.

Women who are pregnant or lactating will be excluded to minimize the confounding effects of reproductive hormones on myocardial metabolism. Women of child-bearing age will be on effective contraception (e.g., stable dose oral contraceptive, IUD), because fibrates are contraindicated in pregnancy. Efforts will be made to study menstruating women at the same point in their cycle.

Subjects with evidence of renal damage (e.g., creatinine > 1.5 mg/dl) will also be excluded, as will individuals with systemic illness or significant anemia (hematocrit < 28), history of biliary or liver disease, or history of recreational drug use or moderate to severe alcohol use (equivalent of > 5 glasses wine/wk). Subjects will also be excluded if they have a history of allergic reaction to fenofibrate.

METHODS

The study coordinator will describe the study to prospective subjects and may meet with them to answer questions. Interested potential subjects will be given the consent form to consider. Those potential subjects who indicate a willingness to participate will be scheduled for a screening appointment in the Center for Applied Research Sciences (CARS) at which time the signed consent form will be obtained prior to interview or any testing. Potential subjects will be asked to fast for 12 hours overnight prior to this visit; this may require a

temporary change in their diabetic medication regimen, which will be directed by a physician. Subjects will otherwise be continued on their stable outpatient medical regimens, including lipid-lowering therapy. We will be able to monitor and treat abnormal glucose level, if necessary, in the CARS. During this screening (VISIT 1) the following evaluation will occur:

a. A general medical history questionnaire will be filled out by the subject.
b. Urine will be obtained for urine pregnancy test (for female subjects).
c. An intravenous (IV) catheter will be placed in an arm vein and blood will be obtained to evaluate for evidence of end-organ damage related to diabetes or systemic illness and to assess glucose and lipid levels (18 ml or approximately 4 teaspoons for HbA1c, complete blood count (CBC), complete metabolic panel (CMP) and lipid panel).
d. Subjects will undergo electrocardiogram (ECG) stress echocardiography in the Cardiovascular Imaging and Clinical Research Core Laboratory or the Cardiac Diagnostic Laboratory (CDL) at Barnes Jewish Hospital to rule out occult coronary artery disease and cardiomyopathy. If the images obtained are not adequate, Definity or Optison contrast will be administered intravenously.

Potential subjects who have had recent blood tests (within 3 weeks of scheduled screening visit) or stress testing (within 12 months of scheduled screening visit) may not need to repeat all of the scheduled screening tests if recent test results are available. They will be asked if they give permission for study investigators to access their medical record for the purpose of obtaining these specific results.

Potential subjects who do not know their triglyceride level or their HbA1c but are otherwise eligible may be asked to come in to the CARS for a pre-screening blood draw to determine eligibility. They would be asked to fast for 12 hours overnight prior to this visit; this may require a temporary change in their diabetic medication regimen, which will be directed by a physician. If their blood tests indicate they are eligible, they would then be scheduled for their Visit 1. Thus, for some potential subjects, screening may be divided into two sessions.

Subjects who qualify for the study will then return on a second day following an overnight (12 hr) fast for study VISIT 2 to undergo metabolic phenotyping and to begin intervention. This visit will involve CARS, the Cardiovascular Imaging and Clinical Research Core Lab and CCIR and includes:

a. Urine will be obtained for biochemical analysis of oxidative stress and for urine pregnancy test (female subjects).
b. An IV catheter will be placed in an arm vein and blood (30 ml or approximately 2 tablespoons) will be obtained to determine lipid biomarkers, genetic markers, markers of oxidative stress and systemic inflammatory markers (hsCRP, TNFalpha, and IL-6).
c. Body fat and fat-free mass will be determined by dual energy x-ray absorptiometry scan (DXA).
d. Cardiac structure and function will be quantified by transthoracic echocardiography using two-dimensional (2D), Doppler, and tissue Doppler imaging. If the images obtained are not adequate, Definity or Optison contrast will be administered intravenously.
e. Subjects will undergo ^{1}H-MRS imaging so that hepatic triglyceride can be quantified.
f. After this baseline testing is complete, subjects will be randomized (using randomization.com to create block randomization) in a blinded fashion to treatment with fenofibrate (160mg/d), a prescription medicine approved by the Food and Drug Administration for treatment of hypertriglyceridemia in adults, or identical-appearing placebo for 12 weeks.
g. Subjects will be instructed by the study coordinator to continue their usual medications, diet and level of physical activity. They will be given a pedometer to wear daily in order to track their physical activity. Study subjects will be instructed to record blood glucose concentrations and distance walked daily in a study-supplied log. They will also note in this log any side effects, major illnesses or stresses, since TGs are acute phase reactants. Subjects will be asked to either send the log by fax or email to the study coordinator each week or to discuss the log by telephone with the coordinator each week, so that this information can be reviewed by the study investigators.

