BIOMEDICAL RESEARCH PROTOCOL UNIVERSITY OF MISSOURI

Project Title: Sex-related differences in arterial stiffness in type 2 diabetics: role of uric acid
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Study purpose: The purpose of the present project is to determine the effects of a low fructose diet (isocaloric or hypocaloric) or allopurinol on arterial stiffness in patients with type 2 diabetes (T2D).

This study is classified as a clinical trial and is registered on Clinicaltrials.gov #NCT03648996

I. RESEARCH OBJECTIVES/BACKGROUND

A. Background and significance

Cardiovascular disease (CVD) is one of the main complications of type 2 diabetes mellitus (T2D) [2, 3]. One third of the US population over age 65 has diabetes [4], increasing the public health burden of diabetes-related CVD. Increased arterial stiffness is a potential mechanism behind the higher incidence of CVD in T2D [5]. Aortic pulse wave velocity (PWV), a measure of arterial stiffness, is a marker and predictor of CVD risk and mortality [6]. A 1 m/s increase in aortic PWV corresponds to a 15% increased risk for total CV events, CV mortality, and all-cause mortality, as adjusted for age, sex, and other risk factors [7]. Stiffening of the vasculature is a hallmark of aging in men and women [8, 9] but its progression is accelerated by hypertension, obesity and T2D [5, 10-12]. Insulin-resistant and T2D-post-menopausal women are at higher risk for stiffening than men [13-16] but the mechanisms behind this differential augmentation remain unknown.

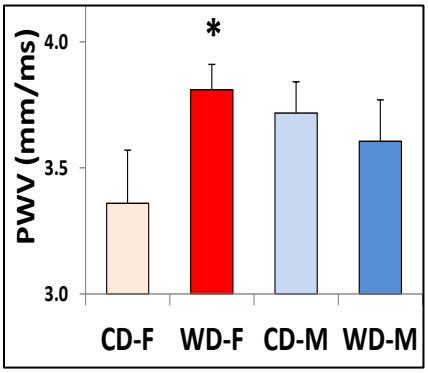

Figure 1. Female C57Bl6/J mice fed a Western diet (WD) are more likely to develop aortic stiffness. CD=control diet. *p<0.05 vs CD, n=7.

Elevated serum uric acid (SUA) levels and xanthine oxidase (XO) activation are linked to increased arterial stiffness in T2D women.

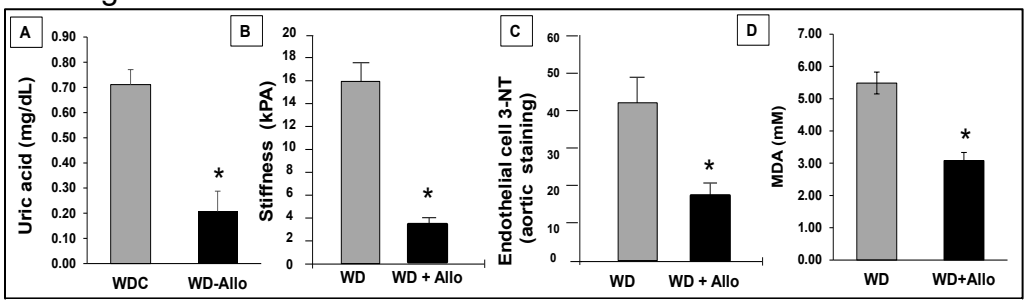

Figure 2. XO inhibition with allopurinol (allo; 0.125 mg/ml) reduced (A) uric acid, (B) vascular stiffness, (C) endothelial cell oxidative stress (3-NT) and (D) serum MDA in WD-fed female C57Bl6/J mice. n=4-8; *p<0.05 [1]

SUA is a product of XO activation. Analysis of the NHANES database found an elevated SUA prevalence of 21.2% in men and 21.6% in women [17]. Elevated SUA is associated with increased morbidity in patients with hypertension [18, 19], CVD and stroke [20], chronic kidney disease (CKD) and insulin resistance [21]. In the Framingham Heart Study, SUA was shown to be independently associated with aortic stiffness [22], particularly in women, as also reported by others [23-30]. In pre-hypertensive subjects, elevated SUA was related to a higher risk of developing hypertension in women than in men [31]. The presence of diabetes significantly increases the risk of arterial stiffness in women [32]. Elevated uric acid (UA) and XO activation can result in chronic inflammation, endothelial dysfunction and increased oxidative stress, key events in the pathogenesis of stiffness [33, 34]. Diet is the main driver of SUA in Western societies. Fructose supplementation elevates SUA in a dose-dependent manner [35]. Reducing SUA with allopurinol, an XO inhibitor, reduces arterial stiffness [36, 37] but whether reducing SUA, by either drug or diet, improves arterial stiffness in T2D subjects is unknown. Importantly, the differential effects of treatment on T2D men and women have not been tested. The proposed study will bridge this knowledge gap.

Distinct contributions of XO activity and SUA. High SUA levels can damage proteins and other macromolecules through mechanisms related to oxidative stress and inflammation as XO simultaneously generates both UA and reactive oxygen species [38]. In the vasculature, elevated UA has been shown to increase expression of inflammatory molecules such as C-reactive protein (CRP) [39, 40], as well as production of reactive oxygen species [41, 42] with parallel decreases in nitric oxide (NO) production [41, 43]. Perez-Pozo et al. suggested that the observed benefits of allopurinol treatment on blood pressure may be the result of either reductions in the deleterious effects of SUA, or from reductions in systemic inflammation following XO inhibition [44]. Consequently, in this proposal we will assess whether XO inhibition, and its direct effects on endothelial function, oxidative stress and inflammation, is superior to dietary UA reduction in improving arterial stiffness.

Dietary fructose and UA. Stanhope et al. found a linear relationship between fructose supplementation and increasing SUA [35]. A recent meta-analysis of the effects of fructose on SUA [45] revealed that most studies were confounded by study designs comparing fructose with sucrose (fructose-containing disaccharide) and allowed for inadvertent weight loss in both groups. Such weight loss would drive down SUA in both arms of the study [46] negating the ability to independently test the impact of fructose on hyperuricemia. With regard to hyper-caloric feeding of fructose, the data are very clear: fructose supplementation was shown to increase SUA concentrations in over 10 studies [45]. None of the long-term studies assessed arterial stiffness as an outcome, nor were there a priori comparisons between T2D men and post-menopausal T2D women in the designs. The proposed study design will allow for an accurate assessment of the effect of dietary fructose reduction on SUA levels and arterial stiffness, without the confounding effect of weight loss.

Endothelial dysfunction and arterial stiffness. Endothelial dysfunction plays a central role in the development and progression of CVD [47], and has been linked to the genesis of arterial stiffening [48-51]. Arterial stiffness has also been associated with microvascular dysfunction [52] and target organ damage [53]. A recent analysis of the Framingham cohort indicates that up to 13% of the relationship between measures of aortic stiffness and CVD events is mediated by impaired microvascular function [54]. Unfortunately, studies of the effect of XO inhibition on endothelial function have produced conflicting results [55-58]. A recent clinical trial in a

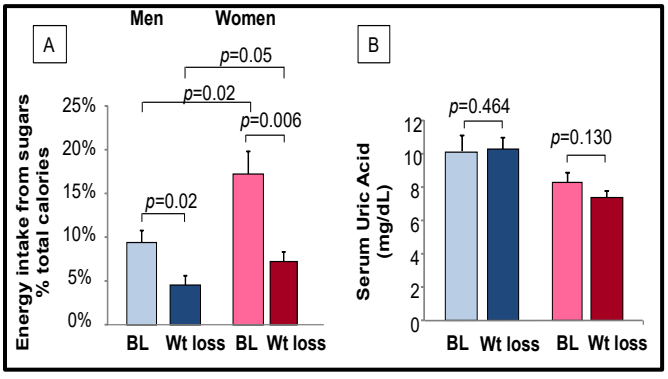

Figure 3. (A) Dietary sugar intake, higher in women at baseline, was significantly reduced after a sugar and weight reduction program. (B) Preliminary data in a small number of subjects indicate that although both sexes lost an equal amount of body weight (10%), SUA trended lower only in females (n=7 men; 9 women).

population of mostly male subjects with stage III CKD did not show a beneficial impact from allopurinol on endothelial function [59]. Nevertheless, a recent meta-analysis, by Cicero et al., of ten published trials, (670 subjects; allopurinol doses from 300-900 mg/day and 2 weeks to 9 months duration) reported improvements in flow-mediated dilation (FMD) with allopurinol therapy [57]. In the proposed study we will evaluate the impact of two SUA-reducing interventions on endothelial function of conduit arteries, assessed via FMD, and on microvascular function, assessed via insulin-stimulated blood flow using Doppler and contrast-enhanced ultrasound. The ultimate goal is to correlate these results with arterial stiffening.

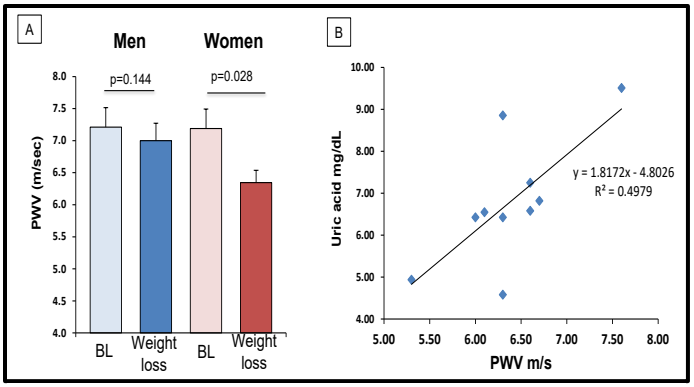

Figure 4. In women, (A) PWV improved significantly, and (B) was significantly correlated to SUA after 4 weeks on a fructose and weight reduction program (n=10 men; 10 women).

