COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title: SGLT2 Inhibition in Older Obese Adults With Pre-diabetes

NCT number: NCT04401904

IRB Approval Date: 10-30-2020

Unique Protocol ID: HSC20190766H

Effect of SGLT2 inhibition on aging-related biomarkers in older obese adults with pre-diabetes

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Funding Sponsor: *San Antonio Older Americans Independence Center, Pilot and Exploratory Studies Core*

Study Product: *Dapagliflozin*

Protocol Number: *HSC 20190766H*

NCT#: NCT04401904

Initial version: v1.0 09/10/2019 last revised v2.4_10-29-2020

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List of Abbreviations

SGLT: Sodium-glucose co-transporter
T2DM: Type 2 Diabetes Mellitus
AGE: Advanced Glycation End products
RAGE: Receptor for Advanced Glycation End products
GFR: Glomerular Filtration Rate
OGTT: Oral Glucose Tolerance Test
CPET: Cardio Pulmonary Exercise Test

Study Summary

Title	Effect of SGLT2 inhibition on aging-related biomarkers in older obese adults with pre-diabetes
Protocol Number	HSC20190766H
Phase	2b Pilot
Rationale	Inhibitors of the sodium-glucose co-transporter (SGLT2) are FDA-approved for the treatment of type 2 diabetes (T2DM). Their mechanism of action involves lowering of blood glucose concentration secondary to increased glucose excretion of glucose by the kidney. These drugs also improve body weight, blood pressure, and cardiac function. Based on these pleiotropic effects, including its calorie restriction-mimetic properties, we hypothesize that SGLT2 drugs will impact several basic aging-related processes, including reductions in oxidative damage to DNA and proteins, advanced glycation end products (AGE) and receptor for AGE (RAGE), cellular senescence, and mitochondrial function.
Study Duration	Treatment duration 12 weeks; complete study participation up to 6 months
Study Center(s)	UTHSCSA (MARC, FORU, BICRC)
Objective	Primary Endpoint*:* Determine if dapagliflozin significantly reduces aging-associated biomarkers (AGE-RAGE, oxidative stress in DNA and protein, cellular senescence, skeletal muscle mitochondrial function.) Secondary Endpoint: Determine healthspan benefits of dapagliflozin treatment (VO_2Max, insulin sensitivity, 6-minute walk test, grip strength, quadriceps strength)
Number of Subjects	20
Inclusion Criteria	Men and post-menopausal women age 60 and above; BMI 30-38 (BMI $\geq$ 29.5 will be rounded up); diagnosis of pre-diabetes (HbA1c 5.7-6.4%); stable body weight (±3% for ≥3 months); Montreal Cognitive Assessment 21 and above; Subjects must be in good general health as determined by physical exam, medical history, blood chemistries, CBC, TSH, lipid profile, EKG and urinalysis.
Exclusion Criteria	Diagnosis of diabetes based on ADA criteria; impaired renal function with eGFR < 45 mL/min/1.73m^2; impaired liver function with labs 3x ULN and above; hematocrit with LLN 30% and below; triglycerides with ULN 600 mg/dL and above; abnormal TSH of 0.3 and below to 10 and above; urinalysis with >5-10 WBC; history of frequent UTI; poorly controlled blood pressure (sysBP >180, diaBP > 100 mmHg); history of recent cardiovascular event in prior 6 months, heart failure, active heart disease; current treatment with drugs known to affect glucose and lipid homeostasis; current treatment with anticoagulants (aspirin <326 mg and clopidogrel permitted if held 7 days prior to biopsies; active inflammatory, autoimmune, gastrointestinal, hepatic, infectious, malignant or uncontrolled psychiatric disease (controlled depression, anxiety, PTSD, etc. enrollment allowed); ; blood donation within 2 months prior to enrollment.
Concomitant Medications	Allowed: Standard of care medications for the management of hypertension, hyperlipidemia and other drugs that at the discretion of the investigator will not interfere with the results of the study. If patient on drugs that affect metabolism like levothyroxine, should be on a stable dose for the last 3 months. Prohibited: Any medications known to adversely affect glucose and lipid metabolism. Additionally, patients on warfarin or other blood thinners except for aspirin up to 325mg and clopidogrel will be excluded.

Study Product, Dose, Route, Regimen	Dapagliflozin, 10mg, Oral, Daily
Duration of administration	12 weeks
Statistical Methodology	Comparisons of means between all the groups will be done by ANCOVA. Associations, within a group, between aging-related biomarkers vs metabolic outcomes and vs. healthspan outcomes will be determined by Pearson's correlation. For tests of correlation coefficients between groups, we will use the Fisher's Z transformation. We will also determine the relationship between aging-related biomarkers vs metabolic outcomes and vs. healthspan outcomes, by using multiple regression analysis. Scatter plots will be done to look for outliers and to verify linearity.

1. Introduction

This document is a protocol for a human research study. This study will be conducted according to Good Clinical Practice guidelines as adopted by FDA, applicable government regulations, and Institutional research policies and procedures.

1.1. Background

Type 2 diabetes mellitus (T2DM) occurs in 9% of the US population, affects ~30 million individuals and its prevalence has increased dramatically (1) due to multiple factors: (i) increased longevity, (ii) increased incidence of obesity, (iii) decreased physical activity, (iv) rapid growth of minority populations at high diabetes risk. T2DM patients experience significant morbidity and mortality from microvascular and macrovascular complications. The cost of treating diabetes and associated complications in 2007 was $174 billion and is predicted to double by year 2030 (2).

The Diabetes Control and Complications Trial (DCCT) (3) and United Kingdom Prospective Diabetes Study (UKPDS) (4,5) have documented that hyperglycemia is the major risk factor for microvascular and, to lesser extent, macrovascular (6,7) complications. Insulin resistance and impaired insulin secretion are characteristic features of T2DM (8,9,10). The earliest expression of this insulin resistance and impaired insulin secretion is called prediabetes. Prediabetes is characterized either by impaired fasting glucose (IFG) or abnormal plasma glucose after a 75gm oral glucose tolerance test (OGTT) or an A1c value of 5.7% to 6.4% There are approximately 84.1 million adults with prediabetes (33.9% of the population) and 23.1 of these adults are 65 years or older (1). Prediabetes has been shown to increase the risk of developing type 2 diabetes, heart disease and stroke. Other classic cardiovascular risk factors are frequently present in prediabetic individuals including classic hypertension, dyslipidemia, procoagulant state, obesity, endothelial dysfunction, insulin resistance, and inflammation (3,4).Since most of the morbidity and mortality in T2DM arises from long term complications, early detection and effective intervention to delay or prevent development of T2DM would be expected to have enormous beneficial clinical, social and economic impact.

The inhibitors of the sodium-glucose co-transporter-2 (SGLT2i) are FDA-approved for the treatment of type 2 diabetes (T2DM). This class of drugs have a unique mechanism of action that involves lowering of plasma glucose concentration secondary to an increase in glucose excretion by the kidney. This glucosuric effect results in a durable reduction in HbA1c, weight loss, improved insulin sensitivity, and enhanced beta cell function (11). Beyond the glucocentric effects of SGLT2i, they have now been shown in patients with cardiovascular disease (CVD) to reduce mortality and hospitalization for heart failure and provide renal protection. A variety of potential mechanisms have been invoked to explain the beneficial effects of SGLT2i on CV mortality and hospitalization for heart failure and these have been the subject of recent reviews (12,13,14). Of the potential mechanisms responsible for the improved CV benefit, hemodynamic factors, including the simultaneous reduction in preload (12) (secondary to mild intravascular volume depletion) and afterload (secondary to reduced blood pressure (12) and improved aortic distensibility (15) most commonly have been cited. These attributes have elevated SGLT2i's to second- or third-line therapy for the treatment of T2DM patients in patients with or without CVD (16,17,18).

