TITLE: THE TESTOSTERONE TRIAL

THE CARDIOVASCULAR TRIAL PROTOCOL

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Study Summary

Title	The Testosterone Trial - The Cardiovascular Trial
Protocol Number	808676
Study Design	Randomized, placebo-controlled, double-blind study
Study Duration	One year
Study Centers	Multi-center trial involving 9 clinical sites geographically distributed across the United States
Objectives	The primary specific aim of the main Testosterone Trials is to test the hypotheses that testosterone treatment of elderly men whose serum testosterone concentrations are unequivocally low – and who have symptoms and/or objectively measured abnormalities in at least one of three areas that could be due to low testosterone (physical or sexual function and vitality), cognition, and anemia) – will result in more favorable changes in those abnormalities than placebo treatment. The primary objective of The Cardiovascular Trial is to determine if testosterone treatment is associated with favorable changes in cardiovascular risk indicators, including atherosclerotic plaque burden by CT angiography and serum markers of cardiovascular and metabolic risk.
Number of Subjects	160
Diagnosis and Main Inclusion Criteria	Subjects who qualify for The Testosterone Trial will be recruited for The Cardiovascular Trial if they have an estimated glomerular filtration rate of >60 mL/min/1.73 m^2 and no history of coronary artery bypass graft.
Study Product, Dose, Route, Regimen	AndroGel®, testosterone in an alcohol-water gel, will be administered transdermally in doses from 5 to 15 grams per day, as necessary to maintain the serum testosterone concentration within the range of normal for young men.
Duration of administration	AndroGel or placebo will be administered to each subject for 12 months.
Reference therapy	The effects of AndroGel on the primary and exploratory end points will be compared to effects of placebo on these end points.
Statistical Methodology	The primary end point for this trial will be the effect of testosterone on change in atherosclerotic plaque burden, compared with placebo. Exploratory end points will be left ventricular mass, calcium score change, serum markers of cardiovascular risk, blood pressure, and central adiposity by CT.

Introduction

This document is a protocol for a human research study to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and institutional research policies and procedures.

1.1. Effects of Aging in Men

As men get older, they experience many conditions, often together, that eventually result in the inability to perform many activities of daily living, an increased propensity to fall, and decreased independence. These conditions include mobility disability and decreased vitality. Elderly men also experience increased rates of anemia and metabolic syndrome, decreased sexual function, and memory impairment. These conditions likely have multiple causes, but one cause that could contribute to all of these conditions is a low serum testosterone concentration. When young hypogonadal men are treated with testosterone, they experience improvements in sexual function, muscle mass and strength, bone mineral density, sense of well-being, and anemia. However, the effects of testosterone therapy on the cardiovascular system are not well described. This Cardiovascular Trial is designed to assess the effects of testosterone treatment on several cardiovascular end points in men ≥65 years and have serum testosterone concentrations <275 ng/dL who are participating in the Testosterone Trial. These end points include coronary atherosclerosis as assessed by CT angiography, and cardiovascular risk factors, such as blood pressure, lipids and lipoproteins, and markers of glucose metabolism, inflammation, coagulation and platelet function, endothelial function, and myocardial damage.

Decrease in Testosterone as Men Age

As men age, their serum testosterone concentration falls gradually from age 20 to over age 80, as demonstrated by both cross-sectional (1) and longitudinal studies (2, 3). By the eighth decade, approximately 30% of men have concentrations of total testosterone lower than normal for young men and 70% have free testosterone concentrations lower than normal for young men (3). Age-related decline in testosterone concentrations is associated with decreases in physical function, sexual function, vitality and, in some studies, decreases in memory and cognitive function. Whether this change contributes to, or is independent of, atherosclerosis is currently unknown.

Significance of Adding a Cardiovascular Trial to the Testosterone Trial

Although testosterone was once considered to be a risk factor for cardiovascular disease, several recent observational studies show an inverse association between serum testosterone concentration and cardiovascular disease, the metabolic syndrome and diabetes. For example, in a study of 40-79 year-old community dwelling men in Rancho Bernardo, the serum testosterone concentrations were inversely correlated with blood pressure (4), prevalence of

diabetes and the risk of future diabetes (5). In the same cohort, men with a testosterone level less than 250 ng/ml had a significantly increased risk of mortality compared to men with higher levels, independent of covariates. In a larger cohort study from England, testosterone levels in men were inversely associated with cardiovascular risk with a graded stepwise association throughout the entire range of testosterone (6). This association was independent of common risk factors.

End Points of Cardiovascular Risk

Coronary Atherosclerosis

Computer tomographic angiography (CTA) using 64+slice multidetector computerized tomography (MDCT) will be the principal imaging modality in this Trial to assess coronary atherosclerosis. The advantages of CT angiography compared to electron beam or multidetector CT to assess coronary calcification are the ability of the former to provide comprehensive information regarding the location, severity, and characteristics not only of calcified and mixed atherosclerotic plaques, but also of non-calcified plaques, which might respond better to intervention.

