

## Testosterone Therapy Prevents Gain in Visceral Adipose Tissue and Loss of Skeletal Muscle in Nonobese Aging Men

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**Background:** Trials of testosterone therapy in aging men have demonstrated increases in fat-free mass (FFM) and skeletal muscle and decreases in fat mass (FM) but have not reported the impact of baseline body composition.

**Objective:** The objective of the study was to determine the effect, in nonobese aging men with symptoms of androgen deficiency and low-normal serum testosterone levels, of testosterone therapy on total and regional body composition and hormonal and metabolic indices.

**Methods:** Sixty healthy but symptomatic, nonobese men aged 55 yr or older with total testosterone (TT) levels less than 15 nM were randomized to transdermal testosterone patches or placebo for 52 wk. Body composition, by dual-energy x-ray absorptiometry (FM, FFM, skeletal muscle) and magnetic resonance imaging (abdominal sc and visceral adipose tissue, thigh skeletal muscle, and intermuscular fat) and hormonal and metabolic parameters were measured at wk 0 and 52.

**Results:** Serum TT increased by 30% ( $P = 0.01$ ), and LH decreased by 50% ( $P < 0.001$ ). Relative to placebo, total body FFM ( $P = 0.03$ ) and skeletal muscle ( $P = 0.008$ ) were increased and thigh skeletal muscle loss was prevented ( $P = 0.045$ ) with testosterone therapy and visceral fat accumulation decreased ( $P = 0.001$ ) without change in total body or abdominal sc FM; change in visceral fat was correlated with change in TT levels ( $r^2 = 0.36$ ;  $P = 0.014$ ). There was a trend to increasing total and low-density lipoprotein cholesterol with placebo.

**Conclusion:** Testosterone therapy, relative to placebo, selectively lessened visceral fat accumulation without change in total body FM and increased total body FFM and total body and thigh skeletal muscle mass. Further studies are needed to determine the impact of these body compositional changes on markers of metabolic and cardiovascular risk. (*J Clin Endocrinol Metab* 93: 139–146, 2008)

Testosterone therapy is increasingly used by middle-aged and older men (1, 2) seeking to ameliorate signs and symptoms of the aging process thought to be causally related to the age-related decline in serum testosterone. Data from randomized placebo-controlled trials are limited (1, 3, 4), and their interpretation is made difficult by the inclusion of cohorts with differing baseline characteristics and testosterone levels (5–12). Only three studies (6, 9, 12) specifically included men with symptoms

consistent with androgen deficiency. Furthermore, whereas some studies used physiological androgen replacement regimens (6–8), others clearly administered supraphysiological doses (13–15).

Testosterone therapy has consistently been associated with favorable changes in body composition, notably decreases in fat mass (FM) and gains in fat-free mass (FFM) (5–8). The magnitude of effect, especially with regard to change in FM, is influ-

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Abbreviations: BMI, Body mass index; calc FT, calculated free testosterone; CV, coefficient of variation; DEXA, dual-energy x-ray absorptiometry; E2, estradiol; FFM, fat-free mass; FM, fat mass; HOMA-IR, homeostasis assessment model insulin resistance index; IPSS, International Prostate Symptom Score; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; PSA, prostate-specific antigen; TT, total testosterone; WC, waist circumference.

enced by baseline body composition and serum testosterone levels, the androgen dose used, and the duration of therapy. Studies have included subjects with a range of body mass indices (BMIs) (5–12, 16–18), but none has reported change in FM as a function of baseline BMI. Accordingly, whether nonobese men experience the same FM and FFM effects of testosterone therapy as do those who are obese is uncertain. The data regarding testosterone effect on regional fat distribution are even more limited. Whereas abdominally obese men lose visceral fat when treated with testosterone (16, 17), results from placebo-controlled trials in cohorts including normal, overweight, and obese men and examining changes in sc and visceral fat with testosterone (18), anabolic steroids (14, 15), and dehydroepiandrosterone (DHEA) (19) are inconsistent. Furthermore, the metabolic consequences of these body composition changes are not well understood (20).

Obesity is an important confounder in the presentation of androgen deficiency with regard to symptomatology and measured testosterone concentrations and is also likely to influence response to testosterone therapy. In this 52-wk randomized, placebo-controlled study of transdermal testosterone therapy, we therefore chose to study nonobese aging men with symptoms of androgen deficiency and low-normal serum testosterone levels and aimed to determine, relative to placebo, the effects of testosterone therapy on body composition, specifically visceral fat, and metabolic indices.

