

## Biomarkers and Noncalcified Coronary Artery Plaque Progression in Older Men Treated With Testosterone

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**Objective:** Recent results from the Cardiovascular Trial of the Testosterone Trials showed that testosterone treatment of older men with low testosterone was associated with greater progression of noncalcified plaque (NCP). We evaluated the effect of anthropometric measures and cardiovascular biomarkers on plaque progression in individuals in the Testosterone Trial.

**Methods:** The Cardiovascular part of the trial included 170 men aged 65 years or older with low testosterone. Participants received testosterone gel or placebo gel for 12 months. The primary outcome was change in NCP volume from baseline to 12 months, as determined by coronary computed tomography angiography (CCTA). We assayed several markers of cardiovascular risk

and analyzed each marker individually in a model as predictive variables and change in NCP as the dependent variable.

**Results:** Of 170 enrollees, 138 (73 testosterone, 65 placebo) completed the study and were available for the primary analysis. Of 10 markers evaluated, none showed a significant association with the change in NCP volume, but a significant interaction between treatment assignment and waist-hip ratio (WHR) ( $P = 0.0014$ ) indicated that this variable impacted the testosterone effect on NCP volume. The statistical model indicated that for every 0.1 change in the WHR, the testosterone-induced 12-month change in NCP volume increased by 26.96 mm<sup>3</sup> (95% confidence interval, 7.72–46.20).

**Conclusion:** Among older men with low testosterone treated for 1 year, greater WHR was associated with greater NCP progression, as measured by CCTA. Other biomarkers and anthropometric measures did not show statistically significant association with plaque progression. (*J Clin Endocrinol Metab* 105: 2142–2149, 2020)

**Key Words:** noncalcified coronary artery plaque, coronary artery disease, testosterone replacement

Lower serum testosterone concentration has been associated with adverse cardiovascular disease (CVD) outcomes (1, 2). There are conflicting reports regarding the effect of testosterone treatment on CVD risk. Some retrospective studies reported more CVD events in men taking testosterone, while others did not (3–7). The Testosterone Trials (TTrials) comprised 7 coordinated placebo-controlled clinical trials designed to assess the effects of testosterone treatment in older men who had low testosterone concentrations for no apparent reason other than age (8). In the Cardiovascular Trial, testosterone treatment for 1 year compared with placebo was associated with significantly greater progression of coronary artery noncalcified plaque (NCP) volume measured by serial coronary computed tomography angiography (CCTA) (9).

Serum markers such as total cholesterol, high-density lipoprotein (HDL), low density lipoprotein (LDL), and hemoglobin A1C (HbA1c), have been recognized as significant risk factors for developing coronary artery plaque and future CVD events (10, 11). There are contradictory reports about the association of biomarkers and the extent, progression of atherosclerosis, and coronary events (12–14). Inflammatory markers such as C-reactive protein (CRP) have been reported to be associated with plaque progression in some studies (15, 16), while other reports found no association (17, 18). Anthropometric measures such as waist-hip ratio (WHR) and waist circumference are predictors of myocardial infarction risk (19, 20). Abdominal obesity can lead to increases in insulin and glucose levels and is a central feature of metabolic syndrome. Several observational studies have shown a link between low endogenous sex hormones and metabolic syndrome (21–23). One large cross-sectional study reported that higher testosterone and sex hormone-binding globulin

(SHBG) levels in older men were independently associated with reduced risk of metabolic syndrome and higher insulin sensitivity (24).

The aim of the current study is to evaluate the impact of baseline anthropometric measures and cardiovascular biomarkers on the progression of coronary artery plaque volume in the 138 men who participated in the Cardiovascular Trial of the TTrials. We also assessed the interaction of anthropometric measures and cardiovascular biomarkers with testosterone treatment for atherosclerotic plaque progression.

## Methods

### Study design

The TTrials comprised 7 double-blind, placebo-controlled randomized controlled trials. The overall study design of the TTrials, as well as that of the Cardiovascular Trial, has been published (8, 25). To qualify for the TTrials overall, a participant had to qualify for at least 1 of 3 main trials (Sexual Function Trial, Physical Function Trial, and Vitality Trial). Qualified men could also participate in any of other trials, if respective eligibility criteria were met. The participants were allocated to receive testosterone or placebo gel for 1 year (8, 9). Institutional review boards of all participating sites approved the TTrials and Cardiovascular Trial protocols. All participants provided written consent. Trial conduct and participant safety were supervised by an independent safety and data monitoring board.