Multidetector CT angiography (MDCTA) has emerged as a promising non-invasive tool to examine directly the coronary artery wall, determine the degree of plaque burden and assess the degree of coronary artery stenosis (7). In addition, based on the tissue specific x-ray attenuation characteristics, MDCTA also provides additional information about atherosclerotic plaque composition. It is able to differentiate plaques that are calcified, predominantly fibrous, or ones that contain a large lipid pool (8, 9). Until recently, the only imaging test used in clinical practice to image the vessel wall was intravascular ultrasound, which risks complications at both the arterial access site and coronary arteries. Plaque characterization (i.e. determining the vulnerability of plaque rupture by examining its tissue components) is now possible using coronary CT angiography. New 64+ slice cardiac CT technology has high accuracy for the detection of lesions obstructing more than 50% of the lumen, with sensitivity, specificity, and positive and negative predictive values all better than 90% in patients without known CAD and has an important role in characterizing the vulnerable non-obstructive plaque (10). Tissue density measured by MDCT can be used to characterize atherosclerotic plaque composition.

We shall collect serum and plasma for the following cardiovascular and metabolic risk factors and consider measuring them at the end of the study depending on which ones are considered most informative.

Lipids and lipoproteins

In order to evaluate the effect of testosterone on lipid metabolism, we shall save sera for lipids and lipoproteins, total, HDL and LDL cholesterol, triglycerides, Lp(a), lipid particle size and number by NMR.

Markers of inflammation

In order to evaluate the effect of testosterone on biomarkers of inflammation, we shall save samples for high sensitivity C reactive protein (hs-CRP), lipoprotein phospholipase A2 (LpPLA2), interleukin 6 (IL-6), matrix metalloproteinase 9 (MMP-9), myeloperoxidase and cell adhesion molecules (ICAM).

- Hs-CRP is a protein made by the liver that increases with inflammation and is a well-validated biomarker of cardiovascular risk.
- $LpPLA_2$ is a calcium-independent phospholipase A2 enzyme, secreted by leukocytes and associated with circulating LDL and macrophages in atherosclerotic plaques. The preponderance of current evidence shows a proatherogenic role of this enzyme. Lp-PLA2 generates two proinflammatory mediators, lysophosphatidylcholine (LPC) and oxidized nonesterified fatty acids (oxNEFAs), which are ultimately responsible for atherosclerotic lesion development and formation of a necrotic core leading to more vulnerable plaques.
- IL-6 is a pro-inflammatory cytokine secreted by T cells and macrophages. This protein is associated with increased risk, especially in older individuals, for cardiovascular events and with decreased survival.
- The matrix metalloproteinases (MMPs) are a large family of zinc-dependent, extracellularly acting endopeptidases, the substrates of which are proteins of the extracellular matrix and adhesion proteins. Matrix metalloproteinase 9 (MMP-9), also known as gelatinase B, 92kDa gelatinase, or 92Da type IV collagenase (which represents the largest and most complex member of this family) has recently been a subject of growing interest in human pathology. MMP-9 is increased in patients with three-vessel coronary artery disease compared with controls or patients with one or two-vessel disease, implying that elevated levels of MMP-9 mirror severity of coronary atherosclerosis.
- Myeloperoxidase (MPO) is a leukocyte-derived enzyme that catalyzes the formation of a number of reactive oxidant species. In addition to being an integral component of the innate immune response, evidence has emerged that MPO-derived oxidants contribute to tissue damage during inflammation. MPO-catalyzed reactions have been attributed to potentially proatherogenic biological activities throughout the evolution of cardiovascular disease, including during initiation, propagation, and acute complication phases of the atherosclerotic process.
- Endothelial recruitment and adhesion of monocytes is the earliest detectable event in the pathogenesis of atherosclerosis. Normally, vascular endothelial cells have low adhesiveness for leucocytes, but when stimulated they express surface adhesion molecules that increase the adhesiveness and rolling of leucocytes along the endothelium. Adhesion molecules, such as ICAM-1 and VCAM 1, are shed into the circulation and can be measured, and increased serum concentrations have been

associated with clinical atherosclerotic disease, acute coronary syndromes and ischemic stroke.

Coagulation-platelet Markers

In order to evaluate the effect of testosterone on coagulation and platelet activation, we shall save plasma for levels of fibrinogen, d-dimer, CD40-ligand and tissue plasminogen activator. All of these biomarkers have been associated with increased risk for cardiovascular events. In addition urinary levels of thromboxane B2 will be measured. Thromboxane A2 (TXA2) is involved in platelet aggregation, vasoconstriction and reproductive functions, but has a half-life of only 37 seconds under physiological conditions. Thromboxane B2 (TXB2) is the stable product of the non-enzymatic hydration of TXA2, thus the production of TXA2 in vivo is typically monitored by measurement of TXB2 and its metabolite 2,3-dinor TXB2. 11-dehydro-TXB2 is a major metabolite of Thromboxane B2 (TXB2) found in urine and plasma produced by its dehydrogenation by the enzyme 11-OH-dehydrogenase.

