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

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Herbst, Karen L., John K. Amory, John D. Brunzell, Howard A. Chansky, and William J. Bremner. 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 HDL_2 and HDL_3. 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 size is potentially mediated by hepatic lipase activity, which hydrolyzes lipoprotein phospholipids and triacylglycerol. To determine how HDL-C and LDL-C particles are affected by testosterone administration to eugonadal men, testosterone was administered as a supraphysiological dose (600 mg/wk) for 3 wk to elderly, obese, eugonadal men before elective hip or knee surgery, and lipids were measured by routine methods and by density gradient ultracentrifugation. Hepatic lipase activity increased >60% above baseline levels, and HDL-C, HDL_2, and HDL_3 significantly declined in 3 wk. In addition, the LDL-C peak particle density and the amount of LDL-C significantly increased. Testosterone is therefore a potent stimulator of hepatic lipase activity, decreasing HDL-C, HDL_2, and HDL_3 as well as increasing LDL particle density changes, all associated with increased cardiovascular risk.

androgen; lipoprotein particle density

TESTOSTERONE (T) administration to men is known to decrease HDL-cholesterol (HDL-C) (6–8, 25, 39), although not necessarily in hypogonadal men (27, 30, 42). When HDL-C declines with T administration in both eugonadal and hypogonadal men, it is unclear whether this decrease is in the subclasses HDL_2 (22) or HDL_3 (32–34) or both. That HDL-C decreases with T administration might be important for long-term use of T in men, because low HDL-C, including low HDL_2 and low HDL_3, is associated with an increased risk for cardiovascular disease (3, 14, 26).

The decrease in HDL-C with T administration is likely mediated by an increase in hepatic lipase (HL) activity. Consistent with this is the fact that HL activity is higher overall in men than in women (4) and is higher with central obesity (11, 12). HL is produced primarily by the liver and is located on the luminal surface of sinusoidal endothelial cells. It catalyzes the hydrolysis of triacylglycerols and phospholipids, mediating the removal of lipoproteins from plasma (23). This hydrolysis converts the more buoyant HDL_2 to the smaller, denser HDL_3, which can be taken up by the liver, thereby decreasing HDL-C. In addition, HL by the same mechanism converts large, buoyant LDL to small, dense LDL (41), the latter also being a risk factor for cardiovascular disease (2, 24, 31). One study demonstrated an increase in one population of small, dense LDL particles with T administration to hypogonadal men (32), but a cross-sectional study demonstrated an inverse association between serum T levels and small, dense LDL (29), although the T levels in that study have been called into question (35).

T is administered to hypogonadal men as replacement as injections, a gel, or a patch and is administered to eugonadal men in hormonal contraceptive regimens. Recently, intramuscular T in supraphysiological dosages has been administered to men to evaluate T effects on body composition and metabolism (10, 29) and to determine whether T could decrease length of hospital stay after knee or hip replacement (1). In the present study, we examined the effect on lipids of supraphysiological T administration (600 mg/wk im) to eugonadal, obese, elderly men. We hypothesized that T administration would rapidly increase HL activity, decrease HDL-C and both HDL_2 and HDL_3 subclasses, and increase LDL particle peak density.

EXPERIMENTAL PROCEDURES

This was a double-blind, randomized study consisting of a period of consent, a 3-wk treatment period, and a 4-wk recovery period. Each participant provided informed consent, and the study was approved by the Human Subjects Committee of the University of Washington and the Department of Veteran Affairs, Puget Sound Health Care System (DVA-PSHCS) Research and Development Committee.

Participants

Twelve men were recruited in succession from a total of 36 men that met entry criteria for randomization to T or placebo

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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 (1). After informed consent was obtained, recruitment for the present study began at enrollment of the 21st subject until 12 subjects were randomized into treatment ($n = 6$) and placebo ($n = 6$) groups by the study pharmacist using a random number sequence. Patients in the treatment group received 600 mg of T enanthate (Schein Pharmaceuticals, New Rahway, NJ) in 3 ml of sterile sesame oil intramuscularly 21, 14, 7, and 1 day before surgery. Patients in the placebo group received 3 ml of sterile sesame seed oil intramuscularly at the same time points.

