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Dose-Dependent Effects of Testosterone on Regional Adipose Tissue Distribution in Healthy Young Men

LINDA J. WOODHOUSE, NIDHI GUPTA, MEENAKSHI BHASIN, ATAM B. SINGH, ROBERT ROSS, JEFFREY PHILLIPS, AND SHALENDER BHASIN

Division of Endocrinology, Metabolism, and Molecular Medicine (L.J.W., N.G., M.B., A.B.S., S.B.), Charles R. Drew University of Medicine and Science, Los Angeles, California 90059; School of Rehabilitation Sciences (L.J.W.), McMaster University, Hamilton, Ontario Canada L8S 1C7; School of Physical and Health Education (R.R.), Queen's University, Kingston, Ontario, Canada, K7L 3N6; and Harbor-University of California, Los Angeles Medical Center (J.P.), Torrance, California 90502

Testosterone supplementation reduces total body adipose tissue (AT), but we do not know whether the effects are uniformly distributed throughout the body or are region specific, or whether they are dose related.

We determined the effects of graded doses of testosterone on regional AT distribution in 54 healthy men (18–35 yr) in a 20-wk, randomized, double-blind study of combined treatment with GnRH agonist plus one of five doses (25, 50, 125, 300, or 600 mg/wk) of testosterone enanthate (TE). Total body, appendicular, and trunk AT and lean body mass were measured by dual-energy x-ray absorptiometry, and sc, intermuscular, and intraabdominal AT of the thigh and abdomen were measured by magnetic resonance imaging. Treatment regimens resulted in serum nadir testosterone concentrations ranging from subphysiological to supraphysiological levels. Dose-dependent changes in AT mass were negatively correlated with TE dose at all sites and were equally distributed between the trunk and appendices. The lowest dose was associated with gains in sc, intermuscular, and intraabdominal AT, with the greatest percent increase occurring in the sc stores. At the three highest TE doses, thigh intermuscular AT volume was significantly reduced, with a greater percent loss in intermuscular than sc depots, whereas intraabdominal AT stores remained unchanged. In conclusion, changes in testosterone concentrations in young men are associated with dose-dependent and region-specific changes in AT and lean body mass in the appendices and trunk. Lowering testosterone concentrations below baseline increases sc and deep AT stores in the appendices and abdomen, with a greater percent increase in sc depots. Conversely, elevating testosterone concentrations above baseline induces a greater loss of AT from the smaller, deeper intermuscular stores of the thigh. (*J Clin Endocrinol Metab* 89: 718–726, 2004)

LOW TESTOSTERONE CONCENTRATIONS are associated with increased total body and visceral adiposity (1, 2). Similarly, induction of androgen deficiency in healthy men by administration of a GnRH agonist has been shown to result in increased adipose tissue (AT) mass (3). Some (4–6), but not all (7–9), studies of testosterone replacement in young, hypogonadal men and older men with low or low-normal testosterone levels have demonstrated a reduction in AT mass; however, these studies have reported a consistent increase in lean body mass (LBM) (6, 8–15). Marin *et al.* (16) have reported that testosterone supplementation of middle-aged men with abdominal obesity reduces visceral adiposity, although these observations have not been confirmed by others (13). In a testosterone dose-response study, we recently demonstrated that in healthy young men, the effects of testosterone administration on total body AT and LBM were linearly associated with testosterone dose and circulating total and free testosterone concentrations during testosterone supplementation (17). However, we do not know whether these testosterone-induced changes in body composition are uniform throughout the entire human body or are region specific.

Increased absolute AT mass is associated with increased risk of atherosclerotic heart disease, hypertension, and dyslipidemia (18, 19). There is a growing body of evidence that the relative distribution of AT may be as important as the absolute quantity. Accumulation of abdominal AT mass (20–24) and AT in deep, extra-sc compartments (*i.e.* intermuscular, intramyocellular, and intraabdominal AT) correlates with increased risk of adverse health outcomes, including insulin resistance, heart disease, stroke, dyslipidemia, and type 2 diabetes mellitus (19, 24–30). The higher risk of cardiovascular disease associated with increased intraabdominal AT exists in nonobese as well as obese individuals (22, 24). Therefore, interventions that decrease accumulation of AT in the intraabdominal and intermuscular depots would be expected to decrease cardiovascular risk.

In the current study, we wished to determine whether testosterone-induced reduction in total body AT mass is associated with proportional losses across all depots or whether the changes are region specific and limited to the intraabdominal or intermuscular compartments. We used a Leydig-clamp model to create and maintain serum testosterone concentrations that ranged from subphysiological to supraphysiological levels over a period of 20 wk. As previously described (17), healthy young men were treated with

Abbreviations: AT, Adipose tissue; DEXA, dual-energy x-ray absorptiometry; LBM, lean body mass; LPL, lipoprotein lipase; MRI, magnetic resonance imaging; TE, testosterone enanthate.

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a long-acting GnRH agonist to suppress their endogenous testosterone production and randomized to receive one of five different doses of testosterone enanthate (TE) ranging from 25–600 mg/wk. We assessed the effects of graded doses of TE on total body AT mass and on the distribution of regional AT masses (appendicular *vs.* trunk and sc *vs.* deeper intraabdominal and intermuscular stores) using total-body dual-energy x-ray absorptiometry (DEXA) scanning and magnetic resonance imaging (MRI) of the thigh and abdomen.

Materials and Methods

The details of the study design and subject characteristics have been previously published (17, 31).