Endothelial Dysfunction Markers

In order to evaluate the effect of testosterone on vascular stress, so called endothelial dysfunction, we shall save platelet poor plasma for circulating levels of microparticles as described below. We hypothesize that testosterone treatment will result in reduced levels of microparticles.

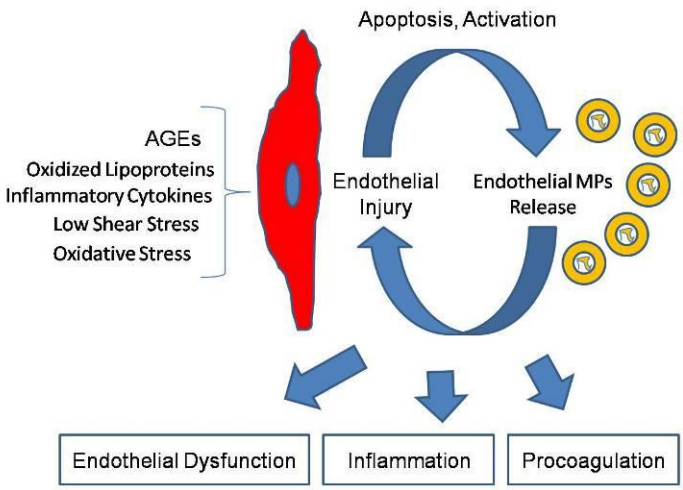

Fig 1. Multiple factors such as oxidative stress secondary to diabetes cause release of microparticles. This leads to endothelial dysfunction, inflammation and procoagulation. AGE indicates advanced glycation endproducts, MPs, microparticles and NO, nitric oxide.

Figure 1*. Microparticles (MPs) as Both Biomarker and Pathological Agent*

There has been a resurgence of interest in circulating MPs from endothelial cells, platelets and leucocytes because of their newly recognized and diverse physiological and pathological functions. MPs are plasma particles of <1 µm diameter that are formed by the exocytic budding of cell membranes. During their formation, the symmetry of the plasma membrane lipid bilayer is altered, resulting in the exposure of a surface that is rich in negatively charged phospholipids. In addition, the MPs bear antigens expressed on the surface of the cells from which they originate. It is this anionic phospholipid surface that can bind coagulation factors, and the expression of functional molecules such as tissue factor or selectins that mediate the biological actions of MPs. Furthermore, elevated levels of MPs have been found in a number of conditions associated with vascular dysfunction, thrombosis and inflammation (Figure 1). Thus, MPs are not innocent byproducts of cell

membranes but pathological vesicles that significantly promote cardiovascular disease.

Glucose Metabolism Markers

In order to evaluate the effect of testosterone on markers of glucose metabolism, we shall measure fasting serum glucose and insulin and hemoglobin A1c.

Myocardial Damage Markers

High sensitivity troponin indicates ongoing cardiac damage and predicts worsening congestive heart failure. Testosterone was recently shown to improve myocardial function in patients with cardiomyopathy. We therefore plan to save samples for troponin.

Study Objectives

The primary specific aim of the Cardiovascular Trial is to test the hypothesis that testosterone treatment of elderly men whose serum testosterone concentrations are unequivocally low will result in more favorable changes in cardiovascular risk markers than placebo treatment.

Primary Specific Aim

The primary specific aim of the Cardiovascular Trial is to test the hypothesis that testosterone treatment for one year, compared with placebo, of men ≥65 years who have an average serum testosterone concentration <275 ng/dL will decrease progression of coronary artery plaque, independent of baseline blood pressure, statin use or other cardiovascular risk factors.

Exploratory Specific Aims

To test the hypotheses that testosterone treatment for one year, compared with placebo treatment, will be associated with:

- Slowing of progression of atherosclerosis, as measured by coronary artery calcification (CAC)
- Decrease in abdominal visceral and subcutaneous fat, as measured by abdominal CT
- Improvement in markers of inflammation, coagulation-platelet activation, endothelial dysfunction and myocardial damage, as described above.
- Improvement in markers of glucose metabolism, including fasting serum glucose and insulin and hemoglobin A1c
- Improvement in fasting serum lipids and lipoproteins (total, HDL and LDL cholesterol, triglycerides, Lp(a), lipid particle size and number by NMR).

Study Design

General Design

This study is a randomized, placebo-controlled, double-blind trial of the effect of testosterone treatment on cardiovascular end points in men ≥65 years who have a low serum testosterone concentration who have enrolled in The Testosterone Trial and who meet additional entry criteria for the Cardiovascular Trial.

The study will be conducted at nine trial sites across the United States. The CT reading center at Los Angeles Biomedical Research Institute at Harbor-UCLA will coordinate the CT angiography studies. The Clinical Research Computing Unit at the University of Pennsylvania will be the Data Coordinating Center, and other central services will be the same as for The Testosterone Trial.

