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Testosterone dose-response relationships in healthy young men

SHALENDER BHASIN,[1] LINDA WOODHOUSE,[1] RICHARD CASABURI,[3] ATAM B. SINGH,[1] DIMPLE BHASIN,[3] NANCY BERMAN,[3] XIANGHONG CHEN,[4] KEVIN E. YARASHESKI,[4] LYNNE MAGLIANO,[2] CONNIE DZEKOV,[1] JEANNE DZEKOV,[1] RACHELLE BROSS,[3] JEFFREY PHILLIPS,[3] INDRANI SINHA-HIKIM,[1] RUOQUING SHEN,[1] AND THOMAS W. STORER[2]

[1]*Division of Endocrinology, Metabolism, and Molecular Medicine, Charles R. Drew University of Medicine and Science, Los Angeles 90059;* [2]*Laboratory for Exercise Sciences, El Camino College, and* [3]*Harbor-University of California Los Angeles Medical Center, Torrance, California 90502; and* [4]*Biomedical Mass Spectrometric Research Resource, Department of Internal Medicine, Washington University, School of Medicine, St. Louis, Missouri 63110*

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Bhasin, Shalender, Linda Woodhouse, Richard Casaburi, Atam B. Singh, Dimple Bhasin, Nancy Berman, Xianghong Chen, Kevin E. Yarasheski, Lynne Magliano, Connie Dzekov, Jeanne Dzekov, Rachelle Bross, Jeffrey Phillips, Indrani Sinha-Hikim, Ruoquing Shen, and Thomas W. Storer. Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab* 281: E1172–E1181, 2001.—Testosterone increases muscle mass and strength and regulates other physiological processes, but we do not know whether testosterone effects are dose dependent and whether dose requirements for maintaining various androgen-dependent processes are similar. To determine the effects of graded doses of testosterone on body composition, muscle size, strength, power, sexual and cognitive functions, prostate-specific antigen (PSA), plasma lipids, hemoglobin, and insulin-like growth factor I (IGF-I) levels, 61 eugonadal men, 18–35 yr, were randomized to one of five groups to receive monthly injections of a long-acting gonadotropin-releasing hormone (GnRH) agonist, to suppress endogenous testosterone secretion, and weekly injections of 25, 50, 125, 300, or 600 mg of testosterone enanthate for 20 wk. Energy and protein intakes were standardized. The administration of the GnRH agonist plus graded doses of testosterone resulted in mean nadir testosterone concentrations of 253, 306, 542, 1,345, and 2,370 ng/dl at the 25-, 50-, 125-, 300-, and 600-mg doses, respectively. Fat-free mass increased dose dependently in men receiving 125, 300, or 600 mg of testosterone weekly (change +3.4, 5.2, and 7.9 kg, respectively). The changes in fat-free mass were highly dependent on testosterone dose ($P = 0.0001$) and correlated with log testosterone concentrations ($r = 0.73$, $P = 0.0001$). Changes in leg press strength, leg power, thigh and quadriceps muscle volumes, hemoglobin, and IGF-I were positively correlated with testosterone concentrations, whereas changes in fat mass and plasma high-density lipoprotein (HDL) cholesterol were negatively correlated. Sexual function, visual-spatial cognition and mood, and PSA levels did not change significantly at any dose. We conclude that changes in circulating testosterone concentrations, induced by GnRH agonist and testosterone administration, are associated with testosterone dose- and concentration-dependent changes in fat-free mass, muscle size, strength and power, fat mass, hemoglobin, HDL cholesterol, and IGF-I levels, in conformity with a single linear dose-response relationship. However, different androgen-dependent processes have different testosterone dose-response relationships.

sexual function; testosterone effects on muscle; cognitive function; plasma lipids; prostate-specific antigen; testosterone effects on insulin-like growth factor I; testosterone and hemoglobin

TESTOSTERONE regulates many physiological processes, including muscle protein metabolism, some aspects of sexual and cognitive functions, secondary sex characteristics, erythropoiesis, plasma lipids, and bone metabolism (7, 50). However, testosterone dose dependency of various androgen-dependent processes is not well understood (6). Administration of replacement doses of testosterone to hypogonadal men (10, 12, 30, 45, 49) and of supraphysiological doses to eugonadal men (9, 22–23, 26) increases fat-free mass, muscle size, and strength. Conversely, suppression of endogenous testosterone concentrations is associated with loss of fat-free mass and a decrease in fractional muscle protein synthesis (33). However, not known are whether testosterone effects on the muscle are dose dependent, or the nature of the testosterone dose-response relationships (6). Androgen receptors in most tissues are either saturated or downregulated at physiological testosterone concentrations (2, 18, 39, 50); this leads to speculation that there might be two separate dose-response curves: one in hypogonadal range, with

Address for reprint requests and other correspondence: S. Bhasin, Professor of Medicine, UCLA School of Medicine, and Chief, Division of Endocrinology, Metabolism, and Molecular Medicine, Charles R. Drew Univ. of Medicine and Science, 1731 E. 120th St., Los Angeles, CA 90059 (E-mail: SBHASIN@UCLA.EDU).

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maximal response at low normal testosterone concentrations, and a second in supraphysiological range, representing a separate mechanism of action (1). However, testosterone dose-response relationships for a range of androgen-dependent functions in humans have not been studied.

