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Principal Investigator: Jaume Padilla, PhD
Project IRB #: 2008181
Application Title: Restoring vasodilator actions of insulin in patients with type 2 diabetes (Role of physical activity in restoring vascular insulin sensitivity in skeletal muscle of patients with type 2 diabetes).

Collaborating Investigators:
Elizabeth Parks, PhD
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Study purpose: The purpose of the present project is to determine the effects of increased walking and shear stress on insulin-stimulated leg blood flow in sedentary patients with type 2 diabetes (T2D).

This study is classified as a clinical trial and is registered on Clinicaltrials.gov #NCT03203694.

Background: The notion that habitual aerobic exercise increases insulin-induced vasodilation is largely founded on rodent studies, hence the urgent need for human studies, especially in T2D. For example, it remains unknown if increased walking, the most common form of physical activity, enhances skeletal muscle vasodilator actions of insulin in T2D. In addition, the molecular mechanisms by which exercise improves vasoreactivity to insulin have not been examined in humans. We propose that in T2D patients who are sedentary (i.e., the vast majority), increased walking signifies a vital strategy to correct the impairment in insulin-stimulated leg blood flow. We hypothesize that this restoration of vascular actions of insulin is associated with a reduction of protein kinase C (PKC) activation and endothelin-1 (ET-1) expression in the vasculature of skeletal muscle. Indeed, we contend that increased physical activity and associated hemodynamic forces (e.g., shear stress) are a direct form of vascular medicine in humans. We capitalize on this concept by proposing that recapitulation of exercise-like hemodynamic signals (i.e., increased shear stress) using a non-exercise stimulus (i.e., lower body heating) will lead to favorable vascular adaptations, including enhanced vascular insulin sensitivity.

Inclusion and exclusion criteria: Men and women with T2D who are overweight and obese (BMI 25-50 kg/m2), 35-65 years of age, and sedentary (<60 minutes structured exercise/week) will be recruited from the local community and the University Hospital Cosmopolitan Diabetes Center. A cohort of non-obese (BMI 19-29kg/m2), recreationally active men and women aged 35-65 will be recruited to serve as healthy control subjects. Patients with T2D will be classified based on physician diagnosis. Exclusion criteria: 1) cardiovascular disease including myocardial infarction, heart failure, coronary artery disease, stroke; 2) renal or hepatic diseases; 3) active cancer; 4) autoimmune diseases; 5) immunosuppressant therapy; 6) excessive alcohol consumption (> 14 drinks/week for men; >7 drinks/week for women); 7) current tobacco use; 8) pregnancy; 9) foot ulcers; 10) diabetic neuropathy; 11) mobility limitations; 12) uncontrolled hypertension (≥180 systolic / 100 diastolic mmHg).

For the experimental studies utilizing an ingestible telemetry sensor to monitor the core temperature of subjects, the following exclusion criteria will also be applied: 13) swallowing or esophageal disorders, or impairment of the gag reflex for experimental studies using the telemetry sensor; 14) gastrointestinal tract diseases or disorders; 15) individuals scheduled for nuclear magnetic resonance (NMR)/magnetic resonance imaging (MRI) scanning while the telemetry sensor is in the body.

Sub-study: A separate cohort of men and women ages 18- 65 will be recruited from the local community. Same exclusion as that listed above will be applied.

Number of subjects to be enrolled and justification: We plan to enroll a total 120 subjects to test hypotheses A, B, and the sub-study described below. This number will allow for subject dropout and provide a sufficient sample size to establish the efficacy of the interventions.

Experimental Studies:

Hypothesis A: Increased walking decreases vascular PKC activation and ET-1 expression, thus leading to an improvement in insulin-stimulated leg blood flow. To test this hypothesis, T2D subjects will be randomized to a control non-exercise group (n=20) or walking group (n=20). Subject randomization will be accomplished by computerized randomization software. The walking program will consist of 45 minutes of walking (at 60% of VO_2 peak) 5 days per week for 8 weeks. Exercise interventions of similar intensity and duration have produced beneficial vascular effects in humans. Three days per week of the exercise intervention will be supervised and conducted at the MU Physical Activity and Wellness (MUPAW) center, a core facility in our department. Subjects will have the option to perform the other two walking sessions per week on their own. Prior to initiating the walking program, subjects will undergo a standardized exercise stress test with assessment of ECG. The workload corresponding to 60% of heart rate reserve will then be identified. Our study physician, Dr. Camila Manrique, oversees these tests and interprets the ECG following American College of Sports Medicine (ACSM) guidelines. These exercise interventions and protocols in patients with chronic disease are routine at MUPAW.

