Targeting the Endothelial Glycocalyx to Enhance Vascular Function and Exercise- Induced Vascular Adaptations in Type 2 Diabetes

NCT# 05205005

Protocol: 2062542

Funding Agency: Veterans Administration

Principal Investigator/Study Chair: Camila Manrique-Acevedo, MD, Jaume Padilla, PhD

Protocol Version Number 12

May 15, 2023

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Abstract

Cardiovascular disease (CVD) is the leading cause of death in Veterans with type 2 diabetes (T2D). Lifestyle modifications, including increased physical activity, are recommended as first-line therapy for the management of T2D. Unfortunately, the efficacy of these lifestyle interventions for preventing CVD morbidity and mortality in patients with T2D is not well-established. Available evidence from our group and others indicates that vascular adaptations to exercise training are impaired in subjects with T2D. We propose that diminished vascular adaptations explain why increased physical activity does not lead to a robust reduction in CVD morbidity and mortality in T2D. However, the mechanisms responsible for this deficit in vascular adaptations to exercise in T2D remain unknown. This is a major limitation for identifying new adjuvant therapeutics to maximize the cardiovascular benefits of exercise in the diabetic population. Exercise exerts direct effects on the vasculature via repetitive exposure to hemodynamic stimuli or shear stress. The increased blood flow and luminal shear stress attendant to each bout of exercise are primary mechanisms contributing to vascular adaptations. Shear stress is detected by mechanosensitive endothelial luminal structures, such as the glycocalyx, that convert mechanical forces into biochemical signals via mechanotransduction. As such, we propose that an intact endothelial glycocalyx is required for the mechanotransduction of increased shear stress and the subsequent chronic vascular adaptations associated with exercise to occur. Notably, glycocalyx degradation is a classic feature of T2D. Accordingly, our overarching hypothesis is that endothelial glycocalyx degradation is a key factor precluding shear stress mechanotransduction and consequent exercise-induced vascular adaptations in T2D. The corollary to this hypothesis is that restoration of the endothelial glycocalyx by dietary supplementation of glycocalyx precursors (DSGP) will improve vascular adaptations to exercise in T2D. Specifically, in Aim 1 (*Proof of Concept Clinical Trial Phase*), we will document that DSGP enhances endothelial glycocalyx integrity in patients with T2D. Although we provide preliminary evidence that DSGP can increase glycocalyx thickness and endothelial function in a mouse model of T2D, this will be the first study to demonstrate these effects in T2D subjects. The effects of DSGP for eight weeks will be studied using a double-blinded randomized placebo control trial. Subsequently, in Aim 2 (*Expended Clinical Trial Phase*), we will demonstrate the permissive role of the endothelial glycocalyx in exercise-induced vascular adaptations in patients with T2D. Having shown that restoration of the endothelial glycocalyx via DSGP is feasible in T2D subjects, we will now investigate whether such supplementation will potentiate exercise training-induced improvements in endothelial function. This will be accomplished in a factorial balanced design in which T2D subjects will be randomized to DSGP or placebo with and without concurrent exercise training for eight weeks. Our team is poised to move cardiovascular and diabetes research forward with a translational project that will exert a sustained, powerful impact across a number of levels of inquiry that are novel conceptually, methodologically, and therapeutically. Indeed, targeting the glycocalyx holds extraordinary promise for achieving optimal exercise-induced vascular adaptations in Veterans with T2D, thus maximizing the cardiovascular benefits of exercise.

List of Abbreviations

AMBP- Ambulatory Monitoring of Blood Pressure

BP- Blood Pressure

CMP- Complete Metabolic Panel

CTSU East/West- Clinical Translational Science Unit, location of in-patient vascular testing for the clinical research study.

CVD- Cardiovascular Disease

DEXA- Dual-energy x-ray absorptiometry, body composition testing

DSGP- Dietary Supplementation of Glycocalyx Precursors, supplement that is being investigated for its effects on endothelial glycocalyx restoration and exercise-induced vascular adaptations

DSMC- Data Safety Monitoring Committee

ECG/EKG- Electrocardiogram

FLP- Fasting Lipid Panel

FMD- Flow Medicated Dilation

HbA1c- Hemoglobin A1c

H&H- Hemoglobin and Hematocrit

HR- Heart Rate

Ht- Height

NDSR- Nutrition Data System for Research

PWV- Pulse Wave Velocity

PT- Prothrombin Time

PTT- Partial Prothrombin Time

SAE- Serious Adverse Event

T2D- Type 2 Diabetes

TSH- Thyroid-Stimulating Hormone

Wt- Weight

Contents

1.0 Study Personnel

- Principal Investigators:
  - Camila Manrique, MD
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    Harry S. Truman VA Hospital
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    University of Missouri, School of Medicine
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  - Jaume Padilla, PhD
    University of Missouri, Department of Nutrition and Exercise Physiology
    Associate Professor of Nutrition and Exercise Physiology
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- Collaborators:
  - Adam Whaley-Connell, DO
    Associate Chief of Staff for Research and Development
    Harry S. Truman VA Hospital
    Professor of Medicine
    University of Missouri, School of Medicine
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    573-814-6551

- Indicate the number of potential participating sites (both VA and non-VA) and if there is any graduated start-up plan for the sites:

  Only site – Harry S Truman VA Hospital

2.0 Introduction

The prevalence of type 2 diabetes (T2D) continues to increase in the US, with 26.8 million adults carrying a diagnosis.[1-3] Importantly, T2D is widespread among our Veterans, and the VA administration spends $1.5 billion annually in diabetes care.[4, 5] This T2D epidemic also contributes to the staggering rates of cardiovascular disease (CVD) and cardiovascular mortality.[6] Indeed, eight out of 10 patients with T2D die from CVD.[7] Mechanistically, endothelial dysfunction, defined as a pathological state typified by oxidative stress, inflammation, reduced nitric oxide bioavailability and impaired endothelium-dependent vasodilation, is the most prevalent vascular characteristic implicated in the increased incidence of CVD in T2D.[8-11]

