Testosterone administration to men increases hepatic lipase activity and decreases HDL and LDL size in 3 wk

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Testosterone administration to men increases hepatic lipase activity and decreases HDL and LDL size in 3 wk. Am J Physiol Endocrinol Metab 284: E1112–E1118, 2003; 10.1152/ajpendo.00524.2002.—Testosterone administration to men is known to decrease high-density lipoprotein cholesterol (HDL-C) and the subclasses HDL2 and HDL3. It also might increase the number of small, dense, low-density lipoprotein cholesterol (LDL-C) particles in hypogonadal men. The decrease in HDL-C and in LDL-C is potentially mediated by testosterone administration might be important for long-term use of androgen; lipoprotein particle density

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administration before knee or hip replacement at the DVA-PSHCS, as described previously (1). Inclusion criteria included male sex, age >55 yr, and a decision by the patient and an orthopedic surgeon to undergo elective hip or knee replacement. Exclusion criteria included known prostate cancer (benign prostatic hyperplasia was acceptable), severe liver or kidney disease, substance abuse (including illicit use of anabolic steroids, androstenedione, DHEA, or growth hormone), or severe musculoskeletal conditions.

Randomization

All male patients at the DVA-PSHCS, Seattle Division, undergoing elective unilateral hip or knee replacement over a 2-yr period were offered enrollment in the original study (21). The present study began at enrollment of the 21st subject until 12 participants in this study were elderly men, average age 73.4 ± 2.4 yr (range 60–85 yr), and many were obese, with an overall weight of 100.4 ± 5.4 kg (range 71.4 to 125 kg) and body mass index (BMI) of 32.8 ± 1.7 kg/m² (range 22.4 to 40.4 kg/m²). There was no significant difference in age, weight, or BMI between treatment groups at the start of the study (Table 1). All 12 men enrolled completed all requirements for this study. The use of lipid-lowering medications was not part of the exclusion criteria, as the study was primarily designed to compare length of hospital stay and measures of functional recovery in patients administered preoperative intramuscular T vs. patients receiving placebo. One subject in the placebo group was on a low-dose hydroxymethylglutaryl-CoA reductase inhibitor (lovastatin 20 mg/day). Exclusion of this patient from data processing did not change results; therefore, this subject was included in the database. One subject in the treatment group was on levothyroxine (0.05 mg/day) with a normal TSH at the time of the study.

Statistical Analysis

Analysis was performed on the 12 subjects who completed the study. FSH, LH, and T were expressed as mean hormone levels ± SE. Cholesterol data from DGUC was normalized individually to the total cholesterol level at the time of sampling, and then the data were averaged as a group. Differences from baseline were measured by ANOVA. Differences between groups were compared by two-way ANOVA for repeated measures and analyzed post hoc using Duncan’s comparison measures. One-sample t-tests were used to determine significance from zero for the difference between treatment and baseline values. P < 0.05 was considered significant.

RESULTS

Participant Characteristics

The participants in this study were elderly men, average age 73.4 ± 2.4 yr (range 60–85 yr), and many were obese, with an overall weight of 100.4 ± 5.4 kg (range 71.4 to 125 kg) and body mass index (BMI) of 32.8 ± 1.7 kg/m² (range 22.4 to 40.4 kg/m²). There was no significant difference in age, weight, or BMI between treatment groups at the start of the study (Table 1). All 12 men enrolled completed all requirements for this study. The use of lipid-lowering medications was not part of the exclusion criteria, as the study was primarily designed to compare length of hospital stay and measures of functional recovery in patients administered preoperative intramuscular T vs. patients receiving placebo. One subject in the placebo group was on a low-dose hydroxymethylglutaryl-CoA reductase inhibitor (lovastatin 20 mg/day). Exclusion of this patient from data processing did not change results; therefore, this subject was included in the database. One subject in the treatment group was on levothyroxine (0.05 mg/day) with a normal TSH at the time of the study.