1.2. Innovation

Investigations into the aging process have identified major cellular dysfunctions that contribute to aging, including but not limited to increased burden of damaged DNA and protein, reduction in mitochondrial respiration, and the development of pro-inflammatory senescent cells (19). Developing and testing interventions that interact with multiple points of this spectrum may delay the aging process. Based on prior investigations, we believe the SGLT2 inhibitor class of drugs may target these basic mechanisms involved in the aging process and propose testing in a high-risk human population to evaluate their effectiveness in

ameliorating aging-associated dysfunctions. Specifically, we hypothesize that SGLT2i drugs will lead to reductions in oxidative damage to DNA and proteins, AGE-RAGE, and cellular senescence, which will be accompanied by improvements in mitochondrial function. If our hypothesis is correct, these findings could lead to the development of new approaches to increase both healthspan and lifespan.

1.3. Preliminary data

SGLT2 inhibitors have shown effects on systemic and tissue low-grade inflammation (20) In the CANTATA-SU study, 52 weeks of canagliflozin 300 mg daily to individuals with T2DM decreased IL-6 by 22% and CRP by 4.4% versus glimepiride (21) (Figure 1). CRP and other inflammatory marker data following SGLT2 administration are relatively scarce in humans, but in animals, several reports have shown reductions in cytokine and chemokine concentrations in parallel with protective effects against progression of atherosclerotic lesions (20). Experimental findings in rodents also suggest that part of the reno-protective effects of SGLT2 inhibition may be related to anti-inflammatory and anti-fibrotic actions at the kidney level. Entry of glucose into the tubule cells may induce inflammatory and fibrosis genes, as well as increase apoptosis. Experimental administration of an SGLT2i prevents tubulointerstitial injury in obese T2DM mice (22). Underlying mechanisms to explain this anti-inflammatory effect are varied, but may involve weight loss, reduction in adipose tissue inflammation and attenuation of oxidative stress (20).

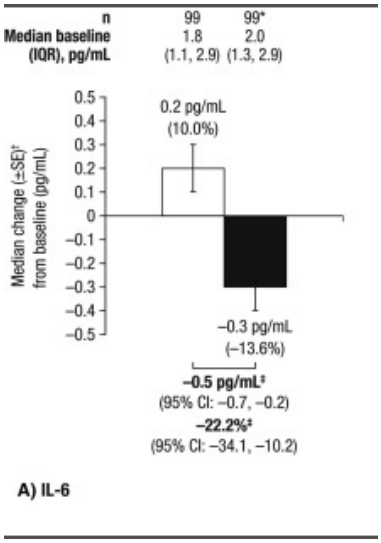


Figure 1. Effects on Inflammatory Biomarkers at Week 52. Canagliflozin 300 mg was associated with a 22%reduction in median serum IL-6 compared to glimepiride. Change from baseline in serum inflammatory biomarkers at Week 52. IL-6, interleukin-6.

It is clear that the elevated glycemia can accelerate the formation of advanced glycation end-products (AGE's) (23). There is also evidence that engagement of the AGEs receptor (RAGE) by AGE's may elicit oxidative stress as well as generation of inflammatory and fibrotic reactions in tissues (23). Importantly, these implicated AGEs inflammatory/fibrotic pathways cause widespread tissue damage including the pancreatic beta-cells, renal cells, and the vascular system. This directly or indirectly increases the risk of progression to T2DM, increases the risk of complications of diabetes such as nephropathy, neuropathy, and retinopathy, and the development of CVD (24). In addition, RAGE may play a critical role in reactive oxygen species (ROS) production. RAGE expression and activation may worsen AGE accumulation through decreased expression of key antiglycation enzymes (23).

The effects of SGLT2i on AGEs have yet to be extensively studied in humans, though we have shown that these drugs additionally increase mitochondrial fatty acid oxidation, cause a small increase in ketone levels, a decrease in uric acid levels and are associated with attenuation of oxidative stress (25). Terami et al., showed in male *db/db* mice given SGLT2i for 12 weeks that SGLT2i reduced renal mesangial matrix expansion and renal fibrosis. Proinflammatory genes, oxidative stress, and apoptosis were all decreased in renal tissue. In addition, SGLT2i restored pancreatic beta-cell mass to non-diabetic (non-diabetic *db/m* mice) levels (26). In streptozotocin-induced diabetic rats (STZ) treated for 4 weeks of SGLT2i versus a placebo buffer, SGLT2i decreased the expression levels of AGEs, and its receptor (RAGE) and other markers of oxidative stress at the kidney level (27) (Figure 2). Additionally, Osorio et al. reported in a similar streptozotocin-induced diabetic rat model that SGLT2i restored several oxidative stress markers to control levels and prevented renal tissue 3-nitrotyrosine accumulation, a tissue marker of oxidative stress (28).

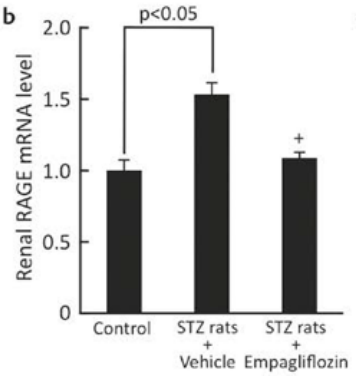


Figure 2. Effects of empagliflozin on AGE-modified protein levels and RAGE immunostaining. Total RNAs extracted from the kidney of control, STZ and STZ rats+empagliflozin with RNAqueous-4PCR kit.

In prediabetes there is limited information regarding the role of SGLT2 inhibitors, but the available data in humans show that prediabetic obese subjects treated with an SGLT2i had significant reductions in weight (29), which is an independent CV risk factor (30) and is associated with insulin resistance,

diabetes, dyslipidemia, hypertension, cancer, stroke and nonalcoholic fatty liver disease (31-34). It is likely that the other known glucose and non-glucose lowering effects of SGLT2i could be extrapolated to prediabetic individuals.

1.4. Dose Rationale and Risk/Benefits

In the DPP trial, weight loss was proportional to diabetes prevention. There are no serious risks to losing weight at a reasonable rate. Dapagliflozin 10mg daily is the maximum daily dose and has been shown to increase glucose urinary excretion more than 5mg daily. Dapagliflozin 10mg/day has been assessed in combination with exenatide treatment in prediabetic patients for one year and shows similar safety and tolerability to previous trials in diabetic patients (35). Dapagliflozin 10mg/day is also in use for the DAPA-HF study in a study population that includes prediabetics (36). Another SGLT2i, empagliflozin, has been assessed in prediabetics at a comparably high dose (25mg/day) for two weeks and did not show AE among treated subjects (37). The SAE's associated with dapagliflozin include acute kidney injury, fall from orthostasis, gangrene of the perineum, and diabetic ketoacidosis. These risks will be mitigated in this study by monitoring subject status with biweekly phone check-ins with patients as well as monthly in-person visits during drug refill visits.

2. Study Objective

The primary objective is to determine whether SGLT2 inhibitors improve biomarkers of aging in older obese adults with pre-diabetes.

The secondary objective is to determine whether changes in aging-related biomarkers resulting from SGLT2i therapy are linked to changes in glucose metabolism and healthspan.

3. Study Design

3.1. Subjects

Recruited subjects for this study will be men and post-menopausal women aged 60 years and above, with a diagnosis of obesity as measured by BMI (30-38 kg/m^2; BMI $\geq$29.5 will be rounded up) and who qualify as pre-diabetic (HbA1c 5.7-6.4%). Screening at visits 1 and 2 will identify participants who meet entry criteria for the study. The target enrollment for this pilot study is 20 completed subjects, split evenly between experimental and control groups. Because this study seeks to identify novel SGLT2i effects on biomarkers of aging, we do not have direct data on the potential impact of dapagliflozin on biomarkers such as cellular senescence, AGE-RAGE, or mitochondrial function to power our study.

