Effects of varying doses of testosterone on atherogenic markers in healthy younger and older men

Christian K. Roberts,1 Brian H. Chen,1 Sandeep Pruthi,1 and Martin L. Lee2
1Exercise and Metabolic Disease Research Laboratory, Translational Sciences Section, School of Nursing, University of California, Los Angeles, California; and 2Department of Biostatistics, School of Public Health, University of California, Los Angeles, California

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MEN EXHIBIT more extensive coronary artery disease (CAD) compared with age-matched women, and consequently there is a widespread perception that testosterone increases the risk of atherosclerotic heart disease. However, this premise is not supported by available data (1, 16, 22, 35). Epidemiological studies suggest that serum total and free testosterone concentrations are inversely correlated with risk of CAD (1). Prospective population-based studies have also not found an association between total testosterone and cardiovascular mortality (4, 41). Because these epidemiological studies examined steady-state testosterone levels, randomized intervention trials are needed to examine whether testosterone supplementation indeed leads to an increased risk of CAD.

Previously, dose-dependent decreases in high-density lipoprotein (HDL) have been reported with testosterone administration (6, 7). Although circulating lipid levels are a good predictor of atherosclerotic risk, recent attention has focused on risk factors such as oxidative stress, inflammation, and endothelial cell activation. For example, levels of the oxidative stress marker 8-isoprostane PGF2α (8-iso-PGF2α) (33), chemokine monocyte chemotactic protein (MCP)-1 (28), and inflammation-associated proteins high-sensitivity C-reactive protein (hs-CRP) and soluble intracellular adhesion molecule (sICAM)-1 (34) all independently predict risk of cardiovascular disease or acute myocardial infarction.

The effects of graded doses of exogenous testosterone on these atherogenic markers in apparently healthy men are poorly understood. Thus the primary objective of the present study was to determine the dose-dependent effects of testosterone on these atherogenic markers. To evaluate whether differences may occur by age, we examined younger (18–35 years) and older (60–75 years) apparently healthy men randomized to one of five testosterone dose regimens after endogenous testosterone production was suppressed with a long-acting gonadotropin-releasing hormone (GnRH) agonist. Based on previously reported dose-dependent decreases in HDL with testosterone in these men (6, 7), we hypothesized that there would be a dose-dependent increase in atherogenic marker concentrations.

METHODS

Experimental design. As described in detail previously (6, 7), this was a double-blind, randomized study that included a 20-wk treatment period. Each participant provided written, informed consent, approved by the institutional ethics and research review boards of Charles R. Drew University of Medicine and Science and Harbor-UCLA Research and Education Institute and all of the study protocols were performed according to the Declaration of Helsinki. A Data Safety Monitoring Board (DSMB) reviewed safety data every 3 mo. In brief, the subjects were healthy, eugonadal men, 18–35 and 60–75 years of age, with normal testosterone levels. Men with prostate cancer, American Urological Association symptom score >7, prostate-specific antigen level >4 ng/ml, hematocrit >48%, diabetes mellitus, congestive heart failure, severe sleep apnea, or myocardial infarction in the preceding 6 mo were excluded. These men had not used any androgenic steroids in the previous year, including dehydroepiandrosterone and androstenedione, or other anabolic agents. Men who participated in resistance exercise training or moderate to heavy endurance exercise training, defined as ≳2 days/wk of structured exercise.
exercised, were also excluded. Study enrollment ended in 2003 and biomarker assays were completed in 2004.

One-hundred and twenty-one eligible men (n = 61 between 18 and 35 years of age, and n = 60 between 60 and 75 years of age) were assigned to one of five groups using a block randomization scheme (block size = 4) within each age category (6, 7). All men received monthly injections of a long-acting GnRH agonist (Lupron Depot, 7.5 mg, TAP, North Chicago, IL) to suppress endogenous testosterone production and weekly injections of one of five doses of testosterone enanthate (200 mg/ml; Delatestryl, Savient Pharmaceuticals, Iselin, NJ): 25 mg (12 younger men, 13 older men), 50 mg (12 younger men, 12 older men), 125 mg (12 younger men, 12 older men), 300 mg (12 younger men, 13 older men), or 600 mg (13 younger men, 10 older men). Testosterone enanthate was selected to raise testosterone concentrations into the supraphysiological range. The 25-mg dose was chosen because this was the smallest dose of testosterone that had been shown to maintain sexual function in men treated with a GnRH antagonist. The 600-mg dose was selected because this is the highest dose that had been administered safely to men in clinical trials (5, 6).