Current standards of medical care for T2D emphasize prioritizing the use of therapies that decrease CVD risk.[12] Lifestyle modifications, such as increased physical activity, are recommended as first-line therapy for the management of T2D.[12] Unfortunately, as shown in the LookAhead trial, the efficacy of these interventions for preventing CVD morbidity and mortality in patients with T2D remains questionable.[13] Evidence from our laboratory and those of others indicate that exercise training in T2D subjects does not elicit optimal vascular adaptations, including improvements in endothelial function.[14-17] In fact, a recent meta-analysis concluded that exercise-induced *"improvements in endothelial function in T2D patients are weakened compared with non-diabetics".*[15] This represents an important scientific premise of this project. Restricted vascular adaptations to exercise training in T2D individuals may also contribute to their poor gains in cardiorespiratory fitness.[18] Importantly, it is likely that such lessened vascular adaptations explain why increased physical activity does not lead to a robust reduction in CVD morbidity and mortality in T2D. A better understanding of the mechanisms responsible for the deficit of vascular adaptations to exercise in T2D is required for identifying new adjuvant therapeutics aimed at maximizing the cardiovascular benefits of exercise.

In the present proposal, we put forth the **idea** that an intact endothelial glycocalyx is a requisite for exercise-induced shear stress mechanotransduction to exert favorable vascular adaptations. The effects of exercise on the vasculature extend beyond improvements of traditional cardiovascular risk factors.[19-22] It is now well-recognized that increased blood flow and associated luminal shear stress, the tangential force caused by the friction of flowing blood on the endothelial surface, are critical exercise-induced signals that lead to enhanced endothelial function,[19, 23-27] another important premise of this project. This concept is supported by results from cell culture and isolated vessels,[28-32] as well as by *in vivo* animal[33, 34] and human experiments.[19, 35-41] It also implies that the endothelium is "equipped" with sensors that transduce such mechanical forces.[42, 43] One such key mechanosensor is the glycocalyx,[42-46] a mesh of negatively charged sialic acid-containing glycosaminoglycans and proteoglycans located on the surface of endothelial cells.[45, 47, 48] Heparan sulfate is the most abundant glycosaminoglycan in the endothelial glycocalyx, followed by chondroitin sulfate and hyaluronic acid. The main proteoglycans are syndecan-1, -2, -4 and glypican-1. Through its interactions with the circulating blood and plasma proteins, membrane ion channels and cortical actin, the glycocalyx operates as the major mechanosensing and mechanotransducing structure in the endothelium.[42, 43, 49, 50] Accordingly, we posit that the endothelial glycocalyx plays a permissive role in shear stress-induced vascular adaptations and that, as such, an intact glycocalyx is vital for exercise-induced vascular adaptations to be fully realized. Unfortunately, T2D is accompanied by glycocalyx degradation, presumably rendering the endothelium unable to respond and optimally adapt to exercise-induced shear stress stimuli.

The primary goal of this project is to establish the **endothelial glycocalyx as a novel target organ for heightening exercise-induced vascular adaptations**. To that end, a dietary supplement that contains glycocalyx precursors (glucosamine sulfate, fucoidan, superoxide dismutase, and high molecular weight hyaluronan) will be used as an innovative "tool" to restore the endothelial glycocalyx in T2D subjects. Demonstration that **dietary supplementation of glycocalyx precursors (DSGP)** is effective at enhancing endothelial glycocalyx integrity in patients with T2D will be accomplished in the *Proof of Concept Clinical Trial Phase* (or Aim 1) of this project. Subsequently, in the *Expended Clinical Trial Phase (*or Aim 2*),* the use of the DSGP will allow us to test the hypothesis that glycocalyx restoration re-sensitizes the endothelium to shear stress mechanotransduction and thus potentiates exercise-induced vascular adaptations. This project will be the first to determine if targeting the glycocalyx is a viable therapeutic strategy for boosting exercise-induced endothelial benefits in diabetes. The present protocol will only describe the experimental strategy for the *Proof of Concept Clinical Trial Phase.*

3.0 Objectives

Our overarching hypothesis is that endothelial glycocalyx degradation is a key factor that precludes shear stress mechanotransduction and consequent exercise-induced vascular adaptations in T2D. (Figure 1). A corollary to this hypothesis is that restoration of the endothelial glycocalyx by DSGP will improve vascular adaptations to exercise in T2D.

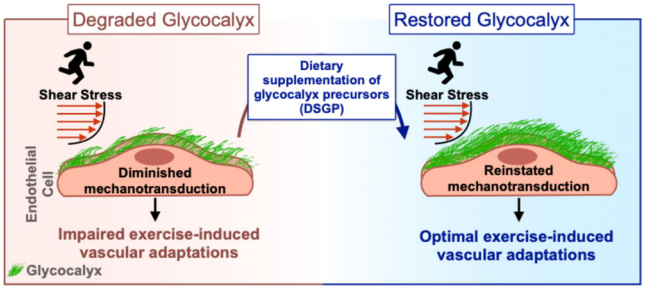

Figure 1. Overarching hypothesis: Endothelial glycocalyx degradation (left side) is a key factor precluding shear stress mechanotransduction (i.e., it renders the endothelium unable to detect shear stress forces) that makes T2D individuals exhibit diminished exercise-induced vascular adaptations. Restoration of the glycocalyx (right side) reinstates shear stress echanotransduction, permitting optimal exercise-induced vascular adaptations.

Specific aims are as follows:

Aim 1 (*Proof of Concept Clinical Trial Phase*): Document that DSGP enhances endothelial glycocalyx integrity in Veterans with T2D. Although we provide preliminary evidence that DSGP can increase glycocalyx thickness and endothelial function in a mouse model of T2D, this will be the first study to demonstrate these effects in T2D subjects. The effects of DSGP for eight weeks on glycocalyx integrity and endothelial function will be studied using a double-blinded randomized placebo control trial.