Animal studies suggest that different androgen-dependent processes have different androgen dose-response relationships (6, 8, 21). Sexual function in male mammals is maintained at serum testosterone concentrations that are at the lower end of the male range (3, 6, 8, 13, 21, 31). However, it is not known whether the low normal testosterone levels that normalize sexual function are sufficient to maintain muscle mass and strength, or whether the higher testosterone concentrations required to maintain muscle mass and strength might adversely affect plasma lipids, hemoglobin levels, and the prostate. This information is important for optimizing testosterone replacement regimens for treatment of hypogonadal men. Also, for the proposed use of testosterone in sarcopenia associated with aging (46, 47) and chronic illness (11, 27), it is important to know whether significant gains in muscle mass and strength can be achieved at testosterone doses that do not adversely affect plasma high-density lipoprotein (HDL) and prostate-specific antigen (PSA) levels.

Therefore, the primary objective of this study was to determine the dose dependency of testosterone's effects on fat-free mass and muscle performance. We hypothesized that changes in circulating testosterone concentrations would be associated with dose-dependent changes in fat-free mass, muscle strength, and power in conformity with a single linear dose-response relationship, and that the dose requirements for maintaining other androgen-dependent processes would be different. We treated young men with a long-acting gonadotropin-releasing hormone (GnRH) agonist to suppress endogenous testosterone secretion, and concomitantly also with one of five testosterone-dose regimens to create different levels of serum testosterone concentrations extending from subphysiological to the supraphysiological range. The lowest testosterone dose, 25 mg weekly, was selected because this dose had been shown to maintain sexual function in GnRH antagonist-treated men (37). The selection of the 600-mg weekly dose was based on the consideration that this was the highest dose that had been safely administered to men in controlled studies (9).

METHODS

This was a double-blind, randomized study consisting of a 4-wk control period, a 20-wk treatment period, and a 16-wk recovery period. Each participant provided informed consent, approved by the institutional review boards of Drew University and Harbor-UCLA Research and Education Institute.

Participants. The participants were healthy men, 18–35 yr of age, with prior weight-lifting experience and normal testosterone levels. These men had not used any anabolic agents and had not participated in competitive sports events in the preceding year, and they were not planning to participate in competitive events in the following year.

Randomization. Sixty-one eligible men were randomly assigned to one of five groups. All received monthly injections of a long-acting GnRH agonist to suppress endogenous testosterone production. In addition, *group 1* received 25 mg of testosterone enanthate intramuscularly weekly; *group 2*, 50 mg testosterone enanthate; *group 3*, 125 mg testosterone enanthate; *group 4*, 300 mg testosterone enanthate; and *group 5*, 600 mg testosterone enanthate. Twelve men were assigned to *group 1*, 12 to *group 2*, 12 to *group 3*, 12 to *group 4*, and 13 to *group 5*. Testosterone and GnRH agonist injections were administered by the General Clinical Research Center staff to assure compliance.

Nutritional intake. Energy and protein intakes were standardized at 36 $kcal \cdot kg^{-1} \cdot day^{-1}$ and 1.2 $g \cdot kg^{-1} \cdot day^{-1}$, respectively. The standardized diet was initiated 2 wk before treatment was started; dietary instructions were reinforced every 4 wk. The nutritional intake was verified by analysis of 3-day food records and 24-h food recalls every 4 wk by use of the Minnesota Nutritional Software.

Exercise stimulus. The participants were asked not to undertake strength training or moderate-to-heavy endurance exercise during the study. These instructions were reinforced every 4 wk.

Outcome measures. Body composition and muscle performance were assessed at baseline and during *week 20*. Fat-free mass and fat mass were measured by underwater weighing and dual-energy X-ray absorptiometry (DEXA, Hologic 4500, Waltham, MA). Total thigh muscle and quadriceps muscle volumes were measured by MRI scanning.

For estimation of total body water, the men ingested 10 g of ^2H_2O (10, 11), and plasma samples were drawn at −15, 0, 120, 180, and 240 min. We measured ^2H abundance in plasma by nuclear magnetic resonance spectroscopy (10, 11), with a correction factor of 0.985 for exchangeable hydrogen. We measured bilateral leg press strength by use of the one-repetition maximum (1-RM) method (11). A seated leg press exercise with pneumatic resistance (Keiser Sport, Fresno, CA) was used for this purpose. Subjects performed 5–10 min of leg cycling and stretching warm-up and received instruction and practice in lifting mechanics before performing progressive warm-up lifts leading to the 1-RM. Seat position and the ensuing knee and hip angles, as well as foot placement, were measured and recorded for use in subsequent testing. To ensure reliability in this highly effort-dependent test, the 1-RM score was reassessed within 7 days, but not sooner than 2 days, after the first evaluation. If duplicate scores were within 5%, the higher of the two values was accepted as the strength score. If the two tests differed by >5%, additional studies were conducted, ≥2 days apart but within 7 days, until the two highest scores were within 5%. No subject required >2 days of strength assessment.

We also measured leg power, because power in the lower extremity is strongly related to performance of functional activities in the elderly (4). The sarcopenia that accompanies aging is due in large part to a loss of the fast-twitch type II fibers and the coincident decrease in explosive force. Muscular power is important in performing such daily activities as rising from a chair, climbing stairs, and walking with speed (4). Leg power was measured with a previously validated (4, 5) Nottingham leg extensor power rig. Subjects performed 10–15 trials of right leg and hip extension, attempting to generate as much force as possible by accelerating the leg rig's weighted flywheel from rest. The power score (in watts) was taken as the highest value observed during these trials with evidence of a plateau. As with the strength tests, power

measurements were preceded by a 5- to 10-min warm-up, stretching, and practice. The power tests were repeated within 7 days, but not sooner than 2 days, after the first tests to reduce the effect of familiarization. To minimize test-retest variability, the angle of knee flexion and the seat position were recorded and maintained constant across tests.