## Subjects and Methods

### Subjects

Men aged 55 yr or older were recruited by community advertisement. Those whose mean (of two samples) serum testosterone level was less than 15 nM and who had both a BMI less than 30 kg/m<sup>2</sup> and waist circumference (WC) less than 102 cm were eligible for randomization. Weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm; waist circumference was measured at the midpoint between the costal margin and iliac crest in the midaxillary line on the dominant side (21). Participants were nonsmokers in good general health but with self-reported, unscaled symptoms consistent with testosterone deficiency in each of three categories: 1) mood and cognition (*e.g.* decreased sense of well-being, low mood, lack of motivation, lethargy, perceived poor short-term memory); 2) body composition (*e.g.* diminished muscle mass or strength, gain in fat mass); and 3) sexual dysfunction (*e.g.* poor libido, decline in sexual performance) based on the manifestations of postpubertal testicular deficiency (22) and the Canadian practice recommendations for screening for androgen deficiency in the aging male (23). Men were excluded if they reported any of the following: intake of more than four standard alcoholic drinks per day, a history of malignancy (excluding nonmelanoma skin cancer), serious chronic medical illness including diabetes mellitus, sleep apnea, or prostate cancer or clinically significant benign prostate disease. Men who had received testosterone therapy in the previous 12 months were not included. Suitable candidates underwent physical examination and laboratory investigations to confirm their health status.

The Southern Health Human Research Ethics Committee approved the project and all subjects gave signed consent. The study was conducted between May 2001 and April 2004.

### Study design

Subjects were treated for 52 wk with transdermal testosterone or a matching placebo with identical constituents (other than the omission of

testosterone) in a double-blinded, randomized protocol. They were instructed not to significantly modify their dietary or exercise patterns during the study and underwent pedometer assessment at wk 0 and 52. Hormonal parameters were studied at wk 0, 4, 12, 26, and 52. Body composition end points were measured at wk 0 and 52, and metabolic indices (lipids, fasting glucose and insulin, insulin resistance) and safety end points were evaluated at wk 0, 12, 26, and 52.

Subjects were randomized in a 1:1 ratio by the clinical trials pharmacist. Compliance was assessed by return of study medication packaging and unused patches and was calculated to be in excess of 95%.

### Study medication

Androderm patches (5.0 mg testosterone) and matching placebo were supplied by Watson Laboratories, Inc. (Salt Lake City, UT). A single patch was applied each evening before retiring. Subjects were instructed to choose a site on the thigh, upper arm, abdomen, or back for patch application and to rotate the sites of application such that there was an interval of (at least) 7 d before the medication was reapplied to any given site. All subjects were advised to pretreat application sites with triamcinolone acetonide cream 0.02% (Sigma Pharmaceuticals Pty. Ltd., Victoria, Australia).

### Outcome measures

#### Hormone assays and metabolic indices

Blood samples were taken between 0800 and 1100 h. Samples were stored at –20°C and assayed at study completion. Total testosterone (TT) was measured on a Beckman Coulter Unicel DXI 800 analyzer using an automated competitive binding immunoassay [coefficient of variation (CV) 5.5% at TT levels of 22.4 nM; normal range for healthy young adult males, 8–28 nM (Beckman Coulter Inc., Fullerton, CA)]. SHBG was measured on an Immulite analyzer (CV 7.9%; Diagnostic Products Co., Los Angeles, CA). Free testosterone (calc FT) was calculated according to the method described by Vermeulen *et al.* (24). Estradiol (E2) was assayed by RIA (CV 14.9%; Diagnostic Products), and LH was measured by microparticle enzyme immunoassay MEIA on an AxSym (Abbott Diagnostics, Abbott Park, IL; CV 6.4%, normal range 1.4–8.0 IU/liter). Assay sensitivities for E2 and LH were 20 pM and 0.5 IU/liter, respectively; where appropriate these values were included during data analysis. Insulin resistance was determined by the homeostasis assessment model; homeostasis assessment model insulin resistance index (HOMA-IR) was calculated as follows: (FPI × FPG)/22.5, where FPI is fasting plasma insulin concentration (milliunits per liter) and FPG is fasting plasma glucose (millimoles).

### Body composition

Total body and regional FFM (CV ~3%) and total body and regional FM (CV ~2%) were measured by dual-energy x-ray absorptiometry (DEXA; Lunar DPX software version 3.6z, in slow mode; Madison, WI), and the skeletal muscle mass component of FFM (excluding lean tissues—skin, tendons, and connective tissue) was estimated (CV ~3%) (25). Thigh skeletal muscle volume and thigh and abdominal regional adipose tissue volumes were measured by magnetic resonance imaging (MRI); initially these were performed on a Magnetom Symphony (Siemens, Melvern, PA) and then, after a change in equipment, on a Genesis-Signa (GE Medical Systems, Milwaukee, WI). Images of 10-mm thickness at 50-mm intervals were taken. An axial T1-weighted spin echo sequence with a repetition time of 80 msec, echo time of 4 msec, and an acquisition matrix of 256 × 256 was used to acquire all images, which were stored in a 16-bit format. Abdominal visceral and sc fat volumes were determined for a 250-mm region beginning 12 cm above the femoral head and extending superiorly (five slices per subject). Thigh skeletal muscle and sc and intermuscular fat were determined over a 250-mm region beginning 12 cm below the femoral head; five slices (including both lower limbs) were analyzed for each subject. Analyses were performed by a single operator (C.A.A.) blinded to treatment group using

customized software (SliceOmatic program software version 4.2; Tomovision, Montréal, Canada) (26). Tissue volume was calculated according to slice thickness and interval and converted to mass according to respective densities. The intraobserver CV was 2% for muscle and sc and intermuscular fat muscle and 5% for visceral fat. Data sets were available for all subjects in the testosterone group and 20 of 25 subjects in the placebo group.