Aim 2 (*Expanded Clinical Trial Phase*): Demonstrate the permissive role of the endothelial glycocalyx for exercise-induced vascular adaptations in Veterans with T2D. Having shown that endothelial glycocalyx restoration via DSGP in T2D subjects is feasible, we will now investigate whether such supplementation potentiates exercise training-induced improvements in endothelial function. This will be accomplished with a factorial balanced design in which T2D subjects will be randomized to DSGP or placebo with and without concurrent exercise training for eight weeks.

RELEVANCE TO VETERANS AND THE VA: Targeting the glycocalyx holds extraordinary promise for achieving optimal exercise-induced vascular adaptations in T2D, thus maximizing the cardiovascular benefits of exercise.

4.0 Resources and Personnel

- Camila Manrique, MD
  Endocrinology Chief
  Harry S. Truman VA Hospital
  Associate Professor of Medicine
  University of Missouri, School of Medicine
  Camila.Manrique-Acevedo@va.gov
  Co-Primary Investigator
  VA Affiliate 5/8s
  Role: Dr. Manrique-Acevedo is a physician-scientist with expertise in the study of the mechanisms of cardiovascular disease in obesity and diabetes. She will be responsible for communication with the VA (contact PI) and the submission of the progress reports. She will maintain IRB and other administrative approvals and assure that staff meet the human subjects and ethic training requirements. She will coordinate the blinding scheme for subjects, investigators and laboratory staff, and communicate with the safety officer. In addition, she will be responsible for the overall medical supervision of the study including subject screening and management of subjects in the supplement/placebo arms. Together with Dr. Padilla, she will be in charge of data interpretation and the preparation of manuscripts. She will have access to study records, including patient PHI.

- Jaume Padilla, PhD
  University of Missouri, Department of Nutrition and Exercise Physiology
  Associate Professor of Nutrition and Exercise Physiology
  Co-Primary Investigator
  VA Affiliate 5/8s
  Role: Dr. Padilla will be in charge of overseeing the study visits in both aims, including all the vascular function measurements and the assessment of glycocalyx integrity. Dr. Padilla will be present during the study visits. Dr. Padilla will review all analyze files by the research assistant, including the off-line videos processed with the edge detection/wall-tracking software, as well as the individual spreadsheets used for calculations. Dr. Padilla will also be responsible for supervising the exercise intervention in Aim 2. In addition, he will assist Dr. Manrique-Acevedo with efforts related to regulatory aspects including IRB requirements, progress reports, and subject screening. Together with Dr. Manrique-Acevedo, he will in charge of data interpretation and the preparation of manuscripts.

- Adam Whaley-Connell, DO
  Harry S. Truman VA Hospital
  Associate Chief of Staff for Research and Development
  University of Missouri, School of Medicine
  Professor of Medicine
  Safety Officer
  VA Affiliate 5/8s

  Role: Dr. Whaley-Connell is the Associate Chief of Staff for Research and Development and a staff Nephrologist at the Truman VA as well as a Professor of Medicine in the Divisions of Nephrology and Endocrinology. He will function as the Safety Officer of this project. He is a clinician-scientist and has extensive experience in clinical intervention studies. As Safety Officer, Dr. Whaley-Connell will review the reports sent by the PIs and 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 study

investigator, the IRB, and the institutional Data Safety Monitoring Board. In addition, the safety officer may comment on whether the study investigator needs to report any specific out-of-range laboratory data to the participant and/or personal physician. He will have access to study records, including PHI.

- Katherine Marie Burr, MS, RD
  University of Missouri, School of Medicine
  Department of Medicine
  Research Coordinator
  Non-VA Affiliate
  Role: Katherine Burr will be involved in subject recruitment and retention, administering survey/interview procedures, and will have access to study records, including patient PHI. She will also participate in the consenting process.

- Tim Morris
  Harry S. Truman Veterans Administration
  Programs Specialist, Grant Manager
  VA Affiliate
- Role: Tim Morris will be involved in study compliance and record keeping, and will have access to study records, including patient PHI. He will serve in a advisory role to the research team.

- Sekinat Mustapha, BSN
  Harry S. Truman Veterans Administration
  Nurse Research Coordinator
  VA Affiliate
  Role: Sekinat Mustapha will be involved in subject recruitment and retention, administering survey/interview procedures, and will have access to study records, including patient PHI. She will also participate in the consenting process.

- Rachel Gilbert, BSN
  Harry S. Truman Veterans Administration
  Nurse Research Coordinator
  VA Affiliate
  Role: Rachel Gilbert will be involved in subject recruitment and retention, administering survey/interview procedures, and will have access to study records, including patient PHI. She will also participate in the consenting process.

- Claudia Simkins, BSN
  Harry S. Truman Veterans Administration
  Nurse Research Coordinator
  VA Affiliate
  Role: Claudia Simkins will be involved in subject recruitment and retention, administering survey/interview procedures, and will have access to study records, including patient PHI. She will also participate in the consenting process.

- Andrea Marie Atkins, BSN
  University of Missouri, School of Medicine
  Department of Medicine
  Research Coordinator
  Non-VA Affiliate
  Role: Andrea Atkins will be involved in subject recruitment and retention, administering survey/interview procedures, and will have access to study records, including patient PHI. She will also participate in the consenting process.

- James Smith, MS
  University of Missouri
  Department of Nutrition and Exercise Physiology
  Staff
  Non-VA Affiliate
  Role: James Smith will be conducting vascular measurements and performing data analysis. He will also have access to PHI in study records and consent documents.

5.0 Study Procedures (*Proof of Concept Clinical Trial Phase)*

5.1 Study Design

Aim 1 (*Proof of Concept Clinical Trial Phase*): Document that DSGP enhances endothelial glycocalyx integrity in Veterans with T2D.

Rationale. Based on our preliminary data showing that DSGP augments endothelial glycocalyx thickness and vasodilatory responses in a mouse model of T2D (**Figure 2**), the purpose of this Aim is to demonstrate that these effects are applicable to Veterans with T2D. Importantly, our results should establish for the first time that glycocalyx restoration is accompanied by improvements in endothelial function, providing support for our proposal that the glycocalyx is a key therapeutic target to combat vascular complications in diabetes. Finally, the results obtained in Aim 1 will set the stage for an expansion of the clinical trial (Aim 2) designed to elucidate if restoration of the endothelial glycocalyx enhances exercise training-induced vascular adaptations in T2D subjects.

