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The Cardiovascular Trial of the Testosterone Trials

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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

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AQ4 ***Background*** **Data from prior studies have yielded inconsistent results on the association of serum testosterone levels with the risk for cardiovascular disease. There are no clinical trial data on the effects of testosterone replacement therapy on plaque progression.**

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) is a double-blind, placebo-controlled trial of 1 year of testosterone therapy in men 65 years or older 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 the effects of testosterone on the progression of the total volume of noncalcified plaques. All scans are evaluated at a central reading center by an investigator blinded to treatment assignment.**

Results **A total of 165 men were enrolled. The average age is 71.1 years, and the average BMI is 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 noncalcified plaque at baseline showed a slight but nonsignificant trend toward lower plaque volume with higher serum testosterone concentrations ($P = 0.12$).**

Conclusion **The Cardiovascular Trial will test the hypothesis that testosterone therapy inhibits coronary plaque progression, as assessed by serial CCTA. *Coron Artery Dis* 00:000–000 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.**

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Keywords: cardiovascular disease, coronary artery plaque progression, coronary computed tomographic angiography, randomized controlled trial, testosterone

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Introduction

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

Whether 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 (CVD), the data are mixed. Several recent studies in men have shown an inverse association between serum testosterone concentration and CVD, metabolic syndrome, and diabetes [4–6], an association independent of traditional CVD risk factors. A recent randomized trial, however, has demonstrated increased CV risk in participants receiving testosterone replacement [7], and recent meta-analyses have shown conflicting results.

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The Cardiovascular Trial, a component of The Testosterone Trials (TTrials), was designed to assess the effects of testosterone treatment on several markers of CVD in men 65 years or older 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, and lipid and lipoprotein levels; and markers of glucose metabolism, inflammation, coagulation and platelet function, endothelial function, and myocardial damage, as assessed by high-sensitivity troponin levels.

Materials and methods

Design

The TTrials are a coordinated group of multicenter, 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 *clinicaltrials.gov* (NCT00799617).

The TTrials include 12 clinical sites geographically distributed across the USA and are sponsored by the National Institute on Aging, National Institute of Neurological Disorders and Stroke, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Heart, Lung, and Blood Institute, and AbbVie Inc. Men who were 65 years or older, with a mean serum testosterone concentration less than 275 ng/dl on two morning measures, with 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 an elevated risk for prostate, cardiovascular, or hematologic problems associated with high levels of testosterone were recruited; those eligible and consenting were assigned to 1 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 nine of the 12 TTrials sites. All the sites have CCTA scanners with 64 slices or more and staff with sufficient experience using them, as determined by a questionnaire completed at each site.

The protocols of TTrials and the Cardiovascular Trial were approved by the Institutional Review Boards of the participating sites. All participants provided written informed consent before the trial-related procedures were conducted. Participant safety and the 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 a matched placebo gel, administered transdermally daily, for 1 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 TTrials 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, participants in TTrials had to have a normal baseline renal function [estimated glomerular filtration rate (eGFR) > 60 ml/min/1.73 m^2] to be eligible for the Cardiovascular Trial (Table 1). Men were excluded if their weights were greater than 300 pounds, they had known allergy to iodinated contrast medium, they were unable to breath-hold for 10 s, they had a prior diagnosis of tachycardia or irregular heart rhythm (e.g. atrial fibrillation), or they had undergone coronary artery bypass graft surgery according to patient report or medical records.

Follow-up

Men enrolled in The Cardiovascular Trial underwent CCTA scanning without and with contrast to allow calculation of noncalcified plaque volume, total plaque volume, subcutaneous fat, and coronary artery calcium (CAC) score. The month 12 visit involved re-evaluation of medical history for the development of allergy to iodinated contrast medium and review of renal function based on month 12 eGFR calculation (>60 ml/min/1.73 m^2). All participants still enrolled at 12 months, with an eGFR greater than 60 ml/min/1.73 m^2 and no new contraindication, underwent a second CCTA scan for the same measures obtained at the baseline visit.

