PDE5 Inhibition for Obesity-Related Cardiometabolic Dysfunction

NCT02819440

Protocol Date: 4/30/2019

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Table of Contents:

1.0 Background

Obesity is a risk factor for nearly all cardiovascular (CV) diseases including coronary artery disease, hypertension, and heart failure. Obesity and its adverse cardiometabolic sequelae represent major and worsening public health burdens. In 2000, the World Health Organization declared obesity as the greatest threat to the health of Westernized nations.[1] In the U.S. Obesity accounts for over 400,000 deaths per year and affects 35% of the adult population.

Increased cardiovascular risk in obese individuals appears to depend largely on the degree of metabolic dysregulation. Obese individuals without other metabolic risk factors (such as glucose intolerance, dyslipidemia, or central adiposity) have cardiovascular outcomes similar to those of lean individuals. In contrast, obese individuals with a high burden of metabolic risk factors are at very high risk of future events. Notably, interventions that improve insulin sensitivity[2] and cardiorespiratory fitness[3] can reduce cardiovascular risk in obese individuals, even in the absence of weight loss.

The poor effectiveness of lifestyle-based interventions highlights the need for new strategies to improve cardiometabolic health in obese individuals. Estimates suggest that over half of all Americans are currently attempting weight loss, and another quarter are attempting to maintain achieved weight loss. Despite these efforts, many people never achieve significant weight reduction and weight loss recidivism at one year approaches 90%.[4,5] Thus, lifestyle-based interventions alone are unlikely to achieve significant reductions in obesity-related cardiovascular risk at the population level. These data underscore the potential value of physiologic interventions directly targeting metabolic fitness in obesity.

2.0 Rationale and Specific Aims

Obesity is frequently accompanied by abnormalities in energy homeostasis, cardiopulmonary function, and peripheral insulin resistance. A large body of experimental and clinical data suggests that these abnormalities contribute to the increased cardiovascular and metabolic risk in obese individuals, making them potentially attractive targets for intervention.

Energy expenditure is a major determinant of weight gain and loss.[6] The primary components of total energy expenditure are physical activity (30%), the thermic effect of food (10%), and resting energy expenditure (REE, 60%). Obese individuals have approximately 50% lower REE, indexed to body weight, compared with normal weight adults.1 Weight loss is associated with a further reduction in REE, an adaptive mechanism that resists maintenance of a reduced body weight and ultimately contributes to weight regain. Adjunctive strategies to complement diet and exercise by safely increasing metabolic rate during rest and physical activity may assist in efforts at weight loss and weight loss maintenance.

Obesity is associated with reduced cardiopulmonary capacity. Maximal oxygen consumption during exercise is inversely related to body mass index and fasting insulin level.[7,8] Furthermore, exercise capacity is strongly related to clinical outcomes in obese individuals, highlighting its value as an endpoint in this population.[9,10]

Increasing cGMP activity can be achieved pharmacologically by inhibiting phosphodiesterase type 5A (PDE5), which catabolizes cGMP. The clinically-available drugs sildenafil and tadalfil are specific inhibitors of PDE5 and multiple studies suggest that they have beneficial cardiometabolic effects.

Strategies to increase energy expenditure may improve the success of lifestyle interventions in obese individuals. In an animal model of obesity, PDE5 inhibition increased energy expenditure for the same amount of physical activity. PDE5 inhibition improves insulin sensitivity and beta cell function in experimental models and small human studies. Prior studies have been limited by small sample size and less sensitive methods for assessing insulin sensitivity and secretion. PDE5 inhibition increases intracellular cGMP tone. Prior studies have used plasma or urine concentrations of cGMP as an index of "target-organ" responsiveness to NP, proposing the ratio of cGMP to natriuretic peptide (NP) concentrations as an index of NP sensitivity.

We propose to evaluate energy expenditure in response to a metabolic intervention in which weight loss is not a direct target. We hypothesize that PDE5 inhibition will increase resting energy expenditure, improve insulin sensitivity, and increase the cGMP/NP ratio in obese adults.

Aim 1. **To examine the effect of PDE5 inhibition on energy expenditure in obese adults.**
Subjects will undergo a 4-hour metabolic chamber protocol at baseline and after 12 weeks of placebo or tadalafil therapy to measure resting and sub-maximal exercise energy expenditure. The primary endpoint is resting energy expenditure at 12 weeks, adjusted for baseline values.

Aim 2. **To examine the effect of PDE5 inhibition on insulin sensitivity.**
Subjects will undergo FS-IVGTTs which will provide a validated estimate of insulin sensitivity and the acute insulin response to glucose. The primary endpoint is insulin sensitivity at 12 weeks, adjusted for baseline values.