Outcome Measures

HL and lipoprotein lipase activity. After a 12-h overnight fast, lipase levels were measured in plasma obtained 10 min after bolus injection of heparin (60 U/kg). Plasma was immediately centrifuged at 4°C at 3,000 rpm for 15 min and then immediately flash-frozen and stored at −80°C. HL and lipoprotein lipase activity were measured as previously described (21). Enzyme activity is expressed as nanomoles of free fatty acid released per minute per milliliter of plasma at 37°C. The intra-assay coefficient of variation of HL is 6%, and the interassay coefficient of variation is 14%. Samples from each subject were run in a single assay.

Hormones. FSH, LH, and T levels were measured by immunofluorometric assay (Delfia, Wallac Oy, Turku, Finland). Samples from each subject were run in a single assay. The sensitivity of the assay for FSH and LH was 0.016 and 0.019 IU/l, respectively. The intra-assay coefficient of variation was 2.9%, and the interassay coefficient of variation was 6.1% for a midrange of pooled values of FSH of 0.96 IU/l. The intra-assay coefficient of variation was 3.2%, and the interassay coefficient of variation was 12.5% for a midrange of pooled values of LH of 1.15 IU/l. The assay sensitivity for T was 0.5 nmol/l. The T intra-assay coefficient of variation was 4.4%, and the T interassay coefficient of variation was 7.3% for a mean of midrange pooled values of 11.4 nmol/l.

Lipids. Total cholesterol, LDL, HDL, HDL_2, apolipoprotein B (apoB), triglyceride, and VLDL levels were determined by standardized methods at the Northwest Lipid Research Laboratories (Seattle, WA; Ref. 37). HDL and HDL_3 were determined in the supernatant after precipitation with dextran sulfate and magnesium chloride (5, 38). The interassay coefficient of variation for apoB was 2.5%. LDL was calculated according to Friedewald's formula (15).

LDL peak buoyancy by density gradient ultracentrifugation. Density gradient ultracentrifugation (DGUC) was performed on plasma samples to calculate the LDL relative flotation rate and density distribution of lipoprotein cholesterol. A discontinuous salt density gradient was created in an ultracentrifuge tube by use of a modification (21) of a previous method (13). Samples were centrifuged at 65,000 rpm for 70 min (total $\omega^2 = 1.95 \times 10^{11}$) at 10°C in a Beckman Vti65.1 (Palo Alto, CA) vertical rotor. Thirty-eight 0.45-ml fractions were then collected from the bottom of the centrifuge tube, and cholesterol was measured in each fraction. The relative flotation rate (Rf), which characterizes LDL peak buoyancy as a continuous variable, was obtained by dividing the fraction number containing the LDL cholesterol peak by the total number of fractions collected. The coefficient of variation of the LDL-Rf value obtained by replicate analysis was 3.6%, as described previously (28).

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 71.3 ± 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/m^2 (range 22.4 to 40.4 kg/m^2). 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*

	Testosterone	Placebo
N	6	6
Age, yr	74.0 ± 8.5	68.7 ± 7.7
Weight, kg	100.4 ± 8.3	102.5 ± 8.9
BMI, kg/m^2	33.2 ± 6.4	32.3 ± 6.1
Total cholesterol, mg/dl	175.2 ± 5.8	190.5 ± 17.6
LDL, mg/dl	93.5 ± 5.7	115.3 ± 13.6
HDL, mg/dl	45.8 ± 10.9	43.2 ± 4.9