Subjects and design

Sixty-one healthy, eugonadal men, 18–35 yr of age, gave written informed consent to participate in this double-blind, randomized, dose-response study. The protocol received approval from the institutional review boards of Charles R. Drew University and Harbor-UCLA Research and Education Institute. Participants were randomized to one of five experimental groups to receive monthly injections of a long-acting GnRH agonist (to suppress endogenous testosterone production) in combination with weekly injections of 25, 50, 125, 300, or 600 mg of TE for 20 wk. The doses of GnRH agonist and TE were started simultaneously on d 1. This strategy allowed us to investigate changes in body AT distribution that occurred when serum testosterone concentrations ranged from subphysiological levels at the lowest dose (25 mg/wk) to supraphysiological levels at the higher doses (300 and 600 mg/wk). This selection of the TE dose range was justified based on previous studies that a dose of 25 mg/wk was enough to maintain sexual function in GnRH antagonist-treated men (32), whereas 600 mg/wk was the highest dose that had been shown to be safe and effective in increasing muscle size and strength in healthy eugonadal men in short-term clinical trials (33). The staff of the General Clinical Research Center administered all TE and GnRH agonist injections. Subjects were excluded if they had taken any anabolic steroids within the previous 12 months. Of the 61 men randomized, 54 completed 20 wk of treatment; 53 had pre- and posttreatment total-body DEXA scans and 50 had pre- and posttreatment MRI scans of the abdomen and thigh.

Nutritional intake and exercise stimulus

Nutritional intake and exercise stimulus were controlled at the outset of the study, as previously described (17). Two weeks before the initiation of the study, subjects were prescribed a diet standardized for energy intake at 150 kJ/kg·d and protein intake at 1.3 g/kg·d. These instructions were reinforced monthly, and compliance with the dietary prescription was verified by analysis of 3-d food records.

Hormone assays

Serum total testosterone was measured by a previously reported radio immunoassay (7, 33–35). Free testosterone was separated by an equilibrium dialysis procedure and measured by RIA (36). The sensitivity of total testosterone assay was 0.6 ng/dl; the intraassay coefficient of variation was 8.2%, and interassay coefficients of variation were 13.2%. For the free testosterone assay, the sensitivity was 0.22 pg/ml, and intra- and interassay coefficients of variation were 4.2 and 12.3%, respectively. Hormone assays were run, in duplicate, at baseline and at the end of 16 wk of GnRH plus TE treatment (*i.e.* 1 wk after the previous injections) to obtain nadir concentrations of total and free testosterone.

Total body, appendicular, and trunk AT and lean body mass by DEXA

DEXA (Hologic QDR 4500A, Waltham, MA) was used to measure total body, appendicular, and trunk AT and LBM before and after GnRH agonist plus TE treatment. Fifty-four participants had DEXA scans before and 53 after the 20 wk of treatment. The DEXA scanner was calibrated weekly using the manufacturer's body composition analysis step phantom and daily using the spine phantom. Appendicular AT and LBM were determined by summing the respective bilateral arm and leg masses (37).

Subcutaneous, intermuscular, and intraabdominal AT volumes by MRI

Multislice MRI of the thigh and abdomen were obtained using a 1.5-tesla whole-body Signa Horizon LX scanner (General Electric Medical Systems, Milwaukee, WI). Fifty participants completed the MRI scans both before and after treatment. Three participants (two in the 125-mg/wk dose group and one in the 600-mg/wk dose group) did not have a posttreatment MRI scan, and one individual in the 600-mg/wk dose group had metal behind his eye and could not undergo MRI scanning. Subjects lay in a supine position and entered the scanner feet first with heels in a neutral position. After a series of scout images were obtained to identify the landmarks, 17 transverse slices (10 mm thick, 15 mm apart) were obtained for the right thigh, starting with the initial slice at the distal border of the lateral femoral condyle. An axial T1-weighted spin-echo sequence with a repetition time of 400 msec, echo time of 14 msec, acquisition matrix of 256 × 256, and a 24-mm field of view was used to acquire all thigh data. The sc and intermuscular thigh AT volumes (AT between the bundles of skeletal muscle fibers that was visible on the MRI images) were determined for five sequential slices of the right thigh, including the slice with the largest cross-sectional area on axial imaging plus two slices immediately above and below. Sixteen abdominal transverse images (10 mm thick, 3 mm apart) were obtained, with the second slice located at the inferior border of L5. An axial T1-weighted spin-echo sequence with a repetition time of 350 msec, minimum echo time, acquisition matrix of 256 × 128, and a 40-mm field of view was used to acquire all abdominal images. Abdominal sc and intraabdominal AT volumes were determined for seven sequential slices extending from one below to four slices above L4-L5 (38). Intraabdominal AT was quantified as the AT that lay in the intraabdominal cavity, delineated at the innermost aspect of the abdominal and oblique muscle walls surrounding the cavity and the anterior aspect of the vertebral body. For each subject, all analyses were done in a blinded fashion for the same slices before and after treatment using customized software (SliceOmatic version 4.2 by TomoVision, Montreal, Canada) as described by others (38, 39).

Statistical analyses

Data were analyzed using SigmaStat software version 2.03 (SPSS Science, Chicago, IL). Univariate statistics were used to examine variables for their distribution characteristics. One-way ANOVA was used to compare the change from baseline for each of the DEXA and MRI measures of AT and LBM among the five treatment doses (25, 50, 125, 300, and 600 mg/wk). Student-Newman-Keuls method was used for multiple comparisons of the individual groups. All variables were also analyzed using a paired *t* test to detect a nonzero change from baseline and to compare the regions of AT loss (*e.g.* appendices *vs.* trunk) within each dose group. Correlations were examined using the Pearson product-moment correlation coefficient. The level of significance was set at $P < 0.05$.

Results

Participant characteristics

Fifty-four of the 61 men randomized completed the 20 wk of treatment; 11 men were randomized to group 1 (25 mg/wk), eight to group 2 (50 mg/wk), 12 to group 3 (125 mg/wk), 10 to group 4 (300 mg/wk), and 13 to group 5 (600 mg/wk). There were no significant differences in baseline characteristics between the five groups (Table 1).