### Participants

The TTrials included men  $\geq 65$  years of age who had symptoms and objective evidence of low libido, physical dysfunction and/or low vitality, and serum testosterone levels that averaged  $< 275$  ng/dL on 2 morning samples. Men who were at moderate or high risk for prostate cancer, who had had a myocardial infarction within the previous 3 months, or who had systolic blood pressure  $> 160$  mm Hg or diastolic blood pressure  $> 100$  mm Hg, were excluded (8).

Exclusion criteria specifically for the Cardiovascular Trial included circumstances that either made coronary computed tomography angiography (CCTA) technically unfeasible (inability to hold breath for 10 seconds, a prior diagnosis of tachycardia or irregular heart rhythm [eg, atrial fibrillation], weight > 136 kg, or a history of coronary artery bypass graft surgery) or that increased the risk of performing the CCTA (estimated glomerular filtration rate < 60 mL/min/1.73 m<sup>2</sup> or known allergy to iodinated contrast) (9, 25).

### Testosterone treatment

Participants were assigned to receive either testosterone as a 1% gel in a pump bottle (AndroGel) or a placebo gel, by a double-blinded method for 1 year. The initial testosterone dose was 5 g/day and was adjusted to maintain the serum concentrations within normal range for young men (280–873 ng/dL) measured at a central laboratory (Quest Clinical Trials) at months 1, 2, 3, 6, and 9. To maintain blinding, whenever dose adjustments were made in a man receiving testosterone treatment, the dose was also changed in a man receiving placebo (8).

### Assessments

The concentrations of cardiovascular biomarkers were measured on serum samples drawn at baseline and months 3 and 12 and stored at –80 C. These assays were performed at the Laboratory for Clinical Biochemistry Research, University of Vermont, and University of Minnesota, as described previously (7, 9). At months 3, 6, 9, and 12, clinical variables were measured.

Details of coronary artery plaque volume by CCTA assessment have been published (25). In brief, coronary artery plaque volume was assessed by CCTA at 9 of the 12 TTrial clinical sites. Precontrast scans for evaluation of coronary artery calcium density and postcontrast scans for evaluation of coronary artery plaque volume were performed at baseline and 12 months. Scans were assessed at a central reading center (Harbor-UCLA Medical Center) by readers who were blinded both to treatment group and date of scan. Quantitative plaque assessment was conducted according to a previously defined protocol (26) using semi-automated plaque analysis software (QAngioCT Research Edition Version 2.0.5; Medis Medical Imaging Systems). Based on the guidelines of the Society of Cardiovascular Computed Tomography, 17-segment coronary artery model vessels greater than 1.5 mm were evaluated (27). The volumes of 4 types of coronary artery plaque (low attenuation, fibrous-fatty, fibrous, and dense calcified) were calculated by Hounsfield unit threshold. The primary outcome was change in NCP volume from baseline to month 12. NCP was defined as the sum of the fibrous, fibrous fatty, and low attenuation plaque. Secondary outcomes were change in calcified plaque volume and change in coronary artery score. Details of intra- and inter-observer variability have been published. The intra-class correlations (ICCs) and coefficient of variation (CVs) were 0.99 and 7.8 %, respectively, for intra-observer variability. The ICC and CV were 0.95 and 19.9 %, respectively, for inter-observer variability (9).

### Statistical analyses

The following markers were available for study: total cholesterol; non-HDL cholesterol; HDL; LDL; total cholesterol/

HDL ratio; triglycerides; HbA1c; glucose, insulin; homeostasis model assessment (HOMA); d-dimer; troponin; CRP; interleukin-6 (IL-6); SHBG; weight; body mass index (BMI); waist circumference; and WHR. We evaluated the inter-correlation of the baseline values of these markers, separately within groups where substantial inter-correlation was expected: lipid markers, metabolic markers, markers of inflammation, and clinical markers. We then excluded from further study the marker showing correlation > 0.5 with the most other markers, and then eliminated any marker with correlation > 0.5 with the selected marker from further consideration. We retained any other markers with correlation < 0.5 with the selected marker. If 2 markers showed high correlation with the same number of other markers, we selected the 1 with the lowest correlation with the remaining markers. We also included SHBG, d-dimer, and troponin without testing for correlation with other markers, since they did not fit into the any of the 4 categories noted above.