Prior to participating in the study, subjects will provide written informed consent, complete a medical health history questionnaire, and receive randomization into experimental groups (walking v. control). Subjects will then be provided with a FitBit pedometer to wear throughout the 8-week period to quantify steps taken. Subsequently, before and after the 8-week period, subjects will be scheduled to come to the University Hospital Clinical Research Center (CRC) for a single testing day (Experimental Visit). On the testing day, subjects will arrive at 7am after an overnight fast, having not taken medications, and not exercised for 24-48 hrs. The cohort of healthy control subjects (n=20) will participate in the experimental visit once, with no exercise intervention.

On the Experimental Visit, body composition will be assessed with dual-energy X-ray absorptiometry. Subjects will then be placed supine in a quiet, climate-controlled room (22–23°C) and flow-mediated dilation in the brachial and popliteal arteries will be assessed as a marker of vascular function. A pressure sensor (tonomter, the size of a pencil) will be placed over the skin of the neck region to obtain the pressure wave form in the carotid artery. Next, the insulin clamp, coupled with measures of leg blood flow, will be performed following standard procedures at the University Hospital CRC. Subjects will be instrumented for measures of heart rate (using standard lead II ECG), arterial blood pressure (using automated sphygmomanometry and beat-to-beat via Finometer), and leg blood flow (using duplex Doppler ultrasound and contrast-enhanced ultrasound, CEU). After a minimum of 20 min supine rest, baseline cardiovascular measurements will be collected, blood samples obtained and the hyperinsulinemic-euglycemic clamp will start. Briefly, insulin diluted in 0.9% saline with 5 ml of the subject's blood is infused with a 10 min priming dose followed by a constant infusion at 40 mU•m−2•min−1, a dose used to mimic postprandial insulin concentrations. Glucose is maintained at fasting values throughout via a variable 20% dextrose infusion. Leg blood flow, arterial blood pressure, and heart rate will be collected for 5-min periods at 20-min intervals throughout the 180-min clamp allowing the magnitude as well as the temporal profile of these variables to be determined. For microvascular blood flow using CEU, measurements will be performed before and during the first 60 min of insulin stimulation. Briefly, assessment of microvascular perfusion utilizing CEU involves ultrasound imaging of the leg skeletal muscle and abdomen during the administration of one vial (1.3mL) of an ultrasound contrast agent diluted in 30mL of 0.9% saline that will be given using a handheld syringe pump through the IV at a rate of 2mL/min. This agent, called Definity, is composed of tiny microbubbles smaller than the size of a red blood cell. These bubbles stay inside the blood vessels and go where the red blood cells go. This contrast agent allows us to evaluate microvascular blood flow. Definity has been FDA approved for use in humans during ultrasound of the heart cavity and has been shown to be safe. The total volume of blood drawn per insulin clamp will be less than 120mL. During the hyperinsulinemic-euglycemic clamp, Dr. Elizabeth Parks will perform the skeletal muscle biopsies from the vastus lateralis muscle, following established procedures at the CRC. Skeletal muscle biopsies will be processed for molecular characterization via immunofluorescence, PCR, and Western blotting. Assessments will include expression/activity of PKC, MAPK, eNOS, and ET-1, among other targets related to insulin signaling and vascular health.

The length of this visit will be 8 hours.

Hypothesis B: Increased leg vascular shear stress using a non-exercise stimulus (i.e., lower body heating) recapitulates the beneficial vascular effects of increased walking, including a reduction in vascular PKC activation and ET-1 expression, as well as an improvement in insulin-stimulated leg blood flow.