Preliminary data. In our previous studies in a rodent model of insulin resistance and obesity, a high fructose/high fat diet (Western diet (WD)) increased aortic stiffness only in females (**Figure 1**), supporting a sex-specific predisposition for the development of arterial stiffness in females. When WD-fed female mice were treated with allopurinol (0.125 mg/mL) (**Figure 2**), SUA (**Figure 2A**), aortic stiffness assessed by atomic force microscopy (AFM) (**Figure 2B**), aortic oxidative stress assessed by 3-nitrotyrosine staining (3-NT) (**Figure 2C**), and systemic oxidative stress assessed by plasma malondialdehyde (MDA) (**Figure 2D**), were all markedly reduced [1]. The proposed study will translate these findings from mice to humans, using similar methodologies, to determine the separate contributions of XO activity and SUA levels to arterial stiffness in men and women with T2D. Data from a weight loss study support the feasibility of our approach and the expertise of our research team. In men, ad libitum diets contained 9.3 ± 3.6% of energy as added sugars, significantly lower than the baseline intake in women (16.6 ± 8.1%, P<0.02). After a 6-month program designed to reduce dietary sugar intake and body weight, weight loss was similar in men (-10 ± 3%) and women (-10 ± 2%), P=0.99, and systolic and diastolic pressure was lower in both (data not shown), but women reduced their sugar intake more than men (**Figure 3A**), highlighting the relevance of dietary sugar intake, particularly in women. Further, even in this relatively small sample size, SUA trended lower with weight loss in women but not in men (**Figure 3B**) exhibiting a significant time-by- sex interaction (P=0.006 by ANOVA). Overall, the data demonstrate that added sugar intake in rodents and humans is a modifiable factor that can be used to impact arterial stiffness. Sexual dimorphism in isocaloric substitution of carbohydrates has yet to be tested in a controlled manner, and the paucity of data in this field leaves a critical gap in our understanding of the role of diet in uric acid-associated arterial stiffness in T2D women at risk for CVD.

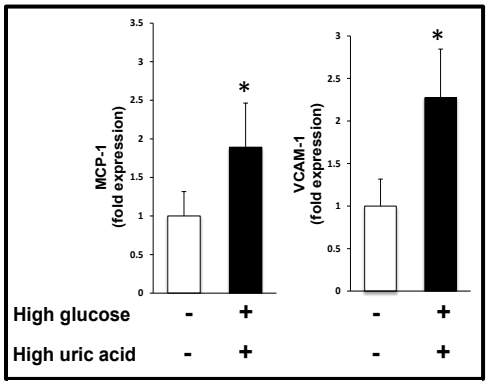

Figure 5. Increased inflammatory marker expression upon exposure of human aortic endothelial cells to high glucose (30mM) and high uric acid (0.1 mg/ml) cells. n=3; *p<0.05 vs. control.

In another recent fructose restriction/weight loss intervention in middle-aged adults, obese women showed a significant improvement in PWV, while men did not (**Figure 4A**). Relevant to this proposal, we found that PWV correlated with SUA in women after 4 weeks of weight loss (**Figure 4B**). TNFα and UA were the only variables that predicted PWV = 3.604 + (0.270*UA) + (0.132*TNFα), r=0.712, p=0.0025 following the weight loss in women, suggesting that SUA is a modifiable risk factor in the development of arterial stiffening in women. In another preliminary study, exposure to high glucose and high UA increased production of inflammatory markers in cultured human aortic endothelial cells

(**Figure 5**). Data from our basic and human studies complement growing epidemiological evidence that hyperuricemia in women is related to increased arterial stiffness, putting women at a high risk for CVD. We propose that the sexual dimorphism in arterial stiffness associated with T2D is mediated, in part, by sex-dependent effects of UA and XO activation.

B. **Summary of Study Objectives**

We will accomplish the following specific aims:

Aim 1: To determine if sex-related differences exist in the relationship between SUA levels and arterial stiffness (via cfPWV) in age-matched T2D men and women, and to what degree these differences are related to augmented oxidative stress, inflammation and endothelial dysfunction. Hypothesis: The relationship between arterial stiffness and elevations in SUA/augmented oxidative stress/endothelial dysfunction/increased inflammatory marker activity seen in T2D women will be weaker or absent in men.

Aim 2: To assess whether XO inhibition is superior to dietary-induced SUA reduction in ameliorating arterial stiffness in T2D subjects. Hypothesis: Although we expect XO inhibition to have the greatest impact, we posit that SUA reduction by either strategy will lead to more improvement in endothelial function and stiffness.

As shown in **Figure 6**, this project has three parts and each subject will participate in only one part. These separate treatment arms are combined into one IRB protocol because they will test an integrated hypothesis regarding uric acid and arterial stiffness in a similar population with identical procedures.

Part 1: Diet treatment

The overall goal of part 1 is to reduce fructose in the diets of T2D women and men at risk for future cardiovascular disease. Dietary fructose will be replaced with starch to keep the research subjects' body weights stable. Subjects will be treated for 6 months and measurements of arterial stiffness and endothelial function will be made before, during, and after treatment.

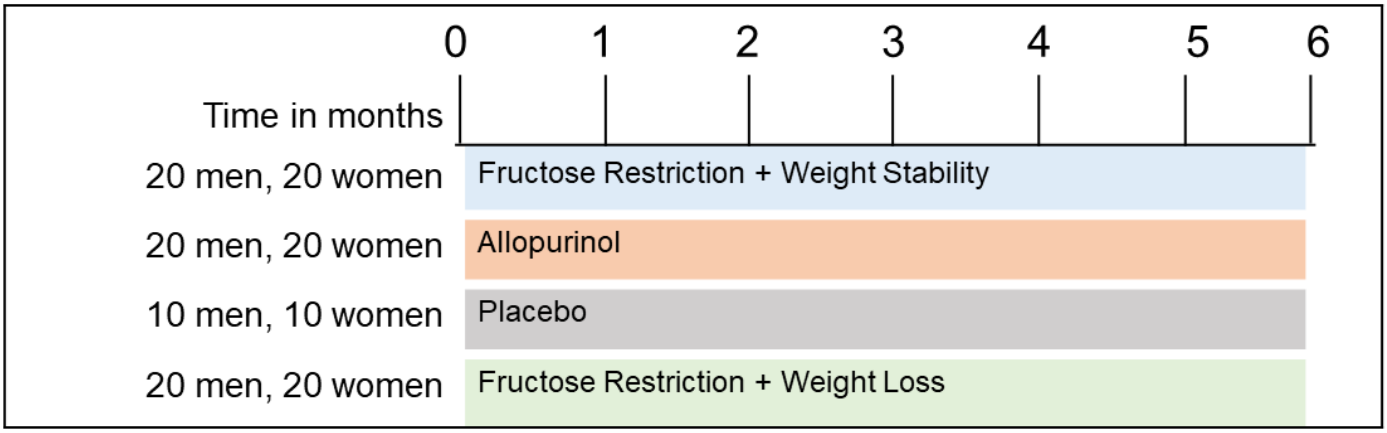

Figure 6. Duration and number of subjects enrolled in each of the four interventions. Interim visits occur at month 3 (12 weeks) for all arms of the study, and final visits occur at month 6 (24 weeks) for all arms of the study.

Part 2: Drug/placebo treatment

The overall goal of part 2 is to lower serum uric acid concentration in T2D women and men. The treatment will be allopurinol administration (or placebo), ramping up the dose as shown below to achieve a target dose of 300 mg/day. Measurements of arterial stiffness and endothelial function will be made before, during, and after.

Subjects that do not qualify based on blood biochemistry to participate in the above interventions will be offered to participate in a hypocaloric low fructose diet intervention.

Part 3. Hypocaloric Low Fructose Diet Treatment

The goal is to restrict fructose and calories in the diets of T2D women and men at risk for future cardiovascular disease to mediate weight loss. Dietary fructose will be reduced, but baseline fat and protein quantities will be held constant. A caloric restriction of 500 kilocalories will be prescribed to subjects. Subjects will be treated for 6 months and measurements of arterial stiffness and endothelial function will be made before, during, and after treatment.

II. DRUGS

A. Drug treatment, dose, route and regimen:

Dr. Velázquez will oversee the drug and placebo treatments in conjunction with principal investigator (Dr. Manrique). They will use standardized clinical protocols to ramp the dosage of allopurinol to a target dose of 300 mg. [60, 61]. The medication will be given orally once a day.

Dose escalation scheme: The dose escalation scheme for allopurinol, **Table 1**, is supported by past work [60, 62]. The doses of drug will be increased according to the scheme presented in **Table 1** until a target dose of 300 mg is reached, while closely monitoring for any adverse effects. Safety visits will be scheduled every 2 weeks during months 1-2 (at weeks 2, 4,6, and 8) and every 4 weeks during months 3-6 (at weeks 12, 16, 20) to monitor blood chemistries. Subjects may have safety labs repeated at the discretion of the safety officer.

Study window	Visit number	Daily dose	How many days
Day -30	v0, screening		
Day 0	v1, day 1		
Day 1-14	v2, day 14	100 mg daily	X 14 days
Day 15-28	v3, day 28	200 mg daily	X 14 days
Day 29-168	v4, day 42	300 mg daily	X 133-147 days

Table 1

B. Rationale for choosing the drug and dose:

Allopurinol was chosen as it is used clinically for management of gout and hyperuricemia. As mentioned before the dose and the escalation scheme for allopurinol, below, is supported by past work and available literature. [60, 62]

C. Risks of not treating hyperuricemia in the placebo group:

A 2013 Cochrane review emphasized the lack of sufficient evidence to support the use of urate-lowering therapy to prevent future gout in hyperuricemic, asymptomatic patients [63]. *A need for further clinical trials to test other benefits of lowering SUA was called for* [64] which strongly supports the rationale for the current study.