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

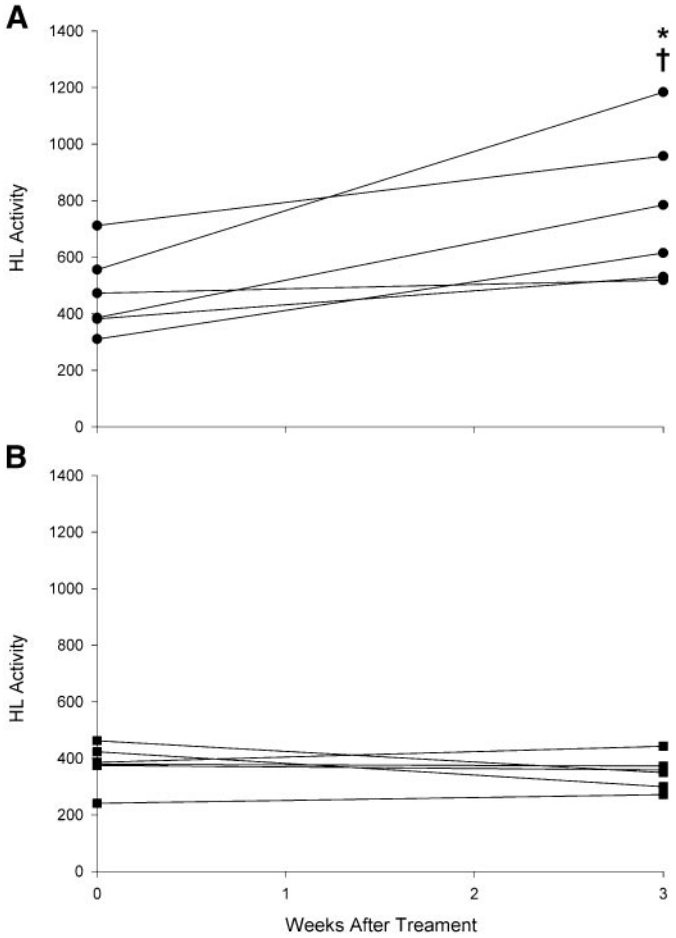

Fig. 1. Individual hepatic lipase (HL) activity at baseline and after 3 wk of treatment in 2 treatment groups: testosterone (T; *A*, ●); and placebo (*B*, ■). $P < 0.05$ for combined data vs. baseline (*) and placebo (†).

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/m^2) to *week 3* of treatment (34.2 ± 2.7 kg/m^2), 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-