Aim 3. **To examine the effect of PDE5 inhibition on cGMP tone and circulating mediators of cardiometabolic risk.**
Subjects will undergo fasting blood draw at baseline and 12 weeks to measure cGMP and NPs. The primary endpoint is cGMP/NP ratio at 12 weeks, adjusted for baseline value

We may perform interim unblinded analyses of select secondary endpoints (e.g. fat mass, blood pressure) after getting approval from the data safety monitor and the study biostatistician. We will not perform any unblinded analyses of primary endpoints until study procedures are complete.

3.0 Animal Studies and Previous Human Studies

Prior experimental work

In a high-fat diet mouse model of obesity and insulin resistance, Dr. Wasserman showed that sildenafil increases resting and ambulatory energy expenditure, independent of activity level. Sildenafil also conferred resistance to weight gain from high fat diet over 12 weeks. Finally, PDE5 inhibition improved insulin sensitivity and muscle glucose uptake assessed by hyperinsulinemic-euglycemic clamp. These observations support measuring energy expenditure and insulin sensitivity as primary endpoints in a human trial of PDE5 inhibition.

Prior observational studies

We have performed metabolomic profiling in more than 3,000 individuals in the Framingham Heart Study and Malmo Diet and Cancer Study. In prior studies, we have shown associations of branched chain amino acids, aromatic amino acids, tryptophan metabolites, nucleotides, glutamine, and glutamate with obesity, insulin resistance, and other metabolic traits.[28] In pre-diabetic individuals, we have shown that branched chain and aromatic amino acids predict incident diabetes up to 12 years later.[29] We have also demonstrated the association of a lysine metabolite, 2-aminoadipic acid, with future diabetes, and shown that 2-aminoadipic acid modulates glucose homeostasis *in vivo*.[33] In the Malmo Diet and Cancer Study, we have demonstrated that many of these metabolites predict cardiovascular events and subclinical atherosclerosis.[30] In analyses of >110 lipid species, we have demonstrated that lipids of lower carbon number and double-bond content are associated with a higher risk of diabetes.[34]

We examined metabolite profiles before and after OGTT in 377 non-diabetic Framingham Offspring cohort participants.[35] Dynamic changes were observed in multiple pathways demonstrating the feasibility of altering metabolomic profiles in response to a metabolic intervention (glucose administration). We identified metabolite responses that distinguished individuals with and without insulin resistance, including in pathways not associated with insulin or glucose action. Findings from these studies support the use of metabolomic profiling to assess the effect of interventions such as PDE5 inhibition on pathways associated with cardiometabolic risk. Exploratory analyses may also allow the identification of additional metabolites related to increased cGMP signaling.

Pilot Studies of PDE5 inhibition

We conducted a pilot study of tadalafil versus placebo in insulin-resistant individuals with BMI > 27 kg/m2. In double-blind fashion, individuals were randomized to receive tadalafil (20mg/day) or placebo for 3 months (n=53). During follow up, 3 subjects from the tadalafil arm compared with 2 subjects from the placebo arm withdrew from the study. Reasons for withdrawal in the tadalafil arm included headache (n=1) and muscle aches (n=2). There were no instances of hypotension, visual disturbances, or serious adverse events among individuals randomized to tadalafil.

In the pilot study, we found improvement in the oral disposition index, a composite measure of insulin release and resistance (p=0.009). We also observed a trend toward improvement in the Matsuda index (p = 0.05). HOMA-IR improved significantly in subjects with BMI above the median (36 kg/m2) assigned to tadalafil (p=0.02 for comparison with placebo), but not in the entire sample (which included overweight individuals). There was no significant change in weight in either group. In additional preliminary work, we have performed hyperglycemic clamps in subjects with impaired glucose tolerance randomized to sildenafil (n=21) 25 mg TID or placebo (n=21) for 3 months. PDE5 inhibition improved the insulin sensitivity index and disposition index, measured by clamp (both p<0.05).

Experience with metabolic techniques

This proposal brings together a multi-disciplinary and collaborative team to leverage some of Vanderbilt's unique institutional and intellectual resources.