Hormone levels

We have previously published a detailed description of the hormonal changes showing that the combined administra-

TABLE 1. Baseline physical characteristics of the participants

	Monthly GnRH agonist plus TE dose (mg/wk)						
	All groups	25 mg	50 mg	125 mg	300 mg	600 mg	*P* value
Age (yr)	27 ± 4	28 ± 4	29 ± 4	28 ± 4	24 ± 5	25 ± 4	0.059
Height (cm)	176 ± 7	174 ± 4	176 ± 6	178 ± 7	176 ± 6	174 ± 9	0.603
Weight (kg)	75.4 ± 10.6	68.0 ± 8.6	77.4 ± 8.2	78.4 ± 9.8	79.0 ± 10.5	74.9 ± 12.4	0.098
BMI (kg/m^2)	24 ± 3	22 ± 3	25 ± 3	25 ± 3	26 ± 3	25 ± 3	0.152
Body fat percentage (%)	12 ± 5	10 ± 5	13 ± 3	14 ± 6	12 ± 5	12 ± 7	0.665
n	54	11	8	12	10	13	

Data represent baseline mean ± SD values for all the participants (n = 54) who completed the study and for each of the randomization groups at baseline. *P* values are results for ANOVA.

tion of GnRH agonist plus graded doses of TE resulted in dose-dependent changes in serum total and free testosterone concentrations (17). This treatment regimen was effective in creating and maintaining graded levels of serum total and free testosterone concentrations ranging from subphysiological levels in the 25-mg/wk group to supraphysiological levels in the 600-mg/wk dose group. There was a significant dose effect ($P < 0.001$) for total serum testosterone levels (mean ± SEM) post treatment (*i.e.* nadir levels at wk 16) with values of 285 ± 121, 318 ± 101, 542 ± 72, 1284 ± 150, and 2535 ± 224 ng/dl (9.9 ± 4.2, 11.0 ± 3.5, 18.8 ± 2.5, 44.5 ± 5.2, and 87.9 ± 7.8 nmol/liter) in the 25-, 50-, 125-, 300-, and 600-mg/wk groups, respectively (17). There was a similar dose effect ($P < 0.001$) for free testosterone levels (mean ± SEM) post treatment with values of 31 ± 8, 30 ± 4, 51 ± 8, 130 ± 18, and 276 ± 27 pg/ml (107.5 ± 27.7, 104.0 ± 13.9, 176.8 ± 27.7, 450.7 ± 62.4, and 956.9 ± 93.6 pmol/liter) in the 25-, 50-, 125-, 300-, and 600-mg/wk groups, respectively (17). Administration of GnRH agonist plus 25 or 50 mg/wk of TE resulted in a significant reduction in both serum total and free testosterone levels to below baseline levels, whereas the 125-mg/wk dose maintained baseline levels, and the two highest doses (300 and 600 mg/wk) resulted in a significant increase above normal baseline levels (17). These values reflect nadir levels measured 1 wk after the previous injection at wk 16 of the 20 wk of treatment. Thus, testosterone levels would have been higher than this throughout the treatment period.

Changes in body weight

Body weight was not significantly different among the five treatment groups at baseline. There was a dose-dependent increase in body weight among the five treatment groups (ANOVA $P = 0.009$) with significant gains compared with baseline within each dose group (Table 2). The change in body weight was positively correlated with testosterone dose ($r = 0.46$; $P = 0.0005$), nadir serum total testosterone concentrations during treatment ($r = 0.42$; $P = 0.0018$), and change in nadir serum total testosterone concentration ($r = 0.44$; $P < 0.0010$).

Changes in total-body AT mass

As previously reported (17), total-body AT mass, measured by DEXA, was not significantly different among the five treatment groups at baseline. The GnRH agonist plus TE administration was associated with dose-dependent changes in total-body AT mass among the groups (ANOVA $P <$ 0.001). Those treated with the two lowest doses experienced a significant increase (change +1.8, $P = 0.029$, and +2.6 kg, $P = 0.032$, at the 25- and 50-mg/wk doses, respectively), whereas those who received the highest dose (600 mg/wk) lost a significant amount (change –1.8 kg, $P = 0.028$) of AT mass. The change in total-body AT mass was inversely correlated with testosterone dose ($r = -0.49$; $P = 0.0002$), nadir serum total testosterone concentrations during treatment ($r = -0.55$; $P < 0.0001$), and change in nadir serum total testosterone concentration ($r = -0.54$; $P < 0.0001$). In relative terms, the percent change in total-body AT mass was also different among the dose groups ($P = 0.004$) with significant increases in percent AT in the two lowest dose groups (change +44.8%, $P = 0.04$, and +19.7%, $P = 0.03$, in the 25- and 50-mg doses, respectively) (Fig. 1).

TABLE 2. Body weight

Testosterone dose (mg)	Body weight (kg)			*P* vs. zero change
	Baseline	Treatment	Change from baseline	
25	68.0 ± 2.5	70.3 ± 2.7[a]	+2.3 ± 0.7[b]	0.006
50	77.0 ± 2.9	80.6 ± 3.3	+3.6 ± 1.1	0.014
125	78.9 ± 3.1	81.6 ± 3.2	+2.4 ± 0.9[b]	0.027
300	78.4 ± 3.2	83.1 ± 3.2	+4.7 ± 0.7	<0.001
600	74.8 ± 3.5	81.2 ± 3.3	+6.3 ± 1.1	<0.001
P value	0.105	0.042	0.009	

Data represent mean ± SEM values at baseline (n = 54) and after 20 wk (n = 53) of GnRH plus TE treatment. Changed scores represent values after 20 wk of treatment minus values at baseline (change from baseline). *P* values are results for ANOVA at each time point: [a] $P < 0.05$ *vs.* 300-mg/wk dose group; [b] $P < 0.05$ *vs.* 600-mg/wk dose group for the multiple comparison tests using Student-Newman-Keuls.