h. Through the Lifestyle Intervention Core, subjects will complete a 24-hour dietary recall to provide insight into dietary exposures.

Subjects will return at 6 weeks following start of the intervention for study VISIT 3 for medical monitoring to ensure medical safety. If this visit cannot be scheduled 6 weeks into the intervention due to staffing/scheduling conflicts, it will take place at 5 weeks so that the medical monitoring can be accomplished in a timely fashion. At this visit:

a. An interim history will be obtained by the study coordinator and reviewed with the study investigator. Study coordinator will also perform pill counts to assess compliance.
b. The log of blood glucose, distance walked, side effects and major illnesses/stress will be reviewed by the study coordinator and discussed with the study investigator.
c. Women will provide a urine sample for a pregnancy test.
d. Phlebotomy will be performed (6 ml or less than 2 teaspoons) to rule out untoward effects of study drug on kidney or liver function (CMP).
e. A dietitian will again assist the subject with completing a 24-hour dietary recall.

Subjects will return 12 weeks following start of intervention for study VISIT 4 at CARS for the following:

a. Urine will be obtained for biochemical analysis of oxidative stress.
b. Fasting (12 hr) phlebotomy (35 ml or less than 2.5 tablespoons of blood) to determine complete metabolic panel, lipid panel, lipid biomarkers, genetic markers, markers of oxidative stress and systemic inflammatory markers (hsCRP, TNFalpha, and IL-6). An IV catheter will be placed in an arm vein to obtain this blood.
c. Subjects will complete a 24-hour dietary recall to provide insight into dietary exposures.
d. Subject's log of blood sugars, distance walked, side effects and major illnesses/stress will be reviewed by study investigators.
e. ^{1}H-MRS imaging of the liver will be repeated to assess the effects of intervention on systemic lipid exposure.
f. Echocardiographic studies will be repeated to assess cardiac function. If the images obtained are not adequate, Definity or Optison contrast will be administered intravenously.

REMUNERATION

Subjects who qualify for the study, choose to enroll, and complete all aspects of this study will receive $250 for their time and inconvenience, in the form of a check, approximately 6 weeks after completion of the study. If a subject qualifies for the study, chooses to enroll, but completes only Visit 1 (screening) and Visit 2 (metabolic phenotyping), the subject will receive $25 in the form of a check.

RISKS OF THIS STUDY

Screening health questions:
Likely: None
Less Likely: None
Rare: Subjects may experience emotional discomfort when answering some questions during the screening interview with study personnel. Subjects do not need to answer questions that make them uncomfortable. However, if they refuse to answer the question regarding implanted metal objects, they will not be able to undergo the magnetic resonance imaging (^{1}H-MRS spectroscopy) and therefore will be excluded from the study.

Overnight fast:
Likely: None

Less Likely: Blood sugar may become too high or too low while fasting. Patients will be counseled ahead of time regarding dosing of anti-diabetic medications and blood sugars will be monitored in CARS and treated appropriately.
Rare: None

Phlebotomy:
Likely: None
Less Likely: Risks associated with sampling of blood include pain, bleeding, and bruising.
Rare: There is a very slight risk of infection or thrombosis of the vein. Sterile technique will be used in all subjects. Some subjects feel lightheaded during blood drawing. Subjects will be counseled not to donate blood during this study and to be hydrated (adequate oral water intake) before all phlebotomy in order to minimize risks.