Given the limited duration of subject participation (6 months), the lack of known risk to the subject for not being treated is balanced by the significant knowledge gained from this study. At the study's end, subjects in the placebo arm will be offered one, no-cost counseling sessions with the dietitian nutritionist, who will have analyzed their ad libitum food intake and body composition. The dietitian nutritionist will make recommendations to these subjects for modifying lifestyle factors to reduce SUA and improve overall health.

D. Risks and safety information

Side effects of allopurinol include hepatitis, rash and pruritus. Our team is aware of the existing recommendations by the American College of Rheumatology in regard to the use of anti-inflammatory agents when starting allopurinol in patients with acute gout [60, 65]. As the present study will not include patients with diagnosis of gout and given that available studies done in subjects with asymptomatic hyperuricemia demonstrate that monotherapy with allopurinol is safe [66, 67], we plan to treat subjects our hyperuricemic subjects only with allopurinol. Dr. Velázquez and Dr. Manrique will closely monitor these subjects and might consider using a low-dose of naproxen in selected cases.

III. RECRUITMENT PROCESS

A. Recruitment Pool

The study population includes male and female subjects with BMI (25.1-50 kg/m2) and age 40-75. We used the MU i2b2 database to determine the recruiting pool for the project this September**.** From a total of 519,153 patients older than 40, after excluding for lack of T2D diagnoses (497,325) and adding the population estimates (overweight/obesity of 65% in Missouri), a potential recruitment pool of 14,188 patients remains. Thus, there are sufficient patients (men and women) in our database to serve as study subjects The number of subjects entered in each study allows for a 10% dropout rate. Our past dietary studies have run from 6 weeks to 6 months and dropout has been 1-10%. In our experience, high public interest in reducing dietary fructose intake suggests that recruiting subjects for this arm will not be difficult.

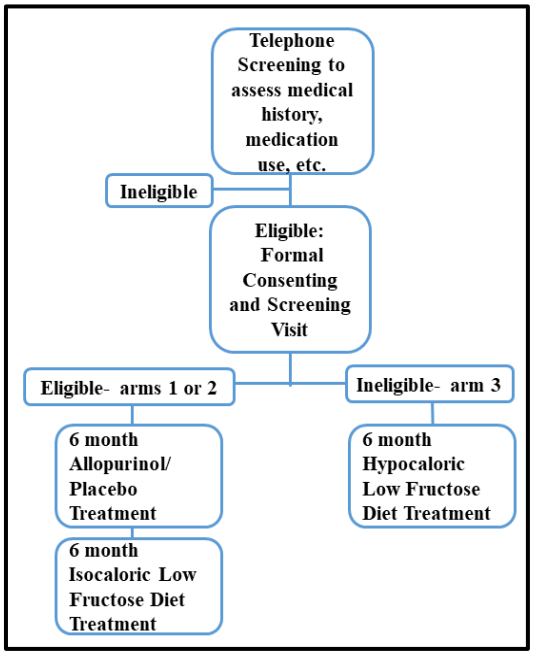

Figure 7. Pathway for subjects to enter into research arms

B. Subjects will be recruited by a number of means:

1. A chart review of clinic patients from the Divisions of Rheumatology, Endocrinology and the Department of Medicine who are between the ages of 40 and 75, and have been diagnosed with type 2 diabetes will be completed in Powerchart by study coordinator/staff based on the study inclusion/exclusion criteria. A letter, chart message, or phone call will be utilized to notifying them of their eligibility to participate in a research study. Study coordinator/staff will work in conjunction with healthcare providers that have a direct patient care relationship to recruit subjects from clinics.
2. Potential subjects may also be identified via use of i2b2 screening. Study coordinator/staff will work in conjunction with healthcare providers that have a direct patient care relationship to recruit these subjects. Subjects may be informed of their eligibility via chart messaging, letter, or phone call.
3. Subjects may also respond to the recruitment flyers or advertisements posted in MU Hospital clinics, MU Hospital television screens, MU Info, MU campus, newspapers, Facebook, or any place generally accessible to the public (i.e grocery stores, community centers, etc). For all recruitment, subjects will be given an email address and/or a phone number to contact. Subjects may also be given a link to complete a screening questionnaire via Qualtrics.

IV. CONSENT PROCESS

Four consent forms will be used:
1) A screening consent form (general consent) describing the isocaloric low fructose diet treatment, the allopurinol treatment, the hypocaloric low fructose diet treatment, and the control arm;
2) A treatment consent form that describes the isocaloric low fructose diet;
3) A treatment consent form that describes the allopurinol/placebo study;
4) A treatment consent form that describes the hypocaloric low fructose diet

A. Preliminary Screening

Subjects who inquire about participation will answer screening questions, with their waiver of written consent. These questions are for preliminary screening only and are not used as study data since the data represent self-report. The questions include queries about health history/habits, age, sex, height, weight, tobacco use, medications/ supplements, over the counter medications use, illness, dietary habits, and chronic conditions. The study design is described in general terms to subjects, with mention of factors most likely to impact subject interest in participating. These factors can include limited food options and the requirement to remain weigh stable, drug treatment or no drug treatment. If the subject is interested in being formally screened for participation, an appointment will be scheduled for a CRC visit for consenting, measurement of fasting blood chemistries, DEXA, vascular measurements, and an Oral Glucose Tolerance Test (OGTT).

B. Formal clinical trial consenting

Subjects who are eligible following the CRC screening visit will be formally consented for the specific arm of the study they are participating in (the isocaloric fructose restriction arm, the allopurinol/placebo treatment arm, or the hypocaloric fructose restriction arm of this project) before their baseline visit. Formal consenting up to 1 hour and during that process, 1) the subject will be asked to describe, in their own words, what the baseline visit will be like, and 2) the subject (in either of the fructose restriction arms) is given a three-day food record and instructed on how to fill it out. They will be provided with the study details. Review of the consent form will occur with study personnel in a quiet, unhurried setting. Comprehension will be assessed by asking the subject to explain the study in their own words. Participants will have adequate opportunity to review the informed consent and to ask any questions they may have about the research protocol, compensation, risks, and benefits of taking part in the study.

V. INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria:

1) Men and women with BMI 25.1-50 kg/m^2, who are 40-75 years of age at randomization.
2) T2D diagnosed > 3 months ago. Patients with T2D will be classified based on physician diagnosis.
3) No vulnerable populations (e.g., prisoners, pregnant, children) will be enrolled.

Exclusion criteria for part 1 and part 2 of the study:

1) Serum uric acid < 5.5 mg/dL
2) Habitual diet containing low amount of sugars < 5% of total energy intake
3) Recent CVD event (stroke, heart failure hospitalization, revascularization or acute coronary event in the last 12 months), abnormal thyroid tests or chronic liver disease, stage IV renal disease (GFR <30) and hyperparathyroidism.
4) Use of azathioprine
5) Active cancer
6) Autoimmune diseases

7) Excessive alcohol consumption (>14 drinks/week for men, >7 drinks/week for women)
8) Current tobacco use
9) Bodyweight change ≥10% within the last 6 months
10) History of gout or uncontrolled hypertension
11) A1C >10.0
12) Pregnancy or lactation in women (or women not using contraceptives)

Medications for glucose control (insulin, metformin, gliptins, GLP-1 agonists, sulfonylureas, sodium glucose co-transporter inhibitors), antihypertensives, hormone replacement therapy, antidepressants, anticoagulants, or statins, will be considered during recruitment, analyses (e.g., using covariate analysis) and interpretation.

Exclusion criteria for hypocaloric low-fructose diet intervention (part 3):

1) Habitual diet containing low amount of sugars < 5% of total energy intake
2) Recent CVD event (stroke, heart failure hospitalization, revascularization or acute coronary event in the last 12 months), abnormal thyroid tests or chronic liver disease, stage IV renal disease (GFR <30) and hyperparathyroidism.
3) Use of azathioprine
4) Active cancer
5) Autoimmune diseases
6) Excessive alcohol consumption (>14 drinks/week for men, >7 drinks/week for women)
7) Current tobacco use
8) Bodyweight change ≥10% within the last 6 months
9) History of gout or uncontrolled hypertension
10) Pregnancy or lactation in women (or women not using contraceptives)

Medications for glucose control (insulin, metformin, gliptins, GLP-1 agonists, sulfonylureas, sodium glucose co-transporter inhibitors), antihypertensives, hormone replacement therapy, antidepressants, anticoagulants, or statins, will be considered during recruitment, analyses (e.g., using covariate analysis) and interpretation.

VI. NUMBER OF SUBJECTS

Sample size calculations are based primarily on satisfying the requirements of Aim 2, i.e. to show that XO inhibition and fructose restriction reduce PWV, and that these interventions are more effective in women than in men. Calculations are based on the paired *t*-test for pre- to post-treatment change, and independent t-tests for comparisons between treatment arms. In both cases, we assume an alpha of .05 and a two-sided alternative. The sample size calculations shown in **Table 2** are derived from the literature as follows: De Angelis *et al* have shown that in men, T2D does not increase PWV in age- and BMI-matched men without T2D [32]. By contrast, in women, T2D significantly increases PWV compared to women without T2D and importantly, compared to men with T2D. Any reduction in PWV represents a significant reduction in cardiovascular risk [7]. However, with 6 months of allopurinol treatment, we have chosen a sample size to be able to detect a reduction in PWV of 0.6 m/s, which Ng *et al* showed to be clinically meaningful [68]. Nine subjects per group (9 men and 9 women) completing the allopurinol arm will allow for 90% power to detect a change of 0.6 m/s and seven subjects/group will allow for 80% power to detect this change **Table 2.** With regard to fructose restriction, our preliminary

Treatments	Power	Alpha	Change PWV	SE of Δ in PWV	# of subjects
Allopurinol	90%	0.05	0.6 m/s	0.4 m/s	9
	80%	0.05	0.6 m/s	0.4 m/s	7
Low-fructose	90%	0.05	0.5 m/s	0.4 m/s	13
	80%	0.05	0.5 m/s	0.4 m/s	10

Table 2

data demonstrate a significant improvement in PWV only in the females. We anticipate that the reduction in PWV with diet may be smaller than that with allopurinol. We would need 13 subjects/group to detect a 0.5 reduction in PWV with 90% power; 10/group would give 80% power. Further, as we do not expect changes in the PWV during 6 month of follow up in the placebo group and to accommodate variability in response and potential dropout, we will include 40 subjects in the low-fructose intervention, 40 subjects in the allopurinol cohort and 20 in the placebo group (half men and half women for all the cohorts). Further and based in our previous weight loss/dietary intervention[69], we have chosen a sample size to be able to detect a reduction in PWV of 0.6 m/s of 20 diabetic males and 20 diabetic females.

