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Older Men Are as Responsive as Young Men to the Anabolic Effects of Graded Doses of Testosterone on the Skeletal Muscle

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Although testosterone levels and muscle mass decline with age, many older men have serum testosterone level in the normal range, leading to speculation about whether older men are less sensitive to testosterone. We determined the responsiveness of androgen-dependent outcomes to graded testosterone doses in older men and compared it to that in young men. The participants in this randomized, double-blind trial were 60 ambulatory, healthy, older men, 60–75 yr of age, who had normal serum testosterone levels. Their responses to graded doses of testosterone were compared with previous data in 61 men, 19–35 yr old. The participants received a long-acting GnRH agonist to suppress endogenous testosterone production and 25, 50, 125, 300, or 600 mg testosterone enanthate weekly for 20 wk. Fat-free mass, fat mass, muscle strength, sexual function, mood, visuospatial cognition, hormone levels, and safety measures were evaluated before, during, and after treatment. Of 60 older men who were randomized, 52 completed the study. After adjusting for testosterone dose, changes in serum total testosterone (change, −6.8, −1.9, +16.1, +49.5, and +101.9 nmol/liter at 25, 50, 125, 300, and 600 mg/wk, respectively) and hemoglobin (change, −3.6, +9.9, +20.9, +12.6, and +29.4 g/liter at 25, 50, 125, 300, and 600 mg/wk, respectively) levels were dose-related in older men and significantly greater in older men than young men (each $P < 0.0001$). The changes in FFM (−0.3, +1.7, +4.2, +5.6, and +7.3 kg, respectively, in five ascending dose groups) and muscle strength in older men were correlated with testosterone dose and concentrations and were not significantly different in young and older men. Changes in fat mass correlated inversely with testosterone dose ($r = −0.54$; $P < 0.001$) and were significantly different in young *vs.* older men ($P < 0.0001$); young men receiving 25- and 50-mg doses gained more fat mass than older men ($P < 0.0001$). Mood and visuospatial cognition did not change significantly in either group. Frequency of hematocrit greater than 54%, leg edema, and prostate events were numerically higher in older men than in young men. Older men are as responsive as young men to testosterone's anabolic effects; however, older men have lower testosterone clearance rates, higher increments in hemoglobin, and a higher frequency of adverse effects. Although substantial gains in muscle mass and strength can be realized in older men with supraphysiological testosterone doses, these high doses are associated with a high frequency of adverse effects. The best trade-off was achieved with a testosterone dose (125 mg) that was associated with high normal testosterone levels, low frequency of adverse events, and significant gains in fat-free mass and muscle strength. (*J Clin Endocrinol Metab* 90: 678–688, 2005)

TESTOSTERONE REPLACEMENT OF older men has been a subject of considerable debate (1, 2). Serum testosterone levels decline with advancing age and are lower in older men than in young men (3–9); however, there is uncertainty about the significance and prevalence of low testosterone levels in older men (1, 2, 10–15). Several age-related changes in men, including loss of muscle and bone mass, body hair, and sexual function and increase in fat mass, are similar to those observed in androgen deficiency (2, 14). However, many middle-aged and older men have serum testosterone levels in the normal range for young men, leading to speculation that older men might be less sensitive to androgen effects than young men (1–4, 6, 8, 9, 12). The small magnitude of changes in muscle mass observed during testosterone supplementation of older men in previous studies (16–25) has also fueled speculation that older men might be resistant to the anabolic effects of androgens on skeletal muscle.

There has never been a direct comparison of the androgen responsiveness of young and older men. Furthermore, published data do not consistently support the idea of age-related resistance to androgen effects. Although androgen receptor number in some organs is lower in older animals than in young animals, most of this decrease in androgen receptor number occurs shortly after puberty and not as a function of advancing age (26, 27). Furthermore, older men are more sensitive to the gonadotropin-suppressive effects of testosterone than young men (28). Therefore, our first ob-

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Abbreviations: FFM, Fat-free mass; HDL, high-density lipoprotein; PSA, prostate-specific antigen; 1-RM, one-repetition maximum.

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jective was to compare directly the responsiveness of young and older men to graded doses of testosterone. Because exogenous testosterone administration suppresses endogenous testosterone concentrations unevenly in different individuals, we used a GnRH agonist to suppress endogenous testosterone production to minimize the heterogeneity in circulating testosterone levels. Previously, we demonstrated that in young men, whose testosterone production had been suppressed by a GnRH agonist, testosterone supplementation engendered dose-dependent gains in fat-free mass (FFM) and muscle strength (29). The present study evaluated the responsiveness of healthy, older men, whose endogenous testosterone production had been similarly suppressed, to graded testosterone doses and compared it to that of young men. We recruited healthy young and older men to minimize the confounding influence of physiological derangements in older men with clinical disorders.

Previous studies reported modest gains in FFM and inconsistent changes in muscle strength after testosterone supplementation of older men (16–20, 23, 25, 29). Because previous studies used relatively small doses of testosterone, we determined whether higher doses would lead to greater FFM and muscle strength gains. We sought to determine the range of testosterone doses that could be safely administered to older men to achieve meaningful gains in FFM and muscle strength.

Subjects and Methods

This randomized, double-blind study consisted of a 4-wk control period, a 20-wk treatment period, and a 16-wk recovery period. The data for young men have been reported previously (29). The protocol was approved by the institutional review boards at Charles Drew University and Research and Education Institute. All participants provided written, informed consent. A Data Safety Monitoring Board (DSMB) reviewed safety data every three months.

Participants

We recruited healthy, eugonadal, 60- to 75-yr-old men. Those with prostate cancer, American Urological Association symptom score above 7, prostate-specific antigen (PSA) levels greater than 4 ng/ml, hematocrit above 48%, diabetes mellitus, congestive heart failure, severe sleep apnea, or myocardial infarction in the preceding 6 months were excluded. All participants performed a maximal cycle ergometer test with 12-lead electrocardiogram monitoring to exclude those with cardiovascular symptoms during exercise. We excluded those who in the previous year had taken androgenic steroids, including dehydroepiandrosterone and androstenedione, GH, or other anabolic agents. Men who were participating in sports events, resistance exercise training, or moderate to heavy endurance exercise training were also excluded

Randomization

Testosterone dose assignment was based on randomization tables, with a block size of four. Sixty older and 61 young men were randomized similarly (29). After DSMB discontinued the 600-mg dose in older men in December 2002, subjects were randomized to one of the lower four doses.