3.2. General Design

This is a single center, open-label, randomized controlled trial. 20 completed subjects are planned. Each subject will be randomized to either the experimental group of dapagliflozin 10mg daily for 12 weeks or the control group of nutritional counseling for weight loss. Healthspan and clinical evaluations will be taken at baseline and at weeks 10-12 of the study.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

The following treatment regimens will be used:

- Dapagliflozin: 10mg per day for 12 weeks. Subjects will return to refill drug medication and perform compliance check at 4 weeks and 8 weeks after beginning treatment.
- Control Group: Subjects will meet with a dietitian every 1-2 weeks throughout the study to discuss nutritional counseling with a goal to lose 3-5% of body weight over 12 weeks to mirror expected SGLT2

inhibitor-derived weight loss over the same period. During these hour-long sessions, volunteers will learn how to adjust their eating habits by following the recommendations of the American Heart Association for reducing dietary fat and substituting complex carbohydrates and reducing energy intake to induce gradual weight loss while maintaining recommended dietary composition. Volunteers also will receive education on portion sizes, meal timing, and meal preparation. Participants receive a notebook of course content and will weigh in prior to each session. Additional counseling visits and/or phone calls may be instituted if desirable weight loss is not achieved. Subjects may be asked to record their dietary intake for a 24-hour period throughout the intervention for review by the dietitian.

Total duration of subject treatment will be 12 weeks. Total duration of the study is expected to be 16-18 weeks.

3.3. Study Endpoints

Primary outcome: The primary endpoint for this study is a reduction in urine AGE-RAGE measurements as a result of dapagliflozin treatment. We will be assessing AGE-RAGE in all subjects at baseline and after intervention

Secondary outcomes: Mitochondrial function: This measurement will assess changes in muscle mitochondrial respiration after dapagliflozin treatment. Measurements will occur using muscle samples obtained at baseline and after intervention.

Insulin Sensitivity: This measurement will be obtained before and after the intervention using the HOMA-IR index derived from the fasting insulin and glucose concentrations.

Oxidative Stress: This measurement will be assessed in muscle samples obtained at baseline and after intervention.

Healthspan Measurements: These assessments will include grip strength, isometric knee extension, 6-minute walking distance, and a VO_2Max obtained from CPET. We will be assessing these outcomes in all subjects at baseline and after intervention. Measurements obtained from these exams will evaluate the effect of dapagliflozin treatment on physical indicators of aging.

Cellular Senescence: Senescent cell burden will be assessed in adipose tissue obtained at baseline and after intervention, using both senescence-associated beta-galactosidase staining as well as senescence-associated gene expression assays.

DNA methylation: DNA methylation, a key biological marker of aging, will be evaluated in peripheral blood cells before and after intervention.

3.4 Potential Risks to Subject Safety

a) Dapagliflozin.

Likely and not serious:
These risks are expected to occur in more than 20 out of 100 subjects: None

Less likely and not serious:
These risks are expected to occur in 5-20 subjects or less out of 100 subjects: Genital mycotic infections (about 4%) Nasopharingytis and UTI (about 1%) and in less than 4 out of 100 individuals low blood sugar (also called Hypoglycemia)

Rare and serious: loss of consciousness (or syncope), decrease in blood pressure, and dehydration (less than 1%)

b) Blood Withdrawal. All studies involve the withdrawal of blood. Any subject who has donated blood in the previous two months will not be studied. The subjects will be instructed not to donate blood for two months after the study. Any subject with a hematocrit of less than 34% will not be studied. The maximal amount of blood to be drawn during the entire study for any given subject will be approximately 200 mL. Hematology comprehensive metabolic panel with lipids and liver function tests will be obtained to assess effects on blood cell count, liver function and lipid metabolism. Pain, bleeding, bruising or swelling may occur at the site of the needlestick. Study participants may also experience light headedness or fatigue. Local hematomas occur in 1-5% of blood draws. Infection and nerve damage are possible (<1%). A qualified phlebotomist will perform venipunctures to minimize these risks.

c) IV lines. Intravenous catheters may be used for venipuncture. Local hematomas occur in about 1% of catheterizations. Infection is possible (<1%), but we have not experienced this complication.

d) Muscle biopsy. At the time of biopsy, subjects may feel pain, discomfort, or pressure (variably described by different subjects) for about 5-10 seconds. Pain or discomfort ceases as soon as the cannula is withdrawn. Some localized bruising is expected after biopsy. Local hematomas occur in <2% of subjects. One patient experienced a moderately painful hematoma that resolved within 2 weeks (0.1%). About 1 in 50 subjects report non-clinically evident numbness or altered sensation at the biopsy site, which is transient. There is the possibility that a future biopsy may not be done at the discretion of the PI in case the subject did not tolerate well a prior biopsy.

e) Adipose tissue biopsy. A burning discomfort will be felt for about 5-10 seconds during the application of lidocaine. Subjects are likely to bruise at the biopsy site, which generally resolves within one week of the biopsy. Mild wound infections occur in <2% subjects.

f) Radiation exposure. Subjects will also be exposed to a very small amount of radiation during the DEXA exam (0.04 mrem), which equals one day of natural background radiation.

g) Home visits. When necessary to perform home visits, study staff will use precautions to reduce the risk of improper disclosure or loss of research records and research specimens. The study staff will not remove any original records from the research site and will only use the minimal amount of PHI necessary to accomplish the home visit. All study records being transported will be secured in a locked file case. Research specimens will be labeled and packed in accordance with the site's laboratory regulations and returned to the research site (FORU) in a carrier regulated for biohazardous materials. Study records and specimens will never be left unattended during the home visit or when being transported to and from the study site.

4. Subject Selection and Withdrawal

4.1. Inclusion Criteria

1) Men or post-menopausal women.
2) Age= 60+ years.
3) All ethnic groups.
4) BMI between 30-38 kg/m^2 (BMI ≥29.5 will be rounded up).
5) Diagnosis of pre-diabetes (HbA1c 5.7-6.4%).
6) Stable body weight (±3% for ≥3 months).
7) Willing to adhere to medication regimen for three months.
8) Montreal Cognitive Assessment score ≥21.

4.2. Exclusion Criteria

1) Diagnosis of diabetes based on ADA criteria
2) Impaired renal function with eGFR < 45 mL/min/1.73m^2. Unstable or rapidly progressing renal failure.
3) Impaired liver function with labs ≥3 times upper limits of normal range
4) Abnormal hematocrit with lower limits of ≤30%
5) Abnormal triglycerides with upper limits ≥600 mg/dL
6) Abnormal TSH values ≤0.3 and ≥10
7) Urinalysis results with >5-10 white blood cell count
8) Current medications known to affect glucose and lipid homeostasis (anti-diabetes medications, glucocorticoids, atypical antipsychotics, anti-transplant rejection medications, anti-retrovirals).
9) Current treatment with anticoagulants (warfarin). Aspirin (up to 325 mg) and clopidogrel will be permitted if these can be held for seven days prior to the biopsies.
10) History of recent cardiovascular event in the last 6 months or Heart Failure (New York Heart Classification greater than class III-IV; recent EKG changes that suggest active heart disease
11) Poorly controlled blood pressure (systolic BP>180, diastolic BP>100 mmHg)
12) Active inflammatory, autoimmune, infectious, hepatic, gastrointestinal, malignant, and uncontrolled psychiatric disease (Subjects with depression, anxiety, PTSD, etc. can enroll if controlled and on stable medication)
13) Blood donation within 2 months prior to enrollment
14) History of frequent UTI

4.3. Subject Recruitment and Screening

We will screen up to 100 potentially eligible candidates to achieve the target enrollment of 20 completes. More candidates will be screened if attrition (est. 20%) requires it.

Subjects will be recruited by advertisement, web-based notices including social media, digital outlets, referrals from the Barshop Clinical Research Call Center, flyers distributed and posted in senior centers, private geriatric offices, and through the outpatient clinics of UT Health San Antonio/UT Medicine Physicians, and University Health System (UHS).