### Safety data

Hemoglobin and hematocrit were measured, and potential adverse prostate events were monitored with digital rectal examination, and prostate-specific antigen (PSA). The International Prostate Symptom Score (IPSS) questionnaire (27) was applied at wk 0, 26, and 52. Urodynamic studies were offered at wk 0 and 52; uroflowmetry was performed on a Urolynx 1000 urine flow meter (Medtronic-Dantec Corp., Österreich, Germany) and postvoiding residual urine volume was measured with a handheld ultrasound device (bladder scanner; Bard, North Ryde, Australia). Any result considered by the investigators to be clinically significant and all abnormal PSA results (a value exceeding 4.0  $\mu\text{g/liter}$  at any time point or an interval increase  $> 1.5 \mu\text{g/liter}$ ) were reviewed by a consultant urologist.

### Statistical analysis

Sample size was calculated using the primary end point of visceral fat mass; to detect a 10% change, a minimum of 10 men were needed in each group to provide 80% power at two-tailed alpha of 5%. Additional enrollments allowed for dropouts and examination of secondary end points. Data were analyzed by both an intention-to-treat and a per-protocol approach. Hormone levels, metabolic indices, and safety data were analyzed by repeated-measures ANOVA with *post hoc* Bonferroni's

multiple comparison tests applied when  $P < 0.05$ . Body composition in subjects completing the study was analyzed with paired *t* tests for within-treatment group change and unpaired *t* tests for differences in responses between the groups. The relationship between change in serum testosterone and visceral adiposity was assessed with linear regression. Significance was set at  $P < 0.05$ ; all values are given as mean  $\pm$  SEM.

### Results

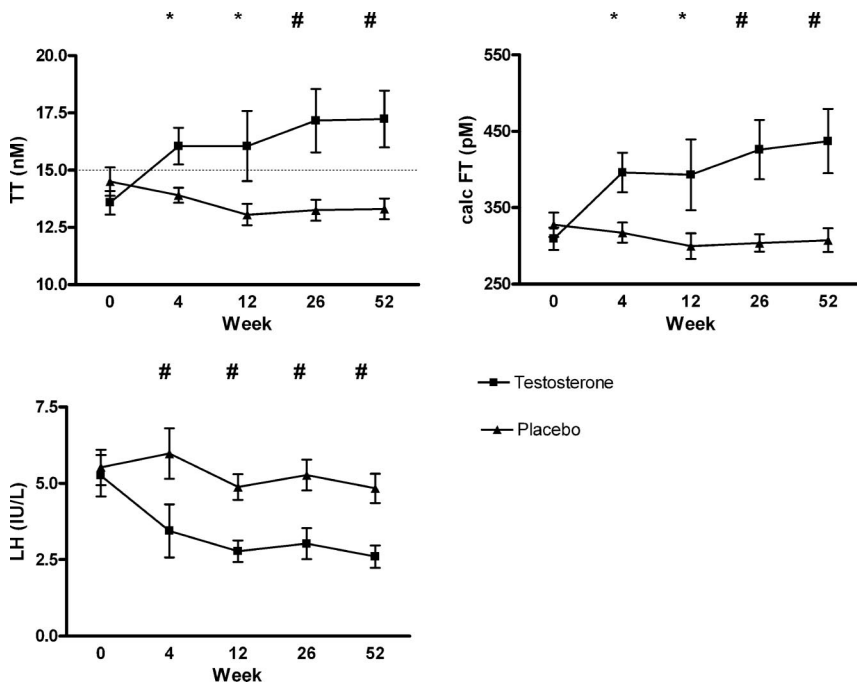
Two hundred twenty-three men were screened to identify the 62 men who were randomized; one subject in each group chose not to commence treatment. Baseline characteristics of the subjects were similar (Table 1). Thirteen subjects in the testosterone group prematurely discontinued treatment, 10 due to skin irritation, one for unrelated illness, and two for personal reasons. Five men in the placebo group withdrew, two for skin intolerance, two due to unrelated intercurrent illness, and one for personal reasons. Subjects who withdrew from the study were of similar age, BMI, and WC to subjects completing the study and had comparable baseline TT and LH levels (data not shown). The number of men remaining in the testosterone group at wk 4, 12, 26, and 52 were 28, 23, 21, and 17, respectively; for the placebo group, the corresponding numbers were 29, 28, 27, and 25. At baseline, the men in each group took approximately 8500 steps per day and this did not change during the study.