Primary endpoint

The primary trial endpoint was percent change in noncalcified coronary plaque volume over the 12-month treatment period. The rationale behind using noncalcified plaque volume is that prior trials have shown significant reductions in the noncalcified plaque volume among statin users; calcified plaques are generally not considered amenable to reversal, and may even be increased by statins [11]. Experienced readers in the CCTA core lab who were blinded to the assigned

Table 1 Inclusion and exclusion criteria for the Cardiovascular Trial of the Testosterone Trials

Inclusion criterion	Normal baseline renal function, as assessed by estimated glomerular filtration rate > 60 ml/min/1.73 m^2 at baseline and month 12 visits
Exclusion criteria	Weight > 300 lb
	Known allergy to contrast medium
	Inability to hold breath for 10 s
	Known diagnosis of tachycardia or irregular heart rhythm (e.g. atrial fibrillation)
	Prior coronary artery bypass grafting by patient report or medical records

treatment visually evaluated the CCTA scans for the presence, extent, and severity of coronary artery plaques. CCTA imaging and measurement of coronary plaques were conducted using standardized procedures and technology for longitudinal measurements of change in atherosclerosis. The baseline and follow-up scans were compared side by side [with blinding to both the treatment arm and the date of the study (baseline vs. 12 month)] to ensure that like segments were compared and measured. The area of each coronary plaque visualized in at least two adjacent slices (reconstructed slice thickness 0.6 mm) was determined on all affected slices [12]. The total plaques per segment was summed over all segments with plaques. The degree of coronary artery stenosis was assessed using axial images, multiplanar 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 four groups (0, 1–29, 30–69, and $\geq$70% stenosis). The most narrowed diameter in each segment was determined, even if the plaque was eccentric. A segment stenosis score was generated on the basis of 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: $\geq$70% stenosis). The extent scores of all 17 individual segments were summed to yield a total stenosis score ranging from 0 to 68 [12]. In addition, each coronary territory (right coronary, left main, left anterior descending, and left circumflex arteries) was also scored according to the presence of the most significant lesion.

The composition of the coronary atherosclerotic plaques was evaluated from both axial source images and multiplanar reconstruction images of the long axis at each site of the coronary arteries. Each coronary segment was classified as either normal (no plaque), containing noncalcified plaques, or containing partially calcified plaques with either predominantly noncalcified plaques (< 50% of the plaque area occupied by calcium) or predominantly calcified plaques (>50% of the plaque area occupied by calcium) [13]. A calcified atherosclerotic plaque was defined as any discernible structure that: (i) had a computed tomography density greater than that of the contrast-enhanced lumen, (ii) was clearly assignable to the coronary artery wall, and (iii) was identified in at least two independent planes. A noncalcified atherosclerotic plaque was defined as any discernible structure that: (i) had a CT density less than that of the contrast-enhanced coronary lumen but greater than that of the surrounding connective tissue, (ii) was clearly assignable to the coronary artery wall, and (iii) was identified in at least two independent planes [13]. Details of the CCTA methods have been described elsewhere [11–16].