Sexual function was assessed by daily logs of sexual activity and desire that were maintained for 7 consecutive days at baseline and during treatment by use of a published instrument (13). Spatial cognition was assessed by a computerized checkerboard test (38) and mood by Hamilton's depression (20) and Young's mania scales (24).

Adverse experiences, blood counts and chemistries, PSA, plasma lipids, total and free testosterone, luteinizing hormone (LH), sex steroid-binding globulin (SHBG), and insulin-like growth factor I (IGF-I) levels were measured periodically during control and treatment periods. Serum total testosterone was measured by an immunoassay (8–11); free testosterone by equilibrium dialysis (43); LH, SHBG, and PSA by immunoradiometric assays (9–11); and IGF-I by acid-ethanol extraction and immunoassay (28). The sensitivities and intra- and interassay coefficients of variation for hormone assays were as follows: total testosterone (0.6 ng/dl), 8.2 and 13.2%; free testosterone (0.22 pg/ml), 4.2 and 12.3%; LH (0.05 U/l), 10.7 and 13.0%; SHBG (6.25 nmol/l), 4 and 6%; PSA (0.01 ng/ml), 5.0 and 6.4%; and IGF-I (80 ng/ml), 4 and 6%, respectively. These assays have been validated previously (8–11).

Statistical analyses. All variables were examined for their distribution characteristics. Variables not meeting the assumption of a normal distribution were log-transformed and retested. An ANOVA was used to compare change from baseline in outcome measures among the five groups. All outcome measures were analyzed using a paired *t*-test to detect a nonzero change from baseline within each group. $P < 0.05$ was considered statistically significant.

To describe the relationship between testosterone concentrations (T) and change in fat-free mass (Y) during testosterone administration, we tested three models: a linear model ($Y = a + bT$); a logarithmic model, $Y = a + b \cdot X$, where $X = \log(T)$, and a and b represent the intercept and slope, respectively; and a growth model, $Y = a/(1 + b \cdot e^{-k \cdot X})$. The logarithmic model provided the best fit for the data and was used to model the effects of testosterone concentrations on the change in other outcome variables. The correlations between testosterone concentrations and change in outcome variables are derived from this model. We also modeled the linear dependence of the change in outcome variables on testosterone dose by use of linear regression.

RESULTS

Participant characteristics. Of 61 men enrolled, 54 completed the study: 12 in *group 1*, 8 in *group 2*, 11 in *group 3*, 10 in *group 4*, and 13 in *group 5*. One man discontinued treatment because of acne; other subjects were unable to meet the demands of the protocol. The five groups did not significantly differ with respect to their baseline characteristics (Table 1).

Compliance. All evaluable subjects received 100% of their GnRH agonist injections, and only one man in the 125-mg group missed one testosterone injection.

Nutritional intake. Daily energy intake and proportion of calories derived from protein, carbohydrate, and fat were not significantly different among the five groups at baseline. There was no significant change in daily caloric, protein, carbohydrate, or fat intake in any group during treatment (data not shown).

Hormone levels. Serum total and free testosterone levels (Table 2), measured during *week 16*, 1 wk after the previous injection, were linearly dependent on the testosterone dose ($P = 0.0001$). Serum total and free testosterone concentrations decreased from baseline in men receiving the 25- and 50-mg doses and increased at 300- and 600-mg doses. Serum LH levels were suppressed in all groups. Serum SHBG levels decreased dose dependently at the 300- and 600-mg doses but did not change in other groups. Serum IGF-I concentrations increased dose dependently at the 300- and 600-mg doses (correlation between log testosterone level and change in IGF-I = 0.55, $P = 0.0001$). IGFBP-3 levels did not change significantly in any group.

Body composition. Fat-free mass, measured by underwater weighing, did not change significantly in men receiving the 25- or 50-mg testosterone dose, but it increased dose dependently at higher doses (Table 3). The changes in fat-free mass were highly dependent on testosterone dose ($P = 0.0001$) and correlated with log total testosterone concentrations during treatment ($r = 0.73$, $P = 0.0001$, see Fig. 2).

Changes in fat-free mass, measured by DEXA scan, were qualitatively similar to those obtained from underwater weighing (Table 3, Fig. 1). The measurements of fat-free mass by DEXA were highly correlated with values obtained from underwater weighing ($r = 0.94$, $P < 0.0001$).

Table 1. *Baseline characteristics of the participants*

GnRH Agonist + Testosterone Enanthate	+ 25 mg	+ 50 mg	+ 125 mg	+ 300 mg	+ 600 mg	*P* Value
Age, yr	28 ± 5	29 ± 5	28 ± 3	24 ± 5	25 ± 4	0.0583
Height, cm	175 ± 5	177 ± 9	178 ± 7	177 ± 7	175 ± 8	0.7230
Weight, kg	68.0 ± 8.4	77.0 ± 8.1	78.9 ± 10.6	78.4 ± 10.1	74.8 ± 12.5	0.1014
Body mass index, kg/m^2	23 ± 3	25 ± 3	25 ± 3	25 ± 3	25 ± 3	0.3680
Serum testosterone levels, nmol/l	593 ± 161	566 ± 220	553 ± 182	654 ± 157	632 ± 228	0.7093
Fat-free mass, kg	59.1 ± 6.7	65.1 ± 5.1	66.0 ± 7.2	67.3 ± 8.9	64.2 ± 8.0	0.1506
Leg press strength, kg	355.5 ± 103.8	407.8 ± 62.2	419.2 ± 86.2	439.8 ± 81.4	431.6 ± 99.3	0.2149
Hemoglobin, g/l	144 ± 12	151 ± 11	142 ± 9	144 ± 8	141 ± 8	0.1428
No. in group	12	12	12	12	13	

Values are means ± SD. GnRH, gonadotropin-releasing hormone.