Screening will be performed during Visits 1 and 2.

4.4. Early Withdrawal of Subjects

4.4.1. When and How to Withdraw Subjects

Subjects have the right to withdraw fully or partially from the study at any time and for any reason without prejudice to his or her future medical care by the physician or at the institution.

Withdrawal of full consent for a study means that the subject does not wish to receive further investigational treatment and does not wish to or is unable to continue further study participation including any follow-up in person, by phone, through third parties including relatives or friends, via discussion with other treating physicians, and by use of medical records; subject data up to withdrawal of full consent will be included in the

analysis of the study. Any subject may withdraw full consent to participate in the study at any time during the study. The Sponsor Investigator or sub-investigator will discuss with the subject appropriate procedures for withdrawal from the study.

4.4.2. Data Collection and Follow-up for Withdrawn Subjects

If subjects are withdrawn prematurely from the study, appropriately designated research staff will make efforts to collect at least survival data throughout the protocol defined follow-up period for that subject.

Investigator will consult with Study Statistician with regard to any incomplete data set as compared to the full data set that fully supports the analysis. If a subject withdraws consent to participate in the study, attempts will be made to obtain permission to record survival data up to the protocol-described end of subject follow-up period.

Investigator and designated research staff make it a high priority to obtain survival data on all subjects lost to follow up. Lost to follow up will be defined as a subject missing 2 or more consecutive visits, not answering or responding to 3 follow up phone calls to subject or emergency contacts, or returned receipt of 1 certified letter.

5. Study Drug

5.1. Description

The inhibitors of the sodium-glucose co-transporter-2 (SGLT2i) are FDA-approved for the treatment of type 2 diabetes (T2DM). This class of drugs have a unique mechanism of action that involves lowering of plasma glucose concentration secondary to an increase in glucose excretion by the kidney. This glucosuric effect results in a durable reduction in HbA1c, weight loss, improved insulin sensitivity, and enhanced beta cell function (11). Beyond the glucocentric effects of SGLT2i, they have now been shown in patients with cardiovascular disease (CVD) to reduce mortality and hospitalization for heart failure and provide renal protection. These attributes have elevated SGLT2i's to second- or third-line therapy for the treatment of T2DM patients in patients with or without CVD (16-18). Approximately 1/3 of older adults are pre-diabetic. In prediabetes there is limited information regarding the role of SGLT2 inhibitors, but the available data in humans show that prediabetic obese subjects treated with an SGLT2i had significant reductions in weight (19), which is an independent CV risk factor (20) and is associated with insulin resistance, diabetes, dyslipidemia, hypertension, cancer, stroke and nonalcoholic fatty liver disease (21-24). It is likely that the other known glucose and non-glucose lowering effects of SGLT2i could be extrapolated to prediabetic individuals. Dapagliflozin is an SGTL2 inhibitor given for the management and treatment of type 2 diabetes and will be the study drug used for these experiments.

Dapagliflozin (Brand name Farxiga) is administered orally as a yellow pill in either 5mg or 10mg doses. The 10mg dose that will be used in this study is a 4-sided yellow pill with 10 and 1428 printed on opposite sides.

5.2. Treatment Regimen

Administration Protocol.

Following the completion of informed consent, screening visits, enrollment into the study, and baseline measurements, subjects will be provided study drug by the Study Coordinator at the conclusion of Visit 5. Subjects will take 1 pill daily (10mg) as directed during the study period. Subjects will self-administer drug according to product packaging, and compliance calls every two weeks from study investigators will ensure proper scheduling of drug administration as well as to monitor AE development. Subjects randomized to treatment group will receive a 30-day supply of drug during their Visit 5 randomization and will be required to return 4 weeks and 8 weeks later for refilling study drug. **Method for Assigning Subjects to Treatment Groups**

The randomization key will be designed by an objective researcher or statistician not on the study team at the Barshop Institute or from Clinical Research Unit and provided to the Clinical Study Coordinator at the site. The Study Coordinator, as assigned, stores the Enrollment key, which is kept in a locked cabinet. Study staff maintains source documents, data records and specimens in a de-identified manner using only the unique Subject ID/Randomization number.

5.3. Administration of Study Drug and Compliance to Regimen

Study Coordinator orders and obtains medication dispensed from the Research Drug Manager (or research pharmacy unit if available). Subjects will self-administer medication and will be instructed to bring back empty or partially empty bottles to each in-person study visit for medication reconciliation and compliance procedures. Subjects will be interviewed by phone every two weeks to ensure compliance with study regimen as well as to note potential AEs.

5.4. Prior and Concomitant Therapy

Exclusionary medications:

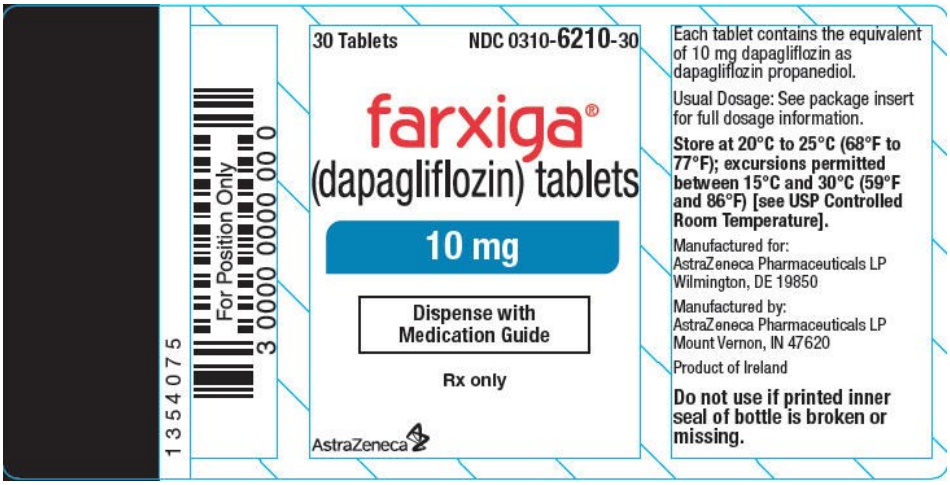
Drugs that affect glucose and lipid homeostasis. Anti-diabetes drugs (including but not limited to

metformin, sulfonylureas, other SGLT2i, DPP inhibitors, etc). Glucocorticoids (prednisone, methylprednisone, hydrocortisone). Atypical antipsychotics (olanzapine, clozapine, etc). Anti-rejection medications (prograf, cellcept). Anti-retrovirals (protease inhibitors).

Treatment with anticoagulants (warfarin). Aspirin (up to 325 mg) and clopidogrel will be permitted if these can be held for seven days prior to the biopsies.

5.5. Packaging

Nature and Contents of the Container – bottle of 30 tablets



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5.6. Handling of Study Drug

Research Drug Manager will be responsible for receipt, storage, dispensing, handling and destruction of Study Drug.

5.6.1. Receipt of Drug Supplies

Any damaged or unusable study drug in a given shipment will be documented by the Research Drug Manager. The Research Drug Manager will notify the Material Sponsor of any damaged study drug.

5.6.2. Storage

The drug product used for clinical studies should be stored at USP recommended room temperature. Excursions from the recommended room temperature will be documented by the Research Drug Manager and acted upon after consultation with Material Sponsor (or AstraZeneca, manufacturer). Expirations on the study product will be honored.

For more details, please see also Farxiga Package Insert or Farxiga Health Care Provider website: https://www.farxiga-hcp.com/

5.6.3. Dispensing of Study Drug

Designated staff from the Research Drug Management unit will maintain the Drug Administration Logs to track how, when and to whom the investigational drug was dispensed and assigned to subjects. Study clinical staff will keep administration records regarding dosing, unused drug, drug damaged, or wasted.

Study drug may be dispensed to participants in person, at home visits, curbside delivery, or by U.S. mail.