Secondary outcomes

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

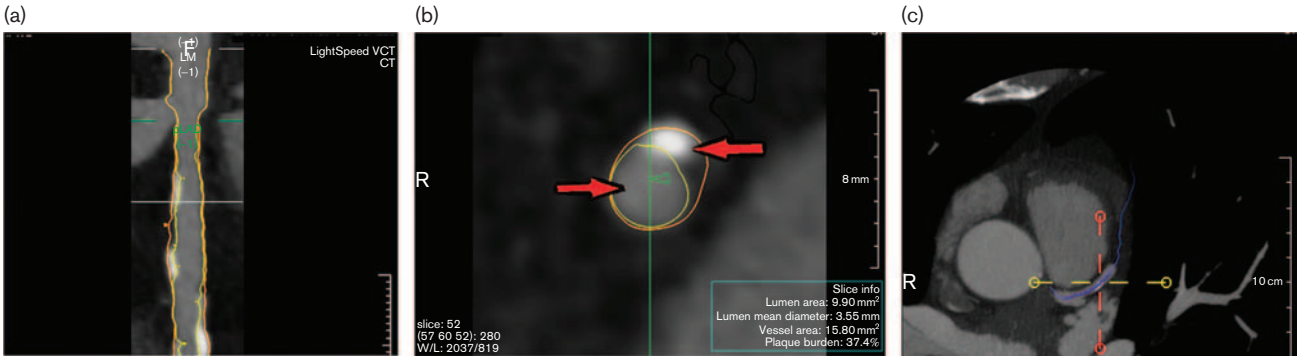
Scanning methodology

Noncalcified plaque volume

Plaque volume in all affected coronary segments was assessed at baseline and follow-up visits by experienced readers at the CCTA core lab by CCTA using semi-automated software (QAngio CT Research Edition 2.1.2; Medis Medical Imaging Systems b.v., the Netherlands; Fig. 1) [17]. First, an automatic tree-extraction algorithm was used to obtain all the three-dimensional centerlines of the coronary tree [18]. On the basis of these centerlines, straightened multiplanar reformatted volumes were created for all vessels. Next, the lumen border contours and vessel wall borders were assessed using spatial first-derivative 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 contours were detected in the individual transverse cross-sections perpendicular to the centerlines, whereby the locations from the longitudinal analyses were taken into account. This method is insensitive to differences in attenuation values between datasets and independent of window and level settings. As previously described [19], 740 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 two adjacent slices (slice thickness 0.6 mm) was determined on all slices and plaque volumes assessed by multiplying the area and slice thickness. The plaque volume was measured in any coronary artery segment 1.5 mm or higher diameter. For each lesion, the

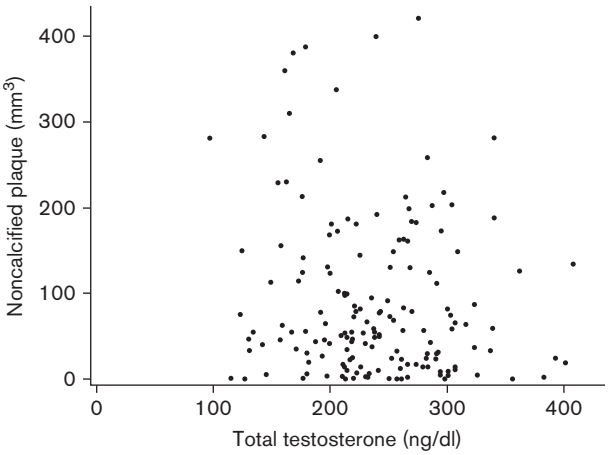
Fig. 1



Cardiac computed tomography angiography image using QAngio CT Research Edition 2.1.2 software. (a) Straightened multiplanar 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) Transverse cross-section perpendicular to the centerlines of (a). One arrow (→) points to the contrast at the lumen of the vessel and the other arrow (←) points to a mixed plaque with calcified and no calcified composition. (c) Axial cross-section demonstrating the left main and proximal left anterior descending coronary artery segment.

AQ8 minimal lumen diameter was summed and reported as noncalcified plaque including fibrous plaque, fibrous fatty plaque, low-attenuation plaque, and dense calcified plaque, all variables of which have been widely used for quantitative plaque assessment in numerous previous AQ9 studies [14,20–22]. Plaque composition is based on predefined fixed-intensity cutoff values in HU. These thresholds are based on studies in our lab and others comparing CCTA with virtual histology by intravascular ultrasound or histological examination [23–27]. The fixed HU cutoff values for classification are as follows: −30 to 30 HU for low-attenuation plaques, 31–130 HU for fibrous fatty plaques, 131–350 HU for fibrous plaques, and greater than 350 HU for dense calcified plaques. 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 interobserver and intraobserver variabilities for lumen and vessel segmentation have been described previously [27,29]. On a per-patient basis, the total plaque volume is calculated as the sum of all plaques measured in available segments at baseline and follow-up. As previously described [19], the total plaque volume, the total plaque burden [(total plaque volume/total vessel volume) × 100], and the normalized total plaque volume (TPV_{Norm}) [(total plaque volume/total segment length) × (mean total segment length in the whole population)] were assessed at baseline and follow-up. The changes in these variables between serial CCTAs were also assessed (Fig. 2).

Fig. 2



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

Coronary artery calcium 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. Scanning parameters were obtained as follows: prospective electrocardiogram triggering (typically 65–80%), 35 cm field-of-view, 512 × 512 matrix size, and peak tube voltage 120 kVp. Slice thickness was 3 mm. The 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, Wisconsin, USA) to quantify coronary artery calcification using the Agatston method [31]. Coronary calcium was identified in three contiguous voxels using a cutoff point of 130 HU, resulting in a minimum calcified lesion area of 1.02 mm^2. 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 and colleagues, ranging from 1 to 4. The total CAC score was obtained by aggregating

individual lesion scores from each of the four anatomic sites (left main, left anterior descending, circumflex, and right coronary arteries).