Table 2. *Serum total and free testosterone, LH, FSH, SHBG, and IGF-I levels*

Testosterone Dose	Baseline	*Week 16*	Change from Baseline	*P* vs. Zero Change
Testosterone (ng/dl) (overall ANOVA P = 0.0001)				
25 mg	593 ± 48	253 ± 66	−340 ± 85	0.0029
50 mg	566 ± 78	306 ± 58	−260 ± 64	0.0037
125 mg	553 ± 53	570 ± 75	57 ± 75	0.7425
300 mg	653 ± 50	1,345 ± 139	691 ± 143	0.0005
600 mg	632 ± 63	2,370 ± 150	1,737 ± 156	0.0001
Free testosterone (pg/ml) (overall ANOVA P = 0.0001)				
25 mg	62 ± 6	29 ± 5	−33 ± 8	0.0014
50 mg	57 ± 6	32 ± 3	−25 ± 5	0.0009
125 mg	49 ± 5	52 ± 8	3 ± 7	0.8601
300 mg	71 ± 7	138 ± 21	67 ± 18	0.0012
600 mg	64 ± 5	275 ± 30	211 ± 31	0.0001
LH (U/l) (overall ANOVA P = 0.8054)				
25 mg	3.5 ± 0.4	0.3 ± 0.1	−3.2 ± 0.4	0.0001
50 mg	3.8 ± 0.3	0.6 ± 0.3	−3.0 ± 0.4	0.0008
125 mg	3.4 ± 0.3	0.5 ± 0.1	−2.8 ± 0.4	0.0001
300 mg	3.7 ± 0.5	0.6 ± 0.1	−3.5 ± 0.5	0.0002
600 mg	3.3 ± 0.3	0.6 ± 0.4	−2.9 ± 0.4	0.0001
SHBG (nmol/l) (overall ANOVA P = 0.0001)				
25 mg	29.1 ± 2.9	28.5 ± 3.6	−0.6 ± 2.9	0.8497
50 mg	24.4 ± 3.4	21.1 ± 3.2	−3.3 ± 1.1	0.0202
125 mg	33.1 ± 4.2	28.9 ± 3.8	−4.2 ± 2.6	0.1410
300 mg	31.4 ± 3.8	22.4 ± 3.9	−9.1 ± 3.7	0.0348
600 mg	40.1 ± 4.9	20.6 ± 3.2	−19.5 ± 2.8	0.0001
IGF-I (ng/ml) (overall ANOVA P = 0.0001)				
25 mg	268 ± 26	261 ± 35	−7 ± 19	0.7462
50 mg	246 ± 14	225 ± 12	−20 ± 10	0.0797
125 mg	299 ± 24	282 ± 31	−18 ± 17	0.3284
300 mg	314 ± 24	388 ± 30	74 ± 28	0.0272
600 mg	227 ± 20	304 ± 21	77 ± 13	0.0001

Values on each day represent the mean (±SE) of all available values on that day. However, the change represents the difference between paired values only. Treatment values represent the *day 113* (*week 16*) values, obtained 1 wk after the previous testosterone injection. We used *week 16* rather than *week 20* values because *week 20* values were not always drawn exactly 1 wk after the previous injection. LH and FSH, luteinizing and follicle-stimulating hormones, respectively; SHBG, sex hormone-binding globulin; IGF-I, insulin-like growth factor I. To convert total testosterone levels to nmol/l, multiply by 0.03467. To convert free testosterone levels to pg/ml, multiply by 3.467.

To determine whether the apparent changes in fat-free mass by DEXA scan and underwater weighing represented water retention, we measured total body water and compared the ratios of total body water to fat-free mass before and after treatment in each group. The ratios of total body water to fat-free mass by underwater weighing did not significantly change with treatment in any treatment group (Table 3), indicating that the apparent increase in fat-free mass measured by underwater weighing did not represent water retention in excess of that associated with protein accretion.

Fat mass, measured by underwater weighing, increased significantly in men receiving the 25- and 50-mg doses but did not change in men receiving the higher doses of testosterone (Table 3, Fig. 1). There was an inverse correlation between change in fat mass by underwater weighing and log testosterone concentrations ($r = -0.60$, $P = 0.0001$, Fig. 2).

Muscle size. The thigh muscle volume and quadriceps muscle volume did not significantly change in men receiving the 25- or 50-mg doses but increased dose dependently at higher doses of testosterone (Table 4, Fig. 1). The changes in thigh muscle and quadriceps muscle volumes correlated with log testosterone levels during treatment ($r = 0.66$, $P = 0.0001$, and $r = 0.55$, $P = 0.0001$, respectively, Fig. 2).

Muscle performance. The leg press strength did not change significantly in the 25- and 125-mg-dose groups but increased significantly in those receiving the 50-, 300-, and 600-mg doses (Table 5). The changes in leg press strength correlated with log testosterone levels during treatment ($r = 0.48$, $P = 0.0005$, Fig. 2) and changes in muscle volume ($r = 0.54$, $P = 0.003$) and fat-free mass ($r = 0.74$, $P < 0.0001$).