Study Endpoints

Cardiovascular Trial Endpoints

Primary Endpoint: Change in atherosclerotic plaque burden from 0 to 12 months, as assessed by cardiac CT angiography

Exploratory Endpoints:

- Coronary artery calcium by CT scan
- Visceral and subcutaneous fat by CT
- Lipids, lipoproteins, and lipid particle size
- Markers of glucose metabolism: fasting glucose and insulin, hemoglobin A1c
- Markers of inflammation, coagulation, endothelial dysfunction, and myocardial damage

Early Withdrawal of Subjects

Because these trials are based on the principle of “intent-to-treat”, every attempt will be made to follow and evaluate all enrolled subjects for the duration of the trials. Therefore, even if treatment is discontinued, the subject will be asked to complete the appropriate evaluations. If subjects develop renal insufficiency or develop a significant contrast allergy prior to follow up CT scan, a follow up contrast scan will not be conducted. These subjects will not be withdrawn from the CV trial, but will undergo a non-contrast calcium scan at 12 months, rather than a contrast study.

Subject Selection and Withdrawal

Number of Subjects

Subjects will be evaluated for study eligibility during their screening process for the main T Trial. Please note that if each of the main trials (Vitality, Physical Function, and Sexual Function) within the Testosterone Trial has met its

enrollment goals, subjects will be required to be eligible for and to participate in either the Bone Trial or the CV Trial, if they are still open to enrollment. At that time, all subjects who are screened for the Testosterone Trial will also be screened for the Cardiovascular Trial. In addition, all subjects who sign the T Trial baseline consent form will also receive details about the Cardiovascular Trial and will consent to the Cardiovascular trial. . The number of subjects will be 160 for CT angiography studies. Each of the nine sites is expected to enroll approximately 20 participants until the goal of 160 is reached. However, sites who are enrolling at a higher rate should continue to enroll in the trial above 20 participants, until the overall recruitment goal is met. It is projected that 85% of subjects allocated to treatment will complete the 12 months of treatment and be available for follow up measures by CT.

Inclusion Criteria for the Cardiovascular Trial

- Normal baseline renal function as assessed by eGFR (estimated glomerular filtration rate) > 60 ml/min/1.73m^2 at Screening Visit 1 and Month 12
- Willingness to consent and participate

Exclusion Criteria for The Cardiovascular Trial

- Weight >300 pounds
- Known allergy to iodinated contrast medium
- Inability to breath-hold for 10 seconds
- Current diagnosis of active atrial fibrillation
- Prior coronary artery bypass grafting by patient report or medical records

Trial Sites

The Cardiovascular Trial will be conducted at 9 of the 12 sites in The Testosterone Trial. These sites all have 64-slice MDCT scanners and sufficient experience using them, as determined by a questionnaire completed by the sites. Subjects will be identified and recruited by coordinators at the 9 sites with sufficient cardiac CT capabilities as identified by prior survey. The coordinators will approach patients for interest in participation in The Cardiovascular Trial.

CT Scanning

CT Reading Center

The Reading Center for CTA will be based at the Los Angeles Biomedical Research Institute at Harbor-UCLA and directed by Dr. Matthew Budoff. The Center will coordinate and oversee the collection of the data, store backup copies of the data, read each scan, send the results to the DCC and maintain quality control.

Subject participation

The Testosterone Trial site staff will communicate with the eligible subjects to explain the cardiac CT measurements and provide information concerning

cardiac CT imaging. The trial site staff will obtain informed consent and provide instructions to and assistance with travel to the CT scanning location.

CT examination

In a single session, each participant will receive sequential scans. A series of noncontrast scans will be performed, including a calcium scan of the heart to measure coronary artery calcium. Three slices through the umbilicus will be performed to measure visceral and subcutaneous fat. Then a contrast-enhanced study will be performed. Assessment of the location and degree of severity of coronary artery stenosis will be performed in each study.

The CT reader will interactively use axial images, multi-planar reconstructions and MIPs to assess the degree of luminal narrowing stenosis in all assessable coronary segments. Standard display settings will be used for the evaluation of the contrast-enhanced CT scans (window width 800 HU; window center 250 HU). Segments with stenosis 1-25% diameter narrowing will be defined as having minimal stenosis, 26%-50% diameter narrowing will be defined as mild stenosis, 51-75% diameter narrowing will be defined as moderate stenosis and those with >75% diameter narrowing will be defined as severe stenosis. We will specifically report ranges of percent diameter stenosis (not area stenosis), since the spatial resolution of CT angiography cannot achieve the precision of quantitative coronary angiography. The most narrowed diameter in each segment will be reported even if the plaque is eccentric. Segment stenosis score will also be generated based on the degree of underlying stenotic disease in each segment (0 = no plaque, 1 = 1-25% stenosis, 2 = 26-50% stenosis, 3 = 51-75% stenosis, 4 = >75% stenosis). The extent scores of all 15 individual segments will be summed to yield a total score ranging from 0 to 60. In addition, each coronary territory (right coronary artery left main, left anterior descending and left circumflex artery) will also be scored according to presence of most significant lesion.