To test this hypothesis, T2D subjects (n=20) will undergo lower body heating for 60 min per day for 7 days at MUPAW. Potential minor deviations from this timeline may occur dependent on subject and study personnel availability. Lower body heating will be accomplished via water bath immersion at a temperature of 40.5°C to target a core temperature of 38.5°C, using a commercially available temperature-controlled water bath. Importantly, this protocol has improved glucose control in patients with T2D (Hooper, 1999) and women with polycystic ovarian syndrome (Ely et al., 2019). Additionally, this heating protocol improved vascular outcomes (blood pressure, endothelial function, and arterial stiffness) in women with polycystic ovarian syndrome (Ely et al., 2019) and sedentary men and women (Brunt et al., 2016). Furthermore, a 20-year longitudinal study showed that whole-body heating (via regular Finnish sauna use) protects against fatal cardiovascular disease and events (Laukkanen et al., 2015). Notably, whole-body heat therapy has been demonstrated to be safe for vulnerable clinical populations, improving prognosis and vascular outcomes of patients with chronic heart failure (Kihara et al., 2009) and peripheral arterial disease (Akermann et al., 2019; Thomas et al., 2017). Hooper (1999) hypothesized that the reason warm-water immersion improved glucose control in T2D and hence can be an effective treatment for the disease is that heating increases blood flow to the muscle. While a large portion of the flow is directed to the skin for heat dissipation, there is firm evidence that muscle blood flow is also markedly increased with local heating. Therefore, assessing the efficacy of passive heating as a potential therapy to improve vascular health and insulin-stimulated leg blood flow in patients with T2D is highly warranted.

During our lower body heating protocol, the subject is seated, and water reaches a level between the navel and the armpit. Additionally, water temperature is below the threshold for pain sensation and has been shown by us and others to produce robust increases in limb blood flow. During each supervised visit the subject is continuously monitored following protocols already published by other groups which include urine sample collection, nude body weight recordings, drinking provided water ad libitum, and frequent recordings of core or tympanic membrane temperature, heart rate, and blood pressures, and perception of thermal strain and exertion. Urine samples will be read using a digital refractometer. A digital scale will automatically log nude body weight; when the subject stands on while alone in a private bathroom while getting changed. Heart rate and blood pressure will be monitored using wearable devices. During the first and last immersion sessions the subject's core temperature will be continuously monitored via ingesting an FDA-approved telemetry pill (CorTemp sensor, HQ Inc., USA) that is used to measure core temperature of different populations (e.g. hospital patients, firefighters, military personnel). As a surrogate marker for core temperature during remaining visits, the subject will have their temperature recorded for 5-seconds from their ear canal using a commercially available infrared tympanic membrane thermometer with single-use probes. Perception of thermal strain and perceived exertion be recorded using validated visual scales. If the subject feels overheated at any time, they will be allowed to exit the water bath.

Prior to participating in the study, subjects will provide written informed consent and complete a medical health history questionnaire. Upon eligibility, subjects will be provided with an accelerometer to wear on their hip for a total of 14 days (7-days before, and 7-days during the heating intervention)- to quantify levels of physical activity in free-living conditions. Additionally, subjects will be provided with a 3-day food recall diary during the weeks they receive the accelerometer and before the experimental visits.. Subsequently, before and after the lower body heating intervention, subjects will be scheduled to come to MU-PAW for a single testing day (Experimental Visit). On testing days, subjects will arrive in the morning after an overnight fast, having not taken morning medications, and not exercised for at least 24hrs. The cohort of healthy control subjects (n=20) will participate in the experimental visit once. This cohort will also wear an accelerometer for 7 days to assess free-living physical activity. The healthy control cohort will not participate in the 7 day lower-body heating intervention.

On the Experimental Visit, body composition will be assessed with dual-energy X-ray absorptiometry. Subjects will then be placed supine in a quiet, climate-controlled room (22–23°C) and flow-mediated dilation in the brachial and superficial femoral arteries will be assessed as a marker of vascular function. Flow-mediated dilation will be measured using an ultrasound machine that images the arteries dilatory response to a blood

pressure cuff inflation of 250mmHg.that is maintained for 5-minutes. A tonometer will be placed over the skin of the neck region to obtain the pressure wave form in the carotid artery. An intravenous catheter will be placed in an elbow crease for blood sampling and collection of endothelial cells. Endothelial cells will be collected using 2-4 sterile J-shaped guidewires that are sequentially advanced ~10 cm through the intravenous catheter and then retracted, and the cells recovered by washing the wires with a dissociation buffer. Subjects will be seated recumbently and given a 75g glucose beverage for the 2hr OGTT, during which, 5mL of blood will be sampled, blood pressure obtained (using an automatic blood pressure monitor), and leg blood flow (using duplex Doppler ultrasound) will be recorded every 15-minutes. At baseline, 10mL will be drawn. Accordingly, the total amount of blood drawn will be 50mL. The length of this visit will be 4-hours long.