Table 3. *Body composition analysis*

Testosterone Dose	Baseline	*Week 20*	Change from Baseline	*P* vs. Zero Change
Fat-free mass (kg) by underwater weighing (overall ANOVA P = 0.0001)				
25 mg	61.1 ± 2.7	58.1 ± 1.7	−1.0 ± 0.5	0.0695
50 mg	66.1 ± 2.5	65.7 ± 2.0	+0.6 ± 0.4	0.1324
125 mg	66.0 ± 2.1	67.9 ± 2.7	+3.4 ± 0.8	0.0024
300 mg	66.9 ± 2.4	72.4 ± 2.8	+5.2 ± 0.8	0.0001
600 mg	64.2 ± 2.2	72.1 ± 2.4	+7.9 ± 0.6	0.0001
Fat mass (kg) by underwater weighing (overall ANOVA P = 0.0001)				
25 mg	8.3 ± 1.4	11.3 ± 1.6	+3.1 ± 0.7	0.0014
50 mg	10.9 ± 1.4	14.3 ± 1.7	+3.5 ± 1.0	0.0096
125 mg	12.2 ± 2.0	10.9 ± 2.1	+0.01 ± 0.5	0.9820
300 mg	11.4 ± 1.6	10.9 ± 1.7	−0.5 ± 0.6	0.4134
600 mg	9.4 ± 1.9	8.8 ± 1.9	−1.1 ± 0.7	0.1132
Fat-free mass (kg) by DEXA scan (overall ANOVA P = 0.0001)				
25 mg	53.6 ± 1.8	53.4 ± 2.0	+0.4 ± 0.3	0.2198
50 mg	58.6 ± 2.3	59.2 ± 2.5	+1.1 ± 0.9	0.2313
125 mg	60.1 ± 2.1	63.1 ± 2.3	+2.9 ± 0.8	0.0054
300 mg	59.0 ± 2.7	64.3 ± 2.2	+5.5 ± 0.7	0.0001
600 mg	57.4 ± 1.9	66.3 ± 2.4	+8.9 ± 0.8	0.0001
Fat mass (kg) by DEXA scan (overall ANOVA P = 0.0004)				
25 mg	10.0 ± 1.8	13.7 ± 1.4	+3.6 ± 1.5	0.0326
50 mg	15.4 ± 1.2	17.9 ± 1.2	+2.6 ± 1.0	0.0324
125 mg	15.2 ± 2.0	15.2 ± 1.9	−0.3 ± 0.8	0.6882
300 mg	16.3 ± 1.2	15.41 ± 1.5	−0.9 ± 0.6	0.1834
600 mg	14.2 ± 1.9	12.0 ± 1.5	−2.0 ± 0.7	0.0141
Ratio of total body water to fat-free mass (percent) (overall ANOVA for change from baseline, P = 0.270)				
25 mg	62.7 ± 2.7	63.7 ± 2.1	+1.1 ± 2.4	
50 mg	62.0 ± 1.9	63.8 ± 2.4	+2.0 ± 2.0	
125 mg	67.0 ± 1.7	63.5 ± 3.0	−3.8 ± 1.6	
300 mg	61.6 ± 2.7	64.6 ± 3.1	+2.1 ± 2.5	
600 mg	65.3 ± 2.4	67.4 ± 2.8	+2.5 ± 1.7	

Values on each day represent the mean (±SE) of all available values on that day. However, the change represents the difference between paired values only. Ratios of total body water assessed by deuterium water dilution to fat-free mass measured by underwater weighing were calculated for each subject and averaged across subjects within each group. DEXA, dual-energy X-ray absorptiometry.