Energy Balance Laboratory. [redacted], an NIH-funded expert in the quantification and interpretation of energy expenditure and physical activity data, directs the lab and Metabolic

Core [redacted]. The group has extensive experience using whole room indirect calorimetry (metabolic chamber) to assess resting and physical activity related energy expenditure and Vanderbilt is one of only 13 institutions in the U.S. with this technology available. [redacted] will also consult on the use of accelerometry to quantify physical activity.[39-41]

Assays of insulin and glucose homeostasis. [redacted], an NIH-funded expert in metabolic studies in humans and mice has extensive experience conducting IVGTTs, hyperinsulinemic-euglycemic clamps, and hyperglycemic clamps.[42,43] He and his team have completed over 200 in the past 5 years and have expertise in the software and statistical analyses for FS-IVGTT studies.

Cyclic GMP signaling and metabolomic profiling. Our group has experience in the quantification of metabolites involved in cGMP signaling and broad-based metabolomics platforms. Dr. Wang has performed several cohort based studies using a targeted mass spectrometry-based platform that quantifies approximately 300 metabolites.[28-30,35] Dr. Brittain has also participated in metabolomics studies using plasma and tissue.

4.0 Inclusion/Exclusion Criteria

Inclusion criteria:

1) Adults (ages 21-50)
2) Obesity (BMI ≥ 30 kg/m_2)
3) Evidence of abnormal glucose metabolism at screening visit, defined as *any* of the following:

- Hemoglobin A1C between 5.7-6.4
- Fasting glucose between 100-125
- HOMA IR greater than 2.5

Rationale for Inclusion Criteria: This study is designed to target individuals at the highest risk for the development of diabetes and subsequent adverse CV outcomes. The upper age cutoff is specified to reduce the likelihood of enrolling individuals with subclinical cardiac disease, which could influence the cardiopulmonary endpoints.

Exclusion criteria:

1. Age <21 or > 50
2. BMI < 30 kg/m_2
3. Systolic blood pressure (SBP) < 100, > 150 mmHg
4. Current anti-hypertensive medication use, including diuretics
5. Current use of organic nitrates
6. Current use of PDE-5 inhibitors (sildenafil, tadalafil, vardenafil)
7. History of reaction to PDE-5 inhibitors
8. Known HIV infection
9. Use of medications that strongly alter CYP3A4 activity
10. History of myocardial infarction, angina, uncontrolled cardiac arrhythmia, stroke, transient ischemic attack, or seizure
11. Known non-arteritic ischemic optic retinopathy (NAIOR)
12. History of hearing loss
13. Estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m_2 by the modified diet in renal disease (MDRD) equation

14. Hepatic transaminase (AST and ALT) levels greater than three times the upper limit of normal
15. Known pregnancy or breastfeeding or those unwilling to avoid pregnancy during the course of the study
16. History of priapism
17. Use in excess of four alcoholic drinks daily
18. History of diabetes mellitus or use of anti-diabetic medications
19. Known anemia (men, Hct < 38% and women, Hct <36%)
20. Menopause
21. Inability to exercise on a bicycle

Rationale for Exclusion Criteria:
1) Given the increasing prevalence of cardiovascular disease and hypertension in older obese subjects, we will exclude individuals over age 50 from the study. Our institutional IRB defines adults as 19 years and older.
2) Given our intent to study the effects of this medication in clinically obese individuals, we will exclude individuals with BMI < 30 kg/m2.
3) Given the potential for mild changes in blood pressure and the potential interaction of tadalafil with antihypertensive medications, we will exclude subjects with low-normal blood pressure and those requiring treatment with anti-hypertensive medication during the study.
4) Given the possibility for hypotension when tadalafil and anti-hypertensive medications are combined, we will exclude subjects currently taking anti-hypertensive medications.
5) Given the potential for severe hypotension when combining tadalafil and nitrates, we will exclude individuals taking nitrates.
6) Given the possibility for interaction between two medications from the same class and the intent to study the native effects of the medication, we will exclude individuals already taking a PDE-5 inhibitor.
7) Given the increased likelihood of a second reaction in those who have already experienced a prior side effect of a PDE-5 inhibitor, we will exclude those who have had a previous reaction to a PDE-5 inhibitor (sildenafil, tadalafil, vardenafil).
8) Given the inhibition of CYP3A4 and subsequent elevation of tadalafil levels by protease inhibitors, which are used only to treat HIV infection, we will exclude individuals with known HIV infection.
9) Given their potential effects on CYP3A4 activity and, as a result, tadalafil levels, we will exclude individuals taking oral ketoconazole, itraconazole, erythromycin, rifampin, phenytoin, or carbamazepine; topical preparations of antifungal or antibiotic medications will not be contraindicated as these do not appear to significantly alter systemic CYP3A4 activity.
10) Given the temporal association of myocardial infarction, angina, uncontrolled cardiac arrhythmia, stroke, transient ischemic attack, or seizure with tadaladil in post-market surveillance (which has not been directly attributed to the drug itself), we will exclude those with a past history of these conditions.
11) Given the temporal association of NAIOR with tadalafil in post-marketing surveillance, we will exclude those with this condition from the study.
12) Given the temporal association of hearing loss with tadalafil in post-marketing surveillance, we will exclude those with this condition from the study.
13) Given the altered metabolism of tadalafil in subjects with renal dysfunction, we will exclude those with abnormal renal function (by eGFR as measured by MDRD).
14) Given the altered metabolism of tadalafil in subjects with hepatic dysfunction, we will exclude those with transaminase levels more than three times the upper limit of normal.