The figure below (Figure 1.) provides a visual representation of the planned timeline of measurements. However, minor deviations from this timeline may occur dependent on subject and study personnel availability:

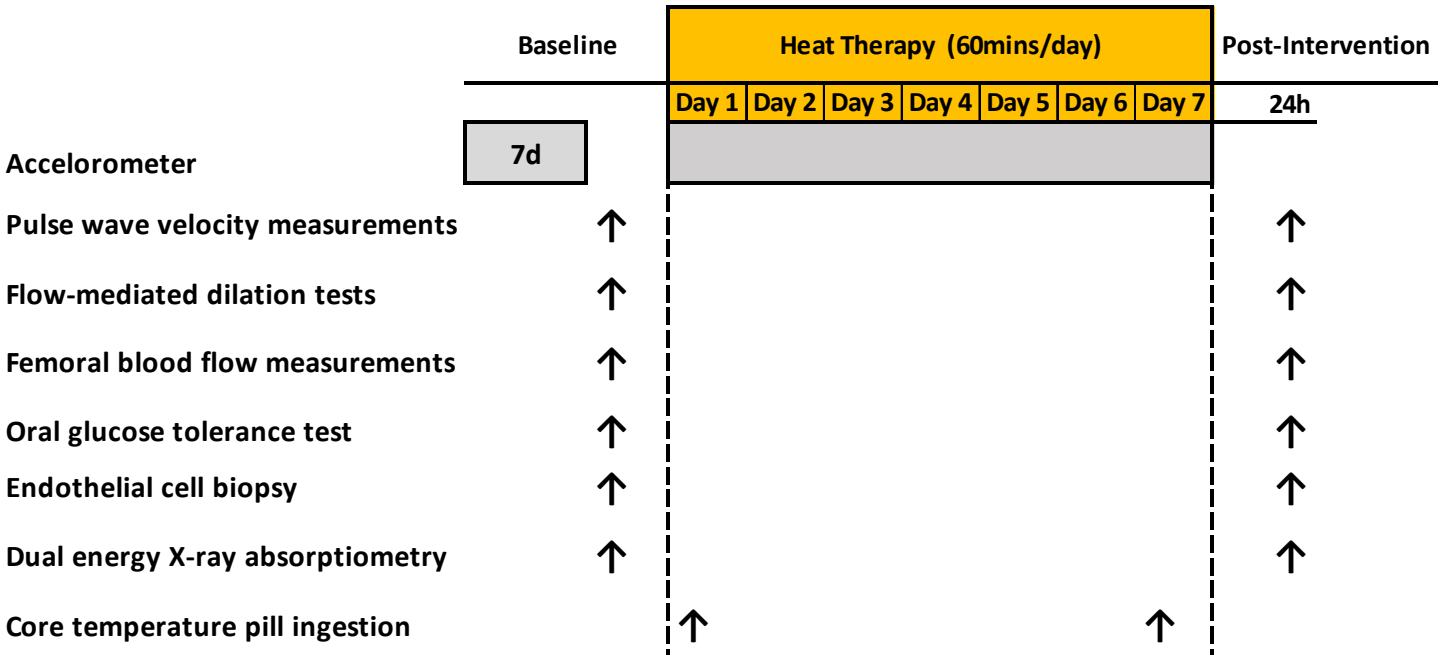

Figure 1. Planned timeline of measurements for Hypothesis B.

Sub-study:
Prior to participating in the study, each subject will provide written informed consent. Subjects will be scheduled to come to the University Hospital Clinical Research Center (CRC) for one testing day. On the testing day, subjects will arrive at 7am after an overnight fast, having not taken medications, and not exercised for 24-48 hrs. Upon arrival to the CRC, height, weight, and waist circumference will be measured.