Plaque quantification

Plaque area will be manually traced per slice in all affected coronary segments. The plaque area of each coronary plaque visualized in at least 2 adjacent slices (reconstructed slice thickness 0.6 mm) will be determined on all affected slices and plaque volume will be assessed by multiplying the area with the slice thickness. The total plaque per segment will be summed. A semi-quantitative plaque score previously utilized will be applied in each subject. Each segment of the coronary arteries and visual semi-quantification of coronary artery calcific and non-calcific plaque will be used. Each plaque will be multiplied by 1 for small plaque volume, 2 for medium plaque volume and 3 for large plaque volume. A small plaque will be defined as <1 mm in diameter perpendicular to the artery, medium as 1-2 mm in diameter and large as >2 mm. “Total Plaque Score” will be determined by summing the number of evaluable coronary segments with individual plaque scores (maximum plaque score = 45 [score of 3 for all 15 segments]). Since there may be difficulty making measurements from vessels

<1.5 mm in diameter, we will use both a 'raw' score (total of 45) and a percent plaque score that normalizes the plaque burden if the total number of measurable segments varies from subject to subject. The "Percent Plaque Score" (%PS) will be calculated as follows: (number of segments with plaque / total number of assessable segments) x 100. Both total plaque score and % plaque score will be evaluated as independent variables compared to other measures obtained in The Testosterone Trial, including demographics, cardiac risk factors, plasma hormone levels, coronary artery calcification, and coronary intimal medial thickness. The relationship between coronary plaque volume and traditional risk factors for cardiovascular disease will be investigated.

Composition of coronary atherosclerotic plaque

Plaque will be evaluated from both axial source images and multi-planar reconstruction images of the long axis at each site of the coronary arteries. Each coronary segment will be classified as either *normal* (no plaque), containing *non-calcified plaque*, containing *mixed plaque* with either predominantly non-calcified plaque (<50% of plaque area occupied by calcium) or containing *calcified plaque* (>50% of plaque area occupied by calcium) (9). Measurements are summarized in Table 1 below.

Calcified atherosclerotic plaque

This will be defined as any discernable structure which 1) has a CT density greater than the contrast-enhanced lumen; 2) is clearly assignable to the coronary artery wall; and, 3) is identified in at least 2 independent planes (11).

Non-calcified atherosclerotic plaque

This will be defined as any discernible structure which 1) has a CT density less than the contrast-enhanced coronary lumen but greater than the surrounding connective tissue; 2) is clearly assignable to the coronary artery wall; and, 3) is identified in at least 2 independent planes (11). Standard display settings will be used for the evaluation of the contrast-enhanced CT scans (window width 800 HU; window center 250 HU).

Cardiac chambers volume and mass

Left ventricular volume and mass will be measured on all CT scans using Simpson's method of discs as previously validated (12). Left atrial volume (and indexed volume) will also be simultaneously calculated on all scans.

Abdominal Subcutaneous and Visceral Adiposity

Abdominal adipose tissue areas will be measured by three CT slices obtained at the same visit as coronary measures. This will require the addition of one abdominal slice to the coronary calcium scanning protocol. After coronary artery scanning, a single CT slice will be obtained at the level of the umbilicus to assess intra-abdominal and subcutaneous fat distribution. Intra-abdominal adipose tissue area is quantified by delineating the intra-abdominal cavity at the internal-

most aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body (Figure 2). Using supine axial CT data at the umbilicus, the body wall of each subject will be manually traced, excluding all subcutaneous fat, using a spline tool available on the image software program. After each spline has been traced, the internal region (visceral tissues including visceral fat) of interest will be selected (Figure 2b). The total area within the visceral fat range will be computed. Fat is considered as pixels with densities between -130 and 0 Hounsfield units. The amount of extra-abdominal (subcutaneous) fat is calculated by subtracting intra-abdominal fat from total abdominal fat. This method limits the radiation exposure to a very minimum (one slice represents <0.01 mSev exposure), and the results are highly correlated with total abdominal fat. This single abdominal CT cut will be used to calculate the visceral, subcutaneous and intermuscular adipose tissue compartments.