VII. STUDY PROCEDURES/ DESIGN/TREATMENT PLAN

Following screening, eligible male and female subjects will be assigned to one of the three treatment arms. In the medication arm of the study, they will be randomized to either the allopurinol group (n=40; 20 men and 20 women), or the placebo (n=20; 10 men and 10 women) for 6 months, with a final drug/placebo allocation ratio of 2:1 using a permuted block design with random variation of block size. In the isocaloric low-fructose arm of the study, 20 women and 20 men will be recruited to enter a dietary treatment of fructose restriction for 6 months. In the hypocaloric low-fructose arm of the study, 20 women and 20 men will be recruited to enter a dietary treatment of calorie and fructose restriction for 6 months.

A. Screening/Initial Consenting Visit

The first visit to the CRC will take approximately 6 hours and include a written consent to be screened. The subject is instructed to hold their glucose lowering AM medications (subject will resume regular medications after blood has been drawn), and not to eat or drink anything besides water for at least 10 hours before this visit. The subject is also told not to exercise the morning of this visit. After signing the screening consent form, medical information is obtained by the study team, including: DOB, gender, ethnic/racial category, height, body weight (history of body weight gain or

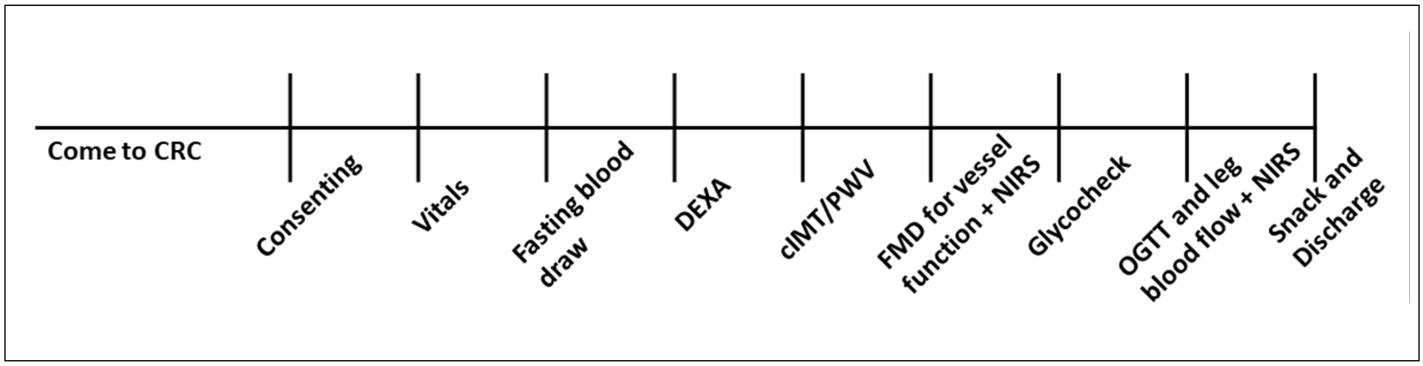

Figure 8. Order of events at the Screening Visit occurring at the clinical research center (CRC).

loss), waist circumference, vitals, and a medical history questionnaire. A fasting blood draw is taken to test for biochemistries. Subjects will also undergo a DEXA scan and vascular measurements including carotid intima media thickness (cIMT), carotid femoral pulse wave velocity (cfPWV), Glycocheck, Near-Infrared Spectroscopy **(**NIRS), Brachial artery FMD, popliteal artery FMD testing, and OGTT with femoral artery blood flow (**Figure 8**).

An introduction to the clinical trial will be done which includes abbreviated descriptions of the various treatment arms and the placebo arm (no treatment). As soon as the blood biochemistry results are available, the subject is contacted, provided with a copy of the results. If eligible for the allopurinol/placebo arm or (isocaloric) low fructose diet intervention arm, study staff discuss with him/her whether they would like to be consented for enrollment in one of the present studies.

Subjects that are ineligible for both the allopurinol/placebo and (isocaloric) low fructose arms or do not want to pursue the 6-month intervention will be offered the opportunity to participate in the hypocaloric low fructose arm. Participants that have previously failed initial screening (were ineligible to participate in arm 1 or 2 of the study) will be offered the opportunity to re-consent to the study and have vascular measurements taken at an additional paid screening visit and to enroll in the 5-month long hypocaloric low fructose arm (arm 3).

B. **Baseline/Formal Consenting Visit**

The subject will arrive to the CRC in the morning to perform further metabolic and vascular tests (**Figure 9**). The subject is instructed to hold their glucose lowering AM medications (subject will resume regular medications after the visit has been completed), and not to eat or drink anything besides water for at least 10 hours before this visit. The subject is also told not to exercise the

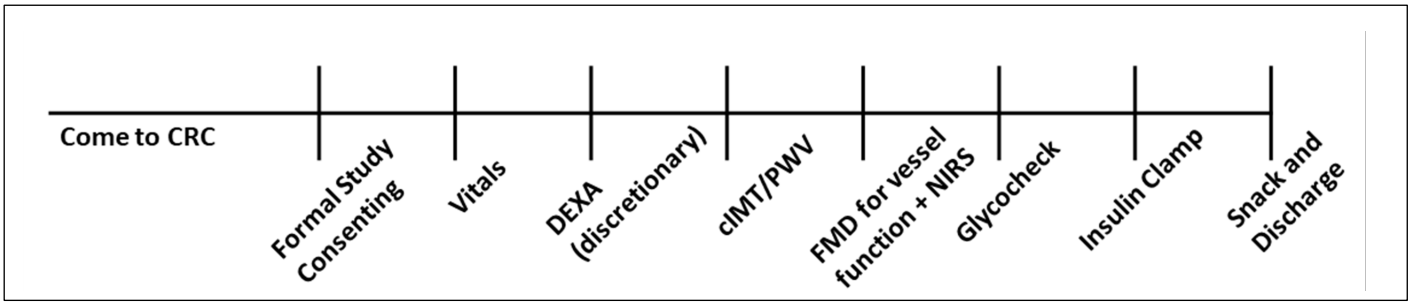

Figure 9. Order of events at the Baseline Visit occurring at the clinical research center (CRC).

morning of this visit. The visit will take up to 8 hours and the subjects must be fasted. Upon arrival, the participant meets with one of the study's physicians to review and sign the study consent forms. The subject then voids and the study staff takes anthropometric measurements and repeats a DEXA scan at the discretion of the investigator. Subjects will then be placed supine in a quiet, climate-controlled room (22–23 ° C) to rest before vascular measurements are repeated by study staff, including: cIMT, PWV, Glycocheck, FMD and NIRS. Vital signs are measured and an IV line is placed in each arm by the nurse in order to draw blood and infuse during a hyperinsulinemic-euglycemic clamp. The CRC vascular procedures will be repeated again at interim and final visits at 3 and 6 months.

C. **Interim visit**

An interim visit will include fasting blood draws. Study staff will assess blood pressure, heart rate, weight, and waist circumference as well as body composition (DEXA), cIMT, FMD, NIRS, Glycocheck, and cfPWV. This visit will take up to 4 hours and must occur in the fasting state.

D. **Final visit**

A final appointment will repeat all baseline measurements including DEXA, cIMT, Glycocheck, cfPWV, FMD, NIRS and insulin clamp with CEU. The study staff will assess the body weight, waist circumference, heart rate, and blood pressure. Blood will be collected at the beginning and throughout the study procedures at this visit. This visit will take up to 8 hours and must occur in the fasting state. Following the final visit, the treatment portions of each arm are over. Subjects in Part 2 will be offered a meeting with the dietitian nutritionist to discuss diet and exercise. Subjects may be asked to complete an exit survey upon completion of the study. Exit surveys will be administered via Qualtrics or in person depending upon subject preference.

E. **Special Consideration in case of unforeseeable hardships such as public health emergencies or weather-related events**

In the event that subjects are not able to complete study visits for the reasons described above, the following remedial steps will be taken:

1. Safety visits #4, 6 and 7 at weeks 8, 16, and 20 (respectively) can be completed via a phone interview during which the subject will answer the safety questionnaire (no changes in compensation).
2. In the case that the subject cannot complete safety visit #3 at week 6, every effort will be made to complete this visit within 7 days of the originally scheduled date. In the case that the visit cannot be completed, the subject will be instructed to discontinue the study medication and his/her participation in the study will be completed. Dr. Velazquez, who oversees the allopurinol/placebo treatment with the Principle Investigator will be informed.
3. In the case that safety visit #4 at week 8 cannot be completed, and providing the subject has completed at least 2 in-person visits since the medication was started, study medication will be mailed by investigational pharmacy for the subject to continue treatment. Dr. Velazquez will be informed.
4. In the case that the subject cannot complete dietary counseling in person, counseling visits may be completed via phone or comparable, secure tele-counseling set-up.
5. In the case that subjects are unable to complete their interim visits at the originally scheduled date, their visits may be scheduled +/-14 days of the originally scheduled date. If the interim visit cannot be completed onsite, safety formulary will be completed via phone.
6. In the case that subjects are unable to complete their final visits at the originally scheduled date, their visits may be scheduled +/-14 days of the originally scheduled date provided that the subject maintains the assigned intervention for the duration of the study.
7. In the case that the Clinical Research Center is unavailable or at the discretion of the primary investigator, safety visits may be completed at MUPAW.
8. In the event of the clinical research center not being available or if it is considered by the study physician that performing studies there can increase risk of exposure to infectious agents or related hazards, the following alternative sites will be made available for screening, safety and study visits:
    - Medical school room NW404
    - University hospital clinic rooms

    These locations are not carpeted and we do not anticipate an increased risk for the subjects given that medical supervision and nursing staff assistance will be unchanged.