Labeling study drug will include FDA required language including "Caution: New Drug--Limited by Federal (or United States) law to investigational use."

Routine Study Drug reconciliation will be performed based on clinical site policy and standard operating procedures.

5.6.4. Return or Destruction of Study Drug

The procedures for final reconciliation of the site's drug supply at the end of the study will be in accordance with local site Drug Management standard operating procedures.

There are no special precautions cited for disposal of dapagliflozin.

6. Study Procedures

6.1. Study visits.

Visits will be performed at the UT Health San Antonio Medical Arts and Research Center (MARC) or at the Barshop Institute Clinical Research Center (BICRC), except for magnetic resonance studies that will be done at the Research Imaging Institute. Participants will arrive fasting (10-12 h) for visits involving lab work, imaging, or biopsies. If the study investigator determines that it is in the greater interest of the safety of the study staff and study participants, the study staff may perform home visits or video telehealth visits. . Participants will be given a Fitbit Bluetooth scale to obtain their weight measurement at home. The data will be transmitted to the research staff electronically through the Fitbit app. The study staff will receive the information in an individual, non-identifiable email account that the study staff will create for each study participant. If the participant does not have access to a mobile device or cannot access the Fitbit app, the study staff will collect the data during home visits or by telephone. The scales will be provided to study participants at no charge. **Visits that require DXA scans and MRI scans may be performed interchangeably due to availability of the DXA and MRI scanners.**

6.1.1. Visit 1 (Day -17 to -21)

Eligible subjects present to the research unit at appointed time for informed consent. Consenting subjects will arrive fasting to undergo vital sign evaluation, medical history, physical exam, safety labs [comprehensive metabolic panel (CMP), complete blood count with differential (CBC), coagulation labs (PT/PTT/INR), HbA1c, TSH, routine urinalysis, lipid panel, insulin and FFA, and the Montreal Cognitive Assessment (participants must score ≥21 to be eligible). Visit results will help determine eligibility for study inclusion (i.e. HbA1c 5.7-6.4%).

Investigators will routinely assess laboratory value excursions for clinical significance.

Adverse events are assessed at every visit once subjects are consented and enrolled to undergo research procedures and study intervention.

6.1.2. Visit 2 (Day --7 to -17 [+/- 2 weeks])[+]

Eligible subjects will present to the MARC research unit for vital signs, walk distance, grip strength, isometric knee extension strength testing, a body composition DXA scan and liver fibroscan.

6.1.3. Visit 3 (Day -7 to -17 [+/- 2 weeks])[+]

Subjects will come to the Research Imaging Institute for intrahepatic liver fat measurement by Magnetic Resonance Spectroscopy/Imaging.

[+] Visits 2 and 3 may be performed interchangeably based on DXA and MRI scanner availability.

6.1.4. Visit 4 (Day -1 to -6) -- "Optional"

Participants who opt in will come to the research unit for vital signs and VO_2max testing.

Cardiorespiratory fitness is determined by a CPET on an electronically braked cycle ergometer per the American College of Sports Medicine (ACSM) Testing Guidelines. Oxygen consumption and carbon dioxide production are measured by indirect calorimetry. ECG (12-lead) recordings and blood pressure are monitored at rest prior to testing and during the testing. A clinician interprets for contraindications to exercise prior to testing. AHA criteria are used to halt exercise testing [43].

6.1.5. Visit 5 (Day 0)

Eligible subjects present to the research unit fasting at appointed time for a vastus lateralis muscle biopsy (39) and subcutaneous fat biopsy (40). We apply local lidocaine for muscle and adipose biopsies. The muscle biopsy is done using a modified Bergstrom technique with suction and the adipose biopsy is done using a mini-liposuction technique. Urine and blood samples also will be collected. These samples will be used for baseline assays of tissue cellular senescence, DNA methylation, oxidative stress and mitochondrial function. After the muscle and adipose tissue biopsies, subjects will be given breakfast and will receive assignment to treatment intervention (randomized) before being discharged to home. Both treatment and control groups will be given a brochure highlighting healthier eating habits. Subjects in the treatment group will be given a 30-day supply of dapagliflozin to begin use the next day. Subjects in the control group will receive a nutritional consultation with a dietician, which may also be conducted by video telehealth visit, with the goal of losing 3-5% body weight during the 3-month period of the study.

- Once intervention phase begins, the study staff will contact subjects assigned to study drug for telephone follow up at Visit 5 +1 week and at or near midpoint between visits to the research unit up to Visit 8.
- Subjects assigned to nutritional counseling group will receive weekly phone calls/video telehealth visits during the intervention phase which will last for 12 weeks.
- Study team members may contact the subjects by phone at any time for AE follow up as needed.

6.1.6. Visit 6 (Week 4)

Subjects will come to the research unit for evaluation of the biopsy sites and undergo blood draw for CMP to monitor kidney function. Subjects will also have current weight checked at this visit. If necessary, the study coordinator may conduct Visit 6 by home visit and perform the biopsy site check by video telehealth visit. Control group subjects will receive nutritional counseling, which may be performed by home visit or a video telehealth visit.

Drug Refill: Subjects in the treatment group will be given a 30-day supply of dapagliflozin by the Research Drug Manager to begin when their current supply runs out. Study coordinators will ensure treatment compliance by asking subjects to bring drug containers and any remaining dapagliflozin tablets to their visits.

6.1.7. Visit 7 (Week 8)

Subjects in the treatment group will return for T+8 drug refill (in person, by mail, or curbside delivery), and control group subjects will be weighed and receive nutritional counseling, which may be performed by home visit or a video telehealth visit. Control group subjects will receive nutritional counseling, which may be performed by home visit or a video telehealth visit.

6.1.8. Visit 8 (Week 10-11 [+/- 2 weeks])$^{+}$

Subjects will undergo post-treatment measurement of cognition (Montreal Cognitive Assessment), walk

distance, grip strength, isometric knee extension strength, DXA scan and liver fibroscan. Control group subjects will receive nutritional counseling, which may be performed by home visit or a video telehealth visit.

6.1.9. Visit 9 (Week 10-11 [+/- 2 weeks])+

Subjects will return to the Research Imaging Institute to undergo post-treatment Magnetic Resonance Spectroscopy/Imaging for intrahepatic liver fat measurement. Control group subjects will receive nutritional counseling, which may be performed by home visit or a video telehealth visit.

+ Visits 8 and 9 may be performed interchangeably based on DXA and MRI scanner availability.

6.1.10. Visit 10 (Week 11-12) -- "Optional"

Participants will come to the research unit for vital signs and VO_2max testing (same as Visit 4) Control group subjects will receive nutritional counseling, which may be performed by home visit or a video telehealth visit.

6.1.11. Visit 11 (Week 11-12)

Subjects will arrive fasting to undergo vastus lateralis muscle biopsy, subcutaneous fat biopsy, and collection of blood and urine samples for research purposes. These samples will be used to evaluate treatment effects on tissue cellular senescence, oxidative stress, DNA methylation and mitochondrial function. Post-intervention blood laboratory assays also will be done for CMP, CBC, HbA1c, and lipid panel. Control group subjects will receive nutritional counseling, which may be performed by home visit or a video telehealth visit.

6.1.12. Visit 12 (Week 13)

Subjects will come to the research unit for evaluation of the biopsy sites. This visit may be conducted by home visit, telephone visit or a video telehealth visit. Control group subjects will receive nutritional counseling, which may be performed by home visit or a video telehealth visit.

