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The Cardiovascular Trial of The Testosterone Trials: Rationale, Design and Baseline Data of a Clinical Trial using Computed Tomographic Imaging to Assess the Progression of Coronary Atherosclerosis

Moshrik Abd alamir^a, Susan S. Ellenberg^b, Ronald S. Swerdloff^a, Nanette K. Wenger^c, Emile R Mohler III^d, Cora E. Lewis^e, Elizabeth Barrett-Conner^f, Rine Nakanishi^a, Sirous Darabian^a, Anas Alani^g, Suguru Matsumoto^a, Negin Nezarat^a, Peter J. Snyder^h, and Matthew J. Budoff^a

^aLos Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, California, USA

^bCenter for Clinical Epidemiology & Biostatistics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

^cDivision of Cardiology, Department of Medicine, Emory University School of Medicine, Emory Heart and Vascular Center, Atlanta, GA, USA

^dDivision of Cardiovascular Disease and Section of Vascular Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

^eDivision of Preventive Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

^fDivision of Epidemiology, Department of Family and Preventive Medicine, University of California San Diego, La Jolla, CA, USA

^gCardiology Department, University of Florida, Gainesville, FL, USA

^hDivision of Endocrinology, Diabetes, and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract

Background—Data from prior studies have yielded inconsistent results regarding the association of serum testosterone levels with the risk of cardiovascular disease (CVD). There are no clinical trial data on the effects of testosterone replacement therapy on plaque progression.

Address for correspondence: Matthew J. Budoff, MD FACC, Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, California, Tel: +1-310 2224107, Fax: +1-3107829652, mbudoff@labiomed.org.

Conflict of Interest Reporting

SSE reports a conference grant from AbbVie during the conduct of the study; RSS reports grants and consulting from AbbVie, Clarus, Ardana, Besins Health, and Endo Pharma; CEL was supported by the National Institute for Diabetes, Digestive and Kidney Diseases, National Institutes of Health (DK079626) to the UAB Diabetes Research and Training Center; PJS reports grants from NIH and AbbVie for the conduct of this study and has consulted for Watson Laboratories; MJB reports grants from NIH and grant support from General Electric

Objective—We designed a study to investigate the effect of testosterone therapy on coronary artery plaque progression using serial coronary computed tomographic angiography (CCTA). In this paper we describe the study design, methods, and characteristics of the study population.

Methods—The Cardiovascular Trial of The Testosterone Trials (TTrials) (NCT00799617), a double-blind, placebo-controlled trial of one year of testosterone therapy in men ≥65 years with clinical manifestations of androgen deficiency and unequivocally low serum testosterone concentrations (<275 ng/dL). CCTA performed at baseline and after 12 months of therapy will determine testosterone effects on the progression of total volume of non-calcified plaque. All scans are evaluated at a central reading center by an investigator blinded to treatment assignment.

Results—One hundred sixty-five men were enrolled. The average age is 71.1 years and average BMI 30.7. About 9% of men had a history of myocardial infarction, 6% angina, and 10% coronary artery revascularization. A majority reported hypertension and/or high cholesterol; 31.8% reported diabetes. Total non-calcified plaque at baseline showed a slight but non-significant trend of lower plaque volume with higher serum testosterone concentrations (p=0.12).

Conclusions—The Cardiovascular Trial will test the hypothesis that testosterone therapy inhibits coronary plaque progression as assessed by serial CCTA.

Keywords

cardiovascular disease; coronary CTA; testosterone; coronary artery plaque progression; randomized controlled trial

Introduction

As demonstrated by both cross-sectional [1] and longitudinal studies [2,3], men's serum testosterone concentration falls gradually, beginning at age 20. While the reported prevalence rates vary in different studies, one widely quoted sentinel study reported that by the eighth decade, approximately 30% of U.S. men have concentrations of total testosterone lower than normal for younger men and 70% have free testosterone concentration lower than normal for younger men [3].

Whether the age-related decline in testosterone concentrations contributes to, or is independent of, atherosclerosis progression with age is currently unknown. Although testosterone was once considered to be a risk factor for cardiovascular disease, the data are mixed. Several recent studies in men show an inverse association between serum testosterone concentration and cardiovascular disease, the metabolic syndrome or diabetes [4–6], an association independent of traditional CVD risk factors. A recent randomized trial, however, demonstrated increased CV risk in participants receiving testosterone replacement [7], and recent meta-analyses have shown conflicting results.