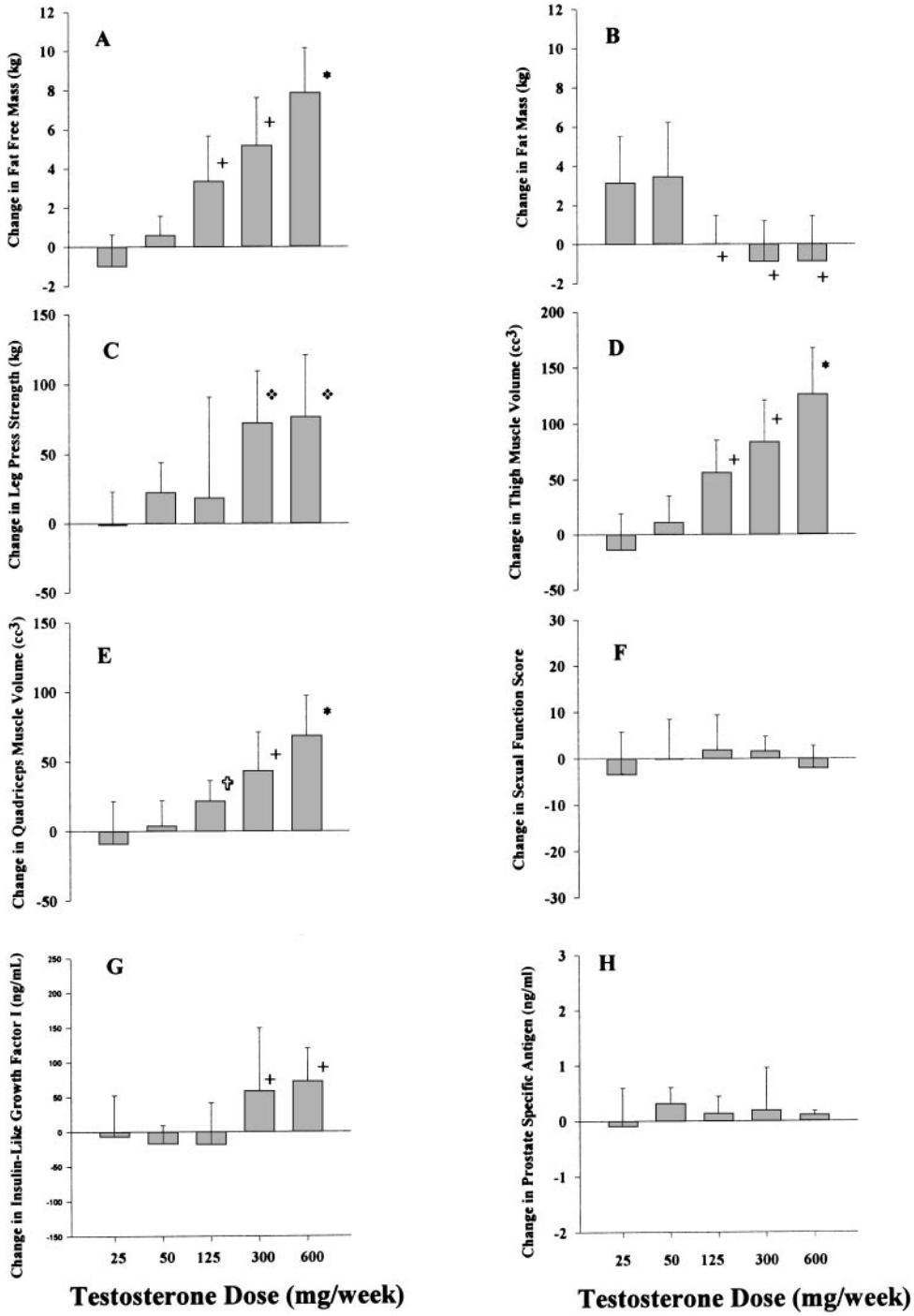

Fig. 1. Change in fat-free mass (*A*), fat mass (*B*), leg press strength (*C*), thigh muscle volume (*D*), quadriceps muscle volume (*E*), sexual function (*F*), insulin-like growth factor I (*G*), and prostate-specific antigen (*H*). Data are means ± SE. *Significant differences from all other groups ($P < 0.05$); ❖ significant difference from 25-, 50-, and 125-mg doses ($P < 0.05$); +significant difference from 25- and 50-mg doses ($P < 0.05$); and ⇧ significant difference from 25-mg dose ($P < 0.05$).

Leg power, measured by the Nottingham leg rig, did not change significantly in men receiving the 25-, 50-, and 125-mg doses of testosterone weekly, but it increased significantly in those receiving the 300- and 600-mg doses. The increase in leg power correlated with log testosterone concentrations ($r = 0.39$, $P = 0.0105$, Fig. 2) and changes in fat-free mass ($r = 0.30$, $P = 0.0392$) and muscle strength ($r = 0.42$, $P = 0.0020$).

Behavioral measures. The scores for sexual activity and sexual desire measured by daily logs did not change significantly at any dose. Similarly, visual-spatial cognition (Table 6) and mood, as assessed by Hamilton's depression and Young's manic scales (data not shown), did not change significantly in any group.

Adverse experiences and safety measures. Hemoglobin levels decreased significantly in men receiving the 50-mg dose but increased at the 600-mg dose; the changes in hemoglobin were positively correlated with testosterone concentrations ($r = 0.66$, $P = 0.0001$) (Table 7). Changes in plasma HDL cholesterol, in contrast, were negatively dependent on testosterone dose ($P = 0.0049$) and correlated with testosterone concentrations ($r =$