6.2. Visit Schedule Summary

	VISIT 1	**VISIT 2****	**VISIT 3****	**VISIT 4**	**VISIT 5**	**VISIT 6**	**VISIT 7**	**VISIT 8*****	**VISIT 9*****	**VISIT 10**	**VISIT 11**	**VISIT 12**
Schedule	-17 days to -21	-7 days to -17, +/- 2wks	-7 days to -17, +/- 2wks	-1 days to -6	Day 0	Week 4	Week 8	Week 10-11, +/- 2wks	Week 10-11, +/- 2wks	Week 11-12	Week 11-12	Week 13
Consent	X											
Vital Signs	X			X						X		
Medical History	X											
Physical Exam	X											
MoCA	X											
Safety Labs (fasting):												
CBC	X										X	
CMP	X					X					X	
Lipid Panel	X										X	
PT/PTT/INR	X											
HbA1c	X										X	
Insulin	X										X	
FFA	X										X	
TSH	X											

Routine UA	X											
DXA		X						X				
Liver Fibroscan		X						X				
MRS/MRI - RII			X						X			
Physical Performance:												
Walk Distance		X						X				
Grip Strength		X						X				
Isom. Knee Ext		X						X				
VO2max CPET*				X						X		
Muscle Biospy					X						X	
SQ Fat Biopsy					X						X	
Research Labs:												
Blood					X						X	
Urine					X						X	
Nutrition consult and weight (controls)					X	X	X					
Nutrition Phone Follow Up					weekly	weekly	weekly					
Treatment dispensed					X	X	X					
Treatment Phone Follow Up					V5+1w	V6+2w	V7+2w					
Biopsy check						X						X
Assess AE		X	X	X	X	X	X	X	X	X	X	X

*CPET is optional (Visits 4 & 10)
**Visits 2 and 3 may be performed interchangeably
***Visits 8 and 9 may be performed interchangeably

6.3. Analyses.

Muscle (frozen): Protein oxidative damage will be evaluated from muscle biopsy tissue using immunoblotting against carbonylated protein side chains. DNA from muscle samples will be extracted and tested for 8-hydroxydeoxyguanosine (8-OHdG) content to evaluate oxidative DNA damage and total DNA methylation will be evaluated.

Muscle (fresh): Tissue obtained will be evaluated by an Oroboros Oxygraph-2k high-resolution respirometer to measure mitochondrial respiration capacity. Skeletal muscle fibers will be mechanically separated and permeabilized in BIOPS as described previously to allow uptake of mitochondrial electron transport chain substrates (25). Tissue will then be tested in MiRO6 buffer using a substrate-uncoupler-inhibitor titration (SUIT) protocol to evaluate oxidative phosphorylation capacity.

Fat (frozen): will be subjected to reverse-transcriptase qPCR for measurement of gene expression levels of senescence and SASP markers, including IL-6, TNFα, p16, p21, and MCP1.

Fat (fresh): tissue will be subjected to beta galactosidase staining to test cellular senescence in the tissue as described previously (42).

Blood/plasma: Circulating factors that include inflammatory markers IL-6 and IL-6R, AGE-RAGE levels, and SASP biomarkers such as plasminogen activator inhibitor (PAI) -1 and 2, intracellular adhesion molecule (ICAM)-1 and 2 will be tested using ELISA kits on blood samples obtained during study. 20mL of blood will be drawn (two (2) 10mL EDTA lavender top tubes), centrifuged to obtain 10mL of plasma, and stored in 0.5-1 ml aliquots to evaluate whether the treatment reduces pro-inflammatory blood markers of aging. Blood samples obtained during the glucose tolerance tests will be analyzed for insulin and free fatty acid concentrations. DNA methylation assays will be done in peripheral blood cells.

Urine: A fasted urine sample will be obtained at baseline and post treatment for urinalysis, microalbumin, excreted IL-6, and AGE-RAGE levels to evaluate changes with treatment. 2-5 ml aliquots will be stored frozen for analysis.

7. Statistical Plan

7.1. Sample Size Determination

The effect of SGLT2 inhibitors on aging-related biomarkers in pre-diabetic subjects is not known. The data generated through this study will guide sample size and power calculations for future studies.

7.2. Statistical Methods

Analytical Approach.

Experimental results will be expressed as means ± SE. Comparisons of means between all the groups will be done by ANCOVA. Associations, within a group, between aging-related biomarkers vs metabolic outcomes and vs. healthspan outcomes will be determined by Pearson's correlation. For tests of correlation coefficients between groups, we will use the Fisher's Z transformation. We will also determine the relationship between aging-related biomarkers vs metabolic outcomes and vs. healthspan outcomes, by using multiple regression analysis. Scatter plots will be done to look for outliers and to verify linearity.

We will use natural logarithmic transformation to evaluate median percent change. This pilot study is not powered to achieve statistical significance with a $p < 0.05$ and is of exploratory nature. The data included in this study will be only of completers. A larger trial in the future based on this pilot data would follow an intention-to-treat analysis.

The Biostatistics Core of the San Antonio Pepper Center, led by Dr. Gelfond, will guide the statistical analyses.

8. Safety and Adverse Events

8.1. Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRSO)

Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- <u>Related or possibly related to participation in the research</u> (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- <u>Suggests that the research places subjects or others at greater risk of harm</u> (including physical, psychological, economic, or social harm).

Adverse Event (AE)

In general, AE is used very broadly and encompasses physical and psychological harms and includes:

> Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not it is considered related to the subject's participation in the research.

Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests

- is considered by the investigator to be of clinical significance

Serious Adverse Event (SAE)
Adverse events are classified as serious or non-serious. A serious adverse event is any AE that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Pre-existing Condition
A preexisting condition is one that is present at the start of the study. A pre-existing condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition.
- Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless

judged by the clinical investigator as worsening or increase in frequency of hospital admissions.

8.2. Recording of Adverse Events

At each contact with the subject, the investigator or study staff will seek information about adverse events by specific questioning and, if appropriate, by examination. Information on all AEs will be recorded immediately in the source document, and also in the appropriate AE section of the case report form (CRF). AEs will be tracked using the HSC IRB AE tracking form or data management tool (See Section 9.3) to be reviewed by Site Investigator on a monthly and ad hoc basis, depending on severity and expected/unexpected nature of the event. All subjects will have study compliance calls every two weeks during which AEs can also be reported.

All AEs occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported per Institutional policy and according to FDA requirements.

8.3. Reporting of Serious Adverse Events and Unanticipated Problems

Each participant will be evaluated for any adverse events (AE). Any incidents, experiences, and outcomes either reported by the subject to the PI or designated research staff or medical staff caring for the subject, and which meet AE criteria will be documented. Any AE reported as serious (SAE) requires submitting a Prompt Report Form to the IRB and, if deemed appropriate by PI, SAE and or unanticipated risks to subjects or others (UPIRSO) may be reportable to the Pepper Center DSMB. A copy of the SAE or UPIRSO prompt report is submitted to the Pepper DSMB for review as well as the NIA Program Officer within 24 hours of notification to PI. All AE that are not serious or UPIRSO will be summarized annually and submitted to the IRB and R&D Committees.

SAE and or UPIRSO will be reported per IRB policy and procedure. Events that do not involve AE or SAE (non-AE UPIRSO), and which are a result of study participation are also promptly reported to the IRB per local policy. The report will include a description of the event, when and how it was reported, as well as any official chart records or documentation to corroborate the event or the reporting of the event. All AE will be graded as mild, moderate, or severe. Any action resulting in a temporary or permanent suspension of this study (e.g. local site IRB actions) will be reported to FDA per IRB stipulations. Additionally, the PI will report SAE to the drug sponsor Astra-Zeneca.

Serious adverse events (SAE) still ongoing at the end of the study period will be followed up to determine the final outcome and or referred to participants' primary care provider. Any SAE that occurs after the study period that is considered to be possibly related to the study treatment or study participation will be recorded and reported per Institutional policy and according to FDA requirements.