The Cardiovascular Trial, a component of the The Testosterone Trials (TTrials), was designed to assess the effects of testosterone treatment on several markers of cardiovascular disease in men ≥65 years old with symptoms and signs consistent with androgen deficiency and low serum testosterone concentrations [8]. These include progression of coronary atherosclerosis as assessed by serial coronary computed tomographic angiography (CCTA);

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 as assessed by high-sensitivity troponin.

MATERIALS AND METHODS

Design

The TTrials are a coordinated group of multi-center, double-blind placebo-controlled trials [8] to evaluate the effects of testosterone treatment in older men with low testosterone levels for no apparent reason other than age, on clinical endpoints that have been shown to improve with testosterone therapy in men with disease-induced hypogonadism. The TTrials are registered at <http://clinicaltrials.gov/> (NCT00799617).

The TTrials include 12 clinical sites geographically distributed across the United States and is sponsored by the National Institute on Aging (NIA), National Institute of Neurological Disorders and Stroke (NINDS), Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Heart, Lung, and Blood Institute (NHLBI), and AbbVie, Inc. Men 65 years who had a mean serum testosterone concentration <275 ng/dL on two morning measures, who had subjective complaints and objective evidence of sexual dysfunction, physical dysfunction and/or reduced vitality (clinical conditions thought to be potentially improved by testosterone supplementation), and who were not at elevated risk for prostate, cardiovascular or hematologic problems associated with high levels of testosterone, were recruited; those eligible and consenting were assigned to one year of treatment with testosterone therapy or placebo [8,9]. The Cardiovascular Trial, designed to determine the effect of testosterone on the progression of coronary atherosclerosis, was conducted at 9 of the 12 TTrials sites. These sites all have 64-slice CCTA scanners and sufficient experience using them, as determined by a questionnaire completed at each site.

The TTrials and the Cardiovascular Trial protocols were approved by institutional review boards of the participating sites. All participants provided written informed consent before trial-related procedures were conducted. Participant safety and trial conduct were overseen by an Independent Data and Safety Monitoring Board appointed by the National Institute on Aging.

Treatment

Participants received AndroGel®, testosterone in an alcohol-water gel, or matched placebo gel, administered transdermally daily, for one year. Treatment allocation used a minimization algorithm with a random component [8,10]. The testosterone dose was adjusted by the (unblinded) data coordinating center as needed to maintain testosterone concentration levels between 500 and 800 ng/ml; similar adjustments were performed on matched placebo participants to maintain blinding. Regular monitoring of participants for potential adverse effects of testosterone was performed.

Eligibility criteria

Inclusion and exclusion criteria for the TTriaIs have been published [8]. Age, serum testosterone concentration, biochemical and clinical conditions that could affect the interpretation of the results, and conditions that could be worsened or exacerbated by testosterone therapy were the major factors used in assessing eligibility. In addition to the testosterone concentration and symptom requirements mentioned earlier, TTriaIs participants had to have a normal baseline renal function (estimated glomerular filtration rate (eGFR) >60 ml/min/1.73m²) to be eligible for the Cardiovascular Trial (Table 1). Men were excluded if their weight was >300 pounds, they had known allergy to iodinated contrast medium, were unable to breath-hold for 10 seconds, or had a prior diagnosis of tachycardia or irregular heart rhythm (eg, atrial fibrillation), or coronary artery bypass graft surgery by patient report or medical records.

Follow-up

Men entered into The Cardiovascular Trial underwent a coronary CT angiography (CCTA) scan without and with contrast to allow calculation of non-calcified plaque volume, total plaque volume, subcutaneous fat and coronary artery calcium (CAC) score. The Month 12 Visit included re-evaluation of medical history for development of allergy to iodinated contrast medium and review of renal function based on month 12 e-GFR calculation (>60 ml/min/1.73m²). All participants still enrolled at 12 months with an eGFR >60 and no new contraindication had a second CCTA scan to repeat the same measures obtained at the baseline visit.