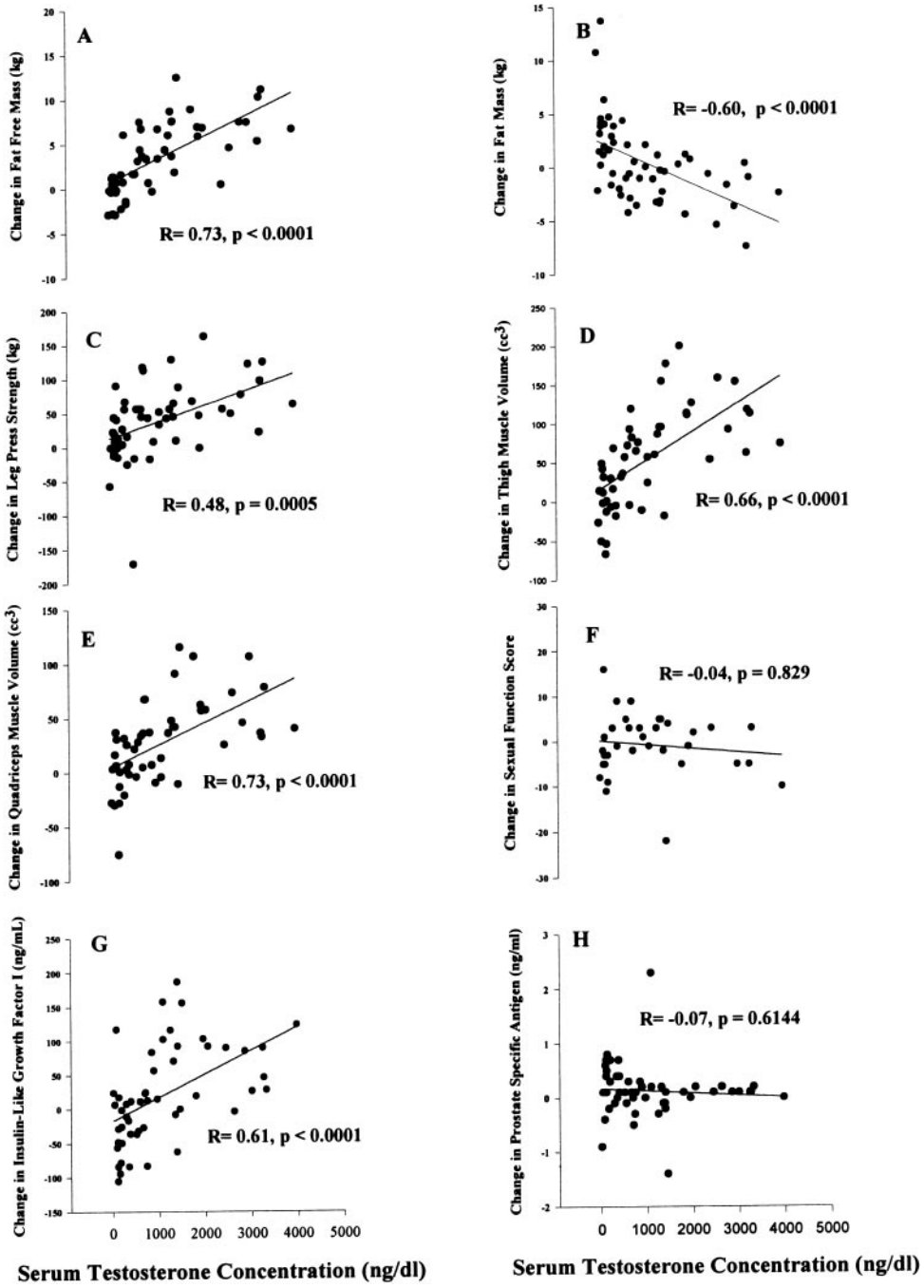

Fig. 2. Relationship between serum testosterone concentrations (T) during treatment (*week 16*) and change in fat-free mass (*A*), fat mass (*B*), leg press strength (*C*), thigh muscle volume (*D*), quadriceps muscle volume (*E*), sexual function (*F*), insulin-like growth factor I (*G*), and prostate-specific antigen (*H*). The correlation coefficient, *r*, was calculated using the logarithmic model, $Y = a + b \cdot X$, where X = log (T), and *a* and *b* represent the intercept and slope.

−0.40, $P = 0.0054$). Total cholesterol, plasma low-density lipoprotein cholesterol, and triglyceride levels did not change significantly at any dose. Serum PSA, creatinine, bilirubin, alanine aminotransferase, and alkaline phosphatase did not change significantly in any group, but aspartate aminotransferase decreased significantly in the 25-mg group. Two men in the 25-mg group, five in the 50-mg group, three in the 125-mg group, seven in the 300-mg group, and two in the 600-mg group developed acne. One man receiving the 50-mg dose reported decreased ability to achieve erections.

DISCUSSION

GnRH agonist administration suppressed endogenous LH and testosterone secretion; therefore, circulating testosterone concentrations during treatment were proportional to the administered dose of testosterone enanthate. This strategy of combined administration of GnRH agonist and graded doses of testosterone enanthate was effective in establishing different levels of serum testosterone concentrations among the five treatment groups. The different levels of circulating testosterone concentrations created by this regimen were associated with dose- and concentration-

Table 4. *Thigh and quadriceps muscle volume measured by MRI*

Testosterone Dose	Baseline	*Week 20*	Change from Baseline	*P* vs. zero change
Thigh muscle volume (overall ANOVA P = 0.0001)				
25 mg	753 ± 46	739 ± 44	−14 ± 10	0.1958
50 mg	833 ± 53	844 ± 58	11 ± 8	0.2332
125 mg	890 ± 49	966 ± 60	56 ± 10	0.0004
300 mg	849 ± 39	933 ± 39	84 ± 12	0.0001
600 mg	802 ± 45	928 ± 48	126 ± 12	0.0001
Quadriceps muscle volume (overall ANOVA P = 0.0001)				
25 mg	436 ± 30	427 ± 27	−9 ± 9	0.3524
50 mg	489 ± 34	493 ± 36	4 ± 7	0.5889
125 mg	508 ± 29	546 ± 36	21 ± 5	0.0027
300 mg	497 ± 25	540 ± 22	43 ± 9	0.0008
600 mg	472 ± 27	540 ± 31	68 ± 8	0.0001

Values (in cm^3) on each day represent the mean (±SE) of all available values on that day. However, the change represents the difference between paired values only.

dependent changes in fat-free mass, fat mass, thigh and quadriceps muscle volume, muscle strength, leg power, hemoglobin, circulating IGF-I, and plasma HDL cholesterol. Serum PSA levels, sexual desire and activity, and spatial cognition did not change significantly at any dose. The changes in fat-free mass, muscle volume, leg press strength and power, hemoglobin, and IGF-I were positively correlated, whereas changes in plasma HDL cholesterol and fat mass were negatively correlated with testosterone dose and total and free testosterone concentrations during treatment.

The compliance with the treatment regimen was high. The participants received 100% of their scheduled GnRH agonist, and 99% of testosterone injections. Serum LH levels were suppressed in all men, demonstrating the effectiveness of GnRH agonist treatment. The treatment regimen was well tolerated. There were no significant changes in PSA or liver enzymes at any dose. However, long-term effects of androgen administration on the prostate, cardiovascular risk, and behavior are unknown.

Table 5. *Change in measures of muscle performance*

Testosterone Dose	Baseline	Treatment	Change from Baseline	*P* vs. Zero Change
Leg press strength (kg) (overall ANOVA P = 0.0003)				
25 mg	355.5 + 31.3	354.2 ± 27.9	−1.2 ± 7.4	0.8701
50 mg	407.8 + 22.0	430.5 ± 22.3	+22.7 ± 7.6	0.0204
125 mg	419.2 ± 24.4	444.6 ± 32.2	+18.4 ± 10.0	0.4195
300 mg	439.8 ± 25.7	525.5 ± 24.9	+72.2 ± 12.4	0.0004
600 mg	431.6 + 27.6	508.1 ± 28.1	+76.5 ± 12.2	0.0001
Leg power (W) (overall ANOVA P = 0.0419)				
25 mg	183.6 ± 10.6	188.9 ± 12.9	5.3 ± 8.4	0.5429
50 mg	234.4 ± 14.2	249.6 ± 17.8	15.2 ± 15.0	0.3468
125 mg	253.8 ± 20.6	265.6 ± 25.2	8.5 ± 15.3	0.5935
300 mg	233.8 ± 20.2	272.4 ± 27.8	38.6 ± 9.4	0.0033
600 mg	212.4 ± 11.0	256.2 ± 13.8	48.1 ± 11.8	0.0015

Values on each day represent the mean (±SE) of all available values on that day. However, the change represents the difference between paired values only.