Hypothesis. We hypothesize that DSGP will enhance endothelial glycocalyx integrity in T2D subjects and that this effect will be associated with increased shear stress mechanotransduction and endothelial function.

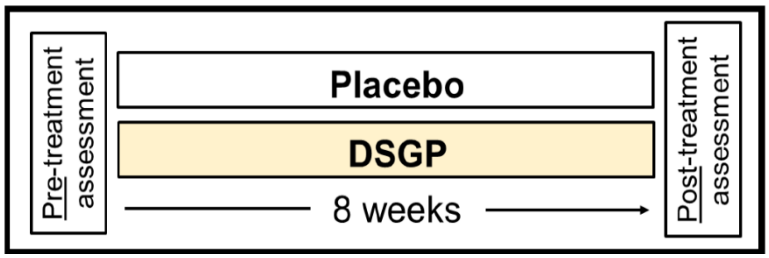

Figure 2. Experimental design in Aim 1. DSGP, dietary supplementation of glycocalyx

Study Design and Recruitment of Participants. We will employ a double-blinded randomized placebo control trial designed to determine the effects of DSGP on endothelial glycocalyx integrity and endothelial function (**Figure 9**). Veterans diagnosed with T2D (n=24), 45- 74years of age, will be recruited from the Harry S. Truman Memorial VA Hospital in Columbia, Missouri. The selected age range aligns with data from the Centers for Disease Control, indicating that the incidence of T2D in Veterans continues to increase after even after 65 years of age.[4] Subjects will be classified as having T2D based on physician diagnosis, following the American Diabetes Association guidelines.[51]

Randomization and Blinding. Participants will be assigned to DSGP or placebo using block randomization to achieve balance in the allocation of participants to treatment arms. Randomization order will be provided to the investigational pharmacy for intervention allocation. Preparation and administration of the supplement/placebo capsules will be directed by the VA Hospital Investigational Pharmacy. All members of the investigative team involved in data acquisition and analysis will be blinded to group assignment, and subjects will not be informed of their group status.

DSGP Intervention and Outcome Measures. Participants will ingest 3,712mg (*i.e.*, six capsules) of DSGP, or placebo capsules, daily for eight weeks (+/-4 days). *Capsules should be swallowed and not crushed and ingested with meals (3 capsules every 12 hours).* DSGP, commercially available as Endocalyx™ (Microvascular Health Solutions LLC, Alpine, UT), include glucosamine sulfate, fucoidan, superoxide dismutase, and high molecular weight hyaluronan. An ongoing study using DSGP in chronic kidney disease patients (NCT03889236), to our knowledge, has not reported serious adverse side effects (*see enclosed letter from Dr. Vink Hans and Robert Long*). Results from our pre-clinical studies in diabetic mice treated with DSGP (100mg/kg/day) for four weeks support that the proposed dosing regimen in humans will be capable of restoring the glycocalyx, particularly when considering the dose conversion from mouse to human. The use of Endocalyx in this study is filed under the research IND 164629 that was submitted on 11/23/2022.

5.2 Recruitment Methods

Dr. Camila Manrique-Acevedo, co-PI in this proposal, is the Chief of Endocrinology at the Harry S. Truman VA facility and will direct recruitment efforts. Approximately 200 participants will be enrolled (consented) to participate in this study to obtain the sample sizes of n=24 (Aim 1) and n=72 (Aim2.) Patients will be recruited from the Endocrinology Clinic as well as Primary Care Clinics at the Truman VA Hospital. During the fiscal year 2020, the Endocrinology Clinic evaluated 1476 patients diagnosed with T2D. Thus, our Endocrinology clinic has sufficient patients to serve as potential study subjects over the duration of this study. Further, we are cognizant that patients with T2D are also frequently evaluated in the primary care setting. Our primary care clinics serve 32,400 Veterans and 9,400 of those have a diagnosis of T2D (29%). To this end, we have established a collaboration with Dr. Lana Zerrer (Chief of Staff and primary care physician at the VA Hospital) to facilitate recruitment efforts in these clinics.

An Informational session about the project will be held with other primary care physicians at the VA who may also be interested in partnering with recruitment efforts.

For patients identified as potentially eligible in the Diabetes Clinic, Dr. Manrique-Acevedo or a designated study team member will approach them in person during their visit with written information (IRB approved) as well as with contact information for the patient to communicate with study coordinator in case of questions or interest in participating. For those potential participants that seem eligible based on this pre-screening review (primary care clinics), recruitment letters (IRB approved) informing them of the opportunity to participate in this research will be mailed or shared through the VA patient portal.

Advertisement fliers and postings will also be placed in MU Info and in publicly accessible areas in the community (i.e. coffee shops, grocery stores, parks, libraries, etc.) to facilitate the recruitment of benefit eligible veterans from the surrounding community.

In the unlikely event that the above recruitment strategies are not successful, we will pursue the following alternative strategies (with IRB and office of research approval):

- Posting of advertisement flyers throughout approved venues at the Harry S. Truman VA Hospital and local primary care clinics (CBOC)s
- Systematic review of charts from primary care and endocrinology clinics with previous approval from the different providers.
- Advertisement in social media outlets, local radio and TV stations.
- Submission a search request to the VA data warehouse (VINCI) for a listing of patients at the Harry S. Truman VA who have a diagnosis of T2D and are within the targeted age range and fulfil inclusion/exclusion criteria.

Screening Procedures:
Interested participants will be instructed, via letters, flyers, or via VA patient portal to contact study coordinator for a 10-minute interview (using a waiver of documentation of consent approved by the local IRB). The interview will be conducted with the goal of determining eligibility (demographics, height, weight, current medical illnesses/ medical history, and use of medications will be obtained). If a subject expresses interest to participate in the study, a screening visit will be scheduled.