Changes in regional (appendices and trunk) AT mass

Appendicular (arms plus legs) and trunk AT mass were not significantly different among the five dose groups at baseline (Table 3). Administration of GnRH agonist plus TE resulted in a significant increase in appendicular AT mass in the lowest dose group (+0.8 kg, $P = 0.037$) and a significant decrease (–1.2kg, $P = 0.014$) in the highest dose group, whereas trunk AT mass increased in the two lowest dose groups (+0.9, $P = 0.027$, and +1.4 kg, $P = 0.011$). Change in both appendicular and trunk AT mass were inversely correlated with testosterone dose ($r = -0.51$; $P = 0.0001$, and $r = -0.47$; $P = 0.0003$, respectively), and with nadir serum total testosterone concentrations during treatment ($r = -0.54$; $P < 0.0001$ and $r = -0.55$; $P < 0.0001$, respectively). In relative terms (Fig. 1), there was a significant ($P = 0.036$) 18.3% gain

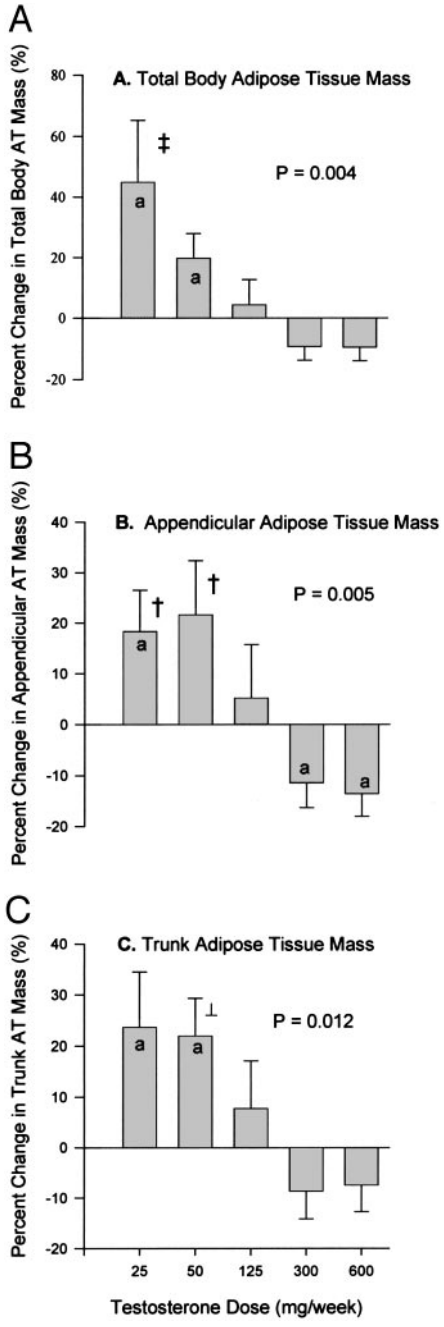

FIG. 1. Effects of 20 wk of GnRH agonist plus TE administration on relative changes (mean ± SEM) in total-body AT mass (A), appendicular AT mass (B), and trunk AT mass (C) (percent change from baseline) measured by DEXA. *P* values are results for ANOVA: ⊥, $P < 0.05$ *vs.* 600-mg/wk dose group; †, $P < 0.05$ *vs.* 300- and 600-mg/wk dose groups; ‡, $P < 0.05$ *vs.* 125-, 300-, and 600-mg/wk dose groups for the multiple comparison tests using Student-Newman-Keuls; [a], $P < 0.05$ *vs.* zero change.

in appendicular AT mass in the lowest dose group and significant losses at the two highest doses (–11.4%, $P = 0.030$, and –13.5%, $P = 0.006$, in the 300- and 600-mg/wk dose groups, respectively). The increase in percent trunk AT mass was statistically significant in the two lowest dose groups (+23.6%, $P = 0.040$, and 21.9%, $P = 0.010$, in the 25- and 50-mg/wk doses, respectively). The gain or loss of total AT mass, measured by DEXA, was evenly divided between the trunk and the extremities. For instance, of the total mean 1.8-kg gain in AT mass in the men treated with 25 mg/wk of TE, there was no significant difference ($P = 0.314$) between the amount of AT gained in the extremities (on average, +0.8 kg) and that gained in the trunk (on average, +0.9 kg). Similarly, the loss of total AT mass in the 600-mg/wk dose group (on average, –1.8 kg) reflected proportional losses ($P = 0.108$) in the extremities (on average −1.2 kg) and the trunk (on average, −0.8 kg).

TABLE 3. Appendicular and trunk AT mass by DEXA

Testosterone dose	AT mass (kg)			*P vs.* zero change
	Baseline	Treatment	Change from baseline	
Appendicular				
25	5.2 ± 0.6	6.0 ± 0.7	+0.8 ± 0.3[a]	0.037
50	7.0 ± 0.6	8.1 ± 0.6	+1.2 ± 0.6[a]	0.078
125	7.0 ± 0.8	6.9 ± 0.8	−0.2 ± 0.4	0.593
300	7.7 ± 0.6	7.0 ± 0.8	−0.7 ± 0.3	0.056
600	7.1 ± 1.1	5.9 ± 0.8	−1.2 ± 0.4	0.014
P value	0.254	0.283	<0.001	
Trunk				
25	5.6 ± 0.7	6.6 ± 0.8	+0.9 ± 0.4[b]	0.027
50	7.1 ± 0.7	8.6 ± 0.8	+1.4 ± 0.4[b]	0.011
125	7.0 ± 1.2	7.1 ± 1.1	−0.1 ± 0.4	0.787
300	6.8 ± 0.7	6.4 ± 0.8	−0.4 ± 0.3	0.213
600	6.0 ± 1.0	5.2 ± 0.8	−0.8 ± 0.4	0.056
P value	0.741	0.157	<0.001	

Data represent mean ± SEM values at baseline (n = 54) and after 20 wk (n = 53) of GnRH plus TE treatment. Changed scores represent values after 20 wk of treatment minus values at baseline (change from baseline). Appendicular AT mass is the sum of AT mass for bilateral arms plus legs. *P* values are results for ANOVA at each time point: [a] $P < 0.05$ *vs.* 300- and 600-mg/wk dose groups; [b] $P < 0.05$ *vs.* 125-, 300-, and 600-mg/wk dose groups for the multiple comparison tests using Student-Newman-Keuls.