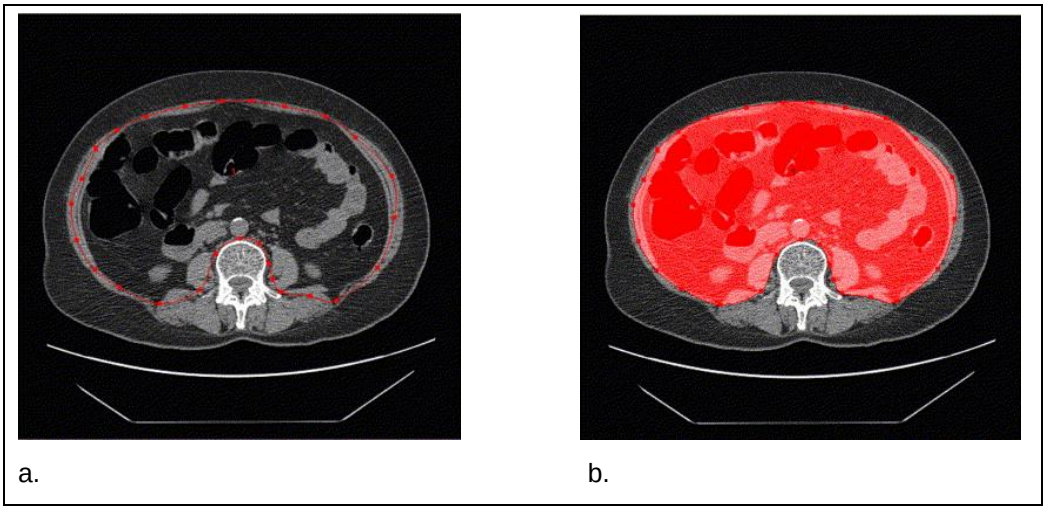

Figure 2. CT scan images showing a slice taken through the umbilicus. **(a)** The tracing of the body wall using the spline tool. Multiple points can be made using the spline tool to accurately outline the visceral fat. **(b)** The highlighted region of interest to be analyzed.

Table 1: Measurements from CTA for each coronary artery segment

Plaque Presence and Coronary Artery Stenosis
Small artery (diameter <1.5 mm, therefore cannot assess)
No stenosis or plaque present
Plaque present - minimally narrowed (stenosis grade 1% to 25%)
Plaque present - mildly narrowed (stenosis grade 26% to 50%)
Plaque present - moderately narrowed (stenosis grade 51% to 75%)
Plaque present - severely narrowed (stenosis grade >75%)
Size of each Plaque
Small (<1 mm in diameter perpendicular to the coronary artery)
Medium (1-2 mm in maximum diameter)
Large (>2 mm in diameter)
Plaque Composition
Non-calcified
Mixed
Calcified
Cardiac Chambers
Left ventricular mass
Left ventricular volume
Left atrial volume

Measurement of Biomarkers

Blood Sampling and Processing for Biomarkers

All subjects will fast from the night before the visit, and blood samples will be drawn between 7 and 10 AM. Blood will be collected for serum and citrated and EDTA plasma that will be stored at -80° C in the central laboratory until assay. Samples will be frozen and thawed only once.

Additional Processing of Blood for Microparticles

For eventual determination of microparticles, 10 mL of citrated blood will be centrifuged at 1,500 *g* for 15 minutes. The supernatant will then be centrifuged at 13,500 *g* for 5 minutes to remove the platelets. The resulting supernatant of platelet-free plasma will frozen, shipped to the central laboratory on dry ice, and stored at -80^0 C until assay. Samples will be frozen and thawed only once.

Cardiovascular Events Ascertainment and Adjudication

See the T Trial Cardiovascular Event Adjudication Manual for a complete description of the ascertainment and adjudication process.

Study Procedures and Visits

Prescreening/Screening Visit 1/Screening Visit 2Men will be screened over the phone and in person to determine if they qualify for the CV study. Screening for the CV Trial will take place over the course of the telephone screen, Screening Visit 1 and Screening Visit 2 of the Main T Trial.
Baseline Visit

- Sign Baseline consent form
- CT scan without and with contrast for coronary artery calcium score, non-calcified plaque volume, and total plaque volume
- CT scan for visceral and subcutaneous on three slices through abdomen at L3-4

Month 12 Visit

- Re-evaluation of medical history for development of allergy to iodinated contrast medium
- Review of renal function based on month 12 e-GFR calculation (> 60 ml/min/1.73m^2)
- CT scan including coronary artery calcium score, non-calcified plaque volume, total plaque volume
- CT scan for visceral and subcutaneous on three slices through abdomen at L3-4

Subject Compensation

Subjects will be compensated during the course of the trial, $50 for the baseline visit and for the month 12 visit, for a total of $100 if a subject completes the trial. In addition, subjects will be compensated for travel and parking and provided meal tickets for study visits.

Statistical Plan

Analytical Methods and Sample Size Estimations

The CV trial sample size has been revised based on new data provided by Dr. Matthew Budoff, the director of the CTA Reading Center for the TTrial. The data were derived from a similar population of evaluated at two time points approximately one year apart. The measurement used was of fatty (noncalcified) plaque volume, which is the primary end point of CT angiography, since fatty plaque volume is what is most likely to change in response to testosterone treatment. We project that about 140 subjects will enroll by the end of enrollment in the main trial and that 85%, or about 120, will have a second scan. However we also expect about a 15% attrition in the TTrial overall, but it is likely that some subjects who remain in the TTrial for 12 months will develop a condition that would exclude them between the baseline and 12 month scans, so we have decided to target enrollment in the CV Trial at 160 in order to be more sure of having 120 subjects complete both baseline and month 12 scans.