Participant 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%. We utilize a validated retention strategy published by Jeffrey et al (Am J Clin Nutr. 2003;78(4):684-689) that has been successful. With respect to subject honoraria, subjects will be paid $25 after completion of the screening visit, $75 after the baseline visit, and $255 after the final visit. Subjects will also receive travel compensation of $0.41/mile

traveled (round trip) from their residence to the Harry S. Truman VA Hospital and Clinics for each of their study visits. If subjects are asked to repeat safety labs, you will be compensated for applicable travel at a rate of $0.41/mile traveled (round trip) from their residence to the Harry S. Truman VA Hospital and Clinics. Thus, a total compensation of $355 plus applicable travel compensation will be provided as study events are completed. Participants will receive study compensation via check.

5.3 Informed Consent Procedures

The study team has obtained Waiver of Documentation of Consent from the IRB for participant fasting prior to the study screening visit. Participants will be asked for verbal confirmation of their willingness to fast overnight prior to the study screening visit.

Screening visits (up to 60 minutes duration) will occur in the morning at the Endocrinology Clinic space at the Harry S Truman VA Hospital. Informed written consent will be obtained by Dr. Manrique-Acevedo in a private and non-rush environment. Dr. Manrique-Acevedo is trained and compliant with IRB and HIPAA education requirements. Sufficient time will be provided for the subject to read and review the documents. Dr. Manrique-Acevedo will answer any questions that the potential participants may have and will assess understanding of the study consent by asking subjects simple questions about the study purpose and the procedures involved. As described in the informed consent, Dr. Manrique-Acevedo will reiterate to the subjects that: 1) participation is completely voluntary, 2) decision to participate or not will not influence their relationship with the physician or any member of the research team; and 3) participant may withdraw from the study at any time for any reason. For all subjects, a HIPAA Authorization form will also be obtained as approved by the VA. One copy of the executed consent and HIPAA Authorization form will be given to the subject and one copy will be stored in a secured file cabinet. Dr. Manrique-Acevedo will perform a pertinent medical history on all subjects.

5.4 Inclusion/Exclusion Criteria

Inclusion:

1) 45-74 years of age at the time of enrollment
2) Diagnosis of T2D by a health care provider, confirmed by chart review
3) HbA1c <9% and fasting blood glucose <200 mg/dL at screening visit
4) Body mass index (BMI) 25–45 kg/m2
5) Women should be postmenopausal (absence of menses for at least 1 year)
6) Sedentary subjects (<2 days/week of vigorous exercise)
7) Willingness to follow up instructions provided by study team

Exclusion:

1) Evidence of cardiac arrhythmias, unstable angina (or other cardiac event), heart failure or stroke in the last 12 months
2) Evidence of chronic kidney disease stage IV or V (GFR <30 mL/min)
3) Evidence of uncontrolled hypertension, systolic blood pressure > 180 mmHg and/or diastolic blood pressure > 110 mmHg on more than 2 occasions in the past 12 months or at screening visit
4) Diagnosis of chronic liver disease
5) Uncontrolled thyroid dysfunction (abnormal TSH within 3 months of study enrollment)
6) Active cancer
7) Current use of hormone replacement therapy
8) Excessive alcohol consumption (>14 drinks/week for men, >7 drinks/week for women)
9) Current pregnancy or intent to become pregnant during the course of the study
10) Inability to swallow capsules
11) Known allergies to any of the compounds in the supplement: glucosamine extract, fucoidan extract, olive extract, artichoke extract, red and white grapes extract, melon concentrate, hyaluronic acid

	Screening Visit	AMBP Fitting (Day -7 to -2)	Pre-Assessment (Day 0)	**Safety Blood Draw (Days 7-14)**	Dosing Window (Days 1-56)	AMBP Fitting (Day 49-54)	Post-Assessment (Day 56)
Informed consent form signing	X						
Medical history	X						
Concomitant medication assessment	X		X		X	X	X
Height assessment	X		X				X
Weight assessment	X		X				X
Waist circumference assessment	X		X				X
Vitals signs (blood pressure, heart rate and respiratory rate, temperature)	X		X				X
12-lead **ECG**	X						
Fasted bloodwork: **HbA1c, FLP, CMP, TSH, H&H**	X						
Bloodwork: **PT, PTT, H&H**	X			X			X
3- Day food diary		X				X	
24-hour Ambulatory Blood Pressure Monitoring (AMBP)		X				X	
24-hour AMBP activity log		X				X	
AM **T2D** medication hold			X				X
AM Tobacco hold	X		X				X
24-hour exercise hold	X		X				X
Overnight fasting	X		X				X
Fasted blood draw			X				X
Glycocheck		X	X				X
Pulse Wave Velocity			X				X
Brachial FMD			X				X
Femoral FMD			X				X
Insulin and glucose infusion with leg blood flow, sphygmomanometry, and beat-to-beat via finometer			X				X
Contrast enhanced ultrasound			X				X
DEXA			X				X
Supplement/placebo dispensing			X				
Daily Supplement/Placebo dosing					X		
Weekly phone check-in					X		

Table 1. Schedule of study activities. Note that after screening visit, once eligibility has been confirmed subject will be randomized.

5.5 Study Evaluations

Screening and Consenting Visits:
This visit will last approximately 60 minutes in duration and will occur in the morning at the Endocrinology Clinic space at the VA Hospital. Subjects will be asked to come after an overnight fast before their arrival at the clinic. Subjects will be asked to avoid tobacco use on the morning of scheduled visits. Informed written consent will be obtained by Dr. Manrique-Acevedo in a private environment. The decision to participate or not will not influence medical care by the co-PI or any other member of the team. After informed consent has been obtained, the following procedures will take place: 1) anthropometric measurements (waist circumference, height, body weight); 2) seated blood pressure measurements (in triplicate) after five minutes of rest, heart rate, temperature, and respirations 3) resting 12-lead ECG; 4) phlebotomy to obtain blood sample for serum chemistries, HbA1c, lipid panel, thyroid, liver, kidney function, hemoglobin and hematocrit and coagulation tests (prothrombin time, and activated partial prothrombin time). Once screening tests have been completed and resulted, enrollment eligibility will be reviewed by co-PIs, Camila Manrique-Acevedo, MD and Jaume Padilla, PhD.