The General Clinical Research Center staff administered all testosterone and GnRH agonist injections to assure compliance.

**Nutritional intake and exercise.** Energy and protein intakes were standardized at 36 kcal·kg⁻¹·day⁻¹ and 1.2 g·kg⁻¹·day⁻¹, respectively. A standardized diet, including recommendations for 15–20% carbohydrate and ~30% fat was initiated 2 wk before treatment was started; dietary instructions were reinforced every 4 wk. Additionally, the participants were asked not to undertake resistance training or moderate-to-heavy endurance exercise during the study. These instructions were reinforced every 4 wk.

**Outcome measures.** Serum total testosterone was measured periodically (7 days after injection, nadir testosterone level) throughout the study with a radioimmunoassay that used iodinated testosterone as tracer (5, 39) and reported previously (6, 7). This assay has a sensitivity of 0.44 ng/dl and intra- and interassay coefficients of variation of 13.2% and 8.2%, respectively (39). Plasma 8-iso-PGF₂α was measured in duplicate using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Serum hs-CRP concentration was measured in duplicate with a specific ELISA kit (Diagnostic Systems Laboratories, Webster, TX), according to the manufacturer’s instructions. CRP was previously determined among the younger men in this trial (38). Statistical analysis. The distributions of variables were evaluated for normality. Differences in baseline characteristics across intervention groups were tested using one-way ANOVA and the Fisher’s exact test using an exact calculation of P value for continuous and categorical variables, respectively. Changes in atherogenic biomarker levels were computed as the postintervention levels minus the preintervention levels. Generalized linear models were used to compute change in biomarker levels across levels of testosterone. We conducted two complementary analyses: 1) intention-to-treat, which analyzes the change in biomarker levels according to assigned treatment; and 2) per-protocol, which examines the change in biomarker levels in relation to change in blood testosterone levels (15, 21). We excluded potential leverage points by excluding individuals whose biomarker levels were greater than 3 standard deviations from that of the entire study population in our per-protocol regression analyses. We conducted stratified analyses by the two age categories. Differences in trend between younger and older men were tested using a two-way ANOVA. Statistical significance was defined as P < 0.05. All analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC) and R software.

**RESULTS**

**Participant characteristics.** The baseline characteristics of the subject population have been described previously (7). Across the five treatment groups of younger or older men, baseline characteristics did not differ significantly, with the exception of baseline MCP-1 levels, which differed across intervention groups among the younger men (Table 1). Prein-

### Table 1. Baseline characteristics by testosterone treatment group in younger and older men

<table>
<thead>
<tr>
<th>Testosterone Dose, im</th>
<th>25 mg</th>
<th>50 mg</th>
<th>125 mg</th>
<th>300 mg</th>
<th>600 mg</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Younger men (ages 18–35 yr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects (n)</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>0.19</td>
</tr>
<tr>
<td>Age, years</td>
<td>27 ± 4</td>
<td>27 ± 5</td>
<td>28 ± 4</td>
<td>24 ± 4</td>
<td>25 ± 4</td>
<td>0.09</td>
</tr>
<tr>
<td>White race/ethnicity, %</td>
<td>7 (58%)</td>
<td>9 (75%)</td>
<td>8 (67%)</td>
<td>9 (75%)</td>
<td>9 (69%)</td>
<td>0.99</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>427 ± 83</td>
<td>702 ± 92</td>
<td>517 ± 80</td>
<td>870 ± 92</td>
<td>559 ± 80</td>
<td>0.01</td>
</tr>
<tr>
<td>sICAM-1, ng/ml</td>
<td>535 ± 238</td>
<td>429 ± 263</td>
<td>685 ± 228</td>
<td>140 ± 263</td>
<td>128 ± 228</td>
<td>0.38</td>
</tr>
<tr>
<td>8-iso-PGF₂α, pg/ml</td>
<td>98 ± 19</td>
<td>93 ± 21</td>
<td>84 ± 18</td>
<td>87 ± 21</td>
<td>116 ± 18</td>
<td>0.76</td>
</tr>
<tr>
<td>Testosterone, ng/dl</td>
<td>603 ± 145</td>
<td>533 ± 207</td>
<td>562 ± 188</td>
<td>607 ± 207</td>
<td>606 ± 216</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Older men (ages 60–75 yr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects (n)</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>10</td>
<td>0.46</td>
</tr>
<tr>
<td>Age, years</td>
<td>64 ± 4</td>
<td>65 ± 4</td>
<td>66 ± 5</td>
<td>67 ± 4</td>
<td>66 ± 4</td>
<td>0.46</td>
</tr>
<tr>
<td>White race/ethnicity, %</td>
<td>12 (92%)</td>
<td>10 (83%)</td>
<td>9 (75%)</td>
<td>11 (85%)</td>
<td>7 (70%)</td>
<td>0.68</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>672 ± 78</td>
<td>740 ± 81</td>
<td>843 ± 78</td>
<td>660 ± 88</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>sICAM-1, ng/ml</td>
<td>365 ± 418</td>
<td>1,315 ± 435</td>
<td>212 ± 435</td>
<td>407 ± 417</td>
<td>1,162 ± 476</td>
<td>0.27</td>
</tr>
<tr>
<td>8-iso-PGF₂α, pg/ml</td>
<td>95 ± 33</td>
<td>82 ± 34</td>
<td>72 ± 34</td>
<td>66 ± 33</td>
<td>30 ± 37</td>
<td>0.91</td>
</tr>
<tr>
<td>Testosterone, ng/dl</td>
<td>349 ± 107</td>
<td>322 ± 69</td>
<td>363 ± 105</td>
<td>305 ± 116</td>
<td>326 ± 78</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values are means ± SD. MCP-1, monocyte chemotactic protein-1; sICAM-1, soluble ICAM-1. *P value for one-way ANOVA testing differences of each continuous variable across intervention arms. P value for Fisher’s exact test of race/ethnicity across intervention arms.