Intervention

Men were treated with monthly injections of a long-acting GnRH agonist (Lupron depot, 7.5 mg; TAP, North Chicago, IL) to suppress endogenous testosterone production, and weekly injections of one of five doses of testosterone enanthate (200 mg/ml; Delatestryl, Savient Pharmaceuticals, Inc., Iselin, NJ): 25 mg (13 men), 50 mg (12 men), 125 mg (12 men), 300 mg (14 men), or 600 mg (10 men). Testosterone enanthate was selected because this is the only formulation that could raise testosterone concentrations into the supraphyiological range. The 25-mg dose was selected because this was the smallest dose of testosterone that had been shown to maintain sexual function in men treated with a GnRH antagonist (30). The 600-mg dose was selected because this is the highest dose that had been administered safely to men in clinical trials (29, 31). The General Clinical Research Center staff administered all drug injections to assure compliance.

Nutritional intake

Subjects were prescribed a diet standardized for energy (150 kJ/kg·d) and protein (1.3 g/kg·d). Dietary instructions were reinforced monthly, and compliance was verified using 3-d food records every 4 wk.

Exercise stimulus

The men were asked not to undertake resistance training or moderate to heavy endurance exercise. These instructions were reinforced every 2 wk.

Outcome measures

Fat-free mass (FFM), fat mass, leg press strength, sexual function, mood, and visuospatial cognition were assessed at baseline and after 20 wk. Hormone levels were measured twice during the control period and every month thereafter during the treatment and recovery periods. Safety measures, including complete blood counts, chemistry panels, plasma lipids, and PSA levels, were assessed twice during the control period and every month thereafter.

Hormone assays

Serum total testosterone was measured by a previously validated RIA (29, 31–35). Free testosterone was separated by an equilibrium dialysis procedure and measured by RIA (32). The sensitivity of the total testosterone assay was 0.02 nmol/liter (0.6 ng/dl), and the lower limit of the normal male range was 9.5 nmol/liter (275 ng/dl); intra- and interassay coefficients of variation were 8.2%, and 13.2%, respectively. For free testosterone assay, the sensitivity was 0.76 pmol/liter (0.22 pg/ml), and intra- and interassay coefficients of variation were 4.2% and 12.3%, respectively. The cross-reactivity of dihydrotestosterone in the testosterone assay was less than 0.1%.

Serum LH and FSH levels were measured by sensitive, two-site-directed, immunofluorometric assays, (Delfia-Wallac, Gaithersburg, MD), as described previously (31). The sensitivity of these assays is 0.04 U/liter for LH and 0.06 U/liter for FSH. The cross-reactivity with TSH, human chorionic gonadotropin, and free α-subunit of pituitary glycoprotein hormones is less than 1%. Serum SHBG levels were measured by an immunofluorometric assay (31, 35).

Body composition assessment

We measured FFM and fat mass by underwater weighing, dual energy x-ray absorptiometry (DEXA; 4500A, Hologic, Inc., Waltham, MA), and ^2H_2O dilution. A Hologic QDR4500A DEXA scanner was used to measure total body and appendicular FFM and lean body mass before and after GnRH agonist plus testosterone enanthate treatment. The DEXA scanner was calibrated weekly using the manufacturer's body composition analysis step phantom (36). Appendicular fat and lean masses were determined by adding the respective bilateral arm and leg masses (37, 38). Skeletal muscle mass was estimated from appendicular muscle mass, using algorithms published by Kim *et al.* (39).

For estimation of total body water, the men ingested 20 g deuterium oxide, and plasma samples were drawn at −15, 0, 120, 180, and 240 min. We measured deuterium abundance in plasma by nuclear magnetic resonance spectroscopy, using a correction factor of 0.985 for exchangeable hydrogen (35, 40). FFM was estimated as total body water divided by 0.73. We also estimated FFM from measurements of body density obtained by underwater weighing (31). During underwater weighing, the men were asked to exhale to the residual volume, as measured by helium dilution.

Muscle strength

We measured maximal voluntary strength in the leg press exercise by the one-repetition maximum (1-RM) method (41); 1-RM was defined as the maximum amount of weight that a subject was able to lift once and only once using a seated leg press machine (Keiser Sport, Fresno, CA) with pneumatic resistance. Because maximal voluntary strength measurements are highly effort dependent, several strategies were used to assure reliability and reproducibility and to minimize the confounding influence of the learning effect. Tests were performed in duplicate or triplicate on different days, with careful attention to positioning so that starting knee flexion (90° by goniometry), the ensuing hip angles, and foot placement on the leg press footplate were standardized and held constant. The 1-RM procedure (41) included a familiarization period in which subjects were instructed in and then practiced the proper execution of the seated leg press exercise. After this familiarization, subjects completed a generalized warm-up consisting of 5 min of cycle ergometer or treadmill exercise plus stretching of the quadriceps, hamstrings, lower back, and triceps surae. Immediately after this warm-up, subjects were positioned on the leg press machine, with position measurements recorded for subsequent testing. The initial load was set at 50% of the subject's estimated 1-RM using reference values established in our laboratory. Subjects were first asked to perform eight repetitions of the leg press exercise at this load. After 1 min of rest, the subjects performed four repetitions at a load that was increased by approximately 20 kg. After a 1-min rest period, the load was increased further, and attempts were then made to identify the 1-RM. Attempts were punctuated with 2-min rest intervals and continued until the 1-RM was identified as the greatest amount of weight lifted through the complete range of motion. Strength tests were repeated within 2–7 d after the first test on separate days, with scores required to be within 5%. Failure to meet this criterion required a third test. Only 15% of our subjects required a third test, and none required a fourth. In all cases, the highest value in the duplicate or triplicate trails was taken as the 1-RM.

Behavioral measurements

Sexual function was assessed by using 7-d logs of sexual activity and desire (42), which have been validated and published previously (29, 43). Visuospatial cognition was assessed by computerized checkerboard test, and mood was assessed by Hamilton depression and Young's mania scales.

Safety monitoring

Blood chemistries, physical examination including prostate examination, and adverse events were evaluated monthly. Serum PSA and lipids were measured during wk 0, 8, 16, and 20. The Data Safety Monitoring Board reviewed the safety data every 3 months. The following rules for treatment discontinuation were established *a priori*: persistent increase in PSA above 4 μg/ml, increase in PSA more than 1.4 μg/ml above the baseline, hematocrit above 54%, aspartate aminotransferase and alanine aminotransferase more than 3 times upper limit of normal, diagnosis of prostate cancer, palpable prostate abnormality, and urinary retention.

Recovery

After treatment discontinuation, subjects were followed monthly to monitor recovery of hormone levels; subjects whose hormone levels did not return to baseline after 4 months were followed until recovery was complete.