Primary endpoint

The primary trial endpoint is percent change in non-calcified coronary plaque volume over the 12 month treatment period. The rationale for using noncalcified plaque volume is that prior trials have shown significant reduction in the amount of noncalcified plaque volume among statin users; calcified plaque is generally not considered amenable to reversal, and may even be increased by statins [11]. Experienced readers in the CCTA core lab blinded to the assigned treatment visually evaluated the CCTA scans for the presence, extent, and severity of coronary artery plaque. CCTA imaging and measurement of coronary plaque were conducted using standardized procedures and technology for longitudinal measurements of atherosclerosis change. The baseline and follow up scans were compared side by side (with blinding to both treatment arm and date of study [baseline vs. 12 month]) to ensure like segments were compared and measured. The area of each coronary plaque visualized in at least 2 adjacent slices (reconstructed slice thickness 0.6 mm) was determined on all affected slices [12]. The total plaque per segment was summed over all segments with plaque. The degree of coronary artery stenosis was assessed by using axial images, multi-planar reconstructions and curved multiplanar images to assess the degree of luminal narrowing in all assessable coronary segments. The coronary artery stenosis severity was classified into 4 groups (0%, 1–29%, 30–69%, and 70% stenosis). The most narrowed diameter in each segment was determined even if the plaque was eccentric. A segment stenosis score was generated based on the degree of underlying stenotic disease in each segment (0: no plaque, 1: 1–29% stenosis, 2: 30–49% stenosis, 3: 50–69% stenosis, 4: 70%

stenosis). The extent scores of all 17 individual segments were summed to yield a Total Stenosis Score (TSS) ranging from 0 to 68 [12]. In addition, each coronary territory (right coronary artery, left main, left anterior descending and left circumflex artery) was also scored according to presence of the most significant lesion.

Composition of coronary atherosclerotic plaque was 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 was classified as either normal (no plaque), containing non-calcified plaque, containing partially calcified 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) [13]. Calcified atherosclerotic plaque was defined as any discernible structure that: 1) had a CT density greater than the contrast-enhanced lumen; 2) was clearly assignable to the coronary artery wall; and, 3) was identified in at least 2 independent planes. Non-calcified atherosclerotic plaque was defined as any discernible structure that: 1) had a CT density less than the contrast-enhanced coronary lumen but greater than the surrounding connective tissue; 2) was clearly assignable to the coronary artery wall; and, 3) was identified in at least 2 independent planes [13]. Details of the CCTA methods are described elsewhere [11–16].

Secondary Outcomes

Secondary CCTA measures include changes in total plaque volume, coronary artery calcium (CAC) score and subcutaneous fat. HgA1C and HOMA-IR will also be assessed as secondary endpoints in participants without diabetes at baseline. CAC measures will include presence of any CAC (dichotomous variable) and CAC score, as well as changes in LV mass between testosterone and placebo.

Scanning Methodology

Non-Calcified plaque volume—Plaque volume was assessed by experienced readers at the CCTA core lab in all affected coronary segments on CCTA using semi-automated software (QAngio CT Research Edition 2.1.2, Medis medical imaging systems b.v., the Netherlands) (Figure 1) [17]; at baseline and follow up visits. First, an automatic tree extraction algorithm was used to obtain all the 3-dimensional centerlines of the coronary tree [18]. Based on these centerlines, straightened multi-planar reformatted (MPR) volumes are created of all vessels. Next, the lumen border contours and vessel wall borders are assessed using spatial first- and second-derivative gradient filters in longitudinal cross sections in combination with knowledge of the expected CCTA intensity values in the arteries [17–19].

Thereafter lumen and vessel contour are detected in the individual transversal cross-sections perpendicular to the centerlines, whereby the locations from the longitudinal analyses are taken into account. This method is insensitive to differences in attenuation values between data sets and independent of window and level settings. As previously described [19], 740HU and 220 HU were used as window and width levels to identify luminal and vessel outlines. Once automated software completed the vessel trace, expert readers manually corrected areas if additionally needed. The plaque area of each coronary plaque visualized in at least 2 adjacent slices (slice thickness 0.6 mm) was determined on all slices and plaque

