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Biomarkers and Non-Calcified Coronary Artery Plaque Progression in Older Men Treated with Testosterone

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Abstract:

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

Methods: The CV part of the trial included 170 men aged 65 years or older with low T. Participants received T 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 CV 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 T, 65 placebo) completed the study and were available for the primary analysis. Of 9 markers evaluated, none showed a significant association with the change in NCP volume, but a significant interaction between treatment assignment and waist-hip ratio p-0.0014) indicated that this variable impacted the testosterone effect on non-calcified plaque volume. The statistical model indicated that for every 0.1 change in the waist-hip ratio, the T-induced 12-month change in non-calcified plaque volume increased by 26.96 mm^3 (95% confidence interval 7.72, 46.20).

Conclusion: Among older men with low T treated for one year, greater waist-hip ratio was associated with greater NCP progression, as measured by CCTA. Other biomarkers and anthropometric measures did not show statistically significant association with plaque progression.

Introduction:

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 seven 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 one year compared with placebo was associated with significantly greater progression of coronary artery non-calcified plaque 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, 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 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], other reports found no association[17,18]. Anthropometric measures such as Waist-Hip ratio 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 link of low endogenous sex hormones and metabolic syndrome[21-23]. One large cross-sectional study reported that higher testosterone and sex hormone binding globulin

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 seven double-blind, placebo-controlled randomized controlled trials. The overall study design of TTrials, as well that of 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 TTrials and Cardiovascular Trial protocols. All participants provided written consent. Trial conduct and participant safety was supervised by an independent safety and data monitoring board.

Participants

The TTrials included men ≥65 years' old who had symptoms and objective evidence of low libido, physical dysfunction and/or low vitality, 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 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 artery CT angiography (CCTA) technically unfeasible (inability to hold breath for 10 seconds, a prior diagnosis of tachycardia or irregular heart rhythm [e.g., atrial fibrillation], weight >136 kg, or history of coronary artery bypass graft surgery) or increased risk of performing the CCTA (estimated glomerular filtration rate <60 mL/min/1.73 m2 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 placebo gel by a double-blinded method for one year. The initial dose was 5 g/d and was adjusted to maintain the serum concentrations within normal range for young men (280-873 ng/dL) measured at central laboratory (Quest Clinical Trials) at months 1, 2, 3, 6, and 9. Whenever dose adjustments were made in a man receiving testosterone treatment, the dose was changed in a man receiving placebo as well to maintain blinding[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 TTrials clinical sites. Pre-contrast scans for evaluation of coronary artery calcium density and post contrast 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 four 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 non-calcified plaque volume from baseline to month 12. Non-calcified plaque 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 % for intra-

observer variability respectively. ICC and CV was 0.95 and 19.9 % for inter-observer variability respectively[9].

Statistical Analyses

The following markers were available for study: total cholesterol; non-HDL cholesterol; HDL; LDL; total cholesterol/HDL ratio; triglycerides; HgA1c; glucose, insulin; homeostatin model assessment(HOMA); d-dimer; troponin; CRP; interleukin-6 (IL-6); weight; BMI; waist; waist/hip ratio. 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 two markers showed high correlation with the same number of other markers, we selected the one with the lowest correlation with the remaining markers. We also included d-dimer and troponin without testing them for correlation with other markers as 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 (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 BMI 30.6 (3.8) in the testosterone group and 30 (3.5) in the placebo group; mean weight was 94 kg and the mean waist-hip ratio was 1.0 in each treatment group. The calculated 10-year risk of cardiovascular events was relatively high as well (a mean risk of 27% [95% CI, 6.4%-47.6%] in the placebo group and 24% [95% CI 2.6%-45.4%] in the testosterone group.

Of the 18 markers initially evaluated, 9 remained for further study after removing those that were highly correlated with other markers, as described above. These 9 remaining markers were HDL cholesterol, non-HDL cholesterol, D-dimer, IL-6, CRP, insulin, HgbA1C, weight and waist-hip ratio (Table-1). Among these 9 measures, only the baseline waist-hip ratio interaction with treatment showed a significant association with the progression of non-calcified plaque volume at 12 months, (Table 2, Figure 1). 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 waist-hip ratio separately for the two 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 waist-hip ratio, the effect of testosterone on the 12-month change in non-calcified plaque volume would increase by 26.96 mm^3 (95% confidence interval 7.72, 46.20).(The baseline values of waist-hip ratio ranged from 0.9 to 1.2).

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

DISCUSSION:

We report that in older hypo gonadal men participating in the Cardiovascular Trial of the TTrials there was a significant association between baseline waist-hip ratio and progression of non-calcified coronary artery plaque volume measured by coronary artery CT angiography after one year of testosterone treatment. Among men taking testosterone, larger waist-hip ratios were associated with greater progression of non-calcified plaque.

There is strong association 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 CT, DXA or MRI 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 waist hip ratio, there was 26 mm^3 greater increase in progression of non-calcified plaque volume in patients treated with testosterone replacement therapy.

Non-calcified plaque volumes as assessed by cardiac CCTA has been associated with CVD events. In a large single center trial 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 CT plaque characteristics (5.5 % for calcified plaque, 22.7% for non-calcified plaque, 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 mechanisms that may account for this inverse relationship may involve increased leptin levels which are hypothesized to interfere with luteinizing hormone stimulating 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, body fat mass and decrease in 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 effects of testosterone replacement on visceral fat. Some studies reported testosterone replacement therapy decreases visceral fat, while other 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 (e.g.

WC, 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[4144]. A previous paper from the TTrials also did not show any changes in WHR, WC and BMI in men treated with testosterone for 12 months compared to those treated with placebo[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 use of a surrogate marker of heart disease, non-calcified plaque, and not a clinical outcome. Another limitation is that the results apply only to men $\underline{\geq 65}$ with low testosterone[9].

Furthermore, this our results may indicate a chance finding. Although we did adjust for multiple comparisons but there remains a possibility abovementioned cardiac risk factors may be related to cardiovascular events with testosterone therapy.

We conclude that among older men receiving testosterone treatment, those with higher vs. lower WHR may experience greater increases in noncalcified coronary plaque volume. Future trials should evaluate the interaction of testosterone treatment and surrogate markers of abdominal obesity and visceral fat stores.

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