Coronary computed tomographic angiography quality assurance/control

Experienced readers in the CCTA core lab read all the CCTA scans, blinded to all demographics, testosterone randomization, and risk factors, in near real-time (within 1 week of acquisition) during enrollment and during the scanning period for stenosis evaluation and assessability. Three level 3 experienced cardiac CT readers evaluated all studies in the trial. The prevalence of slab, blurring, and partial volume averaging artifacts on all scans were also evaluated in the CCTA core lab. 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 used in this trial. Of these men, 35 were taking statins and 28 were not. Scans were performed at two time points ~1 year apart. In this cohort, the SDs of the changes from the first to the second scan were similar in the two groups; we used the larger of the two values (an SD of 26) for our calculations, which indicated that 140 men, 70/arm, would provide 80% power to detect a difference of 12 mm^3 in noncalcified plaque volume, somewhat less than the difference of 14 mm^3 between statin users and nonusers seen in the prior study. As 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 TTrials who would be eligible for and would consent to participate in this trial, we also calculated power for a total sample size of 120, 60/arm; this number would provide 80% power to detect a 13 mm^3 difference between treatment arms after 1 year of treatment.

Statistical analysis

The primary hypothesis was that testosterone treatment would decrease progression of noncalcified coronary artery plaque volume, as assessed by CCTA. Efficacy was measured using a multivariate linear model that adjusts for baseline noncalcified 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 ($\leq$ or $>$ 200 ng/dl), age ($\leq$ or $>$ 75), use of antidepressants, and AQ10 use of PDE inhibitors. All participants with baseline and 12-month plaque volume measurements were included in the analysis. Significance was assessed using 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. β-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

A total of 199 men consented to be screened for the trial. Of these, 170 were eligible, and 165 underwent baseline scanning. Five men could not be scanned because of high heart rates or lack of venous access.

Demographics

A total of 165 men were enrolled in the Cardiovascular Trial and underwent baseline CCTA. Baseline information for these men is listed in Table 2. The average age of the participants was 71 years. Most men were overweight or obese; 61% had a BMI of 30 or higher. Hypertension and/or hyperlipidemia were present in around two to three participants, and approximately one-third of the participants were diabetic. A prior MI was reported by 9% of participants; 10% had had a prior revascularization and 6% had a history of angina. The mean volume of noncalcified plaques was 91.0 mm^3, with an SD of 95.9 mm^3.

Table 2 Baseline characteristics of men in the cardiovascular trial

Characteristics[a]	Mean ± SD or *N* (%)
Age (years)	71.1 ± 5.7
65–74	135 (79.4)
75–84	25 (14.7)
≥ 85	10 (5.9)
BMI (kg/m^2)	30.7 ± 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 ± 59.5
Noncalcified plaque volume (mm^3)	91.0 ± 95.9

COPD, chronic obstructive pulmonary disease; MI, myocardial infarction; TIA, transient ischemic attack.
[a]By self-report.

Association of coronary plaque volume with serum testosterone concentration

We assessed whether there was any notable association between serum testosterone levels and noncalcified coronary plaque volume at baseline. These data are shown in Fig. 2. There was a trend toward higher values of testosterone associated with lower plaque volumes, but this association was found not to be statistically significant on 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 found to be inversely correlated with blood pressure [4], prevalence of diabetes, and the risk for future diabetes [5]. In the same cohort, men with a testosterone level less than 250 ng/dl were found to have a significantly increased risk of mortality compared with men with higher levels of testosterone, independent of other covariates. In a larger cohort study from England, testosterone levels in men were found to be 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 concerns that exogenous testosterone might increase the risk for 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 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 because of 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 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 noninvasive tool to directly examine the coronary artery wall, determine the degree of plaque burden, and assess the degree of coronary artery stenosis [41]. In addition, based on tissue-specific radiographic attenuation characteristics, CCTA also provides additional information on atherosclerotic plaque composition. It is able to differentiate calcified from predominantly noncalcified plaques and those that contain a large lipid pool [11,42] and allows volumetric analysis of plaques. Plaque characterization (i.e. determining the vulnerability of plaque rupture by examining its tissue components) is now possible using CCTA. New CCTA technology with 64 slices or more 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 vulnerable nonobstructive plaques [43]. Tissue density measured by CCTA can be used to characterize atherosclerotic plaque composition.