volume assessed by multiplying the area and slice thickness. The plaque volume was measured in any coronary artery segment 1.5 mm diameter. For each lesion, minimal lumen diameter was summed and reported as non-calcified plaque including fibrous plaque, fibrous fatty plaque, low attenuation plaque, and dense calcified plaque, which all variables have been widely used for quantitative plaque assessment in numerous previous studies [14,20–22]. Plaque Composition is based upon predefined fixed intensity cutoff values on the Hounsfield units (HU). These thresholds are based upon studies by comparing CCTA with virtual histology by intravascular ultrasound or histological examination in our lab and others [23–27]. The fixed HU cut-off values for classifying are: –30 to 30 HU for low attenuation plaque, 31–130 HU for fibrous fatty plaque, 131–350 HU for fibrous plaque, and >350 HU for dense calcified plaque. These values were initially based on a previous report by Brodoefel et al. [28] and empirically optimized using three representative training sets [17,29,30]. The excellent inter- and intra-observer variability for the lumen and vessel segmentation has been described previously [27,29]. On a per-patient basis, the total plaque volume is calculated by the sum of all plaques measured in available segments at baseline and follow-up. As previously described [19], the total plaque volume, total plaque burden $[(\text{total plaque volume (TPV)}/\text{total vessel volume}) \times 100]$ and normalized total plaque volume $(\text{TPV}_{\text{Norm}})$ $[\text{total plaque volume}/\text{total segment length}] \times (\text{the mean total segment length in the whole population})]$ were assessed at baseline and follow-up. The change between serial CCTAs in these variables was also assessed (Figure 2).

CAC Scanning—For CAC scanning, each scan extended from 1 cm below the carina to the bottom of the heart to include the entire coronary tree. Scan parameters were obtained as follows: prospective electrocardiogram-triggering (typically 65–80%), 35 cm field-of-view, 512×512 matrix size, and peak tube voltage of 120 kVp. Slice thickness was 3 mm. Acquired images were then transferred to the CCTA core lab for analysis. All coronary calcium score measurements were performed on a GE AW system (AW Volume Share™, GE Medical Systems, Milwaukee, WI) to quantify coronary artery calcification using the Agatston method [31]. The coronary calcium was identified in 3 contiguous voxels by using a cutoff point of 130 Hounsfield Units (HU) resulting in a minimum calcified lesion area of 1.02 mm². The area density method was used to compute the lesion score, in which the lesion area was multiplied by an attenuation factor. This attenuation factor is derived from the maximal HU within the area as described by Agatston, ranging from 1 to 4. A total coronary artery calcium (CAC) score was obtained by aggregating individual lesion scores from each of 4 anatomic sites (left main, left anterior descending, circumflex, and right coronary arteries).

CCTA quality assurance/control

The CCTA-core lab read all CTA scans, blinded to all demographics, testosterone randomization and risk factors, in near real time (within one week of acquisition) during enrollment and scanning period for stenosis evaluation and assessability. Three level 3 experienced cardiac CT readers evaluated all studies in the trial. The CCTA-core lab also evaluated the prevalence of slab, blurring, and partial volume averaging artifacts on all scans. The contrast-to-noise ratio of the distal left anterior descending artery is calculated for each scan as a quantitative metric of image quality.

Sample size—We reviewed data on 63 older men from an observational study using CT angiography techniques similar to those employed in this trial. Of these men, 35 were taking statins and 28 were not. Scans were performed at two time points approximately one year apart. In this cohort, the standard deviations of the changes from the first to the second scan were similar in the 2 groups; we used the larger of the 2 values (a SD of 26) for our calculations, which indicated that 140 men, 70/arm, would provide 80% power to detect a difference of 12 mm³ in non-calcified plaque volume, somewhat less than the difference of 14 mm³ between statin users and nonusers seen in the prior study. Since it was difficult to know the number of men who would enroll, as this was dependent on the proportion of men to be enrolled in the TTrial who would be eligible for and consent to participate in this trial, we also calculated power for a total sample size of 120, 60/arm; that number would provide 80% power to detect a 13 mm³ difference between treatment arms after one year of treatment.