Experimental Intervention:
We are interested in determining whether a single-bout of leg heating will result in favorable vascular adaptations, specifically enhanced vascular insulin sensitivity. To test this hypothesis, subjects (n=20) will be asked to participate in a single visit involving 1-hour of unilateral leg heating. The contralateral leg will serve as the internal control. Leg assignment will be randomized by flipping a coin. Leg heating will be accomplished via water bath immersion at a temperature of 40-42°C, using our custom-made temperature-controlled water bath. During leg heating, the subject is seated and water reaches the level below the knee. This water temperature is below the threshold for pain sensation and has been shown by us and others to produce robust increases in limb blood flow. Ultrasound measures of leg blood flow will be performed during heating to document the increase in leg blood flow. Next, the insulin clamp, coupled with measures of leg blood flow, will be performed following standard procedures at the University Hospital CRC. Subjects will be instrumented for measures of heart rate (using standard lead II ECG), arterial blood pressure (using automated sphygmomanometry and beat-to-beat via Finometer), and leg blood flow (using duplex Doppler ultrasound and

contrast-enhanced ultrasound, CEU). After a minimum of 20 min supine rest, baseline cardiovascular measurements will be collected, blood samples obtained and the hyperinsulinemic-euglycemic clamp will start. Briefly, insulin diluted in 0.9% saline with 5 ml of the subject's blood is infused with a 10 min priming dose followed by a constant infusion at 40 mU•m−2•min−1, a dose used to mimic postprandial insulin concentrations. Glucose is maintained at fasting values throughout via a variable 20% dextrose infusion. Leg blood flow, arterial blood pressure, and heart rate will be collected for 5-min periods at 20-min intervals throughout the 180-min clamp allowing the magnitude as well as the temporal profile of these variables to be determined. For leg microvascular blood flow using CEU, measurements will be performed before and during the first 60 min of insulin stimulation. Briefly, assessment of microvascular perfusion utilizing CEU involves ultrasound imaging of the leg skeletal muscle during the administration of one vial (1.3mL) of an ultrasound contrast agent diluted in 30mL of 0.9% saline that will be given using a handheld syringe pump through the IV at a rate of 2mL/min. This agent, called Definity, is composed of tiny microbubbles smaller than the size of a red blood cell. These bubbles stay inside the blood vessels and go where the red blood cells go. This contrast agent allows us to evaluate microvascular blood flow. Definity has been FDA approved for use in humans during ultrasound of the heart cavity and has been shown to be safe. The total volume of blood drawn per insulin clamp will be less than 120mL.

The length of this visit will be 6 hours.

Sources of materials: Research materials will include medical history information, blood samples for assessment of glucose, insulin, HbA1c, lipids, liver enzymes, blood urea nitrogen levels, and electrolytes, height, weight, heart rate, blood pressure, leg blood flow and conductance, flow-mediated dilation, whole body insulin sensitivity, gene and protein expression of target proteins from skeletal muscle biopsies. Subject data will be coded, and only the investigators will have access to the codes. All specimens and data will be used for the sole purpose of generating new knowledge and publishing of peer-reviewed articles. The data will not be used for any other purposes.

Potential risks to human subjects:
Exercise testing and training: The risks associated with high physical activity are minimal but potentially include hypertension, cardiac arrhythmias, syncope, and rarely (in patients without known cardiovascular disease) angina pectoris or myocardial infarction. The mortality rate from testing in symptomatic patients is approximately 1 in 10,000 patients. The mortality rate in asymptomatic subjects is several-fold lower. All subjects will have a medical history taken and all subjects with a history of cardiovascular disease will be excluded. The primary risks of exercise are likely to be fatigue or muscle soreness. If subjects experience muscle soreness, we will prescribe light stretching to reduce the soreness. We anticipate that soreness will occur primarily in the first few days but then subside as they become accustomed to the exercise. Subjects will be fully informed of the potential discomfort and risks and will be able to withdraw from the study at any time. Graduate students trained in exercise physiology will be administering the testing and oversee all the training sessions. A physician will be present during stress testing in subjects with T2D or two risk factors according to ACSM guidelines. The exercise tests will be performed at the University Hospital CRC or MU-PAW.