F. **Description of Study Procedures**

Metabolic testing: As described in the background section, hyperuricemia is characterized by increased inflammation and insulin resistance. We will monitor changes in inflammation and oxidative stress markers through fasting blood sample measurement of serum concentrations of XO activity, TNFα, IL6, CRP, PAI-1, MCP-1, E-selectin, MDA, 3-NT, TBARS, oxidized LDL and 8-iso PGF2α. In addition, this blood sample will also be used for measurement of fructose, blood lipids, glucose, insulin, creatinine, estradiol, estrone, testosterone and sex-hormone binding globulin, uric acid, blood lipids, and estrogen, the latter used as a covariate. [70]

DEXA scan: to assess body composition with dual-energy X-ray absorptiometry.

Arterial Stiffness and Blood Pressure: The SphygmoCor XCEL device will be used to assess blood pressure and aortic pulse wave velocity, a marker of arterial stiffness. A blood pressure cuff will be wrapped around the upper leg. It will periodically inflate to ~200 mmHg for less than 60 seconds. A pressure sensor (tonometer) will be placed over the skin of the neck region to obtain the pressure wave form in the carotid artery. The total time for this procedure is 15 min.

Brachial and popliteal artery flow-mediated dilation (FMD): Arterial measurements will be performed by imaging the brachial and the popliteal artery longitudinally using high-resolution duplex ultrasonography. Arterial vasodilatory responses to hyperemia (i.e., FMD) will be examined by inflating a cuff, distal to brachial and the popliteal artery, up to 250 mmHg for 5 minutes. Before, during and after rapid release of the cuff, brachial and popliteal artery blood flow velocity and diameter will be continuously measured. In addition, during the FMD measurements NIRS probes will be placed on the tibialis anterior muscle and the forearm; the probe will be secured via an elastic tensor bandage which will be loosely wrapped around the site. These probes will stay attached to the participant for the duration of testing.

Carotid artery intima-medial thickness (cIMT): Wall thickness and diameter of the common carotid artery will be measured using high-resolution vascular ultrasound with a 7.5-12.0 MHz linear array transducer and longitudinal 2D mode (Logiq P5; GE Medical Systems, Milwaukee, WI). The left common carotid artery will be measured 2 cm proximal to the bulbus and 2 to 3 cm distal to the bifurcation. For this vascular evaluation, we will measure artery diameter and wall thickness for a brief period of time.

OGTT with leg blood flow: After an overnight fast, a catheter will be inserted into an antecubital vein for sampling of venous blood. Blood will be collected every 15-30 minutes over the next 120 minutes after consuming the glucose beverage (75 grams of dextrose). Moreover, before and during the OGTT, we will be using an ultrasound to measure femoral artery blood flow. Throughout the assessment for leg blood flow, a NIRS probe will be placed on the belly of the tibialis anterior muscle in the same conditions previously described. A pneumatic cuff will be placed below the knee. The cuff will be inflated at 20 mmHg above diastolic blood pressure for 60 s to partially occlude blood flow at the time of the NIRS measurement. The total volume of blood drawn per oral glucose tolerance test will be less than 40mL.

Insulin clamp: Coupled with measures of leg blood flow, will be performed following standard procedures at the University Hospital CRC. Subjects will be instrumented for measures of heart rate (using standard lead II ECG), arterial blood pressure (using automated sphygmomanometry and beat-to-beat via Finometer), and leg blood flow (using duplex Doppler ultrasound and contrast-enhanced ultrasound, CEU). After a minimum of 20 min supine rest, baseline cardiovascular measurements will be collected, blood samples obtained and the hyperinsulinemic-euglycemic clamp will start. Briefly, insulin diluted in 0.9% saline with 5 ml of the subject's blood is infused with a 10 min priming dose followed by a constant infusion at 40 mU•m−2•min−1, a dose used to mimic postprandial insulin concentrations. Glucose is maintained at fasting values throughout via a variable 20% dextrose infusion. Leg blood flow, arterial blood pressure, and heart rate will be collected for 5-min periods at 15-min intervals throughout the 120-min clamp allowing the magnitude as well as the temporal profile of these variables to be determined. For microvascular blood flow using CEU, measurements will be performed before and during the first 60 min of insulin stimulation. Briefly, assessment of microvascular perfusion utilizing CEU involves ultrasound imaging of the leg skeletal muscle and abdomen during the administration of one vial (1.3mL) of an ultrasound contrast agent diluted in 30mL of 0.9% saline that will be given using a handheld syringe pump through the IV at a rate of 2mL/min. This agent, called Definity, is composed of tiny microbubbles smaller than the size of a red blood cell. These bubbles stay inside the blood vessels and go where the red blood cells go. This contrast agent allows us to evaluate microvascular blood flow. Definity has been FDA approved

for use in humans during ultrasound of the heart cavity and has been shown to be safe. The total volume of blood drawn per insulin clamp will be less than 140mL.

GlycoCheck: The GlycoCheck video microscope instrument will be placed under the subject's tongue for a brief period (5 min approximately) to measure capillaries, blood vessel density, red blood cell concentration, flow rate, and red blood cell penetration of the glycocalyx lining. The GlycoCheck instrument, computer system, and software will analyze the measurements and produce a Microvascular Health Score (MVHS). GlycoCheck is an FDA Class 1 Medical Device.

G. **Details of treatment visits for the isocaloric low-fructose diet (part 1)**

Figure 10 shows the appointments associated with pick up of study food, inpatient visits, dietary counseling, and the follow-up visit. Subjects will consume a six-month diet with the goal of reducing dietary fructose intake to <5% of energy and keeping their weight stable. Prescribed caloric intake will be based on

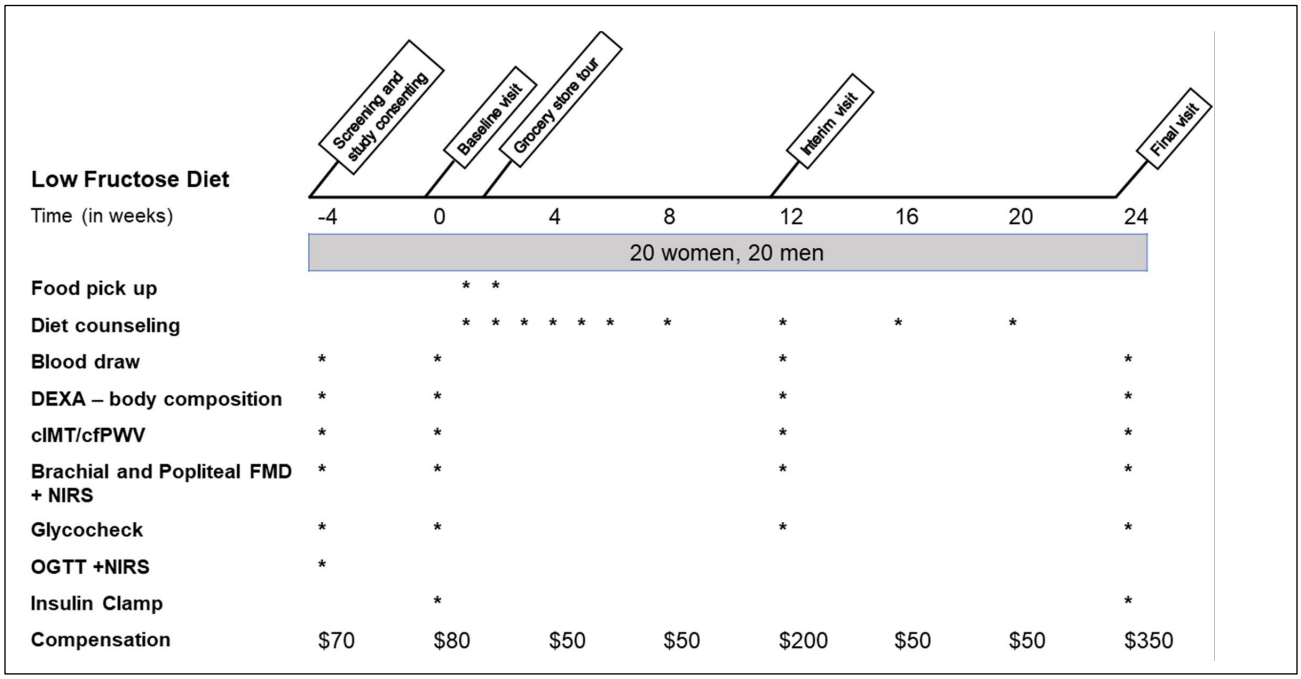

Figure 10

baseline total energy expenditure per day. For the first two weeks, all foods will be provided by the study (made at the MU Nutrition Center for Health, MUNCH) for the subject to consume and he/she is instructed to not eat any other foods. Beverages that may be consumed can include water, non-calorie containing sodas, and regular coffee or tea without nondairy creamer, which may be sweetened with non-caloric sweeteners. A maximum of 2 oz of cream per day may be added to the coffee or tea. Regular sodas and fruit juices that are high in fructose are to be avoided. Habitual alcohol consumption may be continued and subjects will be asked to avoid alcoholic drinks containing fructose and other sugars (e.g., margaritas, fruit-containing drinks). Milk and milk products may be consumed given their low-fructose content. Within week 2, the subject will attend a grocery store tour with study staff to identify high-fructose foods to avoid. After two weeks, the subject will begin to prepare, cook, and eat his/her own foods while following the diet with the assistance of the dietitian nutritionist and study staff.