Changes in thigh sc and intermuscular AT volumes

During GnRH agonist plus TE administration, the gain or loss of appendicular AT occurred in both the sc and intermuscular compartments. Absolute sc AT volume increased significantly in both the 25- and 50-mg/wk dose groups (+106 cc^3, $P = 0.005$, and +81 cc^3, $P = 0.019$, respectively), whereas intermuscular thigh AT increased in the 25-mg/wk dose group (+22 cc^3, $P < 0.001$) and decreased significantly in the three highest dose groups (–21 cc^3, $P = 0.017$; −30 cc^3, $P = 0.005$; and −34 cc^3, $P = 0.006$ at 125, 300, and 600 mg/wk, respectively) (Table 4). Within-dose group comparison of change in thigh sc *vs.* intermuscular AT volume revealed a greater increase in sc AT volume at the two lowest doses (+106 *vs.* +22 cc^3, $P = 0.022$, and +81 *vs.* +15 cc^3, $P = 0.009$, respectively). Although the absolute changes were greater in sc than intermuscular thigh AT stores, the reverse is true when relative changes are considered (Fig. 2). Percent sc thigh AT volume was increased at the two lowest doses (+28%, $P < 0.001$, and +17%, $P = 0.005$, respectively) and significantly reduced at the two highest doses (–9%, $P = 0.002$, and −12%, $P = 0.012$, respectively). Intermuscular thigh AT volume increased 32% ($P < 0.001$) at the lowest dose with reductions of 14, 23, and 28% (all $P < 0.001$) at the three highest doses (125, 300, and 600 mg/wk, respectively). These

TABLE 4. Thigh AT volumes (cc^3) by MRI

Testosterone dose (mg)	Thigh AT volume (cm^3) by MRI scan			*P vs.* zero change
	Baseline	Treatment	Change from baseline	
Subcutaneous				
25	408 ± 52	513 ± 65	+106 ± 29[a,b]	0.005
50	507 ± 66	588 ± 67	+81 ± 27[b]	0.019
125	517 ± 49	542 ± 60	−2 ± 26	0.943
300	585 ± 53	540 ± 57	−45 ± 13	0.590
600	589 ± 118	520 ± 84	−101 ± 46	0.055
P value	0.429	0.956	<0.001	
Intermuscular				
25	112 ± 20	134 ± 20	+22 ± 5[a,b]	<0.001
50	161 ± 17	176 ± 20[b]	+15 ± 15[b]	0.348
125	149 ± 23	131 ± 23	−21 ± 7	0.017
300	119 ± 22	89 ± 17	−30 ± 8	0.005
600	104 ± 18	76 ± 11	−34 ± 10	0.006
P value	0.255	0.005	<0.001	

Data represent mean ± SEM values at baseline (n = 53) and after 20 wk (n = 50) of GnRH plus TE treatment. Changed scores represent values after 20 wk of treatment minus values at baseline (change from baseline). *P* values are results for ANOVA at each time point: [a] $P < 0.05$ *vs.* 125-mg/wk dose group; [b] $P < 0.05$ *vs.* 300- and 600-mg/wk dose groups for the multiple comparison tests using Student-Newman-Keuls.

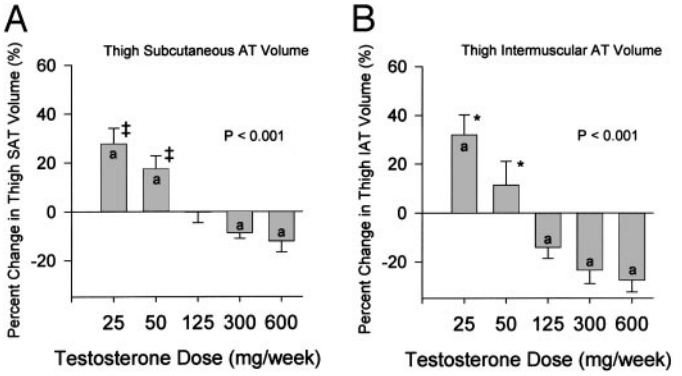

FIG. 2. Effects of 20 wk of GnRH agonist plus TE administration on relative changes (mean ± SEM) in thigh sc AT (SAT) volume (A) and thigh intermuscular AT (IAT) volume (B) (percent change from baseline) measured by MRI. *P* values are results for ANOVA: ‡, $P < 0.05$ *vs.* 125-, 300-, and 600-mg/wk dose groups; *, $P < 0.05$ *vs.* all other dose groups for the multiple comparison tests using Student-Newman-Keuls; [a], $P < 0.05$ *vs.* zero change.

increases in percent thigh intermuscular AT volumes at the three highest doses were significantly greater ($P = 0.014$, $P < 0.001$, and $P < 0.001$, respectively) than those in the more superficial sc stores.