Statistical analysis—The primary hypothesis was that testosterone treatment would decrease progression of non-calcified coronary artery plaque volume, as assessed by computed tomography angiography. Efficacy will be measured through a multivariate linear model that adjusts for baseline non-calcified plaque volume and all balancing variables used in the minimization procedure: study site, indicator variables of participation in each primary efficacy trial, baseline testosterone concentration (< or >200 ng/dL), age (< or > 75), use of anti-depressants, and use of PDE inhibitors. All subjects with a baseline and 12-month plaque volume measurement will be included in the analysis. Significance will be assessed through the two-sided Wald test and confidence interval for the treatment effect.

Safety analysis and evaluation of adverse events

Safety Measures Related to CT Angiography include the amount of radiation absorbed by the body tissues and the exposure to iodinated contrast agents. Beta-blockers were used to control the heart rate and thus maintain the radiation dose as low as reasonably achievable. Other radiation reduction methods included prospective triggering, limited field of view, limited scan length, iterative reconstruction and reduced tube voltage whenever possible based on body habitus [32–35].

Results and Discussion

Recruitment

One hundred ninety-nine men consented to be screened for the trial. Of these, 170 were eligible, and 165 had a baseline scan. Five men could not be scanned due to high heart rates or lack of venous access.

Demographics

One hundred sixty-five men were entered into the Cardiovascular Trial and had a baseline CCTA. Baseline information about these men is displayed in Table 2. The average age of participants was 71. Most men were overweight or obese; 61% had a body mass index > 30. Hypertension and/or hyperlipidemia were present in approximately 2/3 of participants, and approximately one-third were diabetic. A prior MI was reported for 9%; 10% had had a

prior revascularization; and 6% had a history of angina. The mean volume of non-calcified plaque was 91.0 mm³, with a SD of 95.9 mm³.

Association of coronary plaque volume with serum testosterone concentration

We assessed whether there was any notable association between serum testosterone values and non-calcified coronary plaque volume at baseline. These data are shown in Figure 2. There was a trend towards higher values of testosterone associated with lower plaque volume, but this association was not statistically significant using a linear regression model ($p=0.12$).

Discussion

Observational studies have found associations of low testosterone concentrations in older men with adverse cardiovascular outcomes [4–6]. Further, in a study of 40–79 year-old community-dwelling men in Rancho Bernardo, baseline 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/dL had a significantly increased risk of mortality compared to men with higher levels, independent of other 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 traditional risk factors. Some [35,36], but not other [37,38] retrospective studies of testosterone treatment have raised concern that exogenous testosterone might increase risk of CVD events, and results of meta-analysis have been mixed [39–46]. The conclusions of a critical review of 31 placebo-controlled trials of testosterone therapy in older men published in 2004 by the Institute of Medicine (IOM) were that the effects of testosterone therapy, including effects on coronary atherosclerosis and cardiovascular risk in this population, were uncertain [39]. However, a randomized trial of testosterone in older men with limited mobility [7] was stopped early due to the increased frequency of cardiovascular adverse events in the testosterone arm;

Strengths of the Study

The Cardiovascular Trial has many strengths. We selected CCTA as the imaging modality to assess coronary atherosclerosis in this trial. Until recently, estimating the plaque progression was only possible using intravascular ultrasound (IVUS), an invasive approach that shows only proximal coronary artery plaque volumes and correlates poorly with the individual total plaque burden [9,40]. It is also invasive and expensive, with well-defined associated morbidity. Therefore IVUS studies generally recruit patients with acute coronary syndromes, to justify the first invasive angiogram and IVUS. CCTA 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 [41]. In addition, based on the tissue-specific x-ray attenuation characteristics, CCTA also provides additional information about atherosclerotic plaque composition. It is able to differentiate calcified from predominantly non-calcified plaques and ones that contain a large lipid pool [11,42] and allows volumetric analysis of plaque volumes. Plaque characterization (i.e. determining

the vulnerability of plaque rupture by examining its tissue components) is now possible using CCTA. New 64 slice CCTA technology is extremely accurate in detecting 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; it also has an important role in characterizing the vulnerable non-obstructive plaque [43]. Tissue density measured by CCTA can be used to characterize atherosclerotic plaque composition.