15) Given the untested effects of tadalafil on pregnant or breastfeeding women, we will exclude women who are pregnant or breastfeeding or who are able to become pregnant and not practicing an acceptable method of birth control during the study (including abstinence).
16) Given the increased risk of priapism with use of tadalafil, individuals with a prior history of priapism will be excluded.
17) Given that alcohol use in excess of four drinks at one time combined with tadalafil 20mg can precipitate hypotension, individuals who consume more than this amount daily will be excluded.
18) Given that we are interested in determining the effect of tadalafil on insulin resistance and metabolism, we will exclude individuals who are taking or are at risk of starting medication for diabetes treatment, which would affect these measures.
19) Given that blood samples will be taken at three time points over the 3 month study, we will exclude individuals with known anemia.
20) Given the menopause affects energy balance in females, we will exclude menopausal women, determined via interview
21) Given that one of the objectives of our study is to investigate the effect of tadalafil on exercise capacity, we will exclude individuals who are unable to exercise on a bicycle.

5.0 Enrollment

Subjects will be recruited from Vanderbilt's Research Notification Distribution List, Research Match, Subject Locator, and the Vanderbilt Nutrition and Diet Assessment Research Core. Within the distributions, a brief description of the study will be provided and a contact number for further information. Upon contacting the study staff, the potential participant will receive a complete explanation of the protocol procedures by telephone.

6.0 Study Procedures

This study will consist of a screening visit and study testing at 3 time points over the course of 14 weeks. Patients will be consented over the phone prior to their screening visit and will sign the consent in-person during their screening visit at the Clinical Research Center. At screening, to determine if subjects have evidence of abnormal glucose metabolism, the following tests will be conducted: Hemoglobin A1C, fasting glucose, and fasting insulin. HOMA-IR will be calculated from fasting glucose and insulin. The patient will also be screened to determine if C-peptide, metabolic and blood counts are within normal limits.

Subjects meeting exclusion criteria based on abnormal laboratory values due to chronic, stable conditions may be enrolled at the discretion of the PI in consultation with the subject's treating physician.

Urine testing: A urine sample will be collected at screening, and if patient qualifies, at baseline and at 12 weeks, and a urine microalbumin will be measured. Samples may be stored for additional analyses.

Baseline Testing: Baseline testing will take place over 2 half-days. Each half-day visit will last approximately 4 hours. Determined by the randomization outcome, subjects will also receive a 7 week supply of tadalafil or the placebo at the second half-day visit. The study staff will discuss how to take the study medication and answer any questions. The following protocols, tests, and surveys will be performed at baseline:

Metabolic Chamber Protocol: The room calorimeter (2.6 m x 4 m; ~19 m3 volume) assures high-precision measurements of physiological variables (e.g. oxygen consumption, heart rate) in a controlled environment and semi-naturalistic conditions (i.e. not wearing a breathing mask or a mouthpiece). The room is equipped with a bed, a television, a toilet, a sink, and an exercise bicycle**.** Accuracy and precision of the system for measuring EE is tested monthly using an alcohol combustion test. Precision averages 99.2 ± 0.5% (mean ± SD) over 24 hours and 98.6 ± 2.1% over 30 minutes. The system reliably detects short-term changes in metabolic rate to 2.7% over 30 min and 0.6% over a 2 h measurement period.[50,55] In a previous study in adults conducted in our room calorimeter, mean short-term within-subject variability in resting energy expenditure was 3.5±2.2%. The operation of the room calorimeter is controlled and data are collected minute-by-minute using a customized computer program. Subjects will arrive to the CRC after an overnight (~12 hour) fast, have vital signs and a blood sample taken before entering the chamber. Participants will enter the chamber at 8:00 am and remain there until 12:00 pm. After entering the room, subjects will lie on the bed for 30 minutes. Resting energy expenditure will then be averaged over the next 30 minutes while the patient remains on the bed. At approximately 9am, the patient will consume a meal containing approximately 25% of individual daily energy needs and containing 55% of energy from carbohydrates, 30% from fat, and 15% from protein. Diet composition will be determined using the Nutrition Data System for Research (NDSR) software.