Changes in abdominal sc and intraabdominal AT volumes

Lowering of testosterone levels resulted in a gain in both the sc and intraabdominal AT depots of the abdomen. In men receiving the 25-mg/wk dose, there was an increase in both the absolute sc (+261 cc^3, $P < 0.001$) and intraabdominal (+161 cc^3, $P = 0.006$) AT volumes. The percent gains in sc (+32%, $P < 0.001$) and intraabdominal (+33%, $P = 0.002$) AT volumes were approximately equal in men receiving the 25-mg/wk dose. Those receiving 50 mg/wk of TE experienced an increase only in the sc AT volume (+246 cc^3, $P = 0.016$). This gain in abdominal sc AT in men receiving the 50-mg/wk dose was significantly greater, in both absolute (+246 *vs.* +50 cc^3, $P = 0.011$) and relative (+21% *vs.* +3% increase, $P = 0.003$) terms, than that in the intraabdominal stores (Table 5; Fig. 3). Unlike in the appendices, there was no significant reduction of AT volume in either the sc or intraabdominal stores at the higher doses of TE in either absolute or relative terms. Percent changes in sc and intraabdominal AT volumes were negatively correlated with both testosterone dose ($r = -0.51$; $P = 0.0001$, and $r = -0.33$; $P = 0.02$, respectively) and nadir serum testosterone concentration ($r = -0.56$; $P < 0.0001$, and $r = -0.34$; $P = 0.02$, respectively).

Changes in total LBM

LBM measured by DEXA was not significantly different among the five randomized dose groups at baseline. As reported previously (17), the administration of GnRH and TE was associated with dose- and concentration-dependent changes in LBM; the men treated with the highest three doses (125, 300, and 600 mg/wk) experienced significant increases (+2.9, +5.5, and +9.0 kg, respectively) in absolute total LBM. These changes reflect relative increases of 5% ($P = 0.002$), 10% ($P < 0.001$), and 16% ($P < 0.001$) LBM in the 125-, 300-, and 600-mg/wk dose groups, respectively (Fig. 4).

Changes in regional (appendices and trunk) LBM

Appendicular and trunk LBM were not significantly different among the five dose groups at baseline. The pattern of appendicular and trunk LBM responses were similar, with significant increases occurring in the three highest TE dose groups (125, 300, and 600 mg/wk), in both absolute (Table 6) and relative (Fig. 4) terms. There was a proportional increase in LBM in the appendices and the trunk in both the 125- and 300-mg/wk dose groups (125 mg/wk: on average, +1.4 and +1.3 kg, $P = 0.848$; 300 mg/wk: on average, +2.9

TABLE 5. Abdominal AT volume (cc^3) by MRI

Testosterone dose (mg)	Abdominal AT volume (cc^3) by MRI scan			*P vs.* zero change
	Baseline	Treatment	Change from baseline	
Subcutaneous				
25	937 ± 130	1198 ± 143	+261 ± 53[a]	<0.001
50	1219 ± 128	1465 ± 168	+246 ± 78[a]	0.016
125	1251 ± 181	1353 ± 177	−23 ± 57	0.699
300	1349 ± 203	1304 ± 197	−45 ± 46	0.352
600	1169 ± 196	1148 ± 172	−86 ± 53	0.135
P value	0.554	0.734	<0.001	
Intraabdominal				
25	591 ± 73	752 ± 79	+161 ± 47[b,c]	0.006
50	825 ± 49	875 ± 56[d]	+50 ± 53	0.380
125	721 ± 133	766 ± 141	−18 ± 41	0.679
300	478 ± 58	494 ± 55	+16 ± 17	0.346
600	537 ± 74	518 ± 67	−46 ± 39	0.269
P value	0.081	0.014	0.005	

Data represent mean ± SEM values at baseline (n = 53) and after 20 wk (n = 50) of GnRH plus TE treatment. Changed scores represent values after 20 wk of treatment minus values at baseline (change from baseline). *P* values are results for ANOVA at each time point: [a] $P < 0.05$ *vs.* 125-, 300-, and 600-mg/wk dose groups; [b] $P < 0.05$ *vs.* 125-mg/wk dose group; [c] $P < 0.05$ *vs.* 600-mg/wk dose group; [d] $P < 0.05$ *vs.* 300- and 600-mg/wk dose groups for the multiple comparison tests using Student-Newman-Keuls.

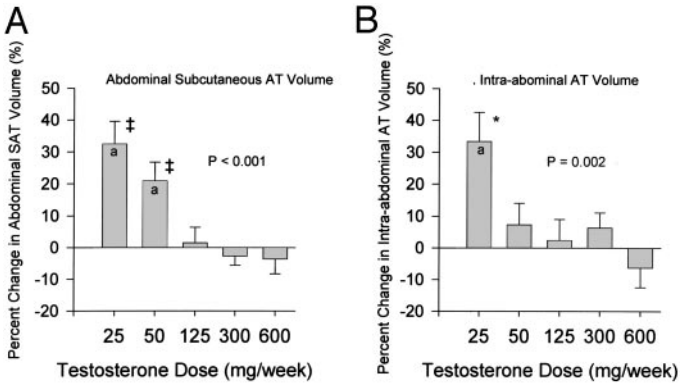

FIG. 3. Effects of 20 wk of GnRH agonist plus TE administration on relative changes (mean ± SEM) in abdominal sc AT (SAT) volume (A) and intraabdominal AT (IAT) volume (B) (percent change from baseline) measured by MRI. *P* values are results for ANOVA: ‡, $P < 0.05$ *vs.* 125-, 300-, and 600-mg/wk dose groups; *, $P < 0.05$ *vs.* all other dose groups for the multiple comparison tests using Student-Newman-Keuls; [a], $P < 0.05$ *vs.* zero change.

and +2.5 kg, $P = 0.395$); however, the average 9.0-kg increase in LBM in those randomized to receive 600 mg/wk was due to greater gains in the appendices than the trunk (on average, +4.7 kg *vs.* +3.9 kg, $P = 0.017$). In relative terms, this difference was not significant (on average, +18 *vs.* +14%, $P = 0.081$). Changes in both appendicular and trunk LBM were correlated with both testosterone dose ($r = 0.79$; $P < 0.0001$, and $r = 0.75$; $P < 0.0001$, respectively) and nadir serum total testosterone concentrations during treatment ($r = 0.63$; $P < 0.0001$, and $r = 0.55$; $P < 0.0001$, respectively).

