Effects of Transdermal Testosterone on Lipids and Vascular Reactivity in Older Men With Low Bioavailable Testosterone Levels

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Background. Sex hormones are known to affect cholesterol levels and vascular tone in women. The effects of testosterone on cholesterol and vascular tone in men are less well understood. Low testosterone levels have been associated with higher cholesterol levels in epidemiologic studies, but testosterone replacement has resulted in variable changes in cholesterol levels. Similarly, clinical studies suggest that testosterone may be vasodilatory, but few studies have directly evaluated the effects of testosterone on vascular tone.

Methods. Sixty-seven men (mean age 76 ± 4 years, range 65–87) with bioavailable testosterone levels below 4.44 nmol/l (lower limit for adult normal range) were randomized to receive transdermal testosterone (2–2.5 mg patches/d) or placebo patches for 1 year. Twenty-three men (34%) withdrew from the study; 44 men completed the trial.

Results. While total cholesterol, triglyceride, and low-density lipoprotein cholesterol levels did not significantly change during the year of therapy, high-density lipoprotein (HDL) levels (p = .004) and, specifically, HDL2 subfraction (p = .02) decreased in men receiving testosterone supplementation. Vascular tone was measured by brachial artery reactivity in 36 men. Endothelium-dependent brachial artery reactivity did not change from baseline measurements in men receiving 1 year of transdermal testosterone (0.3 ± 6.7% to 1.6 ± 4.6%; p = .58) or in the placebo group (3.2 ± 5.5% to 0.7 ± 5.5%; p = .23).

Conclusions. Transdermal testosterone decreased HDL cholesterol but did not affect vascular reactivity in men older than 65 years selected for low testosterone levels. No study to date has addressed the direct relationship between testosterone replacement and cardiovascular events.

THERE is a decline in bioavailable testosterone levels in men with aging due to alterations in the hypothalamic-pituitary axis and the testes (1), and testosterone replacement has numerous beneficial effects on bone, muscle, body composition, and sexual and cognitive function, but the effects on cardiovascular risk factors have been mixed (1,2). Men have a greater propensity for coronary artery disease than women (3). Androgens are postulated as a predisposing risk for coronary artery disease, but data to support this hypothesis have been inconclusive. Cholesterol levels increase and high-density lipoprotein (HDL) levels decrease in boys at puberty (4), and young men taking anabolic steroids have further increases in total cholesterol and decreases in HDL (5,6). In contrast, epidemiologic studies suggest that as men age, lower levels of testosterone are associated with increased cholesterol levels (7,8), more frequent cardiac events (9), and insulin resistance (10). Vascular tone is an important contributor to the risk of cardiac or vascular disease; vasoactive agents can arise from outside the vessel, circulate in the blood, be derived from circulating elements such as platelets, or be derived from vascular epithelium such as nitric oxide and prostacyclin. Sex hormones may play a role in vascular tone through one or more of these mechanisms. Estrogen has been shown to improve endothelial-dependent vasodilation in women (11), but little work has been done to evaluate the endothelial response to testosterone in men and include comparison of vascular reactivity in men with and without androgen deprivation (12), or to assess acute changes in vascular tone in response to acute testosterone infusion (13,14). Clinical studies suggest improvement in vascular tone following testosterone therapy, including studies reporting improvement in angina following testosterone therapy (15–18) and increased time to exercise-induced ischemic changes (19,20). We, therefore, as part of a larger study to assess the effects of testosterone on bone and muscle (21), evaluated the effects of 1 year of transdermal testosterone replacement therapy on lipid profiles and vascular tone, measured by brachial artery reactivity, in older men with low bioavailable testosterone levels.

METHODS

Subjects
Men were recruited to take part in a study to determine the effects of testosterone on bone and muscle (21). As part of that study, we also measured lipid profiles and brachial
artery reactivity. Information on recruitment and eligibility are described elsewhere (21). Men were randomized in a double-masked manner to receive either transdermal testosterone supplementation (two 2.5-mg non-scrotal transdermal patches/d; 5 mg/d total dose) or a matching placebo regimen; all men received 500 mg calcium and 400 IU of vitamin D supplementation. A total of 67 men were randomly assigned to treatment or placebo for participation in the study.