Table 1. Baseline characteristics of groups before treatment

<table>
<thead>
<tr>
<th>Testosterone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
</tr>
<tr>
<td>Age, yr</td>
<td>74.0 ± 8.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>100.4 ± 8.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.2 ± 6.4</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>175.2 ± 5.8</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>93.5 ± 5.7</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>45.8 ± 10.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; N, no. of men in group.
Body Composition

There was a significant gain in weight in the group administered T during the study. At week 1, the group administered T had a significant increase in weight above baseline of 1.6 ± 0.4% (P < 0.02), which increased to a 3.0 ± 0.6% gain in weight at week 3 (P < 0.006). Although there was an increase in BMI in the group administered T from baseline (33.2 ± 2.6 kg/m²) to week 3 of treatment (34.2 ± 2.7 kg/m²), this did not reach statistical significance. BMI and weight did not change significantly in the placebo group.

Hormones

T levels increased significantly in the group administered T from 15.4 ± 3.1 nmol/l at baseline to 92 ± 5.2 nmol/l at week 2 when measured at peak levels (P < 0.02). There was no significant change in the placebo group in T levels obtained at the same times, 11.8 ± 1.7 and 9.5 ± 2.2 nmol/l, respectively. At the end of week 3, trough levels remained significantly elevated above baseline values in the group administered T to 92.3 ± 6.2 nmol/l (P < 0.02) but remained at baseline values in the placebo group (11.7 ± 1.6 nmol/l).

Estradiol levels were significantly elevated after treatment with T (574.5 ± 41 IU/l) compared with baseline (134.2 ± 20 pmol/l, P < 0.002) and placebo. Estradiol levels did not significantly change in the placebo group after treatment (80.5 ± 18.8 IU/l) compared with baseline (84.8 ± 16.9 pmol/l).

Sex hormone-binding globulin (SHBG) levels significantly decreased after T administration from a base-
Table 2. Lipoprotein values and post-HL activity at baseline and at week 3 of treatment with either T or placebo

<table>
<thead>
<tr>
<th></th>
<th>Testosterone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 3</td>
</tr>
<tr>
<td>HL activity</td>
<td>470 ± 59.5</td>
<td>765.3 ± 108.2†</td>
</tr>
<tr>
<td>LPL activity</td>
<td>261 ± 36.5</td>
<td>257.8 ± 27.3</td>
</tr>
<tr>
<td>TC</td>
<td>175.2 ± 5.8</td>
<td>169 ± 8.2</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>177.5 ± 49.1</td>
<td>172.3 ± 39.5</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>41.4 ± 9.7</td>
<td>34.5 ± 7.9</td>
</tr>
<tr>
<td>LDL-C</td>
<td>93.5 ± 5.7</td>
<td>101.3 ± 11</td>
</tr>
<tr>
<td>apoB</td>
<td>89.6 ± 4.4</td>
<td>88.3 ± 24</td>
</tr>
<tr>
<td>HDL-C</td>
<td>45.8 ± 10.9</td>
<td>32.8 ± 7.2†</td>
</tr>
<tr>
<td>HDL2</td>
<td>5.2 ± 2.0</td>
<td>2.8 ± 0.5 †</td>
</tr>
<tr>
<td>HDL3</td>
<td>31.8 ± 5.9</td>
<td>23.4 ± 2.8*</td>
</tr>
</tbody>
</table>
| Lp(a)             | 28.1 ± 14    | 35.3 ± 16.8 | 53.5 ± 25.9 | 56.3 ± 28.3  

Values are means ± SE. Lipoprotein values are mg/dl; post-HL activity values are nmol free fatty acids·min⁻¹·ml⁻¹. HL, hepatic lipase; LPL, lipoprotein lipase; TC, total cholesterol; apoB, apolipoprotein B; Lp(a), lipoprotein(a). *P < 0.05 vs. baseline; †P < 0.05 vs. placebo.

HL Activity

HL activity increased in each participant administered T (Fig. 1). For the group as a whole, HL activity significantly increased to 66.2 ± 17.8% above baseline values (P < 0.01) after 3 wk of treatment with T (Table 2). HL activity was also significantly elevated above placebo at week 3 of T treatment (P < 0.01). There was no significant change in HL activity in the placebo group at week 3 of treatment compared with baseline (Table 2). Lipoprotein lipase activity did not change during the study in either treatment group (Table 2).