The subject will meet one-on-one with the dietitian nutritionist who will counsel him/her on how to eat a diet that matches the study criteria. Subjects' weight, waist circumference and vitals will also be measured at nutrition counseling visits. Throughout the entire study, the subject will be in contact with the dietitian nutritionist by phone or email to have their questions answered and receive social support to maintain adherence. At three months, the subject returns for an interim visit where PWV,

FMD and DEXA will be repeated, while at six months, the subject returns for the final visit which includes vascular measurements and an insulin clamp.

Beverages: The subject may consume non-sugar containing beverages including water, non-calorie containing sodas, and regular coffee or tea without nondairy creamer, which may be sweetened with non-caloric sweeteners. A maximum of 2 ounces of cream per day may be added to the coffee or tea. Regular sodas and fruit juices that are high in fructose are to be avoided. Habitual alcohol consumption may be continued and subjects will be asked to avoid alcoholic drinks containing fructose and other sugars (e.g., margaritas, fruit-containing drinks). Milk and milk products may be consumed given their low-fructose content.

Activity: The subject should not consciously change his/her physical activity while in the study. At the conclusion of the study, subjects may meet with study staff to receive advice on how to increase their activity.

H. Details of treatment visits for the allopurinol/placebo arm (part 2)

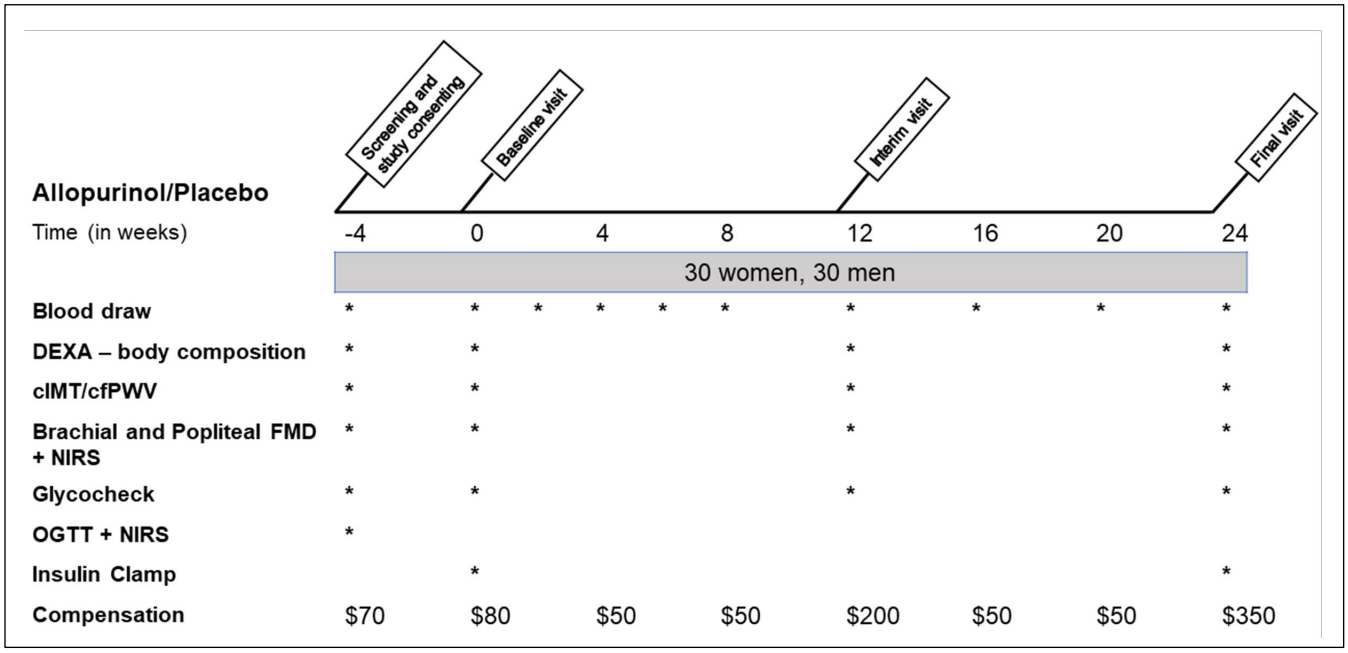

Figure 11

Figure 11 shows the appointments associated with the inpatient visits, drug treatment, and the final visit for this arm. After completion of the baseline visit (procedures described above), subjects participating in the allopurinol treatment/placebo arm will begin with an initial dose of drug of 100 mg/d p.o. daily for 2 wks. The dose is then slowly increased over the next 4 weeks to achieve a target dose of 300 mg/d). Dose escalation will be led by Dr. Celso Velázquez, Professor of Clinical Medicine and Director of the Division of Rheumatology, using his established algorithm, and Dr. Manrique, Internist and Endocrinologist. Once 300 mg/d dose has been reached, the subject stays on this dose; and is seen for the interim visit (3 months), at which time all procedures are repeated except insulin clamp-CEU and OGTT. After this, drug treatment continues for another 3 months and the subject returns for the final visit at 6 months. The DEXA, cIMT/cfPWV, Brachial and Popliteal FMD, and insulin clamp procedures are repeated at this time. The dose of allopurinol will be taken the morning of the final visit.

I. Details of treatment visits for the hypocaloric low-fructose diet (part 3)

Figure 12 shows the appointments associated with pick up of study food, inpatient visits, dietary counseling, and the follow-up visit. Subjects will consume a six-month diet with the goal of reducing

dietary fructose intake to <5% of energy and reducing total daily caloric intake from baseline total energy expenditure by 500 kilocalories per day to promote weight loss. For the first two weeks, all foods will be provided by the study (made at the MU Nutrition Center for Health, MUNCH) for the subject to consume and he/she is instructed to not eat any other foods. Within week 2, the subject will attend a grocery store tour with study staff to identify high-fructose and added sugar foods to avoid. After two weeks, the subject will begin to prepare, cook, and eat his/her own foods while following the diet with the assistance of the dietitian nutritionist and study staff.

The subject will meet one-on-one with the dietitian nutritionist who will counsel him/her on how to eat a diet that matches the study criteria. Subjects' weight, waist circumference and vitals will also be measured at nutrition counseling visits. Throughout the entire study, the subject will be in contact with the dietitian nutritionist by phone or email to have their questions answered and receive social support to maintain adherence. At 3 months, the subject returns for an interim visit where PWV, FMD and DEXA will be repeated, while at 6 months, the subject returns for the final visit which includes vascular measurements and an insulin clamp.

Beverages: The subject may consume non-sugar containing beverages including water, non-calorie containing sodas, and regular coffee or tea without nondairy creamer, which may be sweetened with non-caloric sweeteners. A maximum of 2 ounces of cream per day may be added to the coffee or tea. Regular sodas and fruit juices that are high in fructose and/or added sugar are to be eliminated during the dietary intervention. Habitual alcohol consumption may be continued and subjects will be asked to avoid alcoholic drinks containing fructose and/or added sugars (e.g., margaritas, fruit-containing drinks). Milk and milk products may be consumed given their low-fructose content.

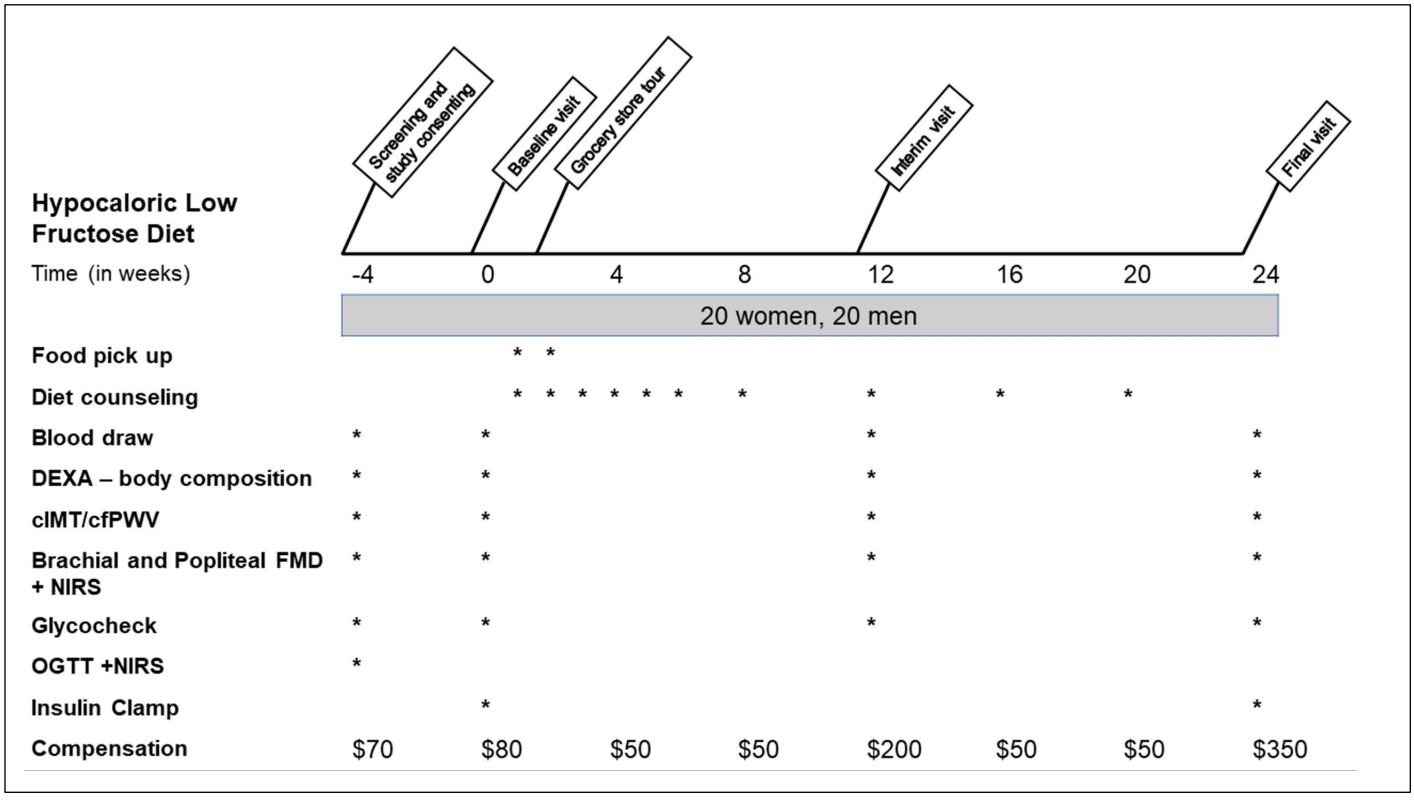

Figure 12

Activity: The subject should not consciously change his/her physical activity while in the study. At the conclusion of the study, subjects may meet with study staff to receive advice on how to increase their activity.