The Cardiovascular Trial applies strict standardization and quality control measures in assessing the imaging data, with all readings made by the same readers, who were blind to treatment assignment. The equivocal results of prior testosterone trials were once thought to be due to selection of men who did not have sufficiently low testosterone concentrations. This pitfall was avoided in the TTrials, as 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 TTrials had a long list of inclusion and exclusion factors [8]; hence, this study will not be representative of all men over the age of 65 years. In particular, men with a recent myocardial infarction, those with serum testosterone within the normal range or less than 100 ng/dl, and those without functional concerns were excluded. In addition, the size of the study and the 1-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 TTrials 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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Conflicts of interest

S.S.E. reports a conference grant from AbbVie during the conduct of the study; R.S.S. reports grants and consulting from AbbVie, Clarus, Ardana, Besins Health, and Endo Pharma; C.E.L. was supported by the National Institute for Diabetes, Digestive and Kidney Diseases, National Institutes of Health (DK-079626) to the UAB Diabetes Research and Training Center; P.J.S. reports grants from

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References

1 Vermeulen A, Rubens R, Verdonck L. Testosterone secretion and metabolism in male senescene. *J Clin Endocrinol Metab* 1972; **34**:730–735.
2 Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, *et al*. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *J Clin Endocrinol Metab* 2002; **87**:589–598.
3 Harman S, Metter E, Tobin J, Pearson J, Blackman M. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J Clin Endocrinol Metab* 2001; **86**:724–731.
4 Barrett-Connor E, Khaw KT. Endogenous sex hormones and cardiovascular disease in men. A prospective population-based study. *Circulation* 1988; **78**:539–545.
5 Oh JY, Barrett-Connor E, Wedick NM, Wingard DL. Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. *Diabetes Care* 2002; **25**:55–60.
6 Smith GD, Ben-Shlomo Y, Beswick A, Yarnell J, Lightman S, Elwood P. Cortisol, testosterone, and coronary heart disease: prospective evidence from the Caerphilly study. *Circulation* 2005; **112**:332–340.
7 Basaria S, Coviello AD, Travison TG, Storer TW, Farwell WR, Jette AM, *et al*. Adverse events associated with testosterone administration. Multicenter Study Randomized Controlled Trial Research Support, N.I.H., Extramural Research Support, U.S. Govt, Non-P.H.S. *N Engl J Med* 2010; **363**:109–122.
8 Snyder PJ, Ellenberg SS, Cunningham GR, Matsumoto AM, Bhasin S, Barrett-Connor E, *et al*. The Testosterone Trials: Seven coordinated trials of testosterone treatment in elderly men. *Clin Trials* 2014; **11**:362–375.
9 Cauley JA, Fluharty L, Ellenberg SS, Gill TM, Ensrud KE, Barrett-Connor E, *et al*. Recruitment and screening for the testosterone trials. *J Gerontol A Biol Sci Med Sci* 2015; **70**:1105–1111.
10 Taves DR. Minimization: a new method of assigning patients to treatment and control groups. *Clin Pharmacol Ther* 1974; **15**:443–453.
11 Zeb I, Li D, Nasir K, Malpeso J, Batool A, Flores F, *et al*. Effect of statin treatment on coronary plaque progression – a serial coronary CT angiography study. *Atherosclerosis* 2013; **231**:198–204.
12 Pagali SR, Madaj P, Gupta M, Nair S, Hamirani YS, Min JK, *et al*. Interobserver variations of plaque severity score and segment stenosis score in coronary arteries using 64 slice multidetector computed tomography: a substudy of the ACCURACY trial. *J Cardiovasc Comput Tomogr* 2010; **4**:312–318.
13 Leber AW, Knez A, Becker A, Becker C, von Ziegler F, Nikolaou K, *et al*. Accuracy of multidetector spiral computed tomography in identifying and differentiating the composition of coronary atherosclerotic plaques: a comparative study with intracoronary ultrasound. *J Am Coll Cardiol* 2004; **43**:1241–1247.
14 Iwasaki K, Matsumoto T, Aono H, Furukawa H, Samukawa M. Prevalence of subclinical atherosclerosis in asymptomatic diabetic patients by 64-slice computed tomography. *Coron Artery Dis* 2008; **19**:195–201.
15 Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, Jacobs DR Jr, *et al*. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. *Radiology* 2005; **234**:35–43.
16 Budoff MJ, Dowe D, Jollis JG, Gitter M, Sutherland J, Halamert E, *et al*. Diagnostic performance of 64-multidetector row coronary computed tomographic angiography for evaluation of coronary artery stenosis in individuals without known coronary artery disease: results from the prospective multicenter ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) trial. *J Am Coll Cardiol* 2008; **52**:1724–1732.
17 de Graaf MA, Broersen A, Kitslaar PH, Roos CJ, Dijkstra J, Lelieveldt BP, *et al*. Automatic quantification and characterization of coronary atherosclerosis with computed tomography coronary angiography: cross-correlation with intravascular ultrasound virtual histology. *Int J Cardiovasc Imaging* 2013; **29**:1177–1190.