Discussion

Our data demonstrate that increasing testosterone concentrations above baseline, by administration of supraphysiological doses of testosterone enanthate in healthy young eugonadal men, leads to a loss of total-body AT. Conversely, lowering of serum testosterone concentrations below baseline is associated with gains in total-body AT mass. The gains in AT mass that occur when testosterone levels are lowered and losses in AT mass when testosterone levels are increased are evenly distributed between the trunk and the appendices in both absolute and relative (percent change) terms. Comparison of the absolute changes in sc *vs.* deep (intermuscular and intraabdominal) AT compartments revealed significantly greater gains in the sc AT stores of the thigh at the lowest two doses and of the abdomen at the 50-mg/wk TE dose. In relative terms, the percent gain in abdominal sc AT volume was significantly greater than for the deeper intraabdominal AT compartment in men receiving the 50-mg/wk TE dose. Conversely, testosterone supplementation was associated with a greater percent loss in the deeper intermuscular than in the sc AT depots of the thigh at the three highest TE doses, with no significant reduction in the abdominal AT stores.

Our study had several unique features. It is the first controlled study in which the effects of testosterone on total-body and regional AT mass have been examined over a wide range of doses and concentrations. In addition, we used a variety of techniques for body composition assessment, including multislide MRI in conjunction with customized software (SliceOmatic by TomoVision) for accurate assessment of changes in sc and deep AT volumes of the thigh and abdomen. Some of the previous studies of testosterone supplementation used DEXA scan alone, which is not as accurate as MRI and does not allow for delineation of sc and intraabdominal AT stores. Finally, we standardized the energy and protein intake and controlled the exercise stimulus.

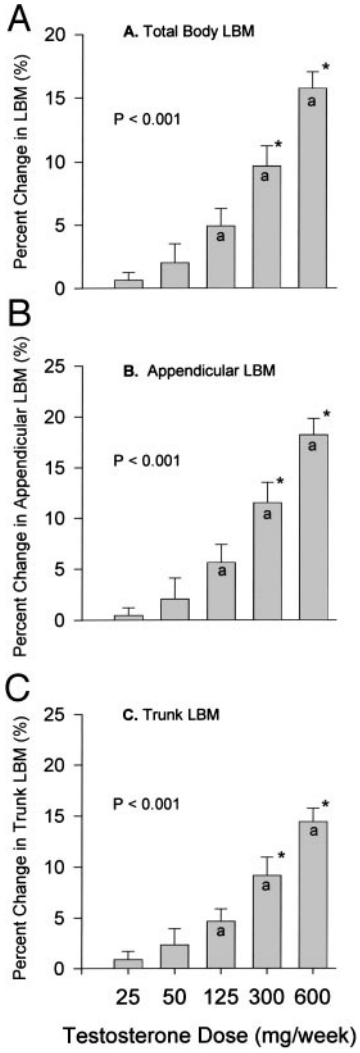

FIG. 4. Effects of 20 wk of GnRH agonist plus TE administration on relative changes (mean ± SEM) in total LBM (A), appendicular LBM (B), and trunk LBM (C) (percent change from baseline) measured by DEXA. *P* values are results for ANOVA: *, $P < 0.05$ *vs.* all other dose groups for the multiple comparison tests using Student-Newman-Keuls; [a], $P < 0.05$ *vs.* zero change.

Not all the studies of testosterone replacement in hypogonadal men have reported a significant decrease in total body AT mass (4–9). The studies that did demonstrate a significant decrease in total body AT mass generally included somewhat older men, with higher AT mass at baseline, in comparison with the studies that did not show a significant change in AT mass. The differences in the methods for body composition assessment may have also contributed to the observed differences in results. Very few studies have examined the effects of testosterone supplementation on regional AT distri-

TABLE 6. Appendicular and trunk LBM by DEXA

Testosterone dose (mg)	LBM (kg) by DEXA scan			*P vs.* zero change
	Baseline	Treatment	Change from baseline	
Appendicular				
25	23.4 ± 0.9	23.5 ± 0.9[a]	+0.1 ± 0.2	0.508
50	26.2 ± 1.0	26.8 ± 1.2	+0.5 ± 0.5	0.359
125	27.3 ± 1.1	28.8 ± 1.2	+1.4 ± 0.5	0.010
300	27.1 ± 1.3	30.0 ± 1.1	+2.9 ± 0.4[b]	<0.001
600	26.0 ± 1.0	30.7 ± 1.2	+4.7 ± 0.5[b]	<0.001
P value	0.095	<0.001	<0.001	
Trunk				
25	25.7 ± 0.8	26.0 ± 1.0[a]	+0.3 ± 0.2	0.212
50	27.7 ± 1.2	28.3 ± 1.2	+0.6 ± 0.4	0.218
125	28.7 ± 0.9	30.0 ± 1.1	+1.3 ± 0.4	0.004
300	28.4 ± 1.2	30.8 ± 1.1	+2.5 ± 0.4[b]	<0.001
600	26.8 ± 0.9	30.7 ± 1.2	+3.9 ± 0.4[b]	<0.001
P value	0.202	0.016	<0.001	

Data represent mean ± SEM values at baseline (n = 54) and after 20 wk (n = 53) of GnRH plus TE treatment. Changed scores represent values after 20 wk of treatment minus values at baseline (change from baseline). Appendicular LBM is the sum of LBM for bilateral arms plus legs. *P* values are results for ANOVA at each time point: [a] $P < 0.05$ *vs.* 125-, 300-, and 600-mg/wk dose groups; [b] $P < 0.05$ *vs.* all other dose groups for the multiple comparison tests using Student-Newman-Keuls.