Lower body heating: While we do not expect any adverse events, the potential risks associated with the heating protocol is dehydration and/or the onset of heat stress. To prevent these risks, all sessions are supervised, and the subject continuously monitored for signs and symptoms that include lightheadedness, dizziness, weak pulse, nausea, headache, feeling unbearably warm, or a tympanic membrane temperature or core temperature above 39.5°C. During each supervised visit the subject is continuously monitored following protocols already published by other groups (Ely et al., 2019; Brunt et al. 2016), which includes urine sample collection, nude body weight recordings, drinking provided water ad libitum, and frequent recordings of core and/or tympanic membrane temperature, heart rate and blood pressures, and perception of thermal strain and exertion. If the participant has signs or symptoms, they are removed from the water bath, placed in a recovery chair with feet elevated, and cooled down with cold packs until tympanic membrane temperature or core temperature returns to pre-immersion values. If signs or symptoms worsen such as fainting, agitation, confusion, seizures, inability to drink, or core temperature will not fall below 39.5°C despite application of cooling packs, immediate medical attention will be sought. To keep the subject's body temperature at 38.5°C, they will be sat with water reaching a level between the naval and armpit, which will help with heat dissipation (Carter et al., 2014; Rivas et al., 2016). To prevent dehydration, if the urine sample has a urine specific gravity

reading >1.02; and/or a bodyweight >1% less post-immersion, they will drink a 500mL bottle of water in the presence of staff. Additionally, the subject will drink bottled water throughout immersion.
Temperature of the water will be continuously monitored.

Insertion of venous catheters: 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 nurse placing the venous catheters.

Endothelial cell collection: This procedure can lead to some discomfort associated with insertion of the venous catheter needle, local bleeding, and a small hematoma (~10% of cases); small risk of blood clot formation; minimal risk of infection or significant external blood loss, risk of fainting. Regarding collecting endothelial cells, there are no reported risks associated with this procedure other than those related to venous catheter placements. Experts at Albert Einstein College of Medicine in New York, who established the technique, reported no adverse events to our knowledge.

Skeletal muscle biopsies: The potential risks associated with the muscle biopsy procedure are acute issues of muscle tightness, soreness, and bruising. A more severe problem can be the risk of infection; however, this is unlikely to occur. To avoid these issues, the biopsy procedures are conducted with sterile techniques and with the use of local anesthesia (lidocaine). This procedure is routinely performed at the University Hospital CRC.

Oral glucose tolerance test: The risks of the OGTT are minimal. This is a regular glucose dose. Some subjects may feel nausea after drinking the glucose, rarely do people experience lightheadedness. Trained personnel conduct this study.

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

Body composition: The amount of radiation received during the DEXA scan is less than that of an airline flight from California to New York and back. Women of childbearing age will undergo a pregnancy test prior to the scan and excluded if pregnancy is confirmed.

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.

Ingestible telemetry pill for core temperature: Manufacturer instructions detail that the CorTemp® pill should not be used by individual's that have any of the following: 1) someone less than 80 pounds, 2) someone diagnosed with but not limited to diverticulitis, inflammatory bowel disease, gag reflex disorders or impairments, previous gastrointestinal surgery, hypomotility of the gastrointestinal tract (such as Ileus), 3) someone undergoing Nuclear Magnetic Resonance (NMR) or MRI scanning while the CorTemp® is within the body,4) someone having a cardiac pacemaker or other implanted electro medical device, 5) people who have difficulty swallowing. Therefore, if potential subjects have anything from the previous list, they will be excluded from the study.

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

Recruitment: Patients with T2D will be recruited from the MU Hospital Cosmopolitan Diabetes Center as well as the Columbia community by Dr. Manrique and our research team. Participants for the sub-study will be recruited from the Columbia community. Subjects will be recruited via flyers and word of mouth. We will also contact our previously recruited subjects from other studies that have indicated they would like to participate in our future research projects. We may also ask our study physician to give our flyer to some of his patients with T2D. We will also utilize MU Info announcements that will reach MU faculty and staff, MU students, and University of Missouri hospital employees with our recruitment ad via email.