Eligibility:
Eligible subjects will be scheduled for four outpatient study visits (2 equipment fitting visits-1 hour duration each, and pre- and post-treatment assessment, eight hours duration each) at the Clinical Translation Science Unit (CTSU) of the University of Missouri, a core facility and one outpatient study visit at the Harry S. Truman VA Hospital (safety labs). The first study visit will be scheduled within four weeks of the screening visit. Participants will be instructed to maintain their habitual physical activity levels and dietary habits throughout the eight-week period. Adherence to the supplement/placebo will be facilitated (and documented) by the following processes: 1) the study coordinator/research nurse will review proper adherence directions at baseline visit; 2) capsule boxes will be provided at the baseline visit and the participant will be asked to bring the box at the post-treatment visit to count any remaining capsules; and 3) participants will be contacted on a weekly basis by the study coordinator to check in and review compliance.

Equipment fitting visits and potential risks:
In the week leading up to the pre and post assessment visits, the subjects will attend an appointment with the purpose of being fitted with an ambulatory blood pressure monitor. The subjects will be instructed on the wear and use of the ambulatory blood pressure system and will take the equipment with them for home use over a 24-hour period. Subjects will also be instructed on the use of the Glycocheck equipment and practice collecting measurements.

Ambulatory blood pressure monitoring: Blood pressure monitoring will be conducted with a Welch Allyn ABPM 7100 Ambulatory Blood Pressure Monitor or equivalent device and adult blood pressure cuff. This procedure is not standard of care. This procedure poses no risks but may cause some discomfort due to cuff inflation.

Assessment visits and potential risks:
At each CTSU visit, subjects will arrive after an overnight fast, having not taken medications the morning of the study, and not exercised for 24 hours. Subjects will be asked to avoid tobacco use on the morning of scheduled visits. Anthropometric (waist circumference, height and body weight), body composition (via DEXA) and blood pressure measurements will be obtained. At this time, a three-day dietary recall will be performed by a registered dietitian at the CTSU and analyzed using NDSR software. Subsequently, subjects will be placed supine in a quiet, climate-controlled room (22–23°C) for measurements to be performed are described below.

Time point	Blood volume (mL)
Screening	20
Baseline visit	70
Safety visit	10
Final Visit	80
Total	**180**
***Unscheduled safety labs** (10)	

Box 1. Expected blood draw volumes during the duration of the study.

Phlebotomy: The total blood withdrawn will not exceed 180 mL throughout the study period (**Box 1**). Hematocrit and Hemoglobin (H&H is measured during screening, safety labs, and at final visits to check that it is within safe limits. 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, fainting 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. The 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.

Safety Labs: Subjects will have safety labs drawn at 7-14 days after beginning study medication. Safety labs will be drawn at the Harry S. Truman VA Hospital. In case of abnormal results in laboratory values obtained during the study, the safety office will determine the clinical significance of those findings and the appropriate course of action.

Additional Safety Visits/unscheduled visits: Additional safety labs/visits can be scheduled at the discretion of the study's safety officer. Amount of blood draw for safety labs will not to exceed 10mL. Unscheduled visits may also occur if safety lab specimens are compromised for any reason or questionable accuracy of the result. Safety labs will then be repeated (amount of blood drawn not to exceed 10 mL).

Glycocheck: The Glycocheck video microscope instrument will be placed under the subject's tongue to measure capillaries, blood vessel density, red blood cell concentration, flow rate, and red blood cell penetration of the glycocalyx lining. Glycocheck is an FDA Class 1 Medical Device. This procedure is not standard of care. This procedure poses no risks.

Pulse wave velocity: A special non-invasive device will be used to assess blood pressure and flow. A blood pressure cuff will be wrapped around the upper arm and upper leg of the participant. The cuffs will periodically inflate to squeeze tightly for less than 60 seconds. A pressure sensor, the size of a pencil will be placed over the skin of the neck region to obtain the pressure wave form in the neck vessel (i.e., carotid artery). This procedure takes about 15 minutes. Risks: 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. This procedure is not standard of care.

Brachial and femoral artery FMD: Arterial measurements will be performed by imaging the brachial or femoral artery longitudinally using high-resolution duplex ultrasonography. Arterial vasodilatory responses to hyperemia (*i.e.*, FMD) will be examined by inflating a forearm or leg cuff up to 250 mmHg for five minutes. Before, during and after rapid release of the cuff, brachial artery blood flow velocity and diameter will be continuously measured. When assessing FMD, the blood pressure cuff will squeeze the arm or leg tightly; however, any discomfort will be alleviated as soon as the pressure in the cuff is released. This procedure is not standard of care.

Insulin and Glucose infusion: A 1-hour insulin and glucose infusion, coupled with measurements of leg blood flow, will be performed following standard procedures at the University Hospital CTSU. Subjects will be instrumented for measures of heart rate (using standard lead II ECG), arterial blood pressure (using automated sphygmomanometry and/or beat-to-beat blood pressure via Finometer), and leg blood flow (using duplex Doppler ultrasound and contrast-enhanced ultrasound, CEU). After a minimum of 20 minutes supine rest, baseline cardiovascular measurements will be collected, blood samples obtained and the insulin infusion will start. Insulin diluted in 0.9% saline with 5 mL of the subject's blood is infused at a constant infusion of 80 mU•m−2•min−1. 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-minute periods at 15-minute intervals throughout a 60-minute insulin infusion 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 minutes of insulin infusion. Briefly, assessment of microvascular perfusion utilizing CEU involves

ultrasound imaging of the leg skeletal muscle during the administration of one vial (1.3 mL) of an ultrasound contrast agent diluted in 30 mL of 0.9% saline that will be given using a handheld syringe pump through the IV at a rate of 2 mL/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 infusion is approximately 52mL.The potential risks during this procedure 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 one hour post insulin infusion. This procedure is routinely performed by Drs. Manrique and Padilla. Dr. Manrique, board-certified endocrinologist, will be available during the procedures. This procedure is not standard of care.