bution, and the few data that exist have been somewhat conflicting. For instance, Marin *et al.* (40–42) have reported in several studies that testosterone supplementation of middle-aged men is associated with a decrease in visceral AT cross-sectional area. Snyder *et al.* (13) found a significant reduction in total-body AT mass after 36 months of testosterone replacement in older men with low testosterone levels, due to a significant reduction in AT mass only in the extremities and not the trunk. Our data demonstrate that the loss of AT is evenly distributed in the trunk and extremities. Although Snyder *et al.* (13) reported an increase in LBM only in the trunk and not in the extremities, we found similar increases in both regions, with greater gains in the appendices at supraphysiological doses of TE. Whether these differences in findings reflect the difference in subject age, level of adiposity at baseline, mode (patch *vs.* injection), or dose of testosterone administered remains to be investigated. Furthermore, we show that when testosterone levels are reduced below baseline, there are greater gains in the sc AT compartments of both the thigh and abdomen; conversely, increasing testosterone levels results in a greater reduction in the deep AT stores of the thigh but not the abdomen. Our data are consistent with data in HIV-infected men with the AIDS wasting syndrome that also demonstrate loss of intermuscular AT during testosterone administration, using single-slice computerized tomography scanning (43).

There is agreement that total-body AT is an important determinant of insulin sensitivity. However, there is still some uncertainty about the contribution of regional AT depots to the pathophysiology of insulin resistance. Epidemiological studies have demonstrated an association between abdominal obesity, assessed by the measurement of waist-to-hip ratio and waist girth, with increased risk of heart disease, hypertension, type 2 diabetes mellitus, and dyslipidemias (22, 44). Some studies suggest that, quantitatively, total body AT mass may be more important in regulating insulin sensitivity than the visceral or intraabdominal AT (18); however, others demonstrate a stronger association of insulin sensitivity with sc (45–47) or deeper thigh intermuscular and abdominal visceral or intraabdominal AT stores (48, 49). There is growing appreciation that the accumulation of adipose in the muscle and in extra-sc compartments has a deleterious effect on insulin sensitivity (50–52). The triglyceride content within the skeletal muscle has been linked to reduced insulin-stimulated glucose uptake and to decreased glycogen synthase activity (53–55). Therefore, interventions such as testosterone administration that decrease intraabdominal and intermuscular AT stores might be expected to have a beneficial effect on insulin sensitivity. Previous studies of testosterone supplementation in middle-aged men with visceral obesity have reported an improvement in insulin sensitivity after testosterone administration (42, 56). However, as we have reported previously, in our study, despite a significant decrease in total-body, intraabdominal, and intermuscular adipose stores, there was no significant change in insulin sensitivity or glucose disposal indices, measured by using the frequently sampled iv glucose tolerance test (57). In contrast to those previous studies of middle-aged men, the participants in our study were lean, young men. It is possible that testosterone supplementation of middle-aged and older men, with significantly higher baseline AT mass and abdominal obesity, might improve insulin sensitivity by decreasing AT mass; this hypothesis should be tested in prospective, adequately powered randomized clinical trials of older men with abdominal obesity.

The study duration was 20 wk. We do not know whether the relative changes in AT mass in various body compartments would be different after long-term testosterone administration. We recognize that the measurement of AT mass by some methods such as underwater weighing, deuterium water dilution, and DEXA is inherently less precise than the measurement of LBM, because the AT mass is calculated by subtraction of the fat-free mass from total-body mass. Also, we did not measure intramyocellular lipid stores, which have been shown to be important determinants of insulin sensitivity. Measurement of intramyocellular lipids requires nuclear magnetic resonance spectroscopy, which was not performed in this study.

The mechanisms by which testosterone administration decreases AT mass are not well understood. Testosterone acts to inhibit lipoprotein lipase (LPL) activity, decrease triglyceride accumulation, and stimulate lipolysis (58, 59). These effects are mediated via the androgen receptor, whose density is up-regulated by testosterone (60). Because androgen receptor density is higher in visceral or intraabdominal than in sc AT in the rat (61) and possibly humans (62), this has led to speculation that the androgen effects should be more pronounced in visceral or intraabdominal than in sc AT stores. Marin *et al.* (16, 56, 63) have reported that testosterone regulates LPL activity in a region-specific manner. These investigators demonstrated that testosterone administration to abdominally obese middle-aged men decreased LPL activity, inhibited triglyceride uptake, and increased the rate of triglyceride turnover in abdominal but not femoral sc AT, resulting in a net loss of abdominal AT.

We have recently demonstrated that testosterone inhibits

the differentiation of pluripotent mesenchymal precursor cells into the adipogenic lineage and promotes their commitment to the myogenic lineage (64). Thus, testosterone down-regulates adipogenic differentiation markers such as peroxisomal proliferator-activated receptor-γ and CCAAT/enhancer-binding protein-α and up-regulates myogenic markers such as desmin and myosin heavy chain type II (64). The hypothesis that testosterone regulates lineage determination of pluripotent mesenchymal stem cells provides a unifying explanation for the reciprocal effects of testosterone supplementation on AT mass and LBM (65). The molecular processes by which testosterone inhibits adipogenesis are not known but likely involve factors crucial to lineage determination in stem cells. The therapeutic applications of androgen effects on total-body and regional AT mass in clinical states characterized by AT redistribution, such as that observed in HIV-infected men with or without some types of antiretroviral therapy, and aging should be investigated.

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Address all correspondence and requests for reprints to: Linda Woodhouse, McMaster University, School of Rehabilitation Science, 1400 Main Street West, Hamilton, Ontario, Canada L8S 1C7. E-mail: woodhou@mcmaster.ca.

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