Statistical analyses

All outcome variables were evaluated for distribution and homogeneity of variance; variables that did not meet the assumptions of homogeneity of variance or normal distribution were log-transformed. The primary analysis was a one-way ANOVA in older men. Secondarily, we also performed a two-way ANOVA to compare the change in outcome measures in older and young men; the two factors were age (young or old) and testosterone dose. If ANOVA revealed a significant effect, then the individual groups were compared using Tukey's multiple comparison procedure. Multiple regression models were used to evaluate the effects of testosterone dose, change in testosterone level, and age. Because testosterone levels during treatment were higher in older men than in younger men, we examined multiplicative interaction of change in testosterone concentration and age to evaluate the parallelism between outcomes and change in testosterone concentrations with respect to age group. Mann-Whitney or Kruskal-Wallis tests compared changes in outcome measures that did not meet assumptions of ANOVA even after transformation. If there was a significant age effect, the values for young and older men for each dose were compared using Tukey's multiple comparison procedure. Similarly, if the linear model revealed a significant dose effect, then different dose groups were compared using Tukey's procedure. $P < 0.05$ for two-tailed comparisons was considered significant.

Assuming a linear relationship between change in FFM, our primary outcome variable, and testosterone concentrations, a sample size of 60 subjects in each age group provided 80% power to detect an effect size (difference between the slopes of the dose-response curves in young and older men) of 0.52 SD and 90% power to detect an effect size of 0.6 SD using a two-sided 5% significance level in a simple two-sample *t* test. We took into account the fact that multivariate models considered in our analyses adjust for a number of covariates, and these analyses would be expected to show reduced within-group variation compared with the unadjusted model and, therefore, would demonstrate greater power for the given effects. Thus, the study had adequate power to detect a medium effect size.

Results

Subjects

We evaluated 205 older men for eligibility; 145 men were excluded, because 89 were ineligible, and 56 declined to participate. Sixty older men were randomized; of these, 52 completed all phases of the study: 13 in the 25-mg group, 12 in the 50-mg group, 11 in the 125-mg group, 10 in the 300-mg group, and six in the 600-mg group (Fig. 1). Eight men did not complete treatment, six because of serious adverse events (three receiving 300 mg and three receiving 600 mg) and one who was lost to follow-up. One subject in the 600-mg group was discontinued when DSMB stopped this study arm.

The characteristics of young men have been described previously (29). Of 61 randomized young men, 54 completed the study: 12 in the 25-mg group, eight in the 50-mg group, 11 in the 125-mg group, 10 in the 300-mg group, and 13 in the 600-mg group. One young man discontinued treatment because of acne, and six stopped treatment for unrelated reasons.

Baseline characteristics

Baseline characteristics did not differ among the five dose groups (Table 1). Older men had greater body and fat masses and lower percent FFM and serum total and free testosterone concentrations than young men (Table 2).

Compliance

All evaluable men took 100% of the scheduled GnRH agonist injections; one young man in the 125-mg group missed one scheduled testosterone injection.

Nutritional intake

Daily energy and percent protein, carbohydrate, and fat intake were not significantly different among the five groups. There were no significant changes in daily caloric or protein intake during treatment (Table 3).

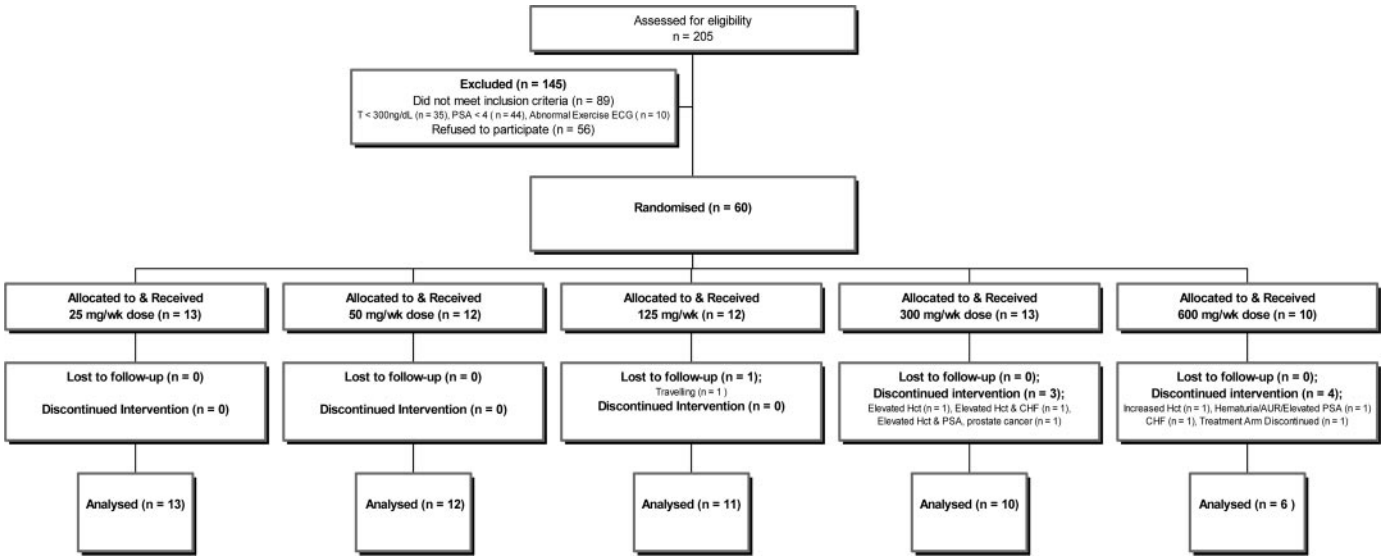


FIG. 1. Flow of subjects through different phases of the study.

Hormone levels

During treatment, significant correlations were observed between testosterone dose and nadir total ($r = 0.94$; $P < 0.0001$) and free ($r = 0.87$; $P < 0.0001$) testosterone levels. Similarly, changes from baseline in total ($r = 0.95$; $P < 0.0001$) and free ($r = 0.83$; $P < 0.0001$) testosterone levels in older men were positively correlated with testosterone dose. Serum total testosterone levels increased dose-dependently in older men receiving the 125-, 300-, and 600-mg doses.

Baseline total and free testosterone levels were lower in older men than in young men (Table 2). Secondary analysis revealed that after adjusting for dose, serum total and free testosterone levels during treatment were significantly higher in older men than young men (age effect, $P < 0.0001$ for each; Table 4 and Fig. 2). Increments above baseline in total and free testosterone levels were significantly greater in older men than young men (age effect, $P < 0.0001$ for each).

Serum LH levels were suppressed in all groups of older men; secondary analysis did not show a significant age effect (Table 4). Baseline SHBG levels did not change in older men with treatment (Table 4).

On d 252, 16 wk after treatment discontinuation, serum LH, total and free testosterone levels were not significantly different from baseline (LH, 5.8 ± 0.5 U/liter; total testosterone, 296 ± 12 ng/dl; free testosterone, 32 ± 2 pg/ml).