Stress Echocardiography:
Likely: Shortness of breath, fatigue
Less Likely: Discomfort from pressure from the echo probe
Rare: For individuals with coronary artery disease, exercise testing is associated with a slight risk of developing serious disturbances in heart rhythm, abnormal increase or fall in blood pressure, fainting, chest discomfort, or very rarely, heart attack (estimate 9 per 10,000 tests or < 0.1%) and death (estimate 2 per 10,000 tests or <0.02%). Stress echocardiographic studies will be performed in the CDL, which is fully equipped with monitoring (blood pressure, 12-lead ECG, pulse oximetry) and emergency equipment (crash cart, intubation, and defibrillation equipment). A physician is immediately available at all times. Optison or Definity injection provides contrast enhancement and may be required for adequate images. Use of Optison or Definity is associated with a less than 1% occurrence of flushing, dizziness, low blood pressure, headache, shortness of breath, or hip or back pain. Allergic reactions (e.g., hives, throat swelling) are rare but possible with Optison or Definity. Allergic reactions can be treated with diphenhydramine or epinephrine.

DXA scan:
Likely: A DXA scan involves exposure to radiation from the X-ray scanner used for the body composition test. The amount of radiation is less than 1 mrem, which is 1% of the amount of radiation exposure all people in St. Louis receive each year from naturally occurring radiation sources. The risk from the radiation exposure in this study is too small to be measured. It is not a big risk when compared with other risks taken every day. Subjects who want to know more about radiation exposure will be referred to the "Radiation Fact sheet" on the Research Participant section of the Human Research Protection Office website at http://hrpo.wustl.edu and/or will be given a copy of the fact sheet.
Less Likely: None
Rare: None

^{1}H-MRS:
Likely: The machine that scans the body during this procedure produces loud noises, which are normal. To protect their hearing, subjects will be required to wear earplugs or earphones during the scan.
Less Likely: None
Rare: The only known risks for MRS are discomfort due to claustrophobia or back/shoulder stiffness from lying flat for up to 15 minutes. Subjects will be screened for claustrophobia in the screening health questionnaire and excluded from the study if unable to participate in this test. Subjects will also be excluded if they have metal implanted hardware, such as a pacemaker or brain aneurysm clips.

Echocardiography:
Likely: None
Less Likely: Discomfort from pressure from the echo probe

Rare: There are no known risks to human beings of ultrasound at the frequencies that will be used in this study. Optison or Definity injection provides contrast enhancement and may be required for adequate images. Use of Optison or Definity is associated with a less than 1% occurrence of flushing, dizziness, low blood pressure, headache, shortness of breath, or hip or back pain. Allergic reactions (e.g., hives, throat swelling) are rare but possible with Optison or Definity. Allergic reactions can be treated with diphenhydramine or epinephrine.

IV catheter:
Likely: None
Less Likely: The insertion of an intravenous catheter for administration of Optison or Definity and/or sampling of blood is associated with a small risk of pain, bleeding, bruising, and infection. Sterile technique will be used in all subjects.
Rare: Some subjects feel lightheaded during the intravenous catheter placement, so this will be carried out with the subject in the supine position. Rarely, an intravenous line may infiltrate, which means that what was being given in the intravenous tube would go into the arm tissue instead of the vein, or an intravenous line may lead to formation of a blood clot. These might be associated with swelling and mild discomfort. If this occurs, the intravenous solution would be stopped and the arm would be elevated and a heating pad would be placed, so that the infiltrated area would resolve as quickly as possible.