Cardiopulmonary Exercise Test Protocol: The test will be conducted inside the metabolic chamber at the CRC according to the American College of Sports Medicine guidelines using an electrically-braked cycle ergometer. We have chosen the ergometer test because it may be better tolerated than the exercise treadmill by obese adults, mechanical efficiency of individuals varies less, measurement of work performed is more accurate, and fatigue-induced change in work efficiency is feasible to measure. Heart rate and rhythm (12-lead electrocardiogram), blood pressure, breath-by-breath gas exchange, and power will be monitored or measured. Peak VO2 uptake will be calculated (ml O2/kg/min) and the anaerobic threshold will be measured. The pedal cadence of ~50 revolutions per minute will be maintained via feedback from a display on the ergometer until voluntary termination of the task (i.e. reaching tolerable fatigue level). Based on our experience the test will last up to 45 minutes. The following measures will be calculated:

- *Energy expenditure (EE) rate (kcal/min):* Oxygen consumption (VO2), carbon-dioxide production, air flow rate, temperature, barometric pressure, and air humidity will be measured minute-by-minute and integrated to calculate respiratory quotient (RQ = VCO2/VO2) and EE using the Weir equation.56,57
- *Resting EE rate (kcal/min; REE):* Calculated as the average EE during a 30-min period while the subject is lying in bed having fasted for ~12 hours.
- *Physical activity-induced EE (kcal/day):* EE related to physical activity will be calculated as total EE above the resting level while performing the ergometer test.

Fasting intravenous glucose tolerance test (FS-IVGTT) Protocol: We will conduct insulin modified FS-IVGTTs, which provide a validated estimate of insulin sensitivity and the acute insulin response to glucose (AIRg).[7] The AIRg is a measurement of insulin secretion (i.e. beta

cell function). The disposition index accounts for the hyperbolic relationship between AIRg and S_I and is associated with risk of diabetes. After an overnight ~12-hour fast, subjects will assume a supine position and we will place an intravenous catheter in each arm, one for sampling and one for infusion, and allowed to rest for 15 minutes.

We will collect baseline samples at t=-15 min and t=-5 min for measurement of glucose and insulin. At t=0, we will bolus 300 mg D-glucose/kg in a 25% glucose-saline solution over 1 minute. At t=20 min, we will give a bolus of 0.02 units/kg of regular insulin followed by a 5 mL 0.9% saline flush (Actrapid, Novo Nordisk, Princeton, NJ) intravenously, to improve the accuracy of the FS-IVGTT in patients in whom insulin responses are reduced or absent.[8-13] Use of low dose insulin avoids saturation of insulin binding sites and maintains insulin concentrations in a range where insulin sensitivity is independent of insulin level.[14] Blood will be collected for measurement of glucose and insulin at t=2, 3, 4, 5, 6, 8, 10, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 90, 110, 130, 150, 170, and 180 min. We will estimate insulin sensitivity (S_I), glucose effectiveness at basal insulin (S_G), glucose disappearance constant (K_g), AIRg, and disposition index using the MINMOD program (MINMOD Millennium v. 6.02).[15-17] Plasma glucose will be determined immediately after bedside plasma centrifugation, using the glucose oxidase method (YSI 2300, Yellow Springs Instruments). Plasma insulin will be measured by RIA using samples stored at -80 °C until assay.

Physical Activity (PA): Total PA in the free-living environment will be measured using tri-axial accelerometers ActiGraph-GT9X+ (Actigraph, Pensacola, FL) for 7 days. Participants will be asked to maintain their regular sleep-wake schedule and wear the monitor for consecutive 7 days. If subject wears the monitor on their wrist, subject will be instructed to wear the monitor on their non-dominant wrist. The raw data will be collected at 30 Hz, preprocessed and converted to vector magnitude counts. After assessing monitor wear/no-wear time, the outcomes of interest will be total number of counts and time and spent in sedentary, light, moderate and vigorous activity intensities. Intra- and inter-individual differences in the amount and patterns of physical activity will be assessed using methods described in previous studies in adults. Subjects will be asked to wear the accelerometer for 7 days after their second baseline half-day, for 7 days before their interim visit, and for 7 days before their 12-week visit.