Adverse events

Older men had 147 adverse and 12 serious adverse events. Twelve serious adverse events occurred in nine older men and included hematocrit greater than 54% (six events), leg edema with shortness of breath (one event), urinary retention (one event), and prostate cancer (two events). There were dose-dependent increases in hemoglobin and hematocrit (dose effect, $P < 0.0001$; see Table 6 and Fig. 3). One older man receiving the 125-mg dose, three receiving the 300-mg dose, and two receiving the 600-mg dose had hematocrits greater than 54%. Leg edema occurred in eight older men: one receiving 50 mg, four receiving 300 mg, and three receiving 600 mg.

Treatment was discontinued because of serious adverse events in three subjects receiving 600 mg and in three receiving 300 mg. In the 600-mg group, treatment was discontinued in one man because of hematocrit above 54%, in one

TABLE 1. Baseline characteristics of the older men

						P (by ANOVA)
Monthly GnRH agonist	+	+	+	+	+	
Weekly testosterone enanthate dose (mg)	25	50	125	300	600	
Age (yr)	65 ± 4	66 ± 4	67 ± 5	68 ± 4	66 ± 4	0.558
Height (cm)	177 ± 6	178 ± 5	176 ± 8	178 ± 6	176 ± 7	0.889
Weight (kg)	84.2 ± 10.6	80.0 ± 6.1	79.6 ± 11.2	89.8 ± 14.8	87.0 ± 17.7	0.204
Body mass index (kg/m^2)	27 ± 3	25 ± 2	26 ± 4	28 ± 4	28 ± 4	0.181
FFM (kg)	61.4 ± 6.8	59.6 ± 5.7	58.6 ± 5.9	62.9 ± 7.3	62.9 ± 7.3	0.232
Body fat (%)	24 ± 6	21 ± 4	22 ± 5	22 ± 6	22 ± 7	0.783
Serum testosterone [ng/dl (nmol/liter)]	372 ± 128 (12.9 ± 4.4)	328 ± 77 (11.4 ± 2.7)	387 ± 123 (13.4 ± 4.3)	312 ± 109 (10.8 ± 3.8)	362 ± 107 (12.6 ± 3.7)	0.445
No. of men	13	12	12	13	10	

Data are the mean ± SD for 60 older men who were randomized into the study. *P* values (determined by ANOVA) for comparison of the five groups are shown. To convert serum total testosterone levels from conventional units (nanograms per deciliter) to Systeme International units (nanomoles per liter), multiply values in nanograms per deciliter by 0.03467.

TABLE 2. Comparison of baseline characteristics of the young and older men

	Old (n = 61)	Young (n = 60)	*P* value
Age (yr)	66 ± 4 (18–35)[a]	26 ± 15 (60–75)[a]	<0.001
Height (cm)	177 ± 6	176 ± 7	0.622
Weight (kg)	84.1 ± 12.7	76.3 ± 11.7	<0.001
Body mass index (kg/m^2)	27 ± 4	25 ± 3	<0.001
Body fat (%)	22 ± 5	12.4 ± 1.6	<0.001
Serum total testosterone [ng/dl (nmol/liter)]	352 ± 111 (12.2 ± 3.9)	585 ± 189 (20.3 ± 6.6)	<0.001
Serum free testosterone [pg/ml (pmol/liter)]	34 ± 10 (117.8 ± 34.7)	60 ± 22 (208.0 ± 76.3)	<0.001

Data are the mean ± SD. *P* values for comparison of young and older men are shown. To convert serum total testosterone levels from conventional units (nanograms per deciliter) to Systeme International units (nanomoles per liter), multiply values in nanograms per deciliter by 0.03467. To convert free testosterone levels from conventional units (picograms per milliliter) to Systeme International units (picomoles per liter), multiply values in picograms per milliliter by 3.467.

[a] Range is in *parentheses*.

because of hematuria and urinary retention, and in another man because of leg edema. In December 2002, one additional subject receiving the 600-mg dose stopped treatment after DSMB discontinued this study arm in older men because of a high frequency of serious adverse events. Three men receiving the 300-mg dose were discontinued from the study: one because of hematocrit above 54%, one because of hematocrit above 54% and leg edema, and one because of hematocrit above 54% and PSA above 4 μg/ml (Fig. 3). Two older men were found to have prostate cancer; one man receiving the 300-mg dose underwent biopsy because of a PSA level greater than 4 μg/ml, and a second man receiving the 50-mg dose underwent biopsy because of prostate irregularity that was palpated on digital rectal examination on the last recovery day.

There were 55 adverse events, but no serious adverse events, in young men (12). The frequency of total and serious adverse events and prostate events by testosterone dose was not statistically different between young and older men, although the total number of adverse events was numerically greater in older men than young men. The older men had significantly greater increments in hemoglobin and hematocrit than young men after adjusting for testosterone levels (age effect, $P = 0.0001$; Table 6 and Fig. 3).

Body composition

Changes in FFM in older men, measured by DEXA, correlated with testosterone dose ($r = 0.77$; $P < 0.001$) and total ($r = 0.74$; $P < 0.001$) and free ($r = 0.72$; $P < 0.001$) testosterone concentrations in older men (Table 5 and Fig. 4). Administration of 125-, 300-, and 600-mg doses in older men was associated with average FFM gains of 4.2, 5.6, and 7.3 kg, respectively; the gains in FFM were significantly greater in older men receiving 125-, 300-, and 600-mg doses than in those receiving 25- or 50-mg doses. Changes in FFM by underwater weighing also revealed a significant dose effect ($P < 0.0001$). Changes in skeletal muscle mass correlated with testosterone dose ($r = 0.76$; $P < 0.001$). The ratio of total body water to FFM did not change significantly at any dose in

TABLE 3. Energy and macronutrient intake at baseline and during treatment

Parameter	Testosterone dose				
	25 mg	50 mg	125 mg	300 mg	600 mg
Energy intake (kcal/kg)					
Baseline	28.8 ± 4.2	26.7 ± 1.6	32.6 ± 3.8	24.8 ± 2.9	26.7 ± 3.3
Treatment	31.7 ± 4.2	28.7 ± 1.0	30.3 ± 1.9	27.4 ± 2.3	28.4 ± 0.1
Protein intake (g/kg)					
Baseline	1.2 ± 0.2	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.2	1.2 ± 0.1
Treatment	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
% Protein					
Baseline	16.8 ± 1.3	18.1 ± 1.3	15.4 ± 1.4	17.9 ± 1.3	19.4 ± 1.2
Treatment	16.9 ± 0.7	16.5 ± 1.1	15.5 ± 0.7	16.0 ± 0.8	15.3 ± 0.5
CHO (g/kg)					
Baseline	3.2 ± 0.5	3.1 ± 0.3	4.1 ± 0.5	2.8 ± 0.3	3.2 ± 0.6
Treatment	3.5 ± 0.3	3.7 ± 0.2	3.6 ± 0.3	3.4 ± 0.3	3.7 ± 0.5
% CHO					
Baseline	46.1 ± 3.2	50.2 ± 2.2	50.6 ± 2.3	45.8 ± 2.8	45.1 ± 3.0
Treatment	47.9 ± 2.6	51.4 ± 2.3	47.1 ± 1.5	51.0 ± 1.8	50.5 ± 0.2
Fat (g/kg)					
Baseline	1.2 ± 0.2	0.9 ± 0.1	1.3 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
Treatment	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
% Fat					
Baseline	35.9 ± 2.5	30.0 ± 2.4	34.5 ± 2.1	33.0 ± 2.1	33.5 ± 2.8
Treatment	33.3 ± 1.8	31.6 ± 1.7	36.8 ± 1.5	32.9 ± 1.3	30.7 ± 2.8