Skeletal muscle 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. This procedure is not standard of care.

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. Only postmenopausal subjects will be included in the study; therefore no pregnancy test will be pursued. Body weight is measured to the nearest 0.1 kg and height to the nearest 0.1 cm. This procedure is not standard of care.

Dietary Recall/Assessment: The study dietitian or a trained research technician will interview research subjects about the foods and beverages that they have recently consumed or asked them to keep a record of the foods that you consume for 3 days. There are no risks with this procedure. Dietary Recall/Assessment will be performed at the preassessment and post assessment visits.

Supplement, Endocalyx: The Dietary Supplementation of Glycocalyx Precursors (DSGP) commercially available as Endocalyx™ (Microvascular Health Solutions LLC, Alpine, UT) which includes: glucosamine sulfate, fucoidan, superoxide dismutase, and high molecular weight hyaluronan is the supplement being compared to placebo in this study. In addition, we will monitor coagulation tests (PT and PTT) at baseline, 7-14 days (this blood check will occur at VA main hospital) and during final visit. There are no known risks associated with supplementation with DSGP. Nevertheless, subjects with known allergies to any of the compounds in the supplement (glucosamine extract, fucoidan extract, olive extract, artichoke extract, red and white grapes extract, melon concentrate, hyaluronic acid) will not be included in the study. If subjects exhibit changes in their health status across any system related or unrelated to the study intervention, Dr. Whaley-Connell (safety officer) will be consulted for advice. Study subjects will be responsible for taking the study supplement or placebo at home, daily for the span of 2 months. The study team will monitor dosing compliance weekly. The use of Endocalyx in this study is filed under the research IND 164629 that was submitted on 11/23/2022.

Randomization and Blinding: Subjects will either take a placebo or a supplement as part of this study. There is an equal but random chance that a subject will be assigned to either the placebo or

supplement treatment. The study team will be blinded to the study treatment. There is no risk associated with randomization or blinding in this study.

Heart rate measurements via ECG: Heart rate will be measured. Some people may have a skin irritation from the patches that connect the wires on your chest to the computer. Skin and hair are pulled slightly when the patches are removed after the test. Research personnel will attach and remove the patches as carefully as possible. Heart rate monitoring will occur at screening, preassessment, and post assessment visits.

Insertion of venous catheters: IVs will be placed to collect blood during study assessments. The potential risks of venous catheterization include infection, swelling and discomfort at the catheter insertion sites. Some bleeding may occur during the insertion of the catheters as well after the catheters have been removed. There is also the possibility of fainting, dizziness, and possible pain and bruising as a result of catheter insertion. These risks will be greatly minimized by using sterile procedures and having an experienced registered nurse placing the venous catheters. Insertion of IVs will occur at preassessment and post assessment.

Medication hold the morning of the study visits: Subjects will be instructed to hold diabetes medications the morning of the CTSU visit to minimize the risk of hypoglycemia. These medications include: insulin, sulfonylureas, gliptins, metformin, acarbose, GLP-1 analogs, inhibitors of the sodium glucose co-transporters. Dr. Manrique-Acevedo will review medications ahead of the visit and instruct subject which medications to hold.

Transport via wheelchair: If research procedures are completed in both CTSU locations during the same visit, participants will be transported via wheelchair between locations. Wheelchair transport will minimize the physical strain on research participants as well as reduce the vascular impact of ambulating during a study visit. Study staff assisting in wheelchair transportation will be trained on common safety practices to minimize risk of injury and/or fall to the participants and themselves.

5.6 Data Analysis

For Aim 1 of the study, twenty-four subjects **(n=24)** will be enrolled in the DSGP/placebo treatment with 12 subjects randomized to each group. For Aim 2 of the study, seventy-two subjects **(n=72)** will be enrolled with eighteen subjects randomized to each of the four interventions.

Statistical analysis, sample size and power calculations:

Aim 1: We will analyze change in PBR (and change in other secondary outcomes) using ANCOVA with baseline PBR as a covariate and dietary supplement as a main effect. We will use residual diagnostic plots to check for violations of assumptions on normality and equal variance among groups. If variance is not equal, separate variance components can be estimated in SAS mixed procedure to account for these differences. Inspection of the variance estimates and a likelihood ratio test can validate the need for this approach. Transformation may be used to correct for skewness and unequal variance. This analysis has two treatment levels to contrast and no need for multiple contrast correction like Bonferroni.
Power estimates are based on preliminary data in PBR (for Aim 1) and FMD (for Aim 2). We observed a mean of 1.742 (standard deviation, SD=0.27) and 1.45 (SD=0.20) in PBR for tyoe 2 diabetes (T2D) and healthy subjects, respectively. Based on our findings in the pre-clinical mouse model of T2D that glycocalyx thickness was completely restored with DSGP, we predict that DSGP in T2D subjects will cause a mean decrease in PBR of 0.3, with no changes in response to placebo. As presented in Figure 3E, we observed a mean of 3.78 (SD=1.86) and 7.06 (SD=2.21) in FMD for T2D and healthy subjects, respectively. We predict that DSGP + exercise will cause a mean increase in FMD of 2, which represents two-thirds of the distance between healthy and T2D subjects. Our pilot data was also

used to estimate the within-subject variance, assuming a correlation of r=0.6 between serial measurements on the same subject.

For Aim 1, assuming pre-treatment PBR SD=0.27, post SD=0.20, and correlation between pre and post is 0.6, then the SD of the pre to post treatment change in PBR is 0.22. A sample size of n=10 per group will provide 82% power when the true mean difference in PBR change is 0.3. Adjustment of sample size for 20% attrition results in 24 subjects (12 per group) for Aim 1 and 72 subjects (18 per group) for Aim 2.