Evaluations

Participants in the study underwent medical history, physical exam, and measurement of fasting total and bioavailable testosterone, sex hormone binding globulin (SHBG), estrone, estradiol, total cholesterol, triglyceride, high-density lipoprotein level, high-density lipoprotein subfraction analysis, and Lp(a). Follow-up evaluations of the hormones and lipids were performed at 6 and 12 months of treatment. Endothelium-dependent vascular reactivity was measured by ultrasound in the brachial artery postcuff occlusion at baseline and 12 months.

Biochemical Measurements

Total and bioavailable testosterone and SHBG measurements were performed at Endocrine Sciences Inc., Calabasas Hills, California. Testosterone levels were measured by radioimmunoassay, SHBG by competitive binding assay, and bioavailable testosterone by competitive binding of the non–SHBG-bound portion of testosterone following ammonium sulfate precipitation of the SHBG-bound steroid as described by Nankin and Calkins (22). Intraassay variability of the testosterone assay is less than 7%, bioavailable testosterone is less than 4%, and SHBG is less than 10%. Total cholesterol, high-density lipoprotein, and triglycerides were measured at the University of Connecticut Health Center Clinical Laboratories. Low-density lipoprotein levels were calculated using the Friedwald equation. HDL cholesterol fractionation and Lp(a) were measured at the Washington University Core Laboratory for Clinical Studies. HDL cholesterol was fractionated into HDL$_2$ and HDL$_3$ by sequential double precipitation using Mg$_{2+}$ dextran sulfate (23); intraassay variability for the fractionation is 4%. The concentration of Lp(a) was measured by immunoturbidimetry on the Hitachi 917 using a kit from Wako Diagnostics (Richmond, VA); intraassay variability of the Lp(a) is 5%. Samples for off-site assay were shipped on dry ice by overnight mail.

Vascular Reactivity

The noninvasive measurement of endothelial function was carried out according to the method of Celermajer and colleagues (24). Endothelium-dependent artery reactivity was measured by the change in brachial artery diameter from before and after cuff occlusion. Brachial artery diameter was measured using a high-resolution, 10-MHz linear array transducer on a GE-Diasonic ultrasound machine (San Jose, CA). All scans were recorded on super VHS tape and digitized, and measurements were done off-line using Image-Pro plus image analysis software (Media Cybernetics, Silver Spring, MD, Version 4.0). Appropriate images for each phase of the study were analyzed on the same computer by the same observer. The intraobserver coefficient of variability for measurement of brachial artery diameter was 1.3%.

Subjects were studied in a fasting state in a temperature-controlled room (22°C). The subjects remained in a supine position for at least 10 minutes prior to study. At that time, a suitable straight portion of the brachial artery was visualized longitudinally, and the transducer position was noted and marked. All subsequent scans were obtained with the transducer and arm in the same position. Measurements were performed at baseline and for 1 minute following 5 minutes of forearm cuff occlusion. Vessel diameters after cuff occlusion are expressed as a percentage of the respective baseline diameters at rest.

Statistical Analysis

Analysis was done on those individuals who completed the study. Baseline and clinical characteristics were reported using means and standard deviations stratified by treatment group. One-way analysis of variance (ANOVA) or chi-square analysis was used to test the difference in baseline characteristics between the treatment groups. For each participant, we calculated the percent change in hormone levels, lipid profiles, and brachial artery diameter changes from baseline to 12 months. Paired $t$ tests were used to assess change within the groups over time. We also compared the percent change of each variable of the groups using one-way ANOVA. We checked variables for normality of distribution and for the impact of outliers. All analyses were done using SPSS, version 10.0 (SPSS Inc., Chicago, IL).

RESULTS

Subject Population and Disposition

Sixty-seven men were recruited for the study. Twenty-three men (33%) discontinued due to intercurrent illness, rash, elevation in prostate-specific antigen level, or personal reasons (Table 1). Forty-four men completed the study: 24 men in the testosterone group and 20 men in the placebo group. A subgroup of 36 men (18 in the testosterone and 18 in the placebo group) had vascular reactivity testing for analysis; eight men had no data due to technical difficulties during data collection. Baseline characteristics of the 44 men who finished the study are presented in Table 2; no significant differences existed in baseline information between