Lipoproteins

Lipoprotein levels were not significantly different between treatment groups at baseline for total cholesterol, triglycerides, VLDL-C, LDL-C, HDL-C, or lipoprotein(a) (Tables 1 and 2). LDL, apoB, LDL-C/apoB ratio as a marker of small, dense LDL (18), total cholesterol, triglycerides, VLDL-C, and lipoprotein(a) did not change significantly in the study in either group (Table 2).

HDL-C

HDL-C decreased significantly in the group administered T compared with baseline at weeks 1, 2, and 3 of treatment (P < 0.05) to a nadir of −25.1 ± 4.7% below baseline values (Fig. 2 and Table 2). HDL-C levels in the treatment group also decreased significantly below those in the placebo group at weeks 2 and 3 of treatment (P < 0.05). HDL2 levels decreased significantly to −32.9 ± 12.1% below baseline values in the group administered T compared with placebo at week 3 of treatment (P < 0.05), and HDL2 decreased significantly vs. placebo at week 2 of treatment (P < 0.05) and to −23.0 ± 5.6% below baseline levels at week 3 of treatment (P < 0.05; Fig. 2 and Table 2), with a trend to decrease vs. the placebo group (P = 0.09).

DGUC

In normal persons, after DGUC for analysis of cholesterol fractions, VLDL-C is found in fractions 30–38, intermediate-density lipoprotein cholesterol in 17–29, LDL-C in 7–16, and HDL-C in 1–6 (4). In this study, after DGUC, individual cholesterol values in each fraction were normalized to total cholesterol at the time samples were drawn for DGUC and then averaged as a group before and after treatment (Fig. 3). There was no significant difference between fractions before or after placebo (Fig. 3A). Cholesterol in fractions 8–10 significantly increased above baseline values in the treatment group (Fig. 3B), suggesting an increase in the amount of LDL particles after treatment with T. In addition, there was a shift in peak LDL particle density from fraction 10 to fraction 9, suggesting an increase in dense LDL particle size. To examine this further, baseline data were subtracted from data obtained after 3 wk of T administration, demonstrating a significant increase in cholesterol in fraction 9 (P < 0.02) and a trend to increase in fraction 8 (P = 0.08) and fraction 10 (P = 0.06), consistent with an increase in dense LDL particles (Fig. 4). There was a small decrease in cholesterol in fractions 12–18 with T treatment, areas that include buoyant LDL and IDL (Fig. 3), but this did not reach significance (P = 0.2). There was also a decrease in cholesterol in fractions 1–6, consistent with a decrease in HDL-C, but this also did not reach significance (P = 0.3).

DISCUSSION

This study demonstrated a rapid increase in HL activity after administration of supraphysiological amounts of T to eugonadal, obese, elderly men. In association with the increased HL activity, there were decreases in HDL-C, and its subclasses HDL2 and HDL3, consistent with the function of HL activity to remove phospholipid and triacylglycerol from lipoprotein levels of 58.6 ± 9.7 to 41.9 ± 7.5 nmol/l at week 1 (P < 0.001) and 25.4 ± 2.8 nmol/l at week 2 of treatment (P < 0.001). There was no significant change in SHBG from baseline (39.5 ± 5 nmol/l) to any time point during treatment in the placebo group (week 2 levels were 29.7 ± 3.3 nmol/l).

**HL Activity**

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**Lipoproteins**

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protein particles. In addition, cholesterol increased when measured by DGUC in fractions consistent with dense LDL.