The reason for the difference between the previous estimate and the current one results from our change in primary end point from total plaque volume to noncalcified plaque volume. The reason for the change in the primary end point from total to noncalcified plaque volume is the growing acceptance in the CTA community that not only is noncalcified plaque more important biologically (it is more likely to rupture than calcified plaque), but also is more likely to change in response to statin or anti-inflammatory treatment than calcified plaque. For example, a study of statin treatment showed a significant reduction in noncalcified plaque volume with statin treatment but not in calcium score (Burgstatler, Invest Radiol 42: 189, 2007). Similarly, a study of the anti-inflammatory drug VIA-2291 showed a significant reduction in noncalcified plaque volume but not in total plaque volume (Tardiff, Circimaging 3: 298, 2010).

Clinical cardiovascular events

Although this Trial will not likely have adequate power to detect a difference between treatment groups in incidence of clinical cardiac events, we shall track and adjudicate cardiovascular events in all subjects in all trials to look for trends. Additionally, although this trial is designed to test whether treatment with testosterone reduces volume of coronary plaque compared with placebo treatment, the supporting data are limited and we recognize that effects in either direction could be observed. Given the increasing use of testosterone in elderly men, data suggesting that testosterone treatment decreases or increases plaque volume would be valuable. As for all other hypothesis tests in The Testosterone Trial, two-sided tests will be performed and will permit conclusions regarding effects in either direction.

Safety and Adverse Events

Definitions

Definitions are per the January 2007 Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events, Office on Human Research Protection (OHRP) Guidance. **http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm**. The requirements and processes for reporting adverse events are described in the corresponding National Institute on Aging (NIA) Guidelines and collected as per the main Testosterone Trial protocols.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality.
- The abnormality suggests a disease and/or organ toxicity.
- The abnormality is of a degree that requires active management.

- Creatinine will be specifically assessed to evaluate safety of contrast administration

Safety Measures Related to CT Angiography.

Use of CT angiography raises two important safety issues: the amount of radiation absorbed by the body tissues and the exposure to iodinated contrast agents, which have the potential to produce allergic reactions and acute renal injury. A number of strategies have now been validated to lower the radiation dose of CT angiography to just above background doses.

Allergy to Contrast Media.

Subjects will be asked at the baseline and 12 month Testosterone Trial visit if they have a known allergy to contrast media. Subjects with known or suspected contrast allergy will not be eligible for the CV trial.

Renal Function.

Subjects will have a serum creatinine measurement as part of a chemistry screening panel at the time of the baseline and 12 month Testosterone Trial visit. If the estimated GFR is <60 mL/min/1.72m^2, CT angiography will not be done.

Radiation Exposure

Total Radiation for the entire cardiac CT protocol is expected to be 3.2-6.5 mSv, including scout imaging, coronary artery calcification and CT angiography, dependent on BMI, cardiac height and heart rate. Beta-blockers will be used to control the heart rate and thus maintain the radiation dose as low as reasonably achievable.

Radiation Reduction Methods:

The primary drawback to the use of CT angiography has been the relatively high radiation doses administered to the participants using previous generations of CT scanners, as high as 20 mSv or more using some protocols. We shall employ several techniques to reduce radiation:

- *Prospective Triggering*. In this technique imaging is performed in only one phase of the cardiac cycle, rather than continuous imaging during the entire cycle. Both Siemens Dual Source and Toshiba 320 scanners have the ability to perform prospective imaging.
- *Limiting the Field of View.* The field of view will be limited to 25 cm. This will both reduce radiation dose by up to 30%, and limit incidental findings related to non-cardiac disease (primarily lung nodules).
- *Limiting the scan length.* Scan length (height of chest imaged) will be limited to 1 cm above the visualized top of the coronary arteries and 1 cm below the distal arteries based on the calcium score. The use of the calcium score anatomical landmarks (rather than the scout image) has

been shown to reduce radiation by 30%, independent of other dose reduction strategies (14).

- *Reducing tube voltage*. Tube voltage will be 100 kVp for subjects <85 kg rather than the typical voltage of 120 kVP (15), which lowers radiation exposure by 40% independently of other techniques.

The CT Reading Center will work closely with sites to ensure average doses remain below 5 mSv. It will use direct measure of radiation. If a site is found to be delivering average doses >5 mSev, the Reading Center will reinstruct the site. If doses are not reduced to below 5 mSev, the site will be asked to stop recruitment until a member of the CT Reading Center can be present for acquisition and protocol adjustment.

Total Radiation.