**TABLE 1.** Baseline characteristics of the subjects according to treatment group

Characteristic	Testosterone (n = 31)	Placebo (n = 31)	P value
Age (yr)	62.1 $\pm$ 1.0	64.5 $\pm$ 1.3	0.15
Anthropometry			
Height (cm)	174.0 $\pm$ 1.0	172.5 $\pm$ 1.4	0.40
Weight (kg)	79.1 $\pm$ 1.6	75.7 $\pm$ 1.7	0.15
WC (cm)	94.7 $\pm$ 1.4	94.5 $\pm$ 1.1	0.87
BMI (kg/m <sup>2</sup> )	26.1 $\pm$ 0.4	25.4 $\pm$ 0.4	0.20
Hormones			
TT (nM)	13.6 $\pm$ 0.5	14.5 $\pm$ 0.6	0.26
Calc FT (pM)	309.3 $\pm$ 14.7	327.6 $\pm$ 16.0	0.40
SHBG (nM)	33.4 $\pm$ 2.0	34.2 $\pm$ 1.7	0.78
E <sub>2</sub> (pM)	50.9 $\pm$ 4.1	53.6 $\pm$ 5.0	0.68
LH (IU/liter)	5.3 $\pm$ 0.7	6.0 $\pm$ 0.7	0.46
Metabolic parameters			
Total cholesterol (mM)	5.1 $\pm$ 0.2	4.9 $\pm$ 0.1	0.45
Triglycerides (mM)	1.4 $\pm$ 0.1	1.5 $\pm$ 0.1	0.53
HDL cholesterol (mM)	1.3 $\pm$ 0.1	1.2 $\pm$ 0.1	0.24
LDL cholesterol (mM)	3.2 $\pm$ 0.2	3.1 $\pm$ 0.1	0.50
Fasting glucose (mM)	5.2 $\pm$ 0.1	5.1 $\pm$ 0.1	0.49
Fasting insulin (mU/liter)	9.4 $\pm$ 0.8	9.4 $\pm$ 0.7	0.97
HOMA-IR	2.2 $\pm$ 0.2	2.1 $\pm$ 0.2	0.84
Hematopoiesis			
Hemoglobin (g/dl)	15.3 $\pm$ 0.1	15.1 $\pm$ 0.2	0.58
Hematocrit (%)	44.6 $\pm$ 0.5	44.1 $\pm$ 0.5	0.57
Prostate			
PSA ( $\mu\text{g/liter}$ )	1.7 $\pm$ 0.2	1.8 $\pm$ 0.4	0.81
IPSS	6.2 $\pm$ 0.9	8.2 $\pm$ 1.0	0.13
Maximum urine flow rate (ml/sec) <sup>a</sup>	15.6 $\pm$ 1.3	13.8 $\pm$ 1.0	0.27
Residual urine volume (ml) <sup>a</sup>	71.8 $\pm$ 11.8	102.6 $\pm$ 23.4	0.24

HDL, High-density lipoprotein.

<sup>a</sup> n = 28 (testosterone), n = 25 (placebo).



**FIG. 1.** TT (left panel), calc FT (right panel), and LH (bottom panel) values (mean ± SEM) for the testosterone and placebo groups at each assessment time point. Each time point includes all men remaining in the study at that time (testosterone group: 31, 28, 23, 21, and 17 and placebo 31, 29, 28, 27, and 25). The dotted line at 15 nM on the TT graph represents the cutoff for inclusion in the study. \*, *P* < 0.05; #, *P* < 0.005 for differences between groups.

**Hormone levels**

TT levels increased by 18% between wk 0 and 4 in the testosterone group (*P* = 0.002) but did not change in the placebo group. TT levels were significantly higher in the testosterone group when compared with the placebo group at each time point during the study (Fig. 1). TT levels increased by 30% (4 nM) in the testosterone group between wk 0 and 52; this was significant when analyzed both by intention to treat (*P* = 0.018) and according to protocol completion (*P* = 0.014). TT levels fell by 10% in the placebo group (*P* = 0.036) with a significant difference between wk 0 and 12 (*P* < 0.05); thereafter TT levels remained stable. calc FT levels increased by 40% with testosterone (*P* = 0.039) between wk 0 and 52 but did not change with placebo (Fig. 1). SHBG and E2 levels did not change significantly in either group. LH levels decreased by almost 50% in response to

testosterone therapy (*P* < 0.001), whereas a trend to decrease in the placebo group was not significant (*P* = 0.07) (Fig. 1).