<table>
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<th>Variable</th>
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<td>4</td>
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Note: PSA = prostate-specific antigen.
the treatment and control groups. None of the men were active smokers: 34% had never smoked, and 66% reported a prior history of smoking. Sixty-three percent (42/67) of the men reported the use of alcohol. Mean alcohol consumption was six drinks/week (range 1–21 drinks/week), using the standard definition of 0.5 oz of ethanol per drink. Medical conditions most commonly reported included coronary artery disease (n = 34), hypertension (n = 34), and osteoarthritis (n = 31). None of the men were receiving treatment for medical conditions that might produce hypogonadism, such as liver disease, alcoholism, hemachromatosis, or Klinefelter’s disease. Commonly used medications included antihypertensives (n = 31), cholesterol-lowering agents (n = 10), thiazide diuretics (n = 12), histamine blockers (n = 9), alpha-adrenergic antagonists (n = 11), and aspirin for cardioprotective reasons (n = 20).

Sex Hormone Changes
Baseline and 12-month data for sex hormones are seen in Table 3. Total testosterone increased in both groups (64% in the testosterone group and 19% in the control group), but because of the variability in measurement, the difference was not significant. Bioavailable testosterone increased by 75% and was within the normal range in 14/24 (58%) of the men in the testosterone treatment group. No changes were seen in SHBG or estradiol levels; estrone increased in the testosterone treatment group.

Lipids
Changes in lipid profiles are outlined in Table 3. There were no differences in lipid levels at baseline between groups. Total cholesterol did not change in either group, but high-density lipoprotein levels, specifically HDL₂ subfraction, decreased from baseline levels in the men receiving testosterone replacement (p = .002). The differences in the changes in HDL and HDL₂ subfraction between groups reached statistical significance (p < .05).

Vascular Reactivity
Endothelium-dependent brachial artery reactivity did not change from baseline measurements in men receiving transdermal testosterone (0.3 ± 6.7% to 1.6 ± 4.6%; p = .58) or in the placebo group (3.2 ± 5.5% to 0.7 ± 5.5%; p = .23). The difference in endothelial-dependent vascular changes at 12 months between groups was not significantly different (p = .63). Further, no differences in brachial artery reactivity were found when the data were analyzed by history of coronary artery disease or history of hypertension rather than treatment group (data not shown).

Discussion
Transdermal testosterone therapy decreased HDL cholesterol but had no effect on endothelium-dependent vasodilation in men older than 65 years selected for low bioavailable testosterone levels. Decreases in HDL cholesterol levels have been found in several other studies of younger men with more severe testosterone deficiency receiving intramuscular testosterone esters (25–27), transdermal patch (28), transdermal testosterone gel (29), or a combination of therapies including oral, intramuscular, and subcutaneous implantation of crystalline testosterone (30) and in young, eugonadal male weight-losers (5,31,32). In addition, a few studies of older men with less severe testosterone deficiency (33) or men selected for study inclusion because of a diagnosis of osteoporosis rather than testosterone deficiency

<table>
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<th>Parameter</th>
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<th>Control</th>
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<tr>
<td>Testosterone (nmol/l)</td>
<td>13.5 ± 6.0</td>
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<td>Bioavailable testosterone (nmol/l)</td>
<td>3.2 ± 1.2</td>
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<td>Estrone (pmol/l)</td>
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<td>Triglycerides (mg/dl)</td>
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<tr>
<td>Lp(a)</td>
<td>29.5 ± 31.7</td>
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Notes: Values are mean ± SD. *p value for analysis of variance to compare changes in means between groups. LDL = low-density lipoprotein; HDL = high-density lipoprotein.
(34) demonstrated decreases in HDL cholesterol following testosterone therapy. In contrast to our study and the study of younger men, in studies of older hypogonadal men receiving testosterone supplementation, total cholesterol remained unchanged (35–37) or decreased with no change noted in HDL cholesterol (38–40). The reason for the differences in the studies is not clear. Others have speculated that the level of HDL cholesterol was already low in the studies that found no change (41). In our study, while HDL cholesterol levels were not significantly different from placebo, levels were somewhat higher than the control group, and the decrease may be explained by regression to the mean.