The CT angiography examination we shall perform has an estimated effective dose of 3.2-6.5 mSv. This value is comparable to the 3-7 mSv people receive annually (based on geography) from natural sources. In comparison, catheter diagnostic coronary angiography delivers effective doses of 7 mSv, while stress nuclear thallium imaging deliver doses of 20-41 mSv. We have tabulated cumulative exposure including estimates for the CT angiography exam. The cumulative average estimated exposure to participants in the MESA study to date is <7 mSv, which is 14% of the participants' cumulative background exposure from natural sources. The Cardiovascular Trial would increase background to 20%. In this study we shall measure radiation, rather than estimate it, as done in previous CT angiography studies.

Quality Assurance of CT Scanning

Acquisition of data from CT angiography is challenging, so this study will be performed only at sites that are very experienced in this technique. The CT Reading Center will instruct each site prior to the initiation of the Trial to ensure protocols are programmed into the computer and the technologists understand the protocols. In addition, each scan will be reviewed at the reading center for compliance to field of view, voltage and power, pitch and other parameters. If the Reading Center finds any scan has been performed in a way that deviates from the protocol, the technologist and the local PI will be advised. If more than two consecutive scans at a site are found to deviate from the protocol, the director of the Reading Center will revisit the site and review the protocols and technique prior to any further imaging at that center.

Data and Safety Monitoring Board

The DSMB of the main Testosterone Trial will monitor the safety of the subjects in the Cardiovascular Trial.

Data Management

Data Management System Components

The Data Coordinating Center (DCC) at the University of Pennsylvania will develop a data management system for the collection, storage and management of data, as consistent with the main Testosterone Trial Protocol.

Manual of Procedures (MOP) – The MOP will describe the sequence of study conduct and provide detailed instruction for the performance of screening, baseline, enrollment, treatment allocation and follow-up procedures. The MOP will provide instruction in case report form completion, use of the electronic data management system, and collection, documentation and transfer of specimens and tests to laboratories and reading centers.

Training and certification procedures – The CT Reading Center, in conjunction with the DCC, will conduct a training session before the study starts to train and certify personnel in the performance of study procedures.

CT technologists should have appropriate knowledge of cross-sectional anatomy, physiology, and pathology related to the heart. Technologists must be certified as RTs in their state. It is recommended that technologists also have at least two years of experience in chest computed tomography. The technologist should also have a basic knowledge of cardiac CT, knowledge of computer software applications, data formatting, and experience with the workstations and data formatting / transmission procedures used.

Each technologist involved in the study should also have a complete understanding of this protocol, be experienced at providing breath-holding instruction, ECG gating, and operation of the CT. To ensure quality control, each site should have designated CT technicians who will perform the examinations.

Training will be performed using a standard training PowerPoint presentation and interactive internet and teleconference system to train technologists. This allows ongoing training and certification of technologists. Other NIH studies have demonstrated that there will be a turnover of technologists, and doing on-site training for each technologist is not possible or practical. Using the standard interactive presentation and teleconference, each site can get specific training and follow-up as needed. Further, quality assurance will be performed on each scan (see below), and necessary feedback given to the specific technologist promptly (within 1-2 days) after the scan is received at the reading center. This will allow for rapid identification of improper scan techniques and has been shown to dramatically decrease inadequate data sets in previous multicenter studies done at this core reading center.

Site visits – Findings from site visits will be used to resolve problems and develop corrective action plans.

External data sources – The DCC will monitor quality control of data received from the CV reading center.

Internal quality control procedures – A data validation plan, rule set specifications, and programming logic to implement data validation rules will be implemented.

Routine reports

The DCC will develop a set of standard enrollment, tracking, quality review, and safety monitoring reports. Adverse event reports, DSMB reports and reports for statistical analysis will be developed and produced on an appropriate schedule.

Urgent Alerts

Several safety alerts (e.g. aortic aneurysm [> 5cm], severe coronary artery disease [defined as left main or 3 vessel disease], non-calcified lung masses [>6 mm] and large pericardial effusions) will require urgent notification and reports forwarded to the local participating clinical centers by the Reading Center PI. The Reading Center PI will be responsible for notification of both the trial site PI and study coordinator, as well as the Data Coordinating Center within 24 hours. The site PI will then be responsible for notifying the subject within the following 24 hours and also notifying the Data Coordinating Center that the subject has been contacted.

Data Security

The data management system will be the same as the main Testosterone Trial Study designed to prevent unauthorized access to trial data and to prevent data loss due to equipment failure or catastrophic events. The procedures to do so encompass user account management, user privilege assignment, data loss prevention (database backup), computer systems validation, performance monitoring, and DMS change management. User access will be controlled by assignment of confidential usernames, passwords and role assignment. The system will meet the applicable Federal regulatory requirements and those described in the E6 Good Clinical Practice Guidelines to ensure the confidentiality of trial subjects.

Maintaining Anonymity of Submitted Medical Records

Clinical site personnel will de-identify all medical records before sending them to the DCC by obliterating any Protected Health Information (PHI). Upon receipt, DCC personnel will review the records to ensure that no PHI is visible.

Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA) of 1996.

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