Quality of Life: Quality of life will be assessed using the Medical Outcomes Study Short-Form Health Survey (SF-36). The SF-36 is a generic, multi-item scale with eight domains that include: physical functioning, bodily pain, mental health, social functioning, vitality, general health perceptions, role limitations-physical, and role limitations-emotional. Individual subscale scores, a mental component summary score, and a physical component score summary score are calculated.[64] The SF-36 has been widely used to measure quality of life among patients with obesity and is sensitive to changes in weight and BMI.[65,66] The International Index of Erectile Function (IIEF) will be used to measure sexual function in men. The IIEF addresses four domains of male sexual function (erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction) and is the gold standard for measuring erectile dysfunction.[67] The Female Sexual Function Index will be used to measure sexual function in women. The FSFI is comprised of five domains: desire and subjective arousal, lubrication, orgasm, satisfaction, and pain/discomfort and is the most widely used measurement of female sexual function.[68] A nurse practitioner [redacted]) with extensive experience administering quality of life surveys will perform these assessments.

Dual-Energy X-ray Absorptiometry (DEXA): Body composition will be assessed at baseline and at 12 weeks. Participants will lie flat on a table while a machine takes pictures of different areas of their bodies. This test will last about 15 minutes.

Interim Visit: This visit will take place approximately 6 weeks after the baseline visits. At this visit, we will discuss any noted side effects, conduct a pill count, perform a blood draw, and give subjects their next 6-week supply of the study medication.

12 Week: The tests performed at baseline (described above) will be performed approximately 12 weeks after beginning the study medication.

7.0 Optional Genetic and Future Research on Biological Samples

As part of the informed consent, participants will be asked whether blood, not used in this analysis, may be stored for future analysis. If the participant does not consent to this, samples will be used only for the present study and excess samples discarded.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Risks and Discomfort

Tadalafil is a medication with proven safety. This drug was initially approved by the FDA in 2003 for the treatment of erectile dysfunction (under the trade name CIALIS) at a starting dose of 2.5mg and a maximum dose of 5mg for daily use and at a starting dose of 10mg and a maximum dose of 20 mg up to once daily for as needed use. Tadalafil 20mg daily has been used without serious adverse effects in a recent study of healthy men with mild erectile dysfunction. Safety data are provided in the package inserts and Physician Desk Reference (PDR through MICROMEDEX) entry and summarized here. In 2009, the FDA approved use of this medication at a dose of up to 40mg daily for management in pulmonary arterial hypertension (under the trade name ADCIRCA) based on Phase III clinical trial data. In pre-clinical safety data, tadalafil 20mg daily produced no significant decrease in supine or standing systolic/diastolic blood pressure when compared to placebo (supine decreased by 1.6/0.8 mm Hg and standing decreased by 0.2/4.6 mm Hg).

Contraindications for the use of tadalafil are concomitant use of organic nitrates (because of severe hypotension) and a history of hypersensitivity reaction to tadalafil; individuals with those characteristics are excluded from this study. Individuals using anti-hypertensive medications are at increased risk of having low blood pressure when using tadalafil, and these individuals are excluded from this study as well. Safety testing and post-marketing surveillance have identified serious potential side effects for which study subjects could be at risk. These include myocardial infarction, unstable angina, heart failure, uncontrolled arrhythmias, stroke, sudden visual loss, sudden hearing loss, and priapism. These conditions are all rare, and whether they are directly related to tadalafil use is unclear. Individuals with a history of these conditions are excluded from the protocol. Study subjects are advised to stop taking tadalafil and seek medical attention immediately should they experience any of the above or symptoms that may indicate any of the above. The most common adverse reactions experienced during pre-clinical safety trials and post-surveillance marketing for individuals taking tadalafil 20mg daily are: headache (15%), dyspepsia (10%), back pain (6%), myalgia (3%), nasal congestion (3%), flushing (3%), and pain in a limb (3%). In a study of otherwise healthy men with mild erectile dysfunction, tadalafil 20mg daily for 9 months was associated with the following adverse events: headache (16%), back

pain (14%), dyspepsia (8%), gastroesophageal reflux disease (8%), myalgia (8%), pain (4%), pain in an extremity (4%), dizziness (3%), and nasopharyngitis (3%) [23].

Because headache is a relatively common side effect of the medication, we will ask subjects to contact us if they experience a persistent headache. If it is determined by the study team that the duration and severity of the subject's symptoms indicate a need to decrease the subject's dosage, the dosage may be decreased to 10mg daily. After adjusting the dose of the medication, study personnel will communicate with subjects and the dose may be readjusted as symptoms decrease. Board certified Internist/Cardiologists (the MPIs) are available by pager and telephone at all times, if not physically present, during the study.