Daily energy and macronutrient intakes were assessed by 3-d food records using Nutrient V software at baseline and every 4 wk during treatment. The values obtained on different treatment days were averaged to obtain the mean intake for each subject and then averaged across subjects within each treatment group. Data are the mean ± SEM. There were no significant dose or treatment effects for either total energy intake or any of the macronutrients. CHO, Carbohydrates.

TABLE 4. Serum total and free testosterone, and LH levels in older men

Testosterone dose (mg)	Baseline	Wk 16	Change from baseline
Total testosterone (ng/dl; overall ANOVA P for change < 0.001)			
25	372 ± 36	176 ± 34	−196 ± 50
50	328 ± 22	274 ± 18	−54 ± 28
125	387 ± 36	852 ± 111	+464 ± 104[a,b]
300	312 ± 32	1784 ± 173	+1429 ± 180[a,b]
600	362 ± 36	3286 ± 271	+2939 ± 228[a,b]
Free testosterone (pg/ml; overall ANOVA P for change < 0.001)			
25	34 ± 3	19 ± 4	−15 ± 4
50	35 ± 3	63 ± 33	27 ± 32
125	36 ± 3	80 ± 11	44 ± 11[b]
300	31 ± 4	215 ± 25	182 ± 23[a,b]
600	33 ± 3	423 ± 35	388 ± 36[a,b]
LH (U/liter; overall ANOVA P for change = 0.24)			
25	5.2 ± 0.7	0.1 ± 0.02	−5.0 ± 0.7[b]
50	5.3 ± 0.7	0.1 ± 0.02	−5.1 ± 0.8[b]
125	6.0 ± 0.7	0.1 ± 0.03	−5.7 ± 0.7[b]
300	5.7 ± 0.7	0.1 ± 0.05	−5.6 ± 0.7[b]
600	4.6 ± 0.6	0.1 ± 0.05	−4.4 ± 0.9[b]
SHBG (nmol/liter; overall ANOVA P for change = 0.94)			
25	57 ± 3	48 ± 2	−12 ± 11
50	53 ± 1	44 ± 1	−11 ± 3
125	54 ± 2	44 ± 1	−12 ± 3
300	47 ± 1	40 ± 2	−12 ± 3
600	51 ± 3	38 ± 2	−12 ± 7

Data represent the mean ± SEM at baseline and after 20 wk of GnRH plus testosterone treatment. Change scores represent wk 20 values minus baseline values for those who completed the study. To convert serum total testosterone levels from conventional units (nanograms per deciliter) to Systeme International units (nanomoles per liter), multiply values in nanograms per deciliter by 0.03467. To convert free testosterone levels from conventional units (picograms per milliliter) to Systeme International units (picomoles per liter), multiply values in picograms per milliliter by 3.467.

[a] Significantly different from all other dose groups at the 0.05 significance level.

[b] Significantly different from baseline at the 0.05 significance level.

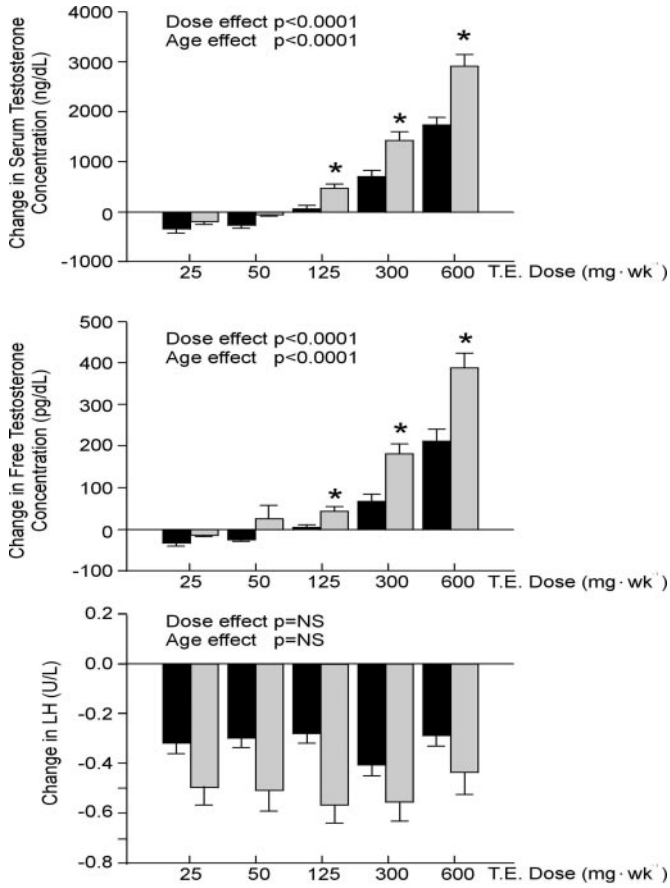


FIG. 2. Changes from baseline in serum total and free testosterone and LH levels in young (■) and older (▨) men in response to graded doses of testosterone enanthate. Healthy, young and older men were randomized to receive a long-acting GnRH agonist plus one of five different doses of testosterone enanthate (25, 50, 125, 300, and 600 mg weekly, im) for 20 wk. Serum testosterone levels were measured 7 d after the previous testosterone injection and represent nadir levels during wk 16. Data are the mean ± SEM. If there was a significant age effect, the values for young and older men for each dose were compared using Tukey's multiple comparison procedure. *, Significant differences between young and older men receiving that dose ($P < 0.05$). Similarly, if the linear model revealed a significant dose effect, then different dose groups were compared using Tukey's multiple comparison procedure. To convert serum total testosterone levels from conventional units (nanograms per deciliter) to Systeme International units (nanomoles per liter), multiply values in nanograms per deciliter by 0.03467. To convert free testosterone levels from conventional units (picograms per milliliter) to Systeme International units (picomoles per liter), multiply values in picograms per milliliter by 3.467.

older men (change from baseline, −0.05 ± 0.05, −0.06 ± 0.03, 0.10 ± 0.04, −0.02 ± 0.03, and −0.09 ± 0.03).