Another explanation for the decrease in HDL cholesterol found in our study could be an interaction of sex hormones with plasma lipolytic enzymes. There is evidence that estrogen inhibits hepatic triglyceride lipase activity (11). Hepatic triglyceride lipase activity increases following treatment with anabolic steroids (3) or testosterone if aromatization to estrogen is blocked (5,42). The increase in hepatic triglyceride lipase activity induced by a nonaromatizable steroid has been shown to precede the decrease in HDL levels (43). Estradiol levels did not increase in our study, while increases in estradiol are typical following supplementation with intramuscular testosterone. We may have provided insufficient substrate for the conversion of testosterone to estrogen, thereby allowing the increase in hepatic triglyceride lipase activity that may have been suppressed if more estrogen were present. We did not measure hepatic triglyceride lipase activity levels.

We found no changes in Lp(a) following 1 year of transdermal testosterone therapy. Lp(a), an independent risk factor for premature coronary artery disease, is low-density lipoprotein-(LDL)-like particle that possesses the atherogenic property of LDL as well as a potential thrombogenic property because of the presence of apo(a), a glycoprotein that is similar to plasminogen. The Lp(a) concentration is largely determined by heredity (44). Most studies have found no association between Lp(a) and testosterone levels (45,46), and few studies have assessed the effects of testosterone manipulation on Lp(a) levels. Henriksen and colleagues (47) found Lp(a) increased following orchietomy in men being treated for prostate cancer, and Berglund and associates (48) found testosterone decreased Lp(a) levels when given to healthy volunteers. In contrast, Ozata and colleagues (49) found no change in Lp(a) concentrations following testosterone therapy in men with hypogonadism.

Vascular reactivity can be affected by many factors, including age (50), cholesterol levels (24,51), the presence of coronary artery disease (52), and smoking (53). Sex hormones are thought to play a role in vascular reactivity, but results from animal and human studies have been variable. Most studies demonstrate estrogen as a vascular dilation agent in both animal (54,55) and human models (11,56), although one study reported no direct vasodilatory response to estrogen in women and a vasoconstrictive response in men (57). The role of testosterone is less well-defined. In animal studies, testosterone has demonstrated a vasodilatory response by endothelium-dependent mechanism (58) and endothelium-independent mechanism (59), as well as impairment in vascular reactivity (60). We found no effect on endothelium-dependent reactivity in men following 1 year of transdermal testosterone therapy compared to men receiving a placebo. In contrast, Herman and colleagues (12) found brachial artery reactivity improved in men with prostate cancer following surgical or chemical castration, as did McCredie and colleagues (61), who found brachial artery reactivity impaired in female-to-male transsexuals receiving long-term high-dose androgens compared to female controls. There have also been reports of a positive vasodilatory response in men receiving supraphysiologic doses of testosterone (13,62).

An explanation for the difference between our study and those that found enhanced vasodilation is not clear. Differences could be due to the acute infusion of testosterone in the studies demonstrating a vasodilatory change compared to the long-term, lower dose delivered in our study. During the year of therapy, we demonstrated a decrease in HDL cholesterol, and low levels of HDL cholesterol are associated with reduced endothelium-dependent vasodilation (63). In addition, long-term effects of testosterone on agents other than lipids may be involved. For example, testosterone increases receptor density and platelet aggregation to a mimetic for thromboxane A2, a vasoconstrictor and platelet proaggregatory (64). Testosterone therapy has also increased human monocyte adhesion to endothelial cells, a key early event in atherosclerosis (65). Finally, monocyte-derived macrophages, an integral component in foam cell formation and early atherosclerotic plaque formation, from male donors demonstrate a dose-dependent increase in intracellular cholesterol ester content to androgen exposure, an increase not seen in female donor monocyte-derived macrophages (66). We may have failed to see enhanced vasodilation following long-term exposure to testosterone because these mechanisms that alter vascular tone may have been affected. Further study of the effects of testosterone will be required to answer these questions.

Our study has several limitations. The sample size is small, the men are heterogenous for coronary disease, hypertension, and hypercholesterolemia, and the various treatments of the individual men may have confounded any hormonal effects on vascular reactivity, such as treatment of hypercholesterolemia. If higher doses of testosterone were required for a treatment effect, as is suggested by acute studies of testosterone infusion, transdermal testosterone would have failed to attain similar peak levels. Further studies using alternate testosterone preparations or dose-response studies will be required to address this question.

Transdermal testosterone decreases HDL₃ cholesterol but did not affect vascular reactivity in men older than 65 years selected for low testosterone levels. No study to date has addressed the direct relationship between testosterone replacement and cardiovascular events.

Acknowledgments

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