Venipuncture

The risks of drawing blood are uncommon and may include bleeding, minor infection and bruising. Commonly, having blood drawn is painful, and rarely can lead to infection at the site of the blood draw. The amount of blood drawn is small, and represents an exceedingly small percentage of the amount of the total blood volume and will not represent a significant risk to the subject.

Subjects will be removed from the study if symptoms or medical issues arise that are deemed clinically significant by the PI or NP. A few examples are listed here:

1) If the participant has or develops clinically significant contraindications to taking the study drug.
2) If the participant has or develops clinically significant lightheadedness, particularly when standing at any blood pressure.
3) If the participant develops systolic blood pressure less than 100 mm Hg when seated or standing.
4) If clinically significant anemia is identified

As stated in the consent form and reviewed at the time of enrollment, subjects may end participation at any time without prejudice.

Adverse Events

Investigators will report all serious, unexpected, and study-related adverse events to the Data Safety Monitor and the local Institutional Review Board in a timely manner according to IRB Policies and Procedures.

Adverse event reporting: It is proposed that events possibly related to the study will be graded by the investigators in the following manner:

Adverse Event (AE) Grading Scale:
0= No Adverse Event or within normal limits
1= Mild Severity: Transient laboratory test alterations; discomforts noted but no disruption of daily activities; no therapy, or only symptomatic therapy required
2= Moderate Severity: Laboratory test alterations indicating injury without long-term risk; discomfort sufficient to modify normal daily activity; specific therapy required (i.e., more than symptomatic)
3= Serious Severity: Laboratory test indicating a serious health threat or permanent injury; incapacity, inability to work, inability to perform normal daily activity; hospitalization required or prolonged; emergency treatment required; life-threatening events; death

f) Breech in confidentiality that may involve risk to that individual or others;
g) Complaint of a participant that indicates an unanticipated risk or which cannot be resolved by the research staff; or
h) Other event that is unanticipated, involved risk to participants or others and was possibly related to the research procedures.

Adverse events of a serious severity thought to be related to the research protocol will be reported to the NIH, the Vanderbilt IRB, the DSM and Drs. Wang and Brittain 24 hours of occurrence and the specific study will cease to perform the suspect procedure until review. The IRB will have the responsibility to confirm the severity of the adverse event, determine if it is likely that the adverse event was related to the study, and make recommendations for continuation, modification or cessation of the study. Data monitoring will routinely be performed by Drs. Wang and Brittain and in response to the reports of serious adverse events.

9.0 Study Withdrawal/Discontinuation

We plan to monitor closely for adverse effects and remove a subject for symptoms deemed related to study drug. The inability to tolerate tadalafil or hypersensitivity to drug will trigger withdrawal. Furthermore, the DSM has the ability to stop the study for safety concerns.
If at any time during the study, participants decide they do want their blood samples or metabolic chamber data analyzed or included in the study results, they can contact the PI via phone or in writing (all contact information is provided in the consent form). At that point, the patient's data would be removed from the electronic database.

10.0 Statistical Considerations

Aim 1:

Statistical analysis: We will test the effects of tadalafil versus placebo on the primary endpoint (resting energy expenditure) and the secondary endpoints, in similar fashion. We will use a generalization of the Wilcoxon test – the proportional odds ordinal logistic model – with the week 12 value of resting energy expenditure as the dependent variable. Based on our pilot study, we will assume dropouts to be infrequent. Predictor variables will include the baseline resting energy expenditure, age, gender, BMI, race, and a treatment group indicator. The effects of the baseline value and age will be assumed to be smooth but not linear by using restricted cubic spline functions. A sensitivity analysis will assign Week 12 non-completers to the worst rank and then apply the proportional odds model. In exploratory analyses, we will also study whether treatment effects differ by patient subgroups. These pre-defined analyses will be performed for sex, race (white or non-white), and BMI. We will also perform sensitivity analyses excluding those non-compliant with study pills (missing ≥ 2/3 doses).

Statistical power: Power analysis is based on an approximation to the proportional odds analysis controlling for baseline by subtracting (analysis of change) instead of regression. Data on the within-individual standard deviation (SD) of resting energy expenditure are limited, particularly from the metabolic chamber. Thus, as an approximation we used the between-individual SD (364 kcal) from our prior studies of 108 obese (BMI 32 kg/m2, 56% female) individuals. We estimate the correlation between pre- and post-treatment measurements to be 0.5 so that the SD of the within-subject change is also 364 kcal. We model several scenarios, up to a dropout rate of 15%, a high estimate. Even with a dropout of 15%, we will have excellent power (84%) to detect a relatively modest 182 kcal difference between tadalafil and placebo groups, with a type I error of 0.025. We have set the p-value threshold at 0.05/2 = 0.025, to account for the 2 primary clinical endpoints in the proposal (corresponding to Specific Aims 1

and 2). 182 kcal represents ~10% of the total daily energy expenditure for an obese adult from our prior studies.