The Cardiovascular Trial involve strict standardization and quality control in assessing the imaging data, with all readings done by the same readers, who were blind to treatment assignment. The equivocal results of prior testosterone trials was once thought to be due to selection of men who did not have sufficiently low testosterone concentrations. This pitfall was avoided in the TTrial since the eligibility criterion for serum testosterone was set at a level low enough to ensure that men were unequivocally testosterone deficient. In addition, the serum testosterone concentration is measured at months 1, 2, 3, 6 and 9 to allow the assessment of medication compliance as well as dosage adjustments to maintain the serum testosterone concentration of the men assigned to the testosterone arm within the normal range for young men.

Limitations of the study

The TTrial had a long list of inclusion and exclusion factors [8], so this study will not be representative of all men over the age of 65. In particular, men with a recent MI, serum testosterone within the normal range or < 100 ng/dL, and men without functional concerns were excluded. In addition, the size of the study and the one-year duration are insufficient to assess the effect of testosterone on clinical cardiovascular outcomes.

The continuing uncertainty over the benefits of testosterone therapy in elderly men emphasizes the need for initiation of randomized trials such as the TTrial and the associated Cardiovascular Trial to test this treatment rigorously. Larger trials focused on clinical outcomes will be needed to fully assess the impact of testosterone therapy on cardiovascular health.

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TTrial Research Group Members

Peter J Snyder MD¹, Susan S Ellenberg, PhD², Glenn R Cunningham MD³, Alvin M Matsumoto MD⁴, Shalender Bhasin MD⁵, Elizabeth Barrett-Connor MD⁶, Thomas M Gill MD⁷, John T Farrar MD PhD⁸, David Cella PhD⁹, Raymond C Rosen PhD¹⁰, Susan M Resnick PhD¹¹, Ronald S Swerdloff MD¹², Jane A Cauley DrPH¹³, Denise Cifelli MS⁸, Laura Fluharty MPH⁸, Marco Pahor MD¹⁴, Kristine E Ensrud MD MPH¹⁵, Cora E Lewis MD MPSH¹⁶, Mark E Molitch MD¹⁷, Jill P Crandall MD¹⁸, Christina Wang MD¹²,

Matthew J Budoff MD¹⁹, Nanette K Wenger MD²⁰, Emile R Mohler 3rd MD²¹, Diane E Bild, MD MPH²², Nakela L Cook, MD MPH²³, Tony M Keaveny PhD²⁴, David L Kopperdahl PhD²⁵, David Lee PhD²⁵, Ann V Schwartz, PhD MPH²⁶, Thomas W Storer PhD²⁷, William B Ershler MD²⁸, Cindy N Roy PhD²⁹, Leslie J Raffel MD³⁰, Sergei Romashkan MD PhD³¹, Evan Hadley MD³¹

¹Division of Endocrinology, Diabetes, and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

²Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

³Division of Diabetes, Endocrinology and Metabolism, Baylor College of Medicine and St. Luke's Episcopal Hospital, Houston, TX, USA

⁴Geriatric Research, Education, and Clinical Center, Division of Gerontology & Geriatric Medicine, Department of Veterans Affairs Puget Sound Health Care System, Department of Internal Medicine, University of Washington School of Medicine, Seattle, WA, USA

⁵Research Program in Men's Health: Aging and Metabolism, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

⁶Division of Epidemiology, Department of Family and Preventive Medicine, University of California San Diego, La Jolla, CA, USA

⁷Division of Geriatric Medicine, Yale School of Medicine, New Haven, CT, USA

⁸Center for Clinical Epidemiology & Biostatistics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

⁹Department of Medical Social Sciences, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

¹⁰New England Research Institutes, Watertown, MA, USA

¹¹Laboratory of Behavioral Neuroscience, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

¹²Division of Endocrinology, Harbor-University of California at Los Angeles Medical Center; Los Angeles Biomedical Research Institute ;Torrance, CA; David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA, USA

¹³Department of Epidemiology, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA, USA

¹⁴Department of Aging & Geriatric Research, University of Florida, Gainesville, FL, USA

¹⁵Department of Medicine, Division of Epidemiology & Community Health, University of Minnesota, Minneapolis VA Health Care System, Minneapolis, MN, USA