J. **Sources of research material**

Sources will include the subject's medical history, physical exam, screening laboratory tests (CMP, lipid panel, uric acid, insulin, A1c), dual-energy X-ray absorptiometry (DEXA, total and regional fat mass and fat-free mass), blood tests, aortic PWV, cIMT, brachial and popliteal artery flow-mediated dilation (FMD), OGTT with femoral artery blood flow results, CEU-insulin clamp results, and food intake. Demographic data (plus other related data: emergency contact person, pregnancy status), blood pressure, and anthropometrics will be measured (height, weight, waist circumference). Each subject in parts 1 or 3 (diet interventions) of the study, will donate up to 410 ml of blood (screening through final visit) which will occur over a period of up to 6 months. For Part 2 drug treatment, each subject will donate up to 470mls of whole blood (screening through final visit) which will occur over a period of 6 months.

K. **Blinding and subject safety**

All of Dr. Manrique' s laboratory staff will be blinded as they perform biochemical assays on serum from the drug/placebo study, the isocaloric fructose-restriction study, or the hypocaloric fructose-restriction study. Dr. Padilla and the assistant performing vascular measurements will be blinded. For the drug study, safety officer cardiologist Brian Bostick, MD, rheumatologist Dr. Celso Velázquez and Dr. Camila Manrique will review screening values to determine subject eligibility. Once the subject is randomized, the subjects themselves, investigators, and all CRC staff working with the subjects will be blinded to treatment group (allopurinol or placebo). During the fructose-restriction studies, the CRC staff and the study dietitian nutritionist will not be blinded because subjects serve as their own control and all subjects will be changing their diets (cannot blind to this treatment). No adverse effects of fructose restriction are anticipated but should subjects exhibit changes in their health status across any system related or unrelated to the dietary treatment, Dr. Bostick or Velázquez will be consulted for advice.

VIII. Potential Risks/Adverse Events

A. Potential Risks to Human Subjects

The following are the potential risks:

Phlebotomy: The total blood withdrawn will not exceed 550 mL in any 8 week period. Hematocrit is measured during screening and before the interim and final visits to check that it is within safe limits (**Table 3** and **Table 4**). All subjects will be advised to refrain from donating blood during public blood drives, during their participation in this project.

These activities are associated with a small risk of phlebitis, bruising and minor pain. Antiseptic technique will be used by nurses who are experts in phlebotomy and catheter placement. If phlebitis occurs, it will be treated conservatively with heat and when appropriate, with antibiotics.

Time point	Volume of whole blood (mls)
Screening	60
Baseline visit	145
SUA concentration	10
SUA concentration	10
SUA concentration	10
Interim visit	35
SUA concentration	10
SUA concentration	10
SUA concentration	10
Final metabolic test	170
Total	470

Table 3. Blood volume taken over the study in the allopurinol/placebo arm

Adherence and serum and urine biochemistries: The SUA response to drug treatment and the pill counts will be used to assess adherence to the drug/placebo arms. Some plasma will be frozen for analysis of potential biomarkers of cardiac and arterial (CA) stiffness. Estimated glomerular filtration rate (GFR) is calculated via the Modification of Diet in Renal Disease Study equation, GFR = 175 × standardized serum creatinine −1.154 × age−0.203 × 1.212 [if black] × 0.742 [if female], where GFR is expressed as mL/min/1.73 m^2 of BSA and creatinine is expressed in mg/dL [71].

Indirect calorimetry: Fasting energy expenditure and substrate oxidation are measured at the beginning of the metabolic test. Calorimetry carries no risk, however, this test may cause discomfort in those who are fearful of confined spaces or claustrophobic. Before the procedure is performed, subjects are allowed to become familiarized with the hood used to collect expired gas.

Aortic pulse wave velocity (cfPWV): This procedure poses not risks.

Carotid artery intima-media thickness (cIMT): This procedure poses no risks.

Time point	Volume of whole blood (mls)
Screening	60
Baseline visit	145
Interim visit	35
Final metabolic test	170
Total	410

Table 4. Blood volume taken over the study in the isocaloric low fructose arm and hypocaloric low fructose arm.

Brachial artery and popliteal flow-mediated dilation (FMD) with NIRS: This is a measurement of endothelial function. There are no risks associated with this procedure.

Oral glucose tolerance test (OGTT): At screening, subjects will have an oral glucose tolerance test performed. This might cause blood sugar levels to be elevated following the completion of the test.

Hyperinsulinemic-euglycemic clamp: For premenopausal female subjects, a urine pregnancy test is administered before the clamp. The potential risks during the clamp include mild nausea or light-headedness, and mild to moderately high or low blood glucose levels. However, blood glucose will be continually monitored during the infusion of insulin and dextrose will be available if needed to counteract hypoglycemia. Also, drinks and snacks will be available for the subjects at the completion of testing should they have low blood sugar or feel nauseous. Subjects are monitored for a minimum of 1 hour post clamp testing. This procedure is routinely performed at the University Hospital CRC by Dr. Manrique and Dr. Padilla. Dr. Manrique, board-certified endocrinologist, will be present during the procedures.

GlycoCheck: This procedure poses no risks

Near-Infrared Spectroscopy (NIRS): This procedure poses no risks.

Skeletal muscle (calf) perfusion assessment using Perflutren (Definity): A potential side effect from the perflutren ultrasound contrast agents is temporary back pain, joint pain, headache, shortness of breath, or flushing. These symptoms occur in about one in 200 subjects and are mild in intensity in 90% of those who experience this reaction. If this happens infusion of the agent will be stopped. The back and joint pain will go away in a few minutes. A serious allergic reaction to ultrasound contrast agents is unlikely (1 in 10,000), but possible. Symptoms of an allergic reaction include: Rash, itching, swelling, severe dizziness, chest pain and trouble breathing. We are using this contrast agent to measure microvascular perfusion in skeletal muscle using a dose approved by the FDA.

DEXA and anthropometrics: Radiation exposure during DEXA is equivalent to about 2% of the average radiation dose from all sources (natural background radiation, consumer appliances, radon gas, medical tests, etc.) that a person in the United States receives each year. Subjects who participated in any other research study or medical procedure involving significant ionizing radiation exposure (e.g., multiple chest x-rays) in the past 12 months will be excluded. For premenopausal

female subjects, a urine pregnancy test is administered before the DEXA. Body weight is measured to the nearest 0.1 kg and height to the nearest 0.1 cm.

Food Intake and dietary control: We do not anticipate that fructose restriction will pose risk to the subjects.

Xanthine oxidase inhibition with allopurinol: Dr. Celso Velázquez is an expert in the drug treatment of gout and he will use standardized clinical protocols to ramp the dosage of allopurinol [60, 61]. Risks: Side effects of allopurinol hepatitis, rash and pruritus. Our team is aware of the existing recommendations by the American College of Rheumatology in regards to the use of anti-inflammatory agents when starting allopurinol in patients with acute gout [60, 65]. As the present study will not include patients with diagnosis of gout and given that available studies done in subjects with asymptomatic hyperuricemia demonstrate that monotherapy with allopurinol is safe [66, 67], we plan to treat subjects our hyperuricemic subjects only with allopurinol. Dr. Velazquez will closely monitor these subjects and might consider using a low-dose of naproxen in selected cases.

Risks of not treating hyperuricemia in the placebo group: A 2013 Cochrane review emphasized the lack of sufficient evidence to support the use of urate-lowering therapy to prevent future gout in hyperuricemic, asymptomatic patients [63]. *A need for further clinical trials to test other benefits of lowering SUA was called for* [64] which strongly supports the rationale for the current study. Given the limited duration of subject participation (6 month), the lack of known risk to the subject for not being treated is balanced by the significant knowledge gained from this study. At the study's end, subjects in the allopurinol/placebo arm will be offered one, no-cost counseling session with the dietitian nutritionist, who will have analyzed their ad libitum food intake and body composition. The dietitian nutritionist will make recommendations to these subjects for modifying lifestyle factors to reduce SUA and improve overall health.

Study exit survey: surveys will be optional for subjects that complete study treatment. This poses no risks.

B. Protection Against Risks

Risks to loss of confidentiality are reduced by assigning all subjects a data identifier code. Hard copies of data are stored in a locked office, and only the PI and study coordinator have access to the locked files. Individual names or initials are not used in any discussions or publications of the data. We have assembled a research team which includes scientists, physicians, and clinician-scientists with significant experience in human research and metabolic diseases to help anticipate and reduce the risks to subjects. The specific protocol to minimize risk associated with each procedure is described below.

Psychological stress: The psychological stress from participation in this study is minimal. However, some of the questions about food intake and physical activity may make the subjects feel uncomfortable. Subjects will be told they may skip any portion of a questionnaire if they feel uncomfortable about answering the questions.

Fructose restriction: Dr. Manrique will be assisted by a dietitian nutritionist to manage the dietary portion of the treatment. The software, NDSR is used to design diets which are balanced and provide the RDAs for all nutrients and micronutrients. Subjects may find it challenging to comply to the dietary restriction, however, the assembled team has performed human nutritional research studies for the past 20 years and, as described in the grant, the diets will be individualized as much as possible, to meet the subject's personal preferences.