Investigator responsibilities - The PI is responsible for:

- Reviewing all incidents, experiences, and outcomes that may represent UPIRSO.
- Determining whether event represents a possible UPIRSO
- Promptly reporting to IRB per local policy
- Contacting institutions involved
- Implementing actions necessary to eliminate immediate hazard
- Submitting follow up reports to IRB
- Submitting amendments to IRB, if applicable or stipulated

Report SAE and UPIRSO immediately by phone and or secure email to:

Carolina Solis-Herrera, MD
Professor, Medicine-Diabetes
210-619-3264
SolisHerrera@uthscsa.edu

Within the following 48 hours, the PI provides further information on the SAE or UPIRSO in the form of a written narrative. This should include a copy of the completed Prompt Report Form, and any other diagnostic information that will assist the IRB to understand of the event. A copy of the SAE or UPIRSO prompt report is submitted to the Pepper DSMB for review as well as the NIA Program Officer.

For further special reporting requirements, please refer to the NIA DSMP document approved by the NIA Program Officer.

Additional reporting requirements

The site PI reports to the regulatory sponsor of the study. The study sponsor (or sponsor-investigator if investigator initiated) is responsible for reporting to FDA when applicable, according to 21 CFR 312 regulations. Contact FDA for guidance.

Reporting Process
Adverse events may be submitted on FDA Form 3500A or in a narrative format. The contact information for submitting safety reports is noted below:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Metabolism and Endocrinology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266
Phone: (301) 796-2290
Fax: (301) 796-9712

8.4. Medical Monitoring

The Sponsor (or investigator initiating the study) will review the safety and progress of this study on a monthly basis or when needed if protocol deviations/violations, SAE or SAE-UPIRSO occurs. The Designated Safety Officer is Devjit Tripathy, MD, PhD a Professor in the Department of *Medicine*, UTHSCSA, and Staff Physician at the Audie L Murphy VA Hospital.

The PI and or Co-PI will review source documentation in the research record and or medical record when study coordinator provides an electronic alert or secure email to review.

8.4.1. Investigator reporting of Protocol Deviations/Violations

Departures during the conduct of a research study constitute a protocol deviation, violation or exception and as such must be reported to the UTHSCSA IRB.

Tracking and reporting of protocol deviations and violations to the IRB is the responsibility of the PI. To determine whether deviations or violations require prompt reporting or other action, refer to the IRB document entitled "Decision Tree – Evaluating Departures" on the IRB website. Failure to report

departures from the protocol according to IRB policy may constitute possible non-compliance, which will require a Prompt Report Form and possible FDA reporting by IRB.

Deviations and violations may be identified in a number of ways including:

- A report by an individual can be made directly to the IRB Office.
- The IRB may learn of event through its continuing review of ongoing research.
- Compliance reviews (audits) conducted by the Office of Regulatory Affairs and Compliance or one of the HSC affiliated institutional compliance offices.
- A report by an individual can be made directly to the Office of Regulatory Affairs and Compliance (Hotline) or one of the HSC affiliated institutional compliance offices.
- A report by another committee, department, institution, or official.
- An audit or report from the study sponsor or sponsor's monitoring entity.

8.4.2. Definitions of Protocol Deviations/Violations

- Protocol deviations
- Protocol violations
- Emergency violations
- For more information , refer to UTHSCSA IRB Policy website: https://research.uthscsa.edu/irb/policy/deviations

8.5. Safety Procedures

See Section 5.8 – If, in the case of SAE or SAE-UPIRSO, the Clinical Study Coordinator or appropriately designated study staff will contact the Designated Safety Officer (Section 8.6) to assess the need for stopping research activities until further information can be gathered.

8.5.1. Designated Safety Officer

- Assess SAE or SAE-UPIRSO to determine if prompt reporting is required and, if yes, notify pharmacist to halt dispensing until further information is obtained.
- Compare event data to intervention/assignment to determine if SAE is related to study intervention
- Assess study and subject safety to determine if stopping rules should be invoked
- Create the Safety Officer record without personal identifiers and communicate to Sponsor Investigator and Clinical Study Coordinator with overall impression and recommended plan of action

8.5.2. Research Drug Manager

- Suspend dispensing of study drug until SAE reporting is complete

8.5.3. Sponsor Investigator

- Receive and review Safety Officer report with Clinical Study Coordinator, Pharmacist and Study Statistician
- Assess and implement stopping rules, if required
- Ensure that IRB, FDA, and funding sponsor are notified as appropriate to local or agency policy
- If applicable, notify Pepper Center DSMB

8.5.4. Clinical Study Coordinator

- Obtain a copy of Safety Officer or DSMB report/record to file in study binder/file and forward a copy to the Regulatory Coordinator
- Ensure appropriate medical care and follow up is scheduled and implemented until SAE resolved

- Create FDA report, if applicable

8.6. Stopping Rules

In the unlikely event that a study-related death or SAE occurs, the decision to stop the trial, either temporarily or permanently, will be the responsibility of the Designated Safety Officer in collaboration with the Principal Investigator.
There are several reasons why the researchers may need to end the subject's participation in the study. Some reasons are:

1. The researcher believes that it is not in the subject's best interest to stay in the study
    a. Adverse drug reaction that does not resolve by dosing titration or if reaction is severe
    b. Subject becomes ineligible to participate due to concomitant use of an exclusionary medication
2. Subject's health condition changes and needs treatment that is not allowed while participating in the study
3. Subject does not follow instructions from the research team.
4. The study is stopped

The research team will discuss options for medical care with the subject when participation in the study ends.

9. Data Handling and Record Keeping

9.1. Confidentiality

Information learned about all subjects will be kept confidential. All data and protected health information in paper form will be kept confidential by assigned uniquely coded identifier and kept secured (password protected and/or double locked). Subjects will not be identified in any way in any publication.

9.2. Registration with Clinicaltrials.gov

As per UT Health San Antonio (UTHSA) Office of Clinical Research policy 1.1.3, this study and other investigator initiated studies that meet criteria of "applicable clinical trial" must be registered by the "Responsible Party" in the Protocol Registration and Results System/PRS (https://register.clinicaltrials.gov). Results must be conveyed within one year of study completion as defined by PRS.

9.3. Source Documents

Source data will be originated both electronically and on paper. Electronic data may be originated in either the medical record or in REDCap (questionnaires answered verbally). The study team will maintain a list of forms to identify where source data are generated for this protocol.

Print all entries legibly in black ink. Erasures and white-out material are prohibited. If any entry error has been made on paper, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.3.1. Research Electronic Data Capture (REDCap) and origination of electronic source data

Contemporaneous medical histories, physical exams, concomitant medications, checklists of consent processing, and documentation of eligibility criteria may be originated electronically in REDCap with date and time stamp and e-signed by the study team member obtaining the data. Then REDCap forms will be downloaded in PDF format containing saved source data and printed to file in the paper participant record at the research site. Other electronically originated data in REDCap include: adverse event (AE) assessments and AE logs, enrollment logs, protocol deviation logs, and other study management checklists. Other

electronic medical record data including pre-existing history, exams, medication lists, and such may be accepted as source data.

All missing data will be routinely queried, corrected, and or explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”.

9.3.2. Paper source data

Paper source data will be collected from handwritten subject diaries, drug management logs, then entered into the REDCap database. All missing data will be routinely queried, corrected, and or explained. If a space is left blank on paper because the procedure was not done or the question was not asked, write “N/D”, initialed and dated by the staff member. If the item is not applicable to the individual case, write “N/A”.

- Lab reports originating from medical records will be printed and filed in paper participant files to facilitate investigator review. Lab data will be entered to REDCap to facilitate analysis.
- Questionnaires and assessments (e.g., cognitive assessments verbally administered according to purchased test booklets and copyrighted material) may be originated electronically in REDCap. Otherwise, it may be necessary to originate survey data on a paper source and transfer the data elements to REDCap for calculation, data management and analysis. Paper sources will be filed in paper subject records.
- Supervising physician investigators will sign and date paper records upon review.

9.3.3. Handwritten entries

All handwritten entries will be created contemporaneously to the visit or phone call, and legibly in blue or black ink. Erasures and white-out material are prohibited. If any entry error has been made on paper, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.3.4. Remote and/or virtual research interactions

This study involves remote and/or virtual research interactions with participants by the research staff. Research activities will be audio and/or video recorded by the conferencing platform, Zoom. Therefore, privacy and confidentiality are not guaranteed due to the nature of the electronic conferencing platform that will be used.