**Body composition**

Body weight did not change. Baseline BMI (21.0–29.0 kg/m<sup>2</sup> in the testosterone group and 20.7–29.0 kg/m<sup>2</sup> in the placebo group) and WC (95 cm in both groups) were not altered by either treatment.

**FFM**

FFM tended to increase in the testosterone group and was unchanged in the placebo group (*P* = 0.026 for the differences in change between groups; Table 2). Regional DEXA studies suggested that the gain in FFM was limited to the appendices with an increase in leg and arm FFM in the testosterone group relative to placebo (*P* = 0.054 and 0.016, respectively; Table 2).

In keeping with the increase in FFM relative to placebo, skeletal muscle mass tended to increase in the testosterone group (*P* = 0.065) but did not change in the placebo group, such that over the study period, the difference between the groups was significant (*P* = 0.008; Table 2).

Thigh skeletal muscle mass determined by MRI was similar between the groups at baseline (testosterone: 5.84 ± 0.17 kg; placebo: 5.57 ± 0.19 kg). There was no change at wk 52 in the testosterone group (5.86 ± 0.20 kg), but a 4.1 ± 1.2% decrease was observed in the placebo group (*P* = 0.003) such that there was a significant difference in the changes between the groups (*P* = 0.045).

**FM**

There was no change in total body or regional FM or percent body fat as determined by DEXA (Table 3).

Neither testosterone nor placebo affected abdominal sc fat mass as determined by MRI (Table 3 and Fig. 2). Visceral adipose tissue mass tended to decrease in the testosterone group and

**TABLE 2.** Total body and regional FFM and skeletal muscle mass in the testosterone-treated and placebo groups

DEXA	Testosterone-treated group (n = 17)			Placebo group (n = 25)			P' value
	Wk 0	Wk 52	P value	Wk 0	Wk 52	P value	
FFM (kg)							
Total body	60.5 ± 1.5	61.3 ± 1.6	0.105	58.2 ± 1.4	57.9 ± 1.4	0.220	0.026
Regional							
Trunk	28.9 ± 0.6	29.1 ± 0.7	0.487	27.8 ± 0.7	27.6 ± 0.7	0.236	0.197
Legs	20.3 ± 0.6	20.6 ± 0.6	0.137	19.2 ± 0.6	19.2 ± 0.6	0.463	0.054
Arms	7.3 ± 0.2	7.5 ± 0.2	0.080	7.2 ± 0.2	7.2 ± 0.2	0.176	0.016
Skeletal muscle mass (kg) <sup>a</sup>							
Total body	29.5 ± 0.9	30.1 ± 0.9	0.065	28.3 ± 0.8	28.1 ± 0.8	0.169	0.008

P' value refers to the difference between groups for the change in study parameter between wk 0 and 52.

<sup>a</sup> Skeletal muscle mass is a subset of FFM by DEXA (25).

**TABLE 3.** Total and regional FM and percent body fat in the testosterone-treated and placebo groups

	Treatment group						
	Testosterone			Placebo			P' value
	Wk 0	Wk 52	P value	Wk 0	Wk 52	P value	
DEXA		(n = 17)			(n = 25)		
Total body FM (kg)	19.8 ± 1.5	19.3 ± 1.6	0.616	18.3 ± 1.0	18.4 ± 0.9	0.969	0.611
Regional FM (kg)							
Trunk	11.4 ± 0.9	11.5 ± 1.0	0.969	10.9 ± 0.6	10.9 ± 0.6	0.816	0.932
Legs	5.6 ± 0.7	5.2 ± 0.5	0.159	4.8 ± 0.3	4.7 ± 0.3	0.482	0.237
Arms	1.7 ± 0.2	1.7 ± 0.1	0.278	1.7 ± 0.1	1.7 ± 0.1	0.260	0.278
Body fat (%)	24.3 ± 1.5	23.6 ± 1.6	0.432	23.8 ± 0.9	23.9 ± 0.9	0.753	0.343
MRI		(n = 16)			(n = 20)		
Abdominal adipose mass (kg)							
Subcutaneous	2.8 ± 0.2	2.7 ± 0.2	0.483	2.6 ± 0.2	2.6 ± 0.2	0.876	0.480
Visceral	2.5 ± 0.2	2.3 ± 0.2	0.217	2.2 ± 0.2	2.7 ± 0.2	0.262	0.001
Thigh adipose mass (kg)							
Subcutaneous	2.4 ± 0.1	2.2 ± 0.1	0.043	2.1 ± 0.1	2.0 ± 0.1	0.2944	0.347
Intermuscular	0.25 ± 0.03	0.30 ± 0.03	0.014	0.23 ± 0.02	0.34 ± 0.03	<0.0001	0.002

P' value refers to the difference between groups for the change in study parameter between wk 0 and 52.

tended to increase in the placebo group such that there was a significant difference in change between the groups ( $P = 0.001$ ; Table 3 and Fig. 2). The change in visceral fat was correlated with the change in TT levels ( $r^2 = 0.36$ ;  $P = 0.014$ ; Fig. 3). Thigh sc fat decreased in the testosterone group ( $P = 0.043$ ) but also tended to decrease in the placebo group with the difference in change between the groups not significant (Table 3 and Fig. 2). Intermuscular fat in the thigh increased in both groups (Table 3 and Fig. 2).