We tested each selected marker separately in a regression model, including treatment as a covariate as well as age (over or under 75), baseline testosterone (over or under 200 ng/mL) and an interaction term of the marker with treatment. Any variable showing a significant association with the change in plaque volume after adjusting for multiple comparisons using the Holm procedure (28) was to be included in a multivariable model, assessing all potentially predictive variables simultaneously.

Secondary analyses included testing association of the selected markers with change in calcified plaque volume and with coronary artery calcium score, using the same approach as above.

## Results

Of 138 men who were enrolled, 73 received testosterone treatment and 65 received placebo. The baseline characteristics of the participants in the Cardiovascular Trial were previously reported (9). At baseline, the mean (standard deviation [SD]) age was 71.2 (5.7) years. The majority of participants were white (81%) and had relatively high rates of cardiovascular risk factors, including hypertension, hyperlipidemia, obesity, and diabetes. At baseline, the mean (SD) BMI was 30.6 (3.8) in the testosterone group and 30 (3.5) in the placebo group; the mean weight was 94 kg and the mean WHR was 1.0 in each treatment group. The calculated 10-year risk of cardiovascular events was relatively high: a mean risk of 27% (95% confidence interval [CI], 6.4%–47.6%) in the placebo group and 24% (95% CI, 2.6%–45.4%) in the testosterone group.

Of the 19 markers initially evaluated, 10 remained for further study after removing those that were highly correlated with other markers, as described above. These 10 remaining markers were HDL cholesterol, non-HDL cholesterol, D-dimer, IL-6, CRP, insulin, HgbA1C, SHBG, weight, and WHR (Table 1). Among these 10 measures, only the baseline WHR

**Table 1. Baseline Values of Anthropometric Measures and Biomarkers**

Assay	Statistic	Testosterone	Placebo
HDL, mg/dL	Mean (SD)	44.2 (11.7)	49.1 (16.0)
	Median (IQR)	43 (36, 51)	46 (39, 56)
Non-HDL, mg/dL	Mean (SD)	116.8 (36.6)	126 (38)
	Median (IQR)	113 (91, 140)	120 (109, 137)
D-dimer, mg/L	Mean (SD)	0.70 (0.52)	0.71 (0.62)
	Median (IQR)	0.58 (0.39, 0.81)	0.51 (0.40, 0.76)
IL-6, pg/mL	Mean (SD)	1.7 (3.0)	1.6 (2.2)
	Median (IQR)	1.2 (0.80, 1.6)	1.1 (0.83, 1.5)
CRP, mg/L	Mean (SD)	5.3 (17.7)	2.8 (4.2)
	Median (IQR)	1.8 (0.78, 2.7)	1.4 (0.82, 2.4)
Insulin, $\mu$ U/mL	Mean (SD)	19.4 (13.8)	17.7 (17.1)
	Median (IQR)	15.6 (10.1, 24.1)	14.3 (10.6, 18.5)
HbA1c, %	Mean (SD)	6.3 (0.79)	6.2 (0.75)
	Median (IQR)	6.0 (5.7, 6.8)	6.0 (5.8, 6.4)
SHBG, nmol/L	Mean (SD)	30.4 (15.1)	29.6 (13.2)
	Median (IQR)	27.2 (20.2, 35.6)	28.5 (20.4, 35.5)
Weight, kg	Mean (SD)	94.8 (14.0)	93.8 (14.6)
	Median (IQR)	91.5 (84, 103)	93 (84, 104)
Waist-hip ratio	Mean (SD)	1.0 (0.06)	1.0 (0.07)
	Median (IQR)	1.0 (0.96, 1.0)	1.0 (0.97, 1.0)

Abbreviations: CRP, C-reactive protein; HbA1c, hemoglobin A1C; HDL, high-density lipoprotein; IL-6, interleukin-6; IQR, interquartile range; SD, standard deviation; SHBG, sex hormone-binding globulin.