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tervention testosterone levels were significantly higher ($P < 0.0001$) among younger men (means ± SD: 585 ± 189 ng/dl) compared with older men (333 ± 97 ng/dl). Additionally, there was no significant change in daily caloric, protein, carbohydrate, or fat intake in any group during treatment (data not shown).

**Intention-to-treat analysis of atherogenic markers.** With the exception of 8-iso-PGF$_{2\alpha}$, which appeared to be inversely associated with assigned testosterone dose among younger men (p-trend = 0.01) (Fig. 1A), no clear relationship between changes in 8-iso-PGF$_{2\alpha}$ (p-trend$_{\text{Older}}$ = 0.79) (Fig. 1B), MCP-1 (p-trend$_{\text{Younger}}$ = 0.20, p-trend$_{\text{Older}}$ = 0.70) (Fig. 2), or sICAM-1 (p-trend$_{\text{Younger}}$ = 0.68, p-trend$_{\text{Older}}$ = 0.24) (Fig. 3) were detected across assigned testosterone doses (p-trend $\geq$ 0.20). We also did not detect any differences in the association between assigned testosterone dose and mean levels of these biomarkers by age groups ($P > 0.20$). Additionally, no linear relation between assigned testosterone dose and levels of hs-CRP was noted for older men (Fig. 4). Similarly, no dose effects were observed for hs-CRP in younger men, as reported previously (38).

![Fig. 1. Mean changes (and standard deviations) in 8-iso-PGF$_{2\alpha}$ from baseline to week 20 in younger (A) and older (B) men in response to graded doses of testosterone enanthate. No significant differences in the linear trends were detected between younger and older men ($P = 0.32$).](image)

![Fig. 2. Mean changes (and standard deviations) in monocyte chemotactic protein-1 (MCP-1) from baseline to week 20 for younger (A) and older (B) men in response to graded doses of testosterone enanthate. No significant differences in the linear trends were detected between younger and older men ($P = 0.21$).](image)

**DISCUSSION**

The perception that testosterone level and testosterone supplementation increases the risk of atherosclerotic heart disease is controversial (1). Administration of high doses of exogenous androgens as well as abuse of orally administered, nonaromatizable androgens results in insulin resistance, dyslipidemia, impaired endothelium-dependent vasodilation, ventricular hypertrophy, and an enhanced tendency for thrombosis (1). Isolated cases of cardiovascular events in bodybuilders using...
extremely high supraphysiological doses of anabolic steroids have also been reported (40). On the other hand, emerging data indicate that testosterone may be protective against atherosclerosis progression and CAD risk (4, 41). Much of this evidence is associative and comes from cross-sectional, case-control, and longitudinal studies. Of 32 cross-sectional studies, 16 demonstrated lower serum testosterone level in CAD patients compared with controls, and the other 16 showed no differences between cases and controls (41). Furthermore, prospective population-based studies (1) have not revealed an association between total testosterone levels and cardiovascular mortality.