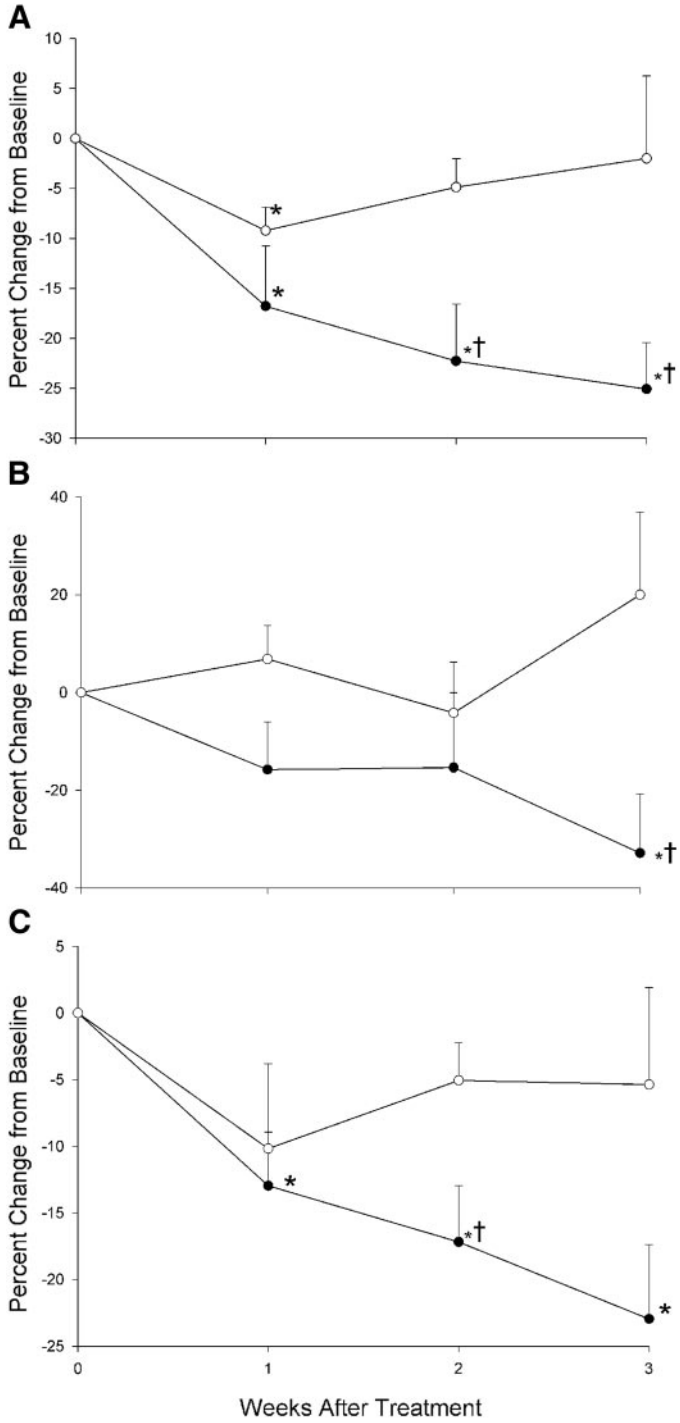

Fig. 2. Percent change from baseline in HDL-C (*A*), HDL_2 (*B*), and HDL_3 (*C*) after treatment with T (●) or placebo (○). $P < 0.05$ vs. baseline (*) and placebo (†).

Table 2. *Lipoprotein values and post-HL activity at baseline and at week 3 of treatment with either T or placebo*

	Testosterone		Placebo	
	Baseline	*Week 3*	Baseline	*Week 3*
HL activity	470 ± 59.5	765.3 ± 108.2*†	377.7 ± 30.5	349.5 ± 24.4
LPL activity	261 ± 36.5	237.8 ± 27.3	272.3 ± 56.8	221.7 ± 104.4
TC	175.2 ± 5.8	169 ± 8.2	190.5 ± 17.6	181.5 ± 15.5
Triglyceride	177.5 ± 49.1	172.3 ± 39.5	157.5 ± 37.2	193.8 ± 56.4
VLDL-C	41.4 ± 9.7	34.5 ± 7.9	31.5 ± 7.5	38.8 ± 11.3
LDL-C	93.5 ± 5.7	101.3 ± 11	115.3 ± 13.6	101.3 ± 10.1
apoB	89.6 ± 4.4	88.3 ± 2.4	105.2 ± 12.2	101.0 ± 10.5
HDL-C	45.8 ± 10.9	32.8 ± 7.2*†	43.2 ± 4.9	41.0 ± 3.4
HDL_2	5.2 ± 2.0	2.8 ± 0.5*†	6.5 ± 1.2	7.3 ± 1.0
HDL_3	31.8 ± 5.9	23.4 ± 2.8*	36.7 ± 3.9	33.7 ± 2.5
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.

line level 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

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 ($P < 0.05$). HDL_2 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 HDL_3 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. 3*A*). Cholesterol in fractions 8–10 significantly increased above baseline values in the treatment group (Fig. 3*B*), 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 HDL_2 and HDL_3, consistent with the function of HL activity to remove phospholipid and triacylglycerol from lipo-