Blood collection and IV lines: Aseptic technique is used to collect blood using butterfly needles and, if necessary, through an IV. Only the amount of blood necessary for analyses is withdrawn and

the staff members performing venipuncture are nurses and trained phlebotomists. Risk of bleeding is reduced by applying pressure at the site of puncture. Bruising is treated with ice. Fainting is prevented by drawing blood in the semi-recumbent position.

Indirect calorimetry: To reduce the feeling of claustrophobia, during the screening visit, the subject is allowed to become familiarized with the hood used to collect expired gas.

Arterial stiffness, carotid intima-medial thickness, flow mediated dilation, and blood pressure: When assessing carotid-femoral PWV, the blood pressure cuff will squeeze the arm and leg tightly; however, any discomfort will be alleviated as soon as the pressure in the cuff is released.

DEXA: Risks include a small radiation for the DEXA, equivalent to about 2% of the average radiation dose from all sources that a person in the U.S. receives each year. Subjects who have participated in other research studies or medical procedures involving significant ionizing radiation exposure in the past 2 months will be excluded. A urine pregnancy test will be performed before the DEXA and insulin clamps for premenopausal women.

There are no alternative methods that will allow us to test our proposed hypotheses.

C. Plan for Reporting Study Deviations:

Any *minor problem/deviation* will be summarized and reported to the IRB within 5 working days of awareness, including any event that does not: 1) increase the risk to the subject, 2) decrease the benefit to the subject, or 3) significantly affect integrity of the research data. Any *major problem/deviation* will be summarized and reported to the IRB within 5 working days of awareness, including any event that: 1) increases the risk to the subject, or 2) significantly affects integrity of the research data.

D. Stopping Rules

We will stop an individual study in the event of an unanticipated serious adverse event. If 4 or more subjects experience adverse events requiring termination of the study, the study will be stopped and the events will be discussed with the IRB to determine whether it is appropriate to continue and/or determine appropriate modifications to the protocol to avoid further adverse events. All adverse events will be submitted for review according to current protocols. The pharmacological treatment will be discontinued in case of severe elevation of liver enzymes, evidence of hypersensitivity to the medication or at the discretion of the safety office Dr. Bostick. Follow up blood work and clinic visits will be scheduled at the discretion of the safety officer in coordination with the investigators.

E. Breach of Confidentiality

Subject confidentiality will be rigorously maintained. The data collected as part of this study will be for research only. It will be de-identified after collection. Confidentiality of data will be assured by coding of unique subject identities and that coding will be known only to the research team, including the use of secure files, locked in an office, and a unique subject coding system. The original study data will be kept in locked in an office (hard copy) or entered into a secure computer database password protected under a secure server space allocated for use by only the study team (electronic). Furthermore, data analysis will be appropriately blinded and any individual data presented in manuscripts will also be presented in an anonymous nature. No identifying information will be disclosed. *Confirming with University of Missouri policy, all research records will be retained for a period of 7-years following completion of the study.*

All protocols and techniques to be used will be approved by the Institutional Review Board (IRB) prior to initiation of any studies. Each subject will give written informed consent after all questions have been answered by a study team member. The consent form will also include a statement guaranteeing confidentiality. Adverse event reports and annual summaries will not include subject-

identifiable material. No information will be given to anyone without permission from the subject. Electronic communication with study team members will involve only coded, unidentifiable information. *Any unanticipated breach of confidentiality will be summarized and reported to the IRB within 5 working days of awareness*

IX. ANTICIPATED BENEFITS

There may be no benefit to the subjects in this study. These data will aid in the understanding of how the characteristics of the T2D and the level of SUA may increase the risk of CVD in women and men. From the screening and baseline tests, all subjects will gain health information about themselves. At the end of the study, results collected will be shared with the subject in face-to-face meetings. At the study's end, subjects in the placebo arm will be offered a no-cost counseling sessions with the dietitian, who will have analyzed their ad libitum food intake, physical activity, and body composition. The dietitian will make recommendations to these subjects for modifying lifestyle factors to reduce SUA and improve overall health.

In summary, the risks of the treatments are minimal, as witnessed by millions of Americans who are treated with similar drugs each year. The health testing offers some risk, which includes the blood draws. Overall, the benefits to the subjects may be substantial, and the new information on characteristics that increase cardiovascular risk in T2D women will benefit the society. There are risks associated with the study, but the experience and medical expertise of the research team should keep these to a minimum, and our track record with these types of studies indicates that this diligence has been effective.

X. COMPENSATION

We have completed multiple clinical studies involving human subjects with adherence rates of greater than 95% and subject retention rates of greater than 85% [72-74]. We utilize a validated retention strategy published by Jeffrey et al [75] that has been successful. With respect to subject honoraria, as shown in **Table 5**, subjects will be paid $70 for a screening test, $80 for a baseline test, $50/month for research participation (taking drug or placebo, or complying with the diet) (6 months = $300), $150 for interim study tests, and $300 for the final. Any additional unscheduled safety visits will be compensated $25. Thus, a total compensation of $900 will be provided as study events are completed not including any additional visits. Subjects that reside more than 25 miles away from the University Hospital CRC will receive an additional compensation of $7 per 25 miles traveled per visit.

Visit	Compensation
Screening test	$ 70
Baseline test	$ 80
$50/month x 6 mo	$ 300
Interim test	$ 150
Final test	$ 300

Table 5. Subject compensation for all arms of the study.

XI. COSTS

You will not be charged for any procedures that are part of this research study. The costs of the study will be covered by a NIDDK R21 grant.

XII. DATA SAFETY AND MONITORING PLAN

Overall framework for safety monitoring and what information will be monitored.

A. Plan for data management:

A password-protected database will be used to manage all study data. To ensure confidentiality only subject ID numbers will be entered into the database. Signed informed consent forms are kept in a locked office. Participants will not be individually identified in any publication. Participants' right to privacy will be protected.

B. Plan for safety monitoring:

Although risks from the *dietary* intervention protocol in our proposal are minimal, the *drug* treatment could pose risk to participants. The data and safety monitoring plan (DSMP) for this trial focuses on close monitoring by the PI and the safety officer.

Frequency of monitoring, including any plans for interim analysis and stopping rules. Monitoring will include enrollment, attrition, efficacy end-points, and adverse events. In addition to monitoring by the PI, study coordinator, and safety officer monitoring, the MU IRB monitors all aspects of the project on an annual basis. The DSMC conducts its reviews on a semiannual basis. The frequency of the structured data review for this study can be summarized in the following table:

Data type	Frequency of review
Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	At the end of each recruitment
Adverse event rates (injuries)	semi-annually
Compliance to treatment	semi-annually
Out of range laboratory data	semi-annually
Stopping rules report regarding statistical power implications of dropouts and missing data	semi-annually
IRB review	annually
DSMC review	semi-annually

Table 6

C. Process for Managing and Reporting Adverse Events, Serious Adverse Events and Unanticipated Problems:

Events that meet the three IRB criteria of being: unexpected, related or possibly related to research participation, and suggesting that research places subjects or others at a greater risk of harm than previously known or recognized will be reported to the IRB. Both serious and non-serious adverse events will be reported to the MU School of Medicine Data and Safety Monitoring Committee (DSMC). For reporting to the DSMC, adverse events will be categorized and classified according to Common Terminology Criteria for Adverse Events Scale (CTCAE v3.0). Safety reports will be sent to the safety officer. The PI will be responsible for assembling the data and producing these reports, as well as assuring that all parties obtain copies of these reports.

D. Qualifications and responsibilities of the Safety Officer:

The safety officer for this trial will be Brian Bostick, MD/PhD. **Dr. Bostick is an Assistant Professor in the Division of Cardiology**. In addition to practicing medicine, he is a clinician scientist. As Safety Officer, Dr. Bostick will review eligibility criteria with Dr. Manrique, and will be unblinded as to treatment assignment. He will review all reports sent by the study coordinator to determine whether

there is any corrective action, trigger of an ad hoc review, or stopping rule violation that should be communicated to the MU IRB and the DSMC. In addition, the safety officer may comment on whether the study PI needs to report any specific out-of-range laboratory data.

E. MU Data and Safety Monitoring Committee (DSMC):

The MU School of Medicine DSMC was formed by the MU Institute for Clinical and Translational Science and activated as an advisory committee within the MU School of Medicine. Its structure and processes are consistent with NIH, NIDDK, and U.S. Food and Drug Administration guidelines. The DSMC helps investigators, and MU research compliance oversight mechanisms protect human-research participants and ensure the integrity and scientific validity of research data. The DSMC provides education for investigators, research teams, and faculty regarding data and safety monitoring; reviews proposed Data and Safety Monitoring Plans; establishes Data and Safety Monitoring Boards; conducts independent, interim reviews of study safety and progress; and makes recommendations concerning the continuation of studies, including recommendations regarding the modification, suspension, or termination of a study. The DSMC has 13 voting members representing: medicine, nursing, health professions, pharmacology, ethics, biostatistics, behavioral science, information technology, social science, and research participant advocacy. There are also seven non-voting members: compliance officers for the health science and campus institutional review boards, compliance officer for the campus conflict of interest committee, managers of the clinical trials offices at University Hospital and Ellis Fischel Cancer Center, director of compliance for the school of medicine, and the executive secretary of the DSMC. Leadership of the DSMC is provided by the chair, Greg Flaker, MD, Brent Parker Professor of Medicine (Cardiology); Vice Chair. David Fleming, MD, Professor of Clinical Medicine and Director, MU Center for Health Ethics; and the Executive Secretary. Don Reynolds, JD, Director, Office for Responsible Research. Administrative Coordination is provided by the School of Medicine Office of Compliance and Quality.

XIII. MULTIPLE SITES

Not applicable

XIV. REFERENCES

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