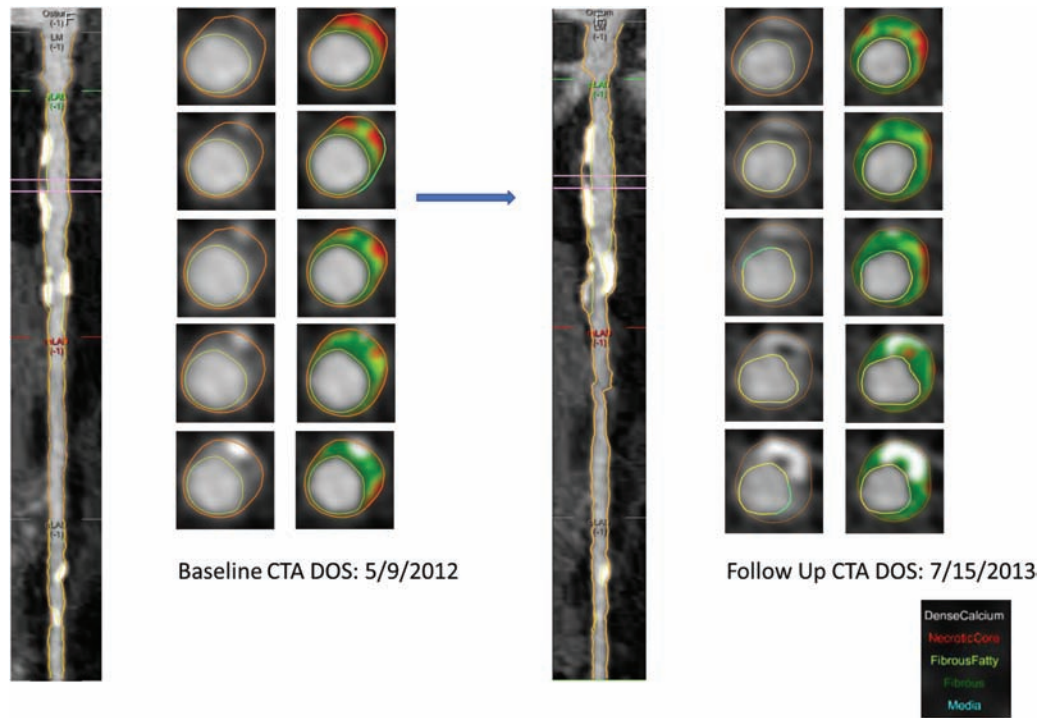
**Table 2. Association of Baseline Anthropometric Measures and Biomarkers with 12-Month Change in NCP Volume. Based on Linear Regression Models Including Terms for Site, Age (over/under 75), Baseline Testosterone Level (over/under 200 ng/dL), Baseline NCP volume, Predictor Variable, and Interaction of Predictor Variable with Treatment. P Values Not Adjusted for Multiple Comparisons**

Predictor variable	Effect Estimate (Standard error)	95% CI	Nominal P value
HDL	0.31 (0.38)	-0.43, 1.06	0.40
HDL $\times$ treatment	-0.75 (0.65)	-2.03, 0.53	0.25
Non-HDL	0.034 (0.16)	-0.29, 0.35	0.84
Non-HDL $\times$ treatment	-0.83 (0.23)	-05.4, 0.37	0.71
D-dimer	-19.4 (9.93)	-39.1, 0.25	0.053
D-dimer $\times$ treatment	15.92 (14.98)	-13.74, 45.58	0.29
IL-6	-2.81 (2.78)	-8.32, 2.69	0.31
IL-6 $\times$ treatment	2.50 (3.32)	-4.07, 9.08	0.45
CRP	-1.48 (1.45)	-4.36, 1.39	0.31
CRP $\times$ treatment	1.30 (1.49)	-1.64, 4.25	0.38
Insulin	0.53 (0.36)	-0.66, 0.76	0.88
Insulin $\times$ treatment	-0.26 (0.57)	-1.40, 0.87	0.65
HbA1c	0.34 (9.00)	-17.5, 18.2	0.97
HbA1c $\times$ treatment	5.15 (11.88)	-18.82, 28.70	0.67
SHBG	0.24 (0.49)	-0.73, 1.21	0.63
SHBG $\times$ treatment	-0.83 (0.60)	-2.02, 0.36)	0.17
Weight	-0.44 (0.42)	-1.27, 0.40	0.30
Weight $\times$ treatment	0.80 (0.59)	-0.37, 1.97	0.18
Waist-hip ratio	-87.7 (93.2)	-272.2, 96.7	0.35
Waist-hip ratio $\times$ treatment	429.4 (131.4)	169.4, 689.5	0.0014

Abbreviations: CRP, C-reactive protein; HbA1c, hemoglobin A1C; HDL, high-density lipoprotein; IL-6, interleukin-6; IQR, interquartile range; SD, standard deviation; SHBG, sex hormone-binding globulin.

interaction with treatment showed a significant association with the progression of NCP volume at 12 months (Table 2, Figure 1). The baseline values of WHR ranged from 0.9 to 1.2. Because it was the interaction term that met the threshold based on the multiple comparisons adjustment ( $P = 0.0014$

compared to threshold value from the Holm multiple comparisons procedure of 0.0056), we evaluated WHR separately for the 2 treatment groups. The association was seen only in the testosterone group ( $P = 0.007$ ). The model indicates that for every 0.1 change in the WHR, the effect of testosterone on the



**Figure 1.** An example of plaque progression in proximal left anterior descending coronary artery over 1 year in participants enrolled in testosterone trial.