Informed Consent: For all subjects, informed written consent will be obtained using Consent Forms approved by the MU IRB. One copy of the executed consent form will be given to the subject and one copy will be stored in a secured file cabinet.

Protection against risk: Trained personnel will be administering all exercise testing. A physician will be present during stress testing according to ACSM guidelines. The exercise tests, muscle biopsies, and insulin clamps will be performed at the University Hospital CRC with supervision of the study physician, Dr. Manrique. Graduate students trained in exercise physiology will oversee the exercise sessions at the MUPAW center. Subjects will be fully informed of the potential discomfort and risks and will be able to withdraw at any time. The informed consent process, which will entail an oral description as well as written description of the study, will attempt to fully inform the subject. 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. This information will only be accessible to the PI, collaborating investigators, or approved research personnel. All records will be kept in a locked filing cabinet which only the research personnel have access to. Computerized records of experimental data will be similarly coded and will be maintained on a password secure system. The only confidential information to be disclosed would relate to the subject's medical history. The purpose of obtaining a careful medical history is to exclude individuals who would be at risk from the experimental procedures. All medical and biographical information will be held in strict confidentiality and no disclosures of personal identity will be allowed unless specifically requested by the subject. Copies of executed consent forms, as well as the experimental log book and any other subject research information, are kept in a locked cabinet in our laboratory.

Drugs:
Ethyl chloride: Used in skeletal muscle biopsy procedure. Ethyl chloride will be sprayed on the biopsy site to numb the skin surface before subcutaneous administration of lidocaine, a local anesthetic. Dosage: Area requiring analgesia will be sprayed until a thin snow film forms.
Lidocaine: Used in skeletal muscle biopsy procedure. Lidocaine, a numbing agent, will be administered subcutaneously. Dosage: Less than 5 mL.
Insulin**:** Used in hyperinsulinemic-euglycemic clamp procedure. Human insulin (Novalin) will be administered to increase circulating levels of insulin while maintaining fasting levels of blood glucose. Dosage: Constant infusion at 40 mU•m−2•min−1, a dose used to mimic postprandial insulin concentrations.
Dextrose: Used in hyperinsulinemic-euglycemic clamp procedure. Dextrose will be infused IV during the insulin clamp to maintain fasting blood glucose. Dosage: Infusion rate will be variable between subjects depending on their levels of insulin sensitivity.
Perflutren (Definity): Contrast agent used in contrast enhanced ultrasound procedure. Definity will be infused IV utilizing a syringe pump at a rate of 2mL/min immediately prior to ultrasound imaging of leg muscle.

Compensation: For hypothesis A experiments, T2D subjects will be compensated $800 for completion of the duration of the study. Healthy control subjects will be compensated $200 for the completion of the single Experimental Visit.

For hypothesis B experiments, subjects will be compensated $800 for completion of the duration of the study. Healthy control subjects will be compensated $200 for the completion of the duration of the study.

For both hypothesis A and B experiments, if the subject or investigator discontinues the study, the subject will be compensated $15 for every hour spent in the laboratory/testing facility.

Subjects will be paid $100 for completion of the sub-study. If the subject or investigator discontinues the study, subject will be compensated $15 for every hour spent in the laboratory/testing facility.

Potential benefits of the proposed research to human subjects and others: Individual subjects may benefit from the proposed study by learning more about their cardiovascular and metabolic health. In addition, subjects may benefit from interventions (exercise or leg heating) designed to improve their vascular function.

Importance of knowledge to be gained: Current literature supports that defects in vascular insulin signaling represent a causal factor in the pathogenesis of T2D and atherosclerosis, underscoring the urgency to identify the molecular mechanisms of vascular insulin resistance. A detailed understanding of the mechanisms underlying the defects in vascular insulin actions is critical for developing effective strategies to correct it. Restoration of vascular insulin sensitivity will in turn lead to improved glycemic control and amelioration of vascular disease in T2D. The proposed research will establish the mechanisms by which T2D causes impaired insulin-induced dilation. Furthermore, this research will determine for the first time if increased walking or increased shear stress, via non-exercise stimulus (i.e., leg heating) improves insulin-stimulated leg blood flow, a viable target to improve glycemic control in T2D patients.

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Supporting literature:

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