Aim 2:
Statistical analysis: The analytic approach will parallel that described for Aim 1 (substituting the endpoints from Aim 2). The primary analyses will focus on the effect of PDE5 inhibition on insulin sensitivity (SI). Secondary analyses include measures of insulin secretion (AIRg, and disposition index), body composition, and quality of life.

Statistical Power: We selected the sample size based on the baseline insulin sensitivity (SI) measured by FS-IVGTT in a prior study of patients with insulin resistance and on the magnitude of effect of PDE5 inhibition on insulin sensitivity in our preliminary data. A higher SI indicates better insulin sensitivity. We plan to enroll 100 subjects per arm and allow up to a 15% dropout rate over 3 months. With 85 completed subjects per arm, the study will have 90% power with alpha 0.025 to detect a 23% increase in SI from a baseline of 5.89±2.75 x10-4•min-1 per mU/L (mean ± SD) versus no change in the placebo arm. We observed a 30% increase in Si in our pilot data using a lower equivalent dose of PDE5 inhibitor over a shorter study duration. Therefore, we will have excellent power to detect a modest effect of PDE5 inhibition. The baseline AIRg from our prior studies in a similar population was 577.3±259.8. With 85 completed subjects per arm, we will have 90% power to detect a 25% increase in AIRg, the other component of disposition index. In prior studies we observed within-subject correlations of 0.7-0.8 for repeated IVGTT measurements (SI, AIRg), but we based our power estimates on a more conservative within-subject correlation of 0.5 for both variables to ensure adequate statistical power. We and others have observed that the acute (AIRg)- and late-phase insulin secretion and disposition index are significantly increased in African American subjects independent of body mass index or insulin sensitivity.[21,22] We will address this by stratifying for race (in addition to gender) at the time of randomization.

Aim 3:
Statistical analysis: The analytic approach will parallel that described for Aim 1 (substituting the endpoints from Aim 3). We will also assess the correlation of the change in cGMP/NP ratio with the change in the primary endpoints (energy expenditure, insulin sensitivity) in the active arm and in the overall sample.

Although the metabolomics studies are exploratory, we will adjust the statistical significance threshold for metabolite comparisons using a conservative Bonferroni correction ($p = 0.05/300$). As a secondary analysis we will do variable clustering to empirically organize the metabolites into clusters such that within-cluster correlations are high and between-cluster correlations of individual metabolites are low. Each cluster will be summarized by its first principal component and these components will be tested for treatment effects using the Wilcoxon test. This reduces multiplicity problems and focuses power.

Statistical power: Based on our pilot study, the within-individual SD of cGMP/NP over 12 weeks in the absence of intervention is 3.6 pmol/pg. With 85 completed subjects per arm (assuming 15% dropout), we will have 90% power with alpha 0.05 to detect a 19% increase in cGMP/NP. We observed an increase of 85% in cGMP/NP in tadalafil-treated subjects in our smaller pilot study, with a smaller sample. Thus, we will have excellent power to detect modest effects. Analysis of metabolites and VEGF-A165b measurements are considered exploratory; therefore, power calculations were not specifically performed for those endpoints.

11.0 Privacy/Confidentiality Issues

Consent forms, medical history data, and study data are stored in secured files, either in locked file cabinets or in a locked room separate from medical records and coded such that all subject identifiers have been removed. As an additional precaution all HIPAA regulated information is stored in an electronic file separate from other study data. Only approved study staff (determined by Drs. Wang and Brittain) will be given authorization to access the database. Bio-specimens are processed and labeled with barcode labels that include the subjects electronically generated study code and date of sample collection. The bio-specimens are stored in locked freezers in the study Laboratory; only approved study staff has access to the keys for each freezer. Access to the electronic freezer inventory of the specimens is kept on a secure password protected computer.

12.0 Follow-up and Record Retention

The duration of this study is estimated to be 4 years. The duration of record retention will be at least 6 years after study completion, but the possibility exists for indefinite archival of study information via the REDCap database. Should the outcome of this study prompt future investigations, the participants may be contacted to obtain follow-up information and invited to participate in additional studies.

13.0 Literature cited

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