18 Yang G, Kitslaar P, Frenay M, Broersen A, Boogers MJ, Bax JJ, *et al*. Automatic centerline extraction of coronary arteries in coronary computed tomographic angiography. *Int J Cardiovasc Imaging* 2012; **28**:921–933.
19 Boogers MJ, Broersen A, van Velzen JE, de Graaf FR, El-Naggar HM, Kitslaar PH, *et al*. Automated quantification of coronary plaque with computed tomography: comparison with intravascular ultrasound using a dedicated registration algorithm for fusion-based quantification. *Eur Heart J* 2012; **33**:1007–1016.
20 Budoff MJ, Gopal A, Gopalakrishnan D. Cardiac computed tomography: diagnostic utility and integration in clinical practice. *Clin Cardiol* 2006; **29** (9 Suppl 1):I4–I14.
21 Ahmadi N, Nabavi V, Hajsadeghi F, Flores F, French WJ, Mao SS, *et al*. Mortality incidence of patients with non-obstructive coronary artery disease diagnosed by computed tomography angiography. *Am J Cardiol* 2011; **107**:10–16.
22 Choi TY, Malpeso J, Li D, Sourayanezhad S, Budoff MJ. Radiation dose reduction with increasing utilization of prospective gating in 64-multidetector cardiac computed tomography angiography. *J Cardiovasc Comput Tomogr* 2011; **5**:264–270.
23 Madaj PM, Budoff MJ, Li D, Tayek JA, Karlsberg RP, Karpman HL. Identification of noncalcified plaque in young persons with diabetes: an opportunity for early primary prevention of coronary artery disease identified with low-dose coronary computed tomographic angiography. *Acad Radiol* 2012; **19**:889–893.
24 Cheng VY, Nakazato R, Dey D, Gurudevan S, Tabak J, Budoff MJ, *et al*. Reproducibility of coronary artery plaque volume and composition quantification by 64-detector row coronary computed tomographic angiography: an intraobserver, interobserver, and interscan variability study. *J Cardiovasc Comput Tomogr* 2009; **3**:312–320.
25 Papadopoulou SL, Garcia-Garcia HM, Rossi A, Girasis C, Dharampal AS, Kitslaar PH, *et al*. Reproducibility of computed tomography angiography data analysis using semiautomated plaque quantification software: implications for the design of longitudinal studies. *Int J Cardiovasc Imaging* 2013; **29**:1095–1104.
26 Motoyama S, Kondo T, Anno H, Sugiura A, Ito Y, Mori K, *et al*. Atherosclerotic plaque characterization by 0.5-mm-slice multislice computed tomographic imaging. *Circ J* 2007; **71**:363–366.
27 Motoyama S, Kondo T, Sarai M, Sugiura A, Harigaya H, Sato T, *et al*. Multislice computed tomographic characteristics of coronary lesions in acute coronary syndromes. *J Am Coll Cardiol* 2007; **50**:319–326.
28 Brodoefel H, Reimann A, Burgstahler C, Schumacher F, Herberts T, Tsiflikas I, *et al*. Noninvasive coronary angiography using 64-slice spiral computed tomography in an unselected patient collective: effect of heart rate, heart rate variability and coronary calcifications on image quality and diagnostic accuracy. *Eur J Radiol* 2008; **66**:134–141.
29 Brodoefel H, Reimann A, Heuschmid M, Tsiflikas I, Kopp AF, Schroeder S, *et al*. Characterization of coronary atherosclerosis by dual-source computed tomography and HU-based color mapping: a pilot study. *Eur Radiol* 2008; **18**:2466–2474.
30 Brodoefel H, Burgstahler C, Tsiflikas I, Reimann A, Schroeder S, Claussen CD, *et al*. Dual-source CT: effect of heart rate, heart rate variability, and calcification on image quality and diagnostic accuracy. *Radiology* 2008; **247**:346–355.
31 Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990; **15**:827–832.
32 Burgstahler C, Reimann A, Beck T, Kuettner A, Baumann D, Heuschmid M, *et al*. Influence of a lipid-lowering therapy on calcified and noncalcified coronary plaques monitored by multislice detector computed tomography: results of the New Age II Pilot Study. *Invest Radiol* 2007; **42**:189–195.
33 Gopal A, Budoff MJ. A new method to reduce radiation exposure during multi-row detector cardiac computed tomographic angiography. *Int J Cardiol* 2009; **132**:435–436.
34 Hausleiter J, Meyer T, Hadamitzky M, Huber E, Zankl M, Martinoff S, *et al*. Radiation dose estimates from cardiac multislice computed tomography in daily practice: impact of different scanning protocols on effective dose estimates. *Circulation* 2006; **113**:1305–1310.
35 Vigen R, O'Donnell CI, Baron AE, Grunwald GK, Maddox TM, Bradley SM, *et al*. Association of testosterone therapy with mortality, myocardial infarction, and stroke in men with low testosterone levels. *JAMA* 2013; **310**:1829–1836.
36 Finkle WD, Greenland S, Ridgeway GK, Adams JL, Frasco MA, Cook MB, *et al*. Increased risk of non-fatal myocardial infarction following testosterone therapy prescription in men. *PLoS One* 2014; **9**:e85805.
37 Shores MM, Smith NL, Forsberg CW, Anawalt BD, Matsumoto AM. Testosterone treatment and mortality in men with low testosterone levels. *J Clin Endocrinol Metab* 2012; **97**:2050–2058.
38 Baillargeon J, Urban RJ, Kuo YF, Ottenbacher KJ, Raji MA, Du F, *et al*. Risk of myocardial infarction in older men receiving testosterone therapy. *Ann Pharmacother* 2014; **48**:1138–1144.