After adjusting for total and free testosterone levels, there was no significant difference in the relationship between testosterone dose and FFM change between young and older men (age effect, $P = 0.54$; multiplicative interaction effect of testosterone concentration and age, $P = 0.76$). There was no significant age effect on changes in FFM by underwater weighing ($P = 0.22$) or in skeletal muscle mass.

Changes in fat mass by DEXA were correlated inversely with testosterone dose ($r = -0.54$; $P < 0.001$) and total ($r = -0.50$; $P < 0.001$) and free ($r = -0.48$; $P < 0.001$) testosterone concentrations in older men (Table 5). Older men receiving 125-, 300-, and 600-mg doses lost greater amounts of fat mass than those receiving the 25-mg dose ($P < 0.05$ for each comparison). There was a significant age effect on change in fat mass ($P < 0.0001$) after adjusting for testosterone levels; young men receiving 25- and 50-mg doses gained more fat mass than older men receiving similar doses ($P = 0.0006$).

Muscle strength

Testosterone administration was associated with dose-dependent gains ($P < 0.001$) in leg press strength in older men (Table 5 and Fig. 4); men receiving 125-, 300-, and 600-mg doses gained more leg press strength than those receiving the 25-mg dose. Changes in muscle strength in older men correlated with total ($r = 0.51$; $P = 0.0001$) and free ($r = 0.44$; $P = 0.001$) testosterone levels. Multiple regression revealed no significant age effect or age by change in testosterone level interactive effect on change in leg press strength ($P = 0.29$).

Behavioral measures

Visuospatial cognition, and mood did not change significantly either in young or older men (data not shown).

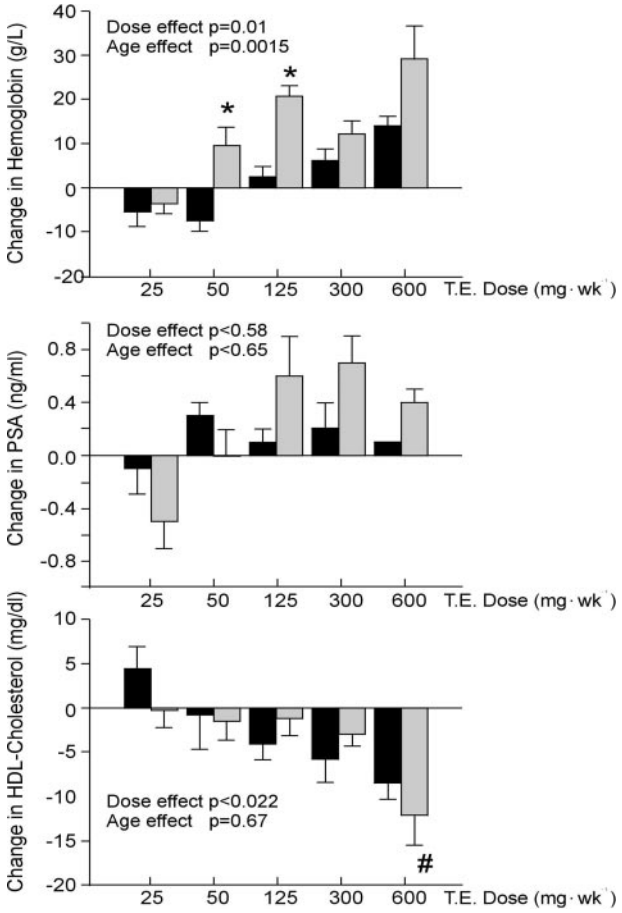


FIG. 3. Changes from baseline in hemoglobin, serum PSA, and HDL cholesterol levels in men in response to graded doses of testosterone enanthate. Healthy, young and older men were randomized to receive a long-acting GnRH agonist plus one of five different doses of testosterone enanthate (25, 50, 125, 300, and 600 mg weekly, im) for 20 wk. Changes in other outcome measures were calculated as the difference between wk 20 and baseline values. Data are the mean ± SEM. *, Significant differences between young and older men receiving that dose ($P < 0.05$). To convert PSA from conventional units (nanograms per milliliter) to Systeme International units (micrograms per liter), multiply values in nanograms per milliliter by 1. To convert HDL cholesterol values from milligrams per deciliter to millimoles per liter, multiply values in milligrams per deciliter by 0.02586.

Blood chemistries

Baseline PSA levels were higher in older men than young men ($P < 0.05$); however, there was no significant dose ($P = 0.58$) or age ($P = 0.65$) effect on PSA levels (Table 6) in older men. Serum aspartate aminotransferase, alanine aminotransferase, and bilirubin did not change significantly at any dose. Serum creatinine increased significantly in correlation with testosterone dose (r = 0.38; $P = 0.005$).

Plasma lipids

Total and high-density lipoprotein (HDL) cholesterol levels decreased dose-dependently in older men; a significant decrease in HDL cholesterol was observed in men receiving the 600-mg dose (Table 6). Changes in low-density lipoprotein (LDL) cholesterol or triglyceride levels were not significant. Secondary analysis revealed no significant age effect on changes in plasma lipids.

TABLE 5. Changes in FFM, fat mass, muscle size, and muscle strength in older men

Testosterone dose (mg)	Baseline	Wk 20	Change from baseline
FFM (kg) by DEXA scan in older men (overall ANOVA P for change < 0.001)			
25	58.7 ± 1.8	58.4 ± 1.7	−0.3 ± 0.5
50	56.9 ± 1.6	58.6 ± 1.8	+1.7 ± 0.4[a]
125	55.8 ± 1.6	60.5 ± 1.4	+4.2 ± 0.6[b]
300	60.2 ± 2.0	64.9 ± 2.1	+5.6 ± 0.5[b]
600	60.8 ± 2.9	66.4 ± 3.6	+7.3 ± 0.4[c]
FFM (kg) by underwater weighing in older men (overall ANOVA P for change < 0.001)			
25	61.9 ± 1.9	61.2 ± 1.8	−0.8 ± 0.3
50	59.8 ± 1.7	61.1 ± 1.6	+1.4 ± 0.5
125	58.2 ± 1.7	62.9 ± 1.5	+4.5 ± 0.6[b]
300	61.4 ± 2.2	64.9 ± 2.1	+4.8 ± 0.7[b]
600	62.2 ± 2.4	67.8 ± 3.5	+6.7 ± 0.9[b]
			SD from 25 & 50 mg/wk[b]
Whole body fat mass (kg) by DEXA in older men (overall ANOVA P for change < 0.001)			
25	22.9 ± 1.62	23.0 ± 1.9	+0.1 ± 0.5
50	20.1 ± 1.0	19.3 ± 1.0	−0.9 ± 0.3[a]
125	20.9 ± 2.4	19.8 ± 2.6	−1.5 ± 0.2[a]
300	26.2 ± 2.4	23.2 ± 2.7	−2.2 ± 0.5[a]
600	23.5 ± 2.9	17.7 ± 3.5	−3.0 ± 0.5[b]
Maximal voluntary muscle strength in leg press exercise (kg; overall ANOVA P for change < 0.001)			
25	300.0 ± 18.7	300.8 ± 19.8	0.8 ± 6.7
50	277.9 ± 12.5	289.4 ± 14.6	11.5 ± 4.7
125	278.1 ± 23.2	309.6 ± 24.2	28.0 ± 6.8[a]
300	301.5 ± 12.7	359.8 ± 15.2	51.7 ± 4.7
600	331.9 ± 17.1	380.0 ± 14.6	29.8 ± 6.4[a]

Data represent the mean ± SEM values at baseline and after 20 wk of GnRH plus testosterone treatment. Change scores represent wk 20 values minus baseline values for those who completed the study.