A decrease in both HDL_{2} and HDL_{3} during T administration has not been demonstrated in other studies of eugonadal or hypogonadal men. The likely explanation for the rapid decline in both subclasses of HDL-C is that serum levels of T were increased above the physiological range for an extended period of time, increasing HL activity to 60% above baseline and leading to greater changes in HDL-C than are seen when T is administered to levels within or slightly above the physiological range in eugonadal men (8). The changes in both HDL-C subclasses are consistent with, but opposite to, data from studies in which gonadotropin and T levels are suppressed into the hypogonadal range by the administration of a gonadotropin-releasing hormone (GnRH) antagonist to eugonadal men. In that case, HDL-C, HDL_{2}, and HDL_{3} increased after administration of the GnRH antagonist because of a significant decrease in T levels (8, 36). Most studies in hypogonadal men do not find a change in HDL_{2} after T administration (6, 33, 39), but some do (9); the difference is likely explained by the amount and length of time T was administered. The decrease in both HDL-C subclasses in our study suggests that HL converts the more buoyant HDL_{2} to HDL_{3}, allowing HDL_{3} to be taken up by the liver, decreasing HDL-C, and therefore the amount of HDL-C in both subclasses.

LDL buoyancy decreased with T administration in this study, consistent with the data from Tan et al. (32), wherein T was administered via a scrotal patch to hypogonadal men for 3 mo. In the Tan et al. study, LDL was divided into three subfractions by DGUC, with subfraction III being the most dense and subfraction I the least dense. Most of the LDL mass before treatment was found in subfractions LDL-II and LDL-III. There was an ~20% increase in the concentration of small, dense LDL-III after treatment with testosterone (P < 0.05).

Because T is aromatized to 17β-estradiol and both T and estradiol increased after T administration to men in our study, it is difficult to determine what hormone is responsible for the change in LDL buoyancy. Giri et al. (16) administered up to 2 mg of 17β-estradiol to elderly men and found a decrease in LDL-C, an increase in LDL size (20.7 ± 0.6 to 20.9 ± 0.6 nm), and a decrease in the number of LDL particles (1,665 ± 483 to 1,513 ± 479 nmol/l). These data are opposite to the data for LDL size found in our study, suggesting that the effect of estradiol in our study on LDL size was minimal. T is also reduced to 5α-dihydrotestosterone (DHT), which could also play a role in lipid metabolism. DHT was not measured in this study.

T is generally accepted to induce HL activity, but some studies have failed to demonstrate an increase in HL activity with T administration (9, 34). Our study demonstrating a rapid and significant increase in HL activity confirms the association. Tan et al. (33) administered 250 mg of T enanthate intramuscularly every 4 wk for a total of 3 mo to hypogonadal Chinese men and demonstrated a significant increase in HL activity at

Fig. 3. Density gradient ultracentrifugation (DGUC) of cholesterol fractions before (○) and after (●) treatment with placebo (A) or T (B). Data are presented as individual cholesterol normalized to total cholesterol and then averaged by group ± SE (see EXPERIMENTAL PROCEDURES). *P < 0.05 vs. baseline.

Fig. 4. Change (Δ) in DGUC cholesterol fractions, treatment minus baseline, for the T-treated group. *P < 0.05 vs. zero.
the end of the study, 3 wk after injection but not 4 wk after injection of T. They concluded that the effect of T on HL activity is transient because of the multiple peaks and troughs of T that are produced when T is administered exogenously, causing a downregulation of HL by the liver. The decreased levels of HDL-C and the decrease in LDL-C size, however, persisted to the end of the study, which is not consistent with this conclusion. In addition, Berg et al. (9) administered an average of 216 mg T enanthate intramuscularly every 17.3 days to hypogonadal men and found a significant increase in HL activity after 6 mo of treatment. After 18 mo of treatment, however, HL activity was no longer significantly increased.

Data from the Women’s Health Initiative suggest that improvements in lipids by estrogen and progesterone do not translate into improved cardiovascular end points (40). We cannot conclude, therefore, that changes in lipids in our study will have effects on cardiovascular end points. Instead, our study points out the necessity of conducting long-term studies of T administration to both eugonadal and hypogonadal men in which not only lipid metabolism is evaluated, but also cardiovascular disease end points.

In conclusion, supraphysiological T administration to elderly, eugonadal men rapidly and significantly decreased HDL-C, HDL2, HDL3, and LDL particle density. Additional studies are needed to evaluate the effects of these changes on cardiovascular disease.

We thank Alegria Albers for conducting the hepatic and lipoprotein lipase assays.

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