To determine whether testosterone is proatherogenic, randomized controlled trials are necessary. In light of the aforementioned controversy, the present study attempted to provide insight using samples from a randomized, double-blind study (6, 7) to investigate the effects of graded doses of testosterone on serum concentrations of markers of oxidative stress, inflammation, and monocyte-endothelial interactions in younger and older men. The present study provided assessment of androgen-induced atherogenic marker changes in the controlled setting of a clinical research center that allowed standardization of energy intake. Combined administration of GnRH agonist and testosterone suppressed luteinizing hormone and consequently endogenous testosterone production; this minimized heterogeneity in testosterone levels due to uneven suppression of endogenous testosterone production by exogenous androgen. Thus circulating testosterone concentrations during treatment were proportional to the administered dose of testosterone enanthate (7). This study provides evidence that different levels of circulating testosterone concentrations created by this regimen did not significantly affect 8-iso-PGF2α, MCP-1, sICAM-1, or hs-CRP in apparently healthy men.

Several lines of evidence support the concept that low testosterone, generally <400 ng/dl (25, 37) is associated with increased CAD risk. First, data from cross-sectional and longitudinal studies reveal that low testosterone is associated with increased body mass index, systolic blood pressure, serum triglycerides (23), total cholesterol, low-density lipoprotein (LDL), apolipoprotein B (37), small dense LDL particles (13), low HDL (43) and apolipoprotein A1 (37), thrombotic factors (low tissue plasminogen activator, elevated plasminogen activator inhibitor-1 and fibrinogen) (8), endothelial dysfunction (10), insulin resistance (12), visceral adiposity (36), diabetes (11), and metabolic syndrome (19). Additionally, Yang et al. (42) noted increased levels of CRP, interleukin-6, sICAM-1, and soluble vascular cell adhesion molecule-1 (sVCAM-1), as well as elevated carotid plaque area in men with low free testosterone. Longitudinal studies in men failed to reveal a significant association between serum testosterone levels and future risk of CAD events (41). An inverse correlation between testosterone levels and the degree of coronary artery (9), abdominal aortic (14), or carotid (29) atherosclerosis has also been reported. Furthermore, it has been suggested that low testosterone predicts increased risk of cardiovascular (18, 20) and all-cause (18) mortality.

A large body of evidence from intervention studies with testosterone supplementation reveal reductions in visceral fat volume, serum glucose, insulin, lipids, blood pressure (27), lipoprotein(a), prothrombotic factors (2), carotid intima-media thickness and CRP (3), leptin (17), and improved insulin sensitivity (26). Previously, the effects of graded doses of testosterone on regional adipose tissue distribution were examined in these healthy younger and older men (7). The changes in whole body and intra-abdominal fat mass were inversely correlated with testosterone dose and concentration. Whole

Fig. 4. Mean changes (and standard deviations) in high-sensitivity C-reactive protein (hs-CRP) from baseline to week 20 in older men in response to graded doses of testosterone enanthate. No linear trend was detected in the older men (P = 0.25).
body and truncal fat mass increased significantly in men who received 25 and 50 mg/wk of testosterone enanthate and decreased in those who received 300 and 600 mg/wk. The present study extends these data to markers of atherosclerotic cardiovascular disease, and suggests that over a wide range of doses, testosterone administration does not affect 8-iso-PGF$_{2\alpha}$, MCP-1, sICAM-1, and hs-CRP.

Few studies have investigated the effects of androgen supplementation on markers of atherosclerosis such as oxidative stress, inflammation, and endothelial cell activation. Ng et al. (31) reported that neither dihydrotestosterone nor recombinant human chorionic gonadotropin administration for 3 mo altered CRP, sICAM-1, or sVCAM-1 in healthy older men with partial androgen deficiency. Nakhai-Pour et al. (30) noted that 26 wk of 160 mg of testosterone undecanoate daily to older men with androgen deficiency did not alter CRP levels. Testosterone also had no adverse effect on insulin sensitivity at any dose in younger men, plasma HDL cholesterol decreased in the 600-mg dose groups (7, 38), but changes in other lipids were not significant. The present study was unable to assess whether the route of administration and aromatization modulated testosterone’s effects on these atherogenic markers or contributed to lack of proatherogenic effects of testosterone. Nevertheless, these data, when taken together with epidemiological data (1, 4, 35, 41), suggest that serum testosterone levels in the normal range for one’s effects on these atherogenic markers or contributed to lack of proatherogenic effects of testosterone. Nevertheless, these data, when taken together with epidemiological data (1, 4, 35, 41), suggest that serum testosterone levels in the normal range for androgen deficiency did not adversely affect atherogenic biomarkers in ap- 

dเสียชีวิต whether testosterone supplementation affects atherosclerosis progression and cardiovascular risk. 

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