5.7 Withdrawal of Subjects

Subjects may be withdrawn from the study at the discretion of the investigator due to a safety concern or if judged non-compliant with trial procedures.

A subject must be withdrawn from treatment if one of the following applies:

- Subject chooses to withdraw from the study at any time
- Pregnancy or intention of becoming pregnant during study duration
- Serious adverse effect occurs
- If a significant violation of the protocol occurs, as defined by the sponsor and the coordinating investigator
- Other circumstances that would endanger the health of the subject if he/she were to continue his/her participation in the trial

Whenever an investigator terminates a subject's participation in research, the investigator must explain to the participant the reasons for the termination and, as appropriate, other treatment options available to the participant.

The PIs will note the number of and summarize the reasons for early withdrawals.

Should a subject withdraw consent to participate in the study, he/she may either:

- Agree to continue releasing information for the study
- Refuse to allow further release of information.

If any subject refuses to return for the defined assessments or is unable to do so, every effort should be made to contact him/her or a knowledgeable informant by telephone. Attempts to contact the subject should be documented in the subject's records. If any subject refuses to be contacted by telephone (e.g. the subject withdrew consent to release further information), every effort should be made to obtain vital status (alive or dead) information through consultation of public databases, wherever allowed by local regulations.

6.0 Reporting

Plan for reporting study deviations

Any minor problem/deviation will be summarized and reported to the IRB within five 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 five working days of awareness, including any event that: 1) increases the risk to the subject, or 2) significantly affects integrity of the research data.

Stopping rules

We will stop an individual study if a serious adverse event occurs. If four or more subjects experience a serious adverse event 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 unanticipated serious adverse events will be submitted for review according to current protocols. The medication will be discontinued at the discretion of the study safety officer.

Definition of serious adverse event (SAE)

A SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria (a – f):

a. Results in death

b. Is life-threatening

The term 'life-threatening' in the definition refers to an event in which the subject was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

A hospitalization or prolongation of hospitalization will not be regarded as a serious adverse event if

at least one of the following exceptions is met:

- The admission results in a hospital stay of less than 12 hours
- The admission is pre-planned
- The admission is not associated with an adverse event related to the study medication or study procedures as determined by the study safety officer and data safety monitoring board.

d. Results in persistent or significant disability / incapacity

Disability means a substantial disruption of a person's ability to conduct normal life's functions.

e. Is a congenital anomaly / birth defect

f. Is another serious or important medical event as judged by the investigator.

The data safety monitoring plan (DSMP) for this study is focused on the supervision performed by the PIs, the Co-Investigator, Dr. Manrique, and the safety officer, Dr. Adam Whaley-Connell. Monitoring will include enrollment, attrition, efficacy end-points, and adverse events.

Data Safety Monitoring Plan

Data and safety monitoring for this study will be provided by the Clinical Science Research & Development (CSR&D) centralized Data Monitoring Committee (DMC). The DMC is provided by CSR&D to ensure independent oversight of the safety and integrity of the project. The DMC is an independent multidisciplinary group, whose members have collectively – through research, education, training, experience, and expertise – the requisite knowledge pertinent to the subject areas to be reviewed. Membership details are available on the CSRD website. The DMC will provide an ongoing independent evaluation of this study focused on safety and feasibility, including participant accrual and retention, adverse events monitoring, and data analyses. Meetings will be held two times per year at which time recommendations will be made to the Director of CSR&D for endorsement. These recommendations will range from approval to continue (unconditionally or with conditions to be addressed) to probation or possibly termination if there are problems with enrollment or safety concerns.

Process for managing and reporting adverse events, serious adverse events and unanticipated problems. Unanticipated events will be reported to IRB, and both serious and non-serious adverse events will be reported to the DMC. For reporting to the DMC, adverse events will be categorized and classified according to Common Terminology Criteria for Adverse Events Scale (CTCAE v6.0). Safety reports will be sent to the safety officer. The PIs will be responsible for assembling the data and producing these reports, as well as assuring that all parties obtain copies of these reports.

Qualifications and responsibilities of the Safety Officer. The safety officer for this trial will be Dr. Adam Whaley-Connell. He is the Associate Chief of Staff for Research and Development and the Research Integrity Officer at the Harry S Truman VA Hospital. In addition, Dr. Whaley-Connell is Professor in the Division of Nephrology at the University of Missouri and a clinician scientist. As Safety Officer, Dr. Whaley-Connell will review eligibility criteria with Dr. Manrique-Acevedo, and be unblinded as to treatment assignment (if needed). 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 VA Research and Development Committee, the IRB and the DSMC. In addition, the safety officer may comment on whether the study investigator needs to report any specific out-of-range laboratory data.

7.0 Privacy and Confidentiality

Protection against risks

The potential risks of the proposed studies will be minimized by: 1) strictly following inclusion and exclusion criteria, and study protocol; 2) close clinical supervision of all study procedures; 3) appropriate training and demonstrated experience of all study personnel (we have assembled a research team which includes scientists and clinician-scientists with significant experience in human research and metabolic diseases to help anticipate and reduce the risks to subjects); and 4) the complete confidentiality of the record keeping process to be employed.

Previous section described specific strategies to minimize risk of planned procedures and interventions. Risks of loss of confidentiality is reduced by assigning all subjects a data identifier code. Hard copies of data are stored in locked file cabinets, and only the PIs, biostatistician and study coordinator have access to the locked files. The health history questionnaire and all subject screening and experimental data and pertinent medical paper records will be placed in individual files and coded for de-identification. Computerized records of experimental data will be similarly coded and will be maintained on a password secure system. Individual names or initials are not used in any discussions or publications of the data. All PIs and key personnel have received HIPAA privacy and security training, and will be the only ones with access to the medical histories. We are confident that the privacy of individuals participating in the study will be effectively maintained.

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 seven 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 five working days of awareness.

8.0 Communication Plan

This study is limited to the local site of the Truman VA Endocrinology and Primary Care Clinics and its core facilities.

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