[a] Significantly different from 25-mg group at the 0.05 significance level.

[b] Significantly different from 25- and 50-mg groups at the 0.05 significance level.

[c] Significantly different from all other dose groups at the 0.05 significance level.

Discussion

Significant gains in FFM and muscle strength, similar in magnitude to those noted in young men, were observed in older men given graded testosterone doses. Thus, even in older men it is possible to induce very substantial skeletal muscle remodeling by androgen administration. However, older men differed from young men in their response to testosterone administration in several respects. Increments in serum total and free testosterone levels above baseline were higher in older men than young men. There were qualitative differences in the types of adverse effects seen in young and older men; the young men had a higher frequency of acne than older men, whereas older men had a higher frequency of hematocrit elevated above 54%, leg edema, and prostate events. Increments in hematocrit during testosterone administration were greater in older men than in young men. The 125-mg dose was associated with high-normal testosterone

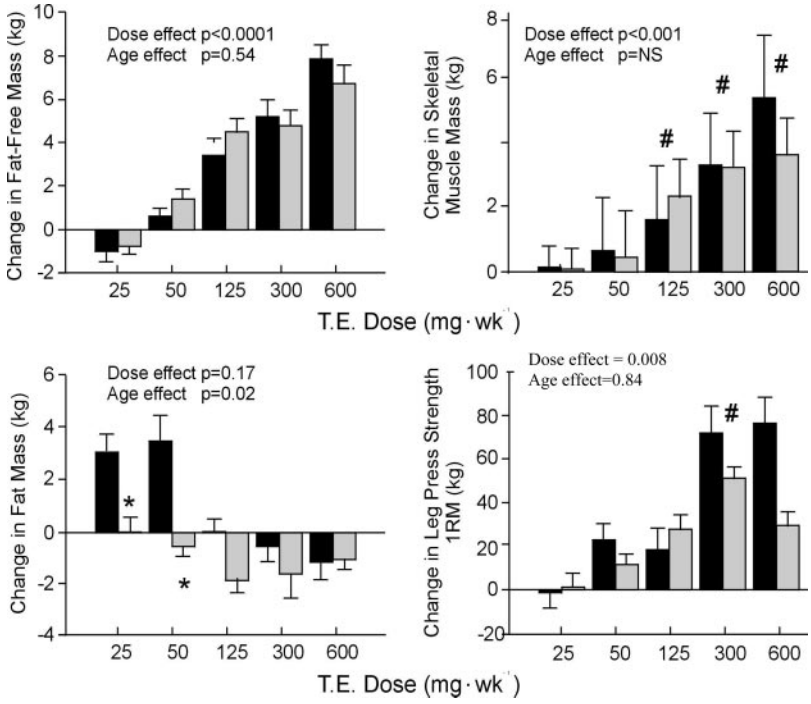


FIG. 4. Changes from baseline in FFM, fat mass, leg press strength, and skeletal muscle mass in young (■) and older (▨) men in response to graded doses of testosterone enanthate. Healthy, young and older men were randomized to receive a long-acting GnRH agonist plus one of five different doses of testosterone enanthate (25, 50, 125, 300, and 600 mg weekly, im) for 20 wk. Changes in other outcome measures were calculated as the difference between wk 20 and baseline values. Data are the mean ± SEM. If there was a significant age effect, the values for young and older men for each dose were compared using Tukey's multiple comparison procedure. *, Significant differences between young and older men receiving that dose ($P < 0.05$). Similarly, if the linear model revealed a significant dose effect, then different dose groups were compared using Tukey's multiple comparison procedure. #, Significant difference from 25- and 50-mg doses ($P < 0.05$).

concentrations and low frequency of adverse events, no serious adverse events, and substantial gains in FFM (+4.2 kg) and leg press strength (+28 kg); thus, this dose provided the best trade-off between anabolic effects and adverse events.

Aging is associated with loss of skeletal muscle mass and strength and impaired physical function (43–51). Age-related sarcopenia increases the risk of falls, fractures, and disability (26–31). Therefore, anabolic interventions that prevent or reverse age-related loss of muscle mass and strength are desirable. Low bioavailable testosterone levels correlate with decreased FFM and muscle strength. Conversely, testosterone supplementation, especially when given in supraphysiological doses, induces remarkable gains in muscle mass and strength in older men, similar to those observed in young men. Gains in leg press strength in older men receiving 125-, 300-, and 600-mg doses averaged 28–50 kg. Thus, skeletal muscle in older men is capable of undergoing considerable hypertrophy in response to androgenic stimulus.

Mechanisms of androgen action on muscle are poorly understood. Testosterone supplementation increases muscle protein accretion (24, 52–57) by increasing fractional muscle protein synthesis and facilitating the reutilization of amino acids by the muscle. Testosterone has also been reported to decrease muscle protein degradation. Testosterone supplementation induces hypertrophy of both type 1 and 2 skeletal muscle fibers (58), associated with a dose-dependent increase in the number of myonuclei and satellite cells (59). The muscle protein synthesis hypothesis does not easily explain the reciprocal change in fat mass during testosterone administration. Emerging data suggest that testosterone promotes commitment of pluripotent, mesenchymal cells into myogenic lineage and inhibits adipogenesis (60, 61).

Although testosterone administration in castrated rats induces salt and water retention, these effects are transient. We have shown in a number of experiments in hypogonadal men (34), healthy eugonadal men (34), and human immunodeficiency virus-infected men (35) that the ratio of FFM determined by DEXA to total body water does not change during testosterone administration. These observations along with the significant, dose-related strength gains indicate that the apparent increase in FFM is not due to water retention in excess of that associated with protein accretion.

This is the first direct comparison of testosterone dose-responsiveness of young and older men. The study provided comprehensive assessment of androgen-induced body composition changes in older men, using multiple methods in the controlled setting of a clinical research center which allowed standardization of energy and protein intake. Combined administration of GnRH agonist and testosterone suppressed LH and consequently endogenous testosterone production; this minimized heterogeneity in testosterone levels due to uneven suppression of endogenous testosterone production by exogenous androgen.