9.4. Data Management

Database Management Software: All data collection for this project will be maintained using the UT Health San Antonio REDCap platform which is managed by the Department of Epidemiology and Biostatistics.

Data System: REDCap is a computing environment developed by Vanderbilt University consisting of a collection of instruments, under the management of UT Health San Antonio’s Information Management systems, policies, and procedures that govern its informatics operations. Data projects are designed to be end-user oriented and constructed to optimize workflow and minimize errors.

All data will be input using a web front-end interface. All users are individually assigned authorization for access to specific components of the database application based on approved study roles and responsibilities. Information that is input is checked for logical and range consistency and mandatory data fields must be entered in order to input a record.

9.5. Records Retention

The regulatory binder is maintained by a designated regulatory coordinator.

The Principal Investigator is responsible for maintaining study essential documents for at least 3 years after the funding grant period ends or at least 2 years have elapsed since the formal discontinuation of clinical

development of the investigational product, whichever is longer. Records retention policy is 6 years after the study is inactivated.

These documents should be retained for a longer period if required by a funding agency, the FDA or other institutional retention policy. In such an instance, it is the responsibility of the sponsor or Principal Investigator to inform the institution as to when these documents no longer need to be retained.

10. Study Monitoring, Auditing, and Inspecting

10.1. Study Monitoring Plan

The Principal Investigator (PI) will be responsible for ensuring the timely monitoring of the data and safety of study participants. The PI will communicate with other members of the study staff to review adverse events and protocol compliance on a weekly basis within 7-10 calendar days of the most recent study visit or phone encounter.

This study will be reviewed by the Pepper Center Data and Safety Monitoring Board (Pepper DSMB) at least annually. The Pepper Center DSMB meets 2-3 times a year, by teleconference call, to review study progress and participants' safety of designated studies. The PI will be required to submit DSMB reports of study progress, adverse events summaries and protocol deviation logs to the Pepper DSMB when requested by the Pepper DSMB Chair.

The Diabetes Division assigns a staff member to conduct quarterly assessments for data quality control/assurance on collected data, which is also reviewed on an annual basis by the designated regulatory coordinator and the PI at the time of preparing continuing review documentation for IRB submission.

The Principal Investigator will ensure that the designated regulatory coordinator or other quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. Research Drug Manager, diagnostic laboratory, etc.), and has adequate space to conduct study monitoring visits as assigned.

10.2. Auditing and Inspecting

The Principal Investigator will permit study-related monitoring, audits, and inspections by the IRB, the funding sponsor, the OAIC Pepper Center Data Safety and Monitoring Board (Pepper DSMB), government regulatory bodies, and University compliance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. drug management unit, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11. Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312) applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to the Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study.

12. Study Finances

12.1. Funding Source

This study is financed through a grant from the San Antonio Older Americans Independence Center Pilot and Exploratory Studies Core.

12.2. Conflict of Interest

UT Investigators are required to submit Conflict of Interest disclosures, if any, with every new study submitted for review by UTHSA IRB and VA Research and Development Committee.

12.3. Subject Stipends or Payments

This study will reimburse subjects for time and transportation. A schedule of payments is shown below. The total potential reimbursement to a subject is $470 for the study for all visits, or payments may be prorated to include the last visit completed if study participation is terminated early. Manual payments for additional visits, if necessary, will be handled on an ad-hoc basis with prior approval from the funding sponsor.

Visit 1	$20
Visit 2	$20
Visit 3	$20
Visit 4A	$20
Visit 4B	$20
Visit 5	$125
Visit 6	$10
Visit 7	$20
Visit 8	$20
Visit 9	$20
Visit 10A	$20
Visit 10B	$20
Visit 11	$125
Visit 12	$10
Manual Payment (unscheduled visit, lab visit, or AE)	$50

13. Publication Plan

The Institution or respective designees may present or publish the results of a scientific investigation involving this Study in accordance with International Committee of Medical Journal Editors (ICMJE). The Sponsor Investigator, funding agency, or PI initiating the study will collaborate to determine who has publication rights for authorship upon review and approval of proposed manuscripts.

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15. Attachments

A. Dapagliflozin Full Prescribing Information Handout

SUMMARY OF PROTOCOL CHANGES

Date	Version	Section	Before	After
09/10/19	1.0	All		IRB submission version
11/07/19	1.1	Section 5.6 Section 8.5 Section 8.5.2	Unblinding of Study Drug Unblinding Procedures Unblinding verbiage	Renamed “Handling of Study Drug” Renamed “Safety Procedures” Removed unblinding verbiage
11/08/19 - 11/15/19	1.2	Section 5 and throughout Throughout document Section 6, Study Procedures and Visits	Research Pharmacy or Pharmacy verbiage STX VA clinical site Redundancies in visit language	Replace with Research Drug Manager or management unit; added FDA required language for labeling Replace with UT MARC site Streamlined verbiage and added fibroscan and MRS/MRI at RII
12/05/19	1.2.1 dated 11-15-19	Cover page SAE Reporting	Administrative update needed Empty cells on pg. 17	Updated IRB# and versioning Removed empty cells
12/18/19	1.3	CTO changes		Approved Stips met - new study by IRB
03/20/20	2.0	Cover sheet Study Summary Eligibility Criteria Stopping Rules Section of Visits	Needed Updates Study duration Safety lab ranges Needs to be added Separate Visits 4 and 10 into 4A-B and 10A-B	Versioning updated Treatment phase v total Exclusionary lab ranges Added rules Relax physical performance burden
05/26/20	2.1	Study Summary Exclusion Criteria	Required updates. Required updates	Study site, exclusion criteria & statistical methodology updated Blood donation in prior 2 months & h/o frequent UTI added; renal failure clarified

		Section 3.4 (Potential risks to Subject Safety) Section 5.6.3 (Dispensing of Study Drug Section 6.1. (Study Visits) Section 6.3 (Analyses) Section 7 (Statistical Methods) Section 8 (Reporting AEs & UPs) 9.3.4 (Telehealth Visits)	Required updates Required updates Required updates & clarification Required clarification ANOVA Required clarification Required updates	Risks for dapagliflozin updated; added potential risks of home visits; Added dispensed at home visit, by mail or curbside delivery Text added to clarify muscle biopsy procedure at Visit 5; added home & video telehealth visits; added BICRC study site; CPET made optional; urine HCG removed Blood/plasma volume clarified ANCOVA will be used for comparisons between groups; the study will only use data from pts. who complete the study Added clarification for reporting SAEs Telehealth visits description added
07/22/20	2.2	Study Summary;Study Design; 4.1 Inclusion Criteria; 6.1.8 3.3, Study Endpoints 6.1, Study Visits 6.1.1; Visit Schedule Summary	Required update Matsuda index using OGTT Required update Required update	Removed OGTT HOMA-IR index using fasting insulin & glucose Added Bluetooth scale Removed OGTT, added insulin & FFA
08/11/20	2.3	Inclusion Criteria 3.2, General Design	BMI 30-37 Correction	BMI 30-38 (≥29.5 will be rounded up) Study treatment period and total study duration corrected.
08/31/20	2.3.1	3.4, Potential Risks to Subject Safety	Blood Withdrawal	Added risks of venipuncture by needlestick w/o catheter
10/16/20	2.3.2	4.3 Subject Recruitment & Screening	will screen up to 40 potentially eligible candidates	will screen up to 100 potentially eligible candidates
10/29/20	2.4	6.1 Study Visits; 6.2 Visit Summary Schedule	Required update	Due to availability of DXA and MRI scanners.Visits 2&3

				will be performed interchangeably, as well as Visits 8&9, Visit windows, also, expanded to +/- 2 weeks.