**Metabolic indices**

Between wk 0 and 52, total ( $5.1 \pm 0.2$  to  $5.2 \pm 0.2$  mM) and low-density lipoprotein (LDL;  $3.2 \pm 0.2$  to  $3.3 \pm 0.2$  mM) cho-

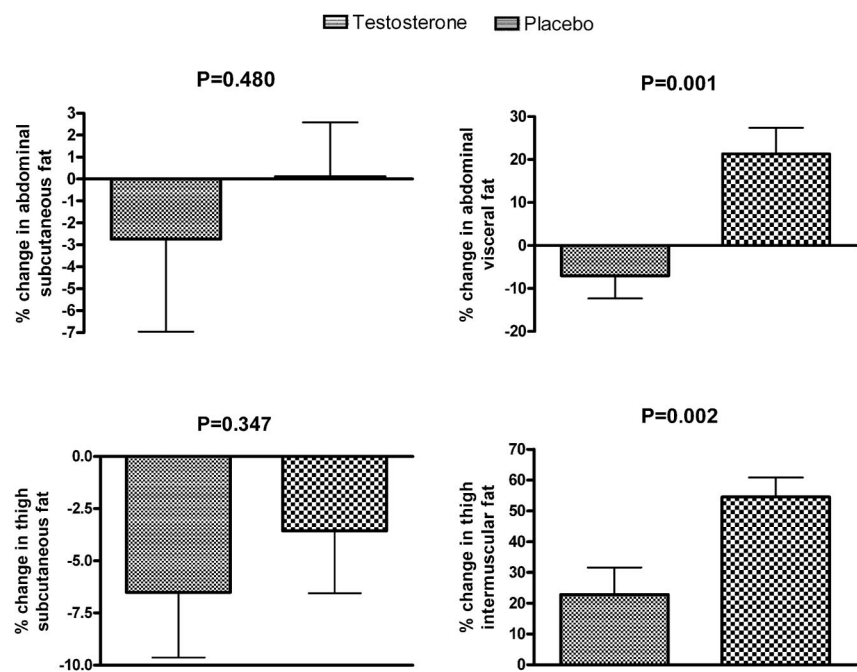
lesterol did not change with testosterone treatment. Total ( $5.0 \pm 0.1$  to  $5.5 \pm 0.2$  mM;  $P = 0.006$ ) and LDL ( $3.1 \pm 0.1$  to  $3.4 \pm 0.2$  mM;  $P = 0.016$ ) cholesterol increased by 10% in the placebo group but the between-group change was not significant. High-density lipoprotein cholesterol and triglyceride levels were unaffected by treatment.

Fasting blood glucose levels (testosterone:  $5.2 \pm 0.1$  to  $5.2 \pm 0.1$  mM; placebo:  $5.1 \pm 0.1$  to  $5.1 \pm 0.1$  mM), fasting insulin levels (testosterone:  $9.7 \pm 1.0$  to  $10.6 \pm 1.4$  mU/liter; placebo:  $9.5 \pm 0.8$  to  $10.2 \pm 1.0$  mU/liter), and HOMA-IR (testosterone:  $2.2 \pm 0.2$  to  $2.5 \pm 0.4$ ; placebo:  $2.2 \pm 0.2$  to  $2.3 \pm 0.2$ ) did not change.

**Safety monitoring**

At wk 52 hemoglobin levels increased with testosterone ( $15.2 \pm 0.2$  to  $15.6 \pm 0.2$  g/dl;  $P = 0.046$ ) but not placebo ( $15.2 \pm 0.2$  to  $15.4 \pm 0.2$  g/dl); the between-group change was not significant ( $P = 0.453$ ). The hematocrit values did not change.

No abnormalities in digital rectal examination were recorded. PSA levels in subjects receiving testosterone and completing the study were  $2.1 \pm 0.4$  μg/liter at wk 0 and  $2.1 \pm 0.5$  μg/liter at wk 52. In the placebo group, the corresponding values were  $1.9 \pm 0.4$  and  $2.2 \pm 0.5$  μg/liter. Mean change in PSA was not different between the groups ( $P = 0.22$ ). IPSS scores did not change in either group. In the 85% of subjects who underwent urodynamic studies, maximum urine flow rates were not different between the groups at either wk 0 (Table 1) or wk 52 (testosterone:  $18.0 \pm 2.0$  ml/sec vs. placebo:  $16.9 \pm 1.3$  ml/sec;  $P = 0.63$ ); rates increased in both the testosterone ( $P = 0.009$ ) and placebo ( $P = 0.020$ ) groups. Residual urine volumes did not change.