39 Liverman C, Blazer D. *Testosterone and aging clinical research directions.* Washington, DC: The National Academies Press; 2004.
40 Newby DE, Fox KA. Invasive assessment of the coronary circulation: intravascular ultrasound and Doppler. *Br J Clin Pharmacol* 2002; **53**:561–575.
41 Cury RC, Pomerantsev EV, Ferencik M, Hoffmann U, Nieman K, Moselewski F, *et al.* Comparison of the degree of coronary stenoses by multidetector computed tomography versus by quantitative coronary angiography. *Am J Cardiol* 2005; **96**:784–787.
42 Achenbach S, Moselewski F, Ropers D, Ferencik M, Hoffmann U, MacNeill B, *et al.* Detection of calcified and noncalcified coronary atherosclerotic plaque by contrast-enhanced, submillimeter multidetector spiral computed tomography: a segment-based comparison with intravascular ultrasound. *Circulation* 2004; **109**:14–17.
43 Hirai N, Horiguchi J, Fujioka C, Kiguchi M, Yamamoto H, Matsuura N, *et al.* Prospective versus retrospective ECG-gated 64-detector coronary CT angiography: assessment of image quality, stenosis, and radiation dose. *Radiology* 2008; **248**:424–430.
44 Corona G, Maseroli E, Rastrelli G, Isidori AM, Sforza A, Mannucci E, *et al.* Cardiovascular risk associated with testosterone-boosting medications: a systematic review and meta-analysis. *Expert Opin Drug Saf* 2014; **13**:1327–1351.
45 Xu L, Freeman G, Cowling BJ, Schooling CM. Testosterone therapy and cardiovascular events among men: a systematic review and meta-analysis of placebo-controlled randomized trials. *BMC Med* 2013; **11**:108.
46 Fernandez-Balsells MM, Murad MH, Lane M, Lampropulos JF, Albuquerque F, Mullan RJ, *et al.* Clinical review 1: adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2010; **95**:2560–2575.

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