Increments in total and free testosterone levels above baseline were higher in older men than young men. Higher testosterone levels suggest that testosterone clearance is lower in older men than young men. The mechanisms of decreased testosterone clearance in older men are unknown.

Sexual function did not change significantly at any dose in either age group. Thus, these data are consistent with previous observations that sexual function in men (29, 42) and male rats (62) is maintained at testosterone concentrations at the lower end of the male range. Testosterone dose-response relationships differ for different androgen-dependent outcomes; sexual function and PSA levels are maintained at lower testosterone concentrations than those required to induce muscle accretion.

The best trade-off between anabolic effects and adverse effects was achieved with the 125-mg dose. These data suggest that in efficacy trials for aging-associated sarcopenia, serum

TABLE 6. Hemoglobin, hematocrit, PSA, and HDL cholesterol levels in older men

Testosterone dose (mg)	Baseline	Wk 20	Change from baseline
Hemoglobin (g/liter; overall ANOVA P for change < 0.001)			
25	137.2 ± 2.8	132.9 ± 2.8	−3.6 ± 2.1
50	132.9 ± 4.8	142.8 ± 5.1	+9.9 ± 4.1[a]
125	141.6 ± 4.3	162.5 ± 5.0[b]	+20.9 ± 2.4[b]
300	146.0 ± 2.7	156.9 ± 3.4[b]	+12.6 ± 2.7[b]
600	139.7 ± 4.7	168.8 ± 7.8[b]	+29.4 ± 7.5[c]
Hematocrit (liter/liter; overall ANOVA $P = 0.00002$)			
25	0.40 ± 0.01	0.43 ± 0.02	+0.01 ± 0.01
50	0.39 ± 0.01	0.42 ± 0.01	+0.05 ± 0.01[a]
125	0.41 ± 0.01	0.48 ± 0.01[a]	+0.07 ± 0.01[a]
300	0.43 ± 0.01	0.47 ± 0.01[a]	+0.07 ± 0.01[a]
600	0.40 ± 0.01	0.50 ± 0.01[a]	+0.08 ± 0.02[a]
PSA (μg/ml)			
25	1.8 ± 0.3	1.5 ± 0.2	−0.5 ± 0.2
50	1.4 ± 0.2	1.5 ± 0.3	+0.0 ± 0.2
125	1.4 ± 0.3	2.1 ± 0.3	+0.6 ± 0.3
300	1.7 ± 0.2	2.6 ± 0.4	+0.7 ± 0.2[a]
600	0.9 ± 0.2	0.9 ± 0.2	+0.4 ± 0.1
HDL cholesterol (mg/dl; overall ANOVA P for change 0.022)			
25	48 ± 5	50 ± 5	−0.08 ± 2.0
50	45 ± 5	43 ± 4	−1.4 ± 2.1
125	45 ± 5	46 ± 5	−1.1 ± 1.9
300	37 ± 2	35 ± 2	−2.9 ± 1.4
600	47 ± 4	37 ± 2	−12.0 ± 3.7[d]
Creatinine (mg/dl; overall ANOVA P for change < 0.001)			
25	1.05 ± 0.05	1.05 ± 0.04	−0.04 ± 0.02
50	1.03 ± 0.05	1.09 ± 0.07	+0.03 ± 0.02
125	1.12 ± 0.10	1.19 ± 0.09	+0.08 ± 0.04[a]
300	0.97 ± 0.04	1.10 ± 0.05	+0.15 ± 0.03[a]
600	1.03 ± 0.05	1.17 ± 0.03	+0.16 ± 0.06[a]

Data represent the mean ± SEM values at baseline and after 20 wk of GnRH plus testosterone treatment. Change scores represent wk 20 values minus baseline values for those who completed the study. To convert cholesterol, HDL and low-density lipoprotein cholesterol (LDL-C) concentrations from milligrams per deciliter to millimoles per liter, multiply concentrations in milligrams per deciliter by 0.02586.

[a] Significantly different from 25-mg group at the 0.05 significance level.

[b] Significantly different from 25- and 50-mg groups at the 0.05 significance level.

[c] Significantly different from 25-, 50-, and 300-mg groups.

[d] Significantly different from all other dose groups at the 0.05 significance level.

testosterone levels should be raised into high end of the normal male range to maximize anabolic effects; the long-term safety of such an approach has not been tested. Also, lower testosterone levels might be sufficient for efficacy trials in men with sexual dysfunction. These data should not be interpreted to justify the 125-mg dose as the replacement dose in clinical practice. Because older men have lower plasma testosterone clearance than young men, it is likely that older men would need lower doses of testosterone than younger men to achieve the desired serum testosterone levels.

Administration of 300- and 600-mg testosterone doses was associated with a high frequency of serious adverse events in older men. An increase in hematocrit was the most frequent dose-limiting adverse event in older men. Testosterone stimulates erythropoietin production and erythropoietic stem cell replication (63–67). The reasons for greater hematocrit increment in older men are unknown. High hematocrit levels are associated with increased plasma viscosity and risk of stroke and hypertension. Leg edema developed in some older men receiving 300- or 600-mg doses. Testosterone administration to castrated male rats causes transient salt and water retention (57). In older men with preexisting heart disease, high testosterone doses may induce edema. Androgen administration induces myocardial hypertrophy (68); we do not know whether androgen-induced myocardial hypertrophy is beneficial or deleterious.

Two older men were diagnosed with prostate cancer (Gleason grade 4 in one man in whom information was available). There is concern that testosterone administration may induce subclinical prostate cancers to grow (69). More intensive PSA monitoring during testosterone administration might lead to the detection of a greater number of prostate cancers.

Because supraphysiological doses of testosterone (300 and 600 mg) were associated with a high frequency of adverse events, it is unlikely that these doses can be used in long-term human trials. However, these data provide compelling rationale for the development of selective androgen receptor modulators with anabolic properties that are free of dose-limiting adverse effects of testosterone (70). The Institute of Medicine committee on assessing the need for clinical trial of testosterone replacement therapy recommended studies of testosterone replacement in older men with low testosterone levels and symptoms attributable to androgen deficiency, such as sexual dysfunction, sarcopenia, or depression (71). Our study was designed to compare the androgen responsiveness of healthy, young and older men, and was not an efficacy trial. The study did not have adequate power to demonstrate improvements in clinical outcomes or risks of testosterone supplementation. We do not know whether testosterone-induced gains in muscle mass and strength translate into improved physical function or quality of life, or whether these gains in muscle mass and strength obtained in the controlled setting of a clinical research center can be replicated in a community setting. Thus, no claims about the efficacy or long-term risks of testosterone replacement in older men can be based on these results. However, these data provide evidence that some age-related changes in body composition and muscle strength are reversible, and that remarkable alterations in muscle mass and strength and fat mass are achievable in older men with androgen administration.

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