**FIG. 2.** Percent change in abdominal sc (top left panel) and visceral (top right panel) fat and thigh sc (bottom left panel) and intermuscular (bottom right panel) fat as determined by MRI. Testosterone, n = 16; placebo, n = 20.

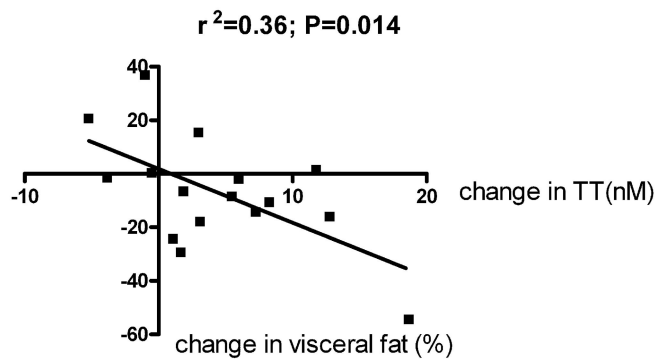


FIG. 3. Change in visceral fat as a function of change in serum TT in the testosterone-treated group.

## Discussion

We recruited subjects representative of those men likely to present to primary care physicians to discuss, and possibly be considered as candidates for, testosterone treatment. The inclusion criteria therefore required the presence of symptoms consistent with (although not diagnostic of) androgen deficiency and a serum testosterone in the low-normal range. To exclude the confounding effects of coexistent illness, only those in good general health were randomized. Given the relationship between obesity (generalized or abdominal) and a lowering in serum total (and free) serum testosterone (28–30), we also excluded those with a BMI greater than 30 kg/m<sup>2</sup> and/or WC greater than 102 cm. The studied cohort thus consisted of healthy nonobese but symptomatic aging men whose baseline TT levels of less than 15 nM represented approximately the lowest tertile of the range for reproductively healthy young adult males as measured in our laboratory (31) and also corresponded to 1 SD below the mean for men aged 20–39 yr based on cross-sectional data in healthy nonobese men (32). We demonstrated that testosterone therapy, relative to placebo, selectively lessened visceral fat accumulation without change in total body fat mass and increased total body FFM and total body and thigh skeletal muscle mass.

Men were randomized if their mean (of two) morning serum TT levels at screening were less than 15 nM (4.3 ng/ml), yet at wk 0, seven of 31 and 10 of 31 men had values of 15 nM or greater in the testosterone and placebo groups, respectively. This is consistent with the known variability of serum TT over time (33) and data from other randomized controlled trials (34, 35). Testosterone treatment led to a 30% increase in serum TT into the middle of the healthy young adult male reference range at wk 52 (serum TT level  $17.7 \pm 1.2$  nM). The reciprocal 50% decline in serum LH serves as a biomarker of this increase in serum TT. A similar 30–40% increase in serum TT has been reported in studies using the same testosterone preparation (6, 10, 11). In contrast, there was a (greater than expected) 10% fall in TT levels in the placebo group evident at 12 wk, with TT levels maintained thereafter.

The mean BMI at baseline in each group lay just within the overweight range, and none was obese as an entry criterion. Subjects in other studies of testosterone therapy in which body composition was assessed had mean baseline BMIs of 26–29

kg/m<sup>2</sup> (5, 7–9, 11) but must have included obese subjects, given SDs of a minimum  $\pm 3$  kg/m<sup>2</sup>. Furthermore, none provided WC data, the best anthropometric index of visceral adiposity (21).

Our subjects had baseline FFM 2–6 kg greater than previous cohorts in randomized controlled trials of equivalent or longer duration (5, 7–9). Whereas the observed increase in FFM ( $0.8 \pm 0.4$  kg) was similar to that seen by Kenny *et al.* (8), it was only approximately half that seen in other studies assessing FFM (or lean body mass) at 12 months (7, 9), perhaps reflecting either our subjects' higher baseline FFM and/or their smaller increase in serum TT. The trend for progressive loss of FFM in placebo-treated men (presumably reflecting aging effect) when combined with the upward trend in the testosterone-treated men led to significant group difference. The only comparable cohort with respect to baseline FFM (10) demonstrated a significant 1.4 kg increase in FFM using the same testosterone regimen but administered for 2 yr. The loss of FFM in the placebo group, although not significant, is consistent with the 0.4 kg loss per year seen in men beyond the fifth decade (36).

Regional DEXA studies suggested that the change in FFM was limited to the appendices, in contrast to a previous study wherein an increase in lean body mass was seen only in the trunk (7). Concomitant with the changes in FFM, DEXA estimates of skeletal muscle mass showed a tendency to increase in the testosterone group that was significant relative to placebo. This finding is of particular relevance, given that skeletal muscle mass is preserved until the fifth decade, and thereafter the age-associated loss is greater in men than women (37).