12-month change in NCP volume would increase by 26.96 mm<sup>3</sup> (95% CI, 7.72-46.20).

None of the cardiovascular risk markers were statistically significantly associated with change in calcified plaque or coronary artery calcium score when applying the multiple comparisons correction.

**Discussion**

We report that in older hypogonadal men participating in the Cardiovascular Trial of the TTrials there was a significant association between baseline WHR and progression of NCP volume measured by CCTA after 1 year of testosterone treatment. Among men taking testosterone, larger WHRs were associated with greater progression of NCP.

There are strong associations among presence of visceral adipose tissue, insulin sensitivity, dyslipidemia, and increase in inflammation and hypertension (29, 30). Visceral adipose tissue stores can be measured by computed tomography, dual-energy X-ray absorptiometry, or magnetic resonance imaging, but these modalities are too expensive and time consuming for day-to-day use (31, 32). WHR is closely related to visceral fat and commonly measured in clinical practice (33). Meta-analyses of 28 114 patients from 15 prospective studies showed that for every 0.01 increase in WHR, there was a 5% increase in risk of future CVD events (33). Our

data indicate that for every 0.1 increase in WHR, there was 26 mm<sup>3</sup> greater increase in progression of NCP volume in patients treated with testosterone replacement therapy.

NCP volume, as assessed by CCTA, has been associated with CVD events. In a large single-center trial conducted by Zu et al (34), the cumulative probability of 3-year major adverse cardiovascular events (including cardiac death, nonfatal myocardial infarction, or coronary revascularization) increased across the strata for cardiac computed tomography plaque characteristics (5.5% for calcified plaque, 22.7% for NCP, and 37.7% for mixed plaque; *P* < 0.001)

WHR and waist circumference, measures of central obesity or abdominal obesity, have been associated with reduced total testosterone levels (35, 36). A potential mechanism for this inverse relationship may involve increased leptin levels, which are hypothesized to interfere with luteinizing hormone stimulation of androgen production and decreased SHBG in central obesity (37). Another plausible mechanism of decreased testosterone in obese individuals is increased aromatase activity in visceral adipose tissue, which leads to higher conversion of testosterone to estradiol (38). Androgen deprivation therapy, as given to patients with prostate cancer, has shown to significantly increase BMI, total weight, and body fat mass and to decrease lean body mass (39, 40). Hence, several studies have investigated the hypothesis that testosterone replacement therapy may decrease

visceral fat stores and improve the metabolic profile in men. However, there are conflicting reports on the effects of testosterone replacement on visceral fat. Some studies reported testosterone replacement therapy decreases visceral fat, while others showed no association (41, 42). In a study of 261 patients in a prospective longitudinal registry, testosterone replacement was associated with a significant reduction in obesity parameters (eg, waist circumference, BMI) and cholesterol values over the 5-year study period (43). However, randomized controlled clinical trials reported no impact of testosterone replacement on weight, BMI, and metabolic syndrome (41, 44). A previous paper from the TTrial also did not show any changes in WHR, waist circumference, and BMI in men treated with testosterone for 12 months compared with placebo-treated men (7).

These results are hypothesis-generating and warrant further investigation of the interaction of visceral adipose tissue stores and testosterone treatment. To our knowledge, no other studies have examined the interaction of testosterone replacement therapy and central obesity on CVD outcomes. The strengths of our trial included requiring all men to have unequivocally low testosterone at baseline, a placebo-controlled design, and blinded central review of baseline and 12 month scans. An important limitation of our study is the use of a surrogate marker of heart disease, NCP, rather than a clinical outcome. Another limitation is that the results apply only to men  $\geq 65$  years of age with low testosterone (9).

Our results are exploratory and do not conclusively demonstrate that the markers we assessed have no associations with NCP progression; however, the lack of statistically significant findings suggest that if such associations do exist, they are not likely to be strong. We conclude that among older men receiving testosterone treatment, those with higher vs lower WHR may experience greater increases in NCP volume. Future trials should evaluate the interaction of testosterone treatment and surrogate markers of abdominal obesity and visceral fat stores.

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## Additional Information

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**Data Availability:** All data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

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