Randomization:
Likely: None
Less Likely: As mentioned before, this study has two groups. Because chance decides which group each subject will be in, the treatment a subject receives as part of this study may not be what his/her own doctor would choose.
Rare: None

Fenofibrate:
Likely: None
Less Likely: Minor side effects include abdominal symptoms (abdominal pain, nausea, vomiting, diarrhea, constipation and flatulence), photosensitivity, myositis or myopathy, and headache. Subjects will be monitored for all of these symptoms and for elevation of liver function tests (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) as these can become elevated in some patients. Should any of these side effects develop, the subject will be withdrawn from the study.
Rare: There is a minimal risk of allergic reaction (1%). Medication interactions are rare but can occur with oral anticoagulants, HMG-CoA reductase inhibitors, bile acid sequestrants, and cyclosporin. Subjects on anticoagulants or cyclosporine will be excluded. Subjects on bile acid sequestrants and HMG-CoA reductase inhibitors will be monitored closely. Allergic reactions can be treated with diphenhydramine or epinephrine.

24-hour food recall:
Likely: None
Less Likely: None
Rare: Subjects may experience some emotional discomfort or frustration in recalling everything they ate and drank over 24 hours.

Blood glucose and event log:
Likely: None
Less Likely: None
Rare: Subjects may experience some emotional discomfort or frustration from recording their blood glucose levels, distance walked, major illnesses/stresses/side effects, and from reporting this information to the study coodinator each week.

Placebo:
Likely: None
Less Likely: None
Rare: None

SUBJECT CONFIDENTIALITY

All data will be used specifically for research purposes, and will be kept confidential. Subjects will be referred to by an ID code, and all data and biological samples will be tracked by this ID code. Co-investigators, advisors, and research assistants will have access to clinical and imaging data, but only the principal investigators and the study coordinator will have the code for subject identification. Paper copies of data will be housed in a locked cabinet in the locked office of the principal investigator. Data collected in the study will be entered into a password- protected database, which will be accessible to investigators only, which will be maintained in the Department of Medicine infrastructure. The database will contain only subjects' unique ID codes, and not names. Names will not be used in any publications. No study results will become part of patients' medical records. The data will be available for review by the FDA or any other authorized governmental agencies. Although subjects may request that the results of studies be made available to primary care physicians, the research nature of these studies will be emphasized. Strict adherence to the guidelines published by the HRPO will be followed.

FOLLOW-UP

The results of the following tests will be made available to the subjects or their physicians if subjects request it in writing: lipid levels, blood chemistries, body composition, heart function and structure, and stress testing.

DATA AND SAMPLE STORAGE

All data generated in this project will be stored in an electronic password-protected database. The Department of Medicine maintains an infrastructure for archiving and analyzing clinical data on an in-house server and will provide support for storing, processing, retrieving and analyzing the data.

ALTERNATIVES

Apart from non-participation there is no specific alternative to this study. Should the need for medical attention arise, all the resources of a large teaching hospital are available for subject evaluation and treatment.

DATA AND SAFETY MONITORING

The Data and Safety Monitoring Plan for this protocol will include adverse event reporting by the PIs on the CARS Data and Safety Monitoring Forms, with oversight and monitoring by the PIs and the Research Subject Advocate (RSA). The PIs and research coordinator will meet weekly to review any study issues, and a Data and Safety Monitoring Board consisting of Edward Geltman, MD (Professor of Medicine and Medical Director of the Heart Failure Program at Washington University) and Eric Novak, MS (Biostatistician in the Division of Cardiology) will meet every six months with the study coordinator and Study Investigators to review study data, discuss any safety issues, ensure compliance with the protocol, and ensure the subjects' protection against risk.

Serious Adverse Events (SAEs) from this protocol will be reported to the CARS Advisory Committee via the CARS RSA, to the HRPO, and to the NIH. The study will be discontinued if there is clear evidence of harm or

harmful side effects of the procedures. In the event that a SAE occurs that increases the risks to participants, the study will be stopped, an investigation will be conducted, and a findings report generated to the NIH, HRPO, and CARS via the RSA before the study is resumed. Should there be SAEs or AEs that occur at a frequency greater than 5%, they will be added to the consent document, if not already addressed, and enrollment will be halted while a determination is made regarding the potential risks to participants. A report summary will be generated and sent to CARS and the RSA every 6 months. The summary report will include (but is not limited to) who monitored the study, SAEs, AEs, compliance, compliance with data collection, dropouts, and recruitment/enrollment.