MRI studies of the thigh documented a 4% loss of skeletal muscle over 12 months with the placebo group ( $P = 0.003$ ), with no change in the testosterone group, suggesting that elevation of serum testosterone from the low-normal into the mid-normal young male range may ameliorate age-related sarcopenia. Similar effects on FFM have been reported in healthy older men (with comparable baseline FFM) rendered hypogonadal with a GnRH agonist and then given exogenous testosterone to return serum TT to baseline levels for 20 wk (38). Subphysiological testosterone doses resulted in loss of FFM and supraphysiological doses led to clear gains in FFM. Our finding that testosterone prevented loss of thigh skeletal muscle is significant, given that, as a function of age in men, skeletal muscle is lost more rapidly than nonskeletal muscle mass (39) and that there is relatively greater loss of skeletal muscle in the lower compared with the upper limbs (40).

In keeping with the study's BMI and WC inclusion criteria, the mean baseline FM of our subjects (20 kg) was 4–8 kg less than in previous trials (7–10). No effect of testosterone was seen on total body FM (by DEXA) in contrast to the 5–15% (up to 4.5 kg) decrease at 12 months in other studies (5, 7–9); in those studies a greater magnitude of change in FM was seen with larger increases in serum TT. We did not observe changes in regional FM (by DEXA) in the trunk, leg, or arm, whereas other studies have shown a decrease in limb but not trunk FM (7) or at both sites (5). The lack of effect in this current study may result from the subjects' lower baseline FM, higher baseline serum TT, and/or the more modest rise in TT with testosterone treatment.

Assessment of regional fat distribution by MRI, however,

demonstrated the prevention of accumulation of visceral fat in men treated with testosterone relative to placebo, whereas abdominal sc fat mass did not change in either group. These findings are consistent with those from studies of testosterone supplementation in eugonadal middle-aged abdominally obese men (mean BMI 29 kg/m<sup>2</sup> and WC 105 cm) in which decreases in visceral adipose tissue cross-sectional area of 5–10% (0.4–0.6 kg) were shown based on computed tomography over 8–9 months (16, 17). In agreement with that study, we also observed no effect of treatment on total body fat or sc adipose tissue mass.

Our study is the first to describe the effects of testosterone treatment on abdominal fat distribution (visceral *vs.* sc) as a function of baseline adiposity. Despite limiting our cohort to nonobese men, we were able to demonstrate that modest increases in serum testosterone effectively target the visceral adipose tissue compartment with the change in visceral fat mass negatively correlated to the change in serum TT. It is the visceral adipose tissue (measured by computed tomography scan) that has been reported to continue to increase with advancing age (41), whereas abdominal sc fat mass plateaus after the age of 30 yr (42).

Testosterone therapy did not lower serum cholesterol, in contrast with trials documenting falls of 10% in total and LDL cholesterol (43–45); however, our subjects had lower baseline lipid levels and a smaller increase in serum testosterone than in studies showing an effect (5, 7, 16, 17). Our findings are consistent with data from a cohort with comparable baseline TT levels (46) also studied for 12 months. The small but significant rises in total and LDL cholesterol in the placebo group parallel the increase in visceral fat.

Our exclusion of abdominally obese men likely accounts for the lower baseline insulin and HOMA-IR values, compared with adults of similar age and BMI (47), and may explain the lack of change after testosterone treatment. Indeed, the only studies examining the relationship of androgens to insulin resistance to find a beneficial effect studied middle-aged men selected for abdominal adiposity (16, 20) or type 2 diabetes (48).

A limitation of the current study is the higher-than-expected dropout rate in the men receiving testosterone. Our choice of testosterone preparation was determined by availability and access to a suitable placebo preparation. Nonscrotal testosterone patches are associated with high rates of skin intolerance (49) and discontinuation rates in routine clinical practice (50). We are not aware that older men are more likely to experience skin irritation; nonetheless, we advised all subjects to pretreat with a corticosteroid cream (51). The higher dropout rate in the testosterone group (30 *vs.* 6% in placebo) remains unexplained because the active component (testosterone) is the only difference between the transdermal systems.

Given the strong association of obesity, and visceral adipose tissue specifically, with metabolic syndrome (52) and excess cardiovascular morbidity and mortality (21, 53, 54), the question as to whether testosterone therapy in older men with low/low-normal testosterone levels will modify metabolic and cardiovascular risk is an important one (55). Our findings suggest a role for testosterone in modifying the age-related increase in visceral adipose tissue (and possibly associated adverse metabolic changes)

and provide a basis for further studies examining surrogate markers of cardiovascular risk and insulin sensitivity in men at risk for metabolic and cardiovascular disorders, specifically those with abdominal obesity.

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