- ¹⁶Division of Preventive Medicine, University of Alabama at Birmingham, Birmingham, AL, USA
- ¹⁷Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA
- ¹⁸Divisions of Endocrinology and Geriatrics, Albert Einstein College of Medicine, Bronx, NY, USA
- ¹⁹Division of Cardiology, Los Angeles Biomedical Research Institute, Harbor- University of California at Los Angeles Medical Center, Torrance, CA, USA
- ²⁰Division of Cardiology, Department of Medicine, Emory University School of Medicine, Emory Heart and Vascular Center, Atlanta, GA, USA
- ²¹Division of Cardiovascular Disease and Section of Vascular Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
- ²²Division of Cardiovascular Sciences, National Heart, Lung, and Blood Institute, Bethesda, MD, USA
- ²³Clinical Applications and Prevention Branch, Division of Cardiovascular Sciences, Centers for Cardiovascular Outcomes Research, National Heart, Lung, and Blood Institute, Bethesda, MD, USA
- ²⁴Departments of Mechanical Engineering and Bioengineering, University of California and ON Diagnostics, Berkeley, CA, USA
- ²⁵O.N. Diagnostics, Berkeley, CA, USA
- ²⁶Department of Epidemiology and Biostatistics, University of California, San Francisco, CA, USA
- ²⁷Section of Endocrinology, Diabetes, and Nutrition, Boston Medical Center, Boston, MA, USA
- ²⁸Division of Hematology/Oncology, Institute for Advanced Studies in Aging, Falls Church, VA, USA
- ²⁹Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
- ³⁰Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA
- ³¹Division of Geriatrics and Clinical Gerontology, National Institute on Aging, Bethesda, MD, USA

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Figure 1.

Cardiac computed tomography angiography image using QAngio CT Research Edition 2.1.2 software.

A – Straightened multi-planar reformatted volume of the vessel with lumen border contours and vessel wall borders of the of the left main and proximal left anterior descending coronary artery segment.

B – Transversal cross-section perpendicular to the centerlines of figure 1A. One arrow ($\rightarrow$) points to the contrast at the lumen of the vessel and the other arrow ($\leftarrow$) points to a mixed plaque with calcified and no calcified composition.

C – Axial cross-section demonstrating the left main and the proximal left anterior descending coronary artery segment.

Figure 2.

Association of serum testosterone with noncalcified plaque volume in men participating in the Cardiovascular Trial of the Testosterone Trials.

Table 1

Inclusion and Exclusion Criteria for The Cardiovascular Trial of The Testosterone Trials.

Inclusion Criterion	<ul style="list-style-type: none">• Normal baseline renal function, as assessed by estimated glomerular filtration rate >60 mL/min/1.73m² at Baseline and Month 12 Visits
Exclusion Criteria	<ul style="list-style-type: none">• Weight >300 lb• Known allergy to contrast medium• Inability to hold breath for 10 sec• Known diagnosis of tachycardia or irregular heart rhythm (eg atrial fibrillation)• Prior coronary artery bypass grafting by patient report or medical records

Table 2

Baseline Characteristics of Men in The Cardiovascular Trial

Characteristic *	Mean $\pm$ SD or N(%)
Age (Years)	71.1 $\pm$ 5.7
65–74	135 (79/4%)
75–84	25 (14.7%)
85	10 (5.9%)
BMI (kg/m ²)	30.7 $\pm$ 3.6
30	104 (61.2%)
<30	66 (38.8%)
Prior MI	15 (8.9%)
Angina	10 (5.9%)
Heart failure	1 (0.6%)
Atrial fibrillation	2 (1.2%)
Revascularization	17 (10.0%)
Stroke	3 (1.8%)
TIA	3 (1.8%)
Peripheral Arterial Disease	2 (1.2%)
Leg pain/cramping	13 (7.7%)
Angioplasty/stent	2 (1.2%)
Hypertension	112 (65.9%)
High cholesterol	111 (65.3%)
Diabetes	54 (31.8%)
Taking antidiabetics	47 (27.7%)
Ever smoker: cigarettes	108 (63.5%)
Current smoker: cigarettes	11 (6.5%)
Current smoker: cigars	6 (3.5%)
COPD	12 (7.1%)
Serum Total Testosterone (ng/dL)	237.3 $\pm$ 59.5
Non-calcified plaque volume (mm ³)	91.0 $\pm$ 95.9

*
by self-report