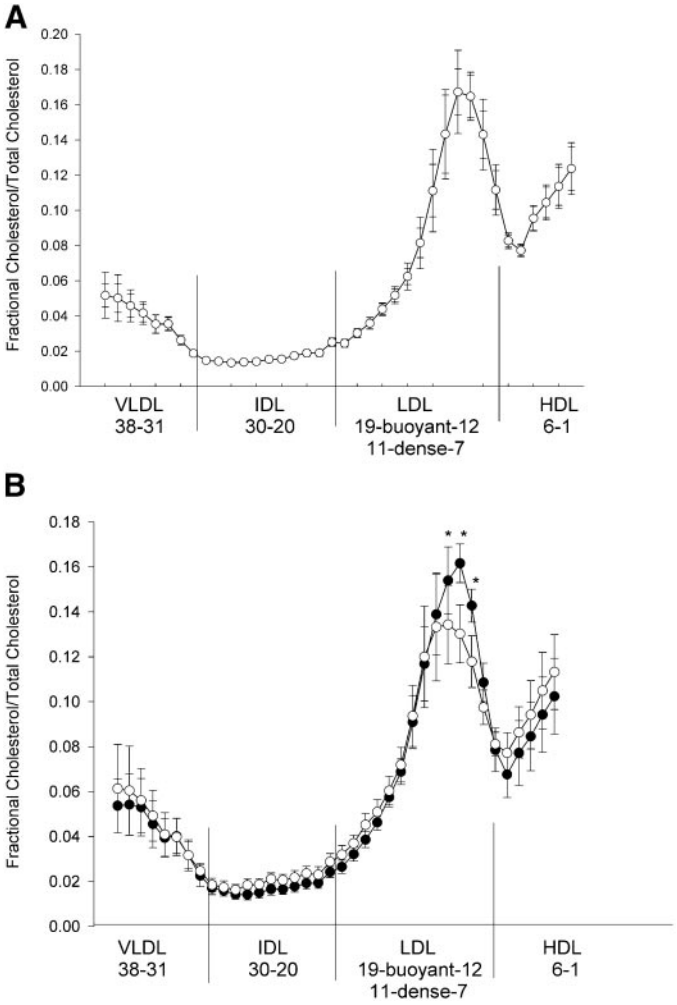

Fig. 3. Density gradient ultracentrifugation (DGUC) of cholesterol fractions before (●) and after (○) treatment with placebo (*A*) or T (*B*). Data are presented as individual cholesterol normalized to total cholesterol and then averaged by group ± SE (see EXPERIMENTAL PROCEDURES). *$P < 0.05$ vs. baseline.

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_3 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

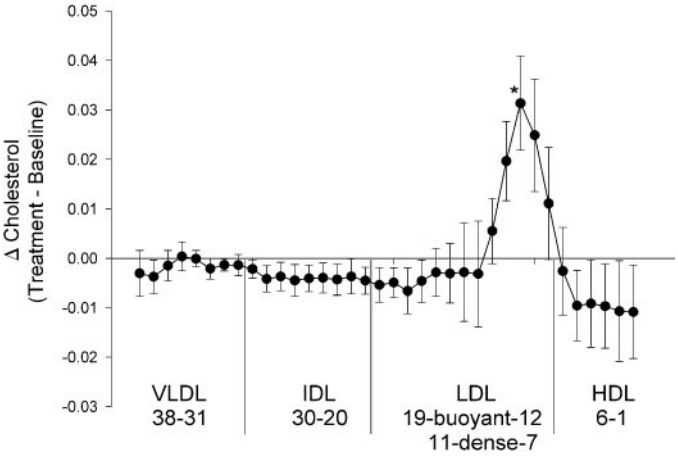

Fig. 4. Change (Δ) in DGUC cholesterol fractions, treatment minus baseline, for the T-treated group. *$P < 0.05$ vs. zero.

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, HDL_2, HDL_3, and LDL particle density. Additional studies are needed to evaluate the effects of these changes on cardiovascular disease.

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