Table 6. *Change in scores for sexual activity, sexual desire, and spatial cognition*

Testosterone Dose	Baseline	Treatment	Change from Baseline	*P* vs. zero change
Sexual activity scores (overall ANOVA P = 0.7842)				
25 mg	10.7 ± 1.7	8.2 ± 2.9	−2.5 ± 3.2	0.4729
50 mg	14.1 ± 2.1	13.7 ± 1.8	−0.4 ± 2.8	0.9017
125 mg	9.8 ± 2.7	12.0 ± 2.9	2.2 ± 3.1	0.5151
300 mg	11.6 ± 1.6	12.0 ± 1.9	0.7 ± 0.9	0.4761
600 mg	16.1 ± 3.7	15.6 ± 0.5	0.7 ± 2.2	0.7891
Intensity of sexual desire scores (overall ANOVA P = 0.477)				
25 mg	1.9 ± 0.1	1.3 ± 0.4	−0.6 ± 0.4	0.2253
50 mg	2.3 ± 0.1	2.2 ± 0.3	−0.0 ± 0.3	0.9615
125 mg	2.1 ± 0.1	2.0 ± 0.3	−0.1 ± 0.4	0.9078
300 mg	2.2 ± 0.2	2.4 ± 0.2	0.1 ± 0.1	0.3559
600 mg	2.7 ± 0.2	2.2 ± 0.1	0.2 ± 0.2	0.4075
Spatial cognition scores				
1. No. of trial levels on the checkerboard test that the participant reached before the test was terminated (overall ANOVA P = 0.235)				
25 mg	6.8 ± 0.3	6.4 ± 0.3	−0.4 ± 0.3	0.284
50 mg	6.7 ± 0.3	6.7 ± 0.3	0.3 ± 0.3	0.284
125 mg	6.6 ± 0.3	6.6 ± 0.2	0.0 ± 0.4	1.0
300 mg	7.3 ± 0.2	6.7 ± 0.2	−0.6 ± 0.3	0.103
600 mg	6.6 ± 0.2	6.9 ± 0.2	0.3 ± 0.3	0.278
2. No. of checkerboard squares correctly marked in all trials (overall ANOVA P = 0.6309)				
25 mg	28.6 ± 2.2	30.4 ± 2.1	1.8 ± 2.1	0.4272
50 mg	30.0 ± 2.3	34.7 ± 4.9	2.7 ± 3.5	0.5236
125 mg	27.3 ± 3.0	28.1 ± 2.2	0.9 ± 3.8	0.7292
300 mg	32.6 ± 2.1	33.3 ± 1.8	0.7 ± 3.1	0.8241
600 mg	26.7 ± 2.7	32.5 ± 2.1	5.8 ± 2.2	0.0265

Values are means ± SE.

Serum testosterone levels were measured 7 days after previous injection; they reflect the lowest testosterone levels after an injection. Testosterone concentrations were higher at other time points. Weekly injections of testosterone enanthate are associated with fluctuations in testosterone levels (44). Although nadir testosterone concentrations were highly correlated with testosterone enanthate dose, it is possible that sustained testosterone delivery by a patch or gel might reveal different dose-response relationships, particularly with respect to hemoglobin and HDL cholesterol (19).

There were no significant changes in overall sexual activity or sexual desire in any group, including those receiving the 25-mg dose. Testosterone replacement of hypogonadal men improves frequency of sexual acts and fantasies, sexual desire, and response to visual erotic stimuli (3, 13, 15, 17, 31, 41). Our data demonstrate that serum testosterone concentrations at the lower end of male range can maintain some aspects of sexual function (3, 13). Testosterone has been shown to regulate nitric oxide synthase activity in the cavernosal smooth muscle (32), and it is possible that optimum penile rigidity might require higher testosterone levels than those produced by the 25-mg dose.

This study demonstrates that an increase in circulating testosterone concentrations results in dose-de-

Table 7. *Changes in hemoglobin, plasma HDL cholesterol, and PSA*

Testosterone Dose	Baseline	Week 20	Change from Baseline	*P* vs. Zero Change
Hemoglobin (g/l), (overall ANOVA P = 0.0001)				
25 mg	143.5 ± 3.5	139.0 ± 2.5	−5.2 ± 3.5	0.1759
50 mg	150.8 ± 3.3	146.6 ± 2.0	−7.4 ± 2.3	0.0153
125 mg	141.9 ± 2.6	146.1 ± 3.1	2.5 ± 2.4	0.3061
300 mg	143.5 ± 2.2	149.6 ± 3.1	6.1 ± 2.9	0.0639
600 mg	141.5 ± 2.3	155.7 ± 2.2	14.2 ± 2.0	0.0001
PSA (ng/ml), (overall ANOVA P = 0.5290)				
25 mg	1.0 ± 0.2	1.0 ± 0.2	−0.1 ± 0.2	0.6870
50 mg	0.8 ± 0.1	1.1 ± 0.2	0.3 ± 0.1	0.0186
125 mg	0.7 ± 0.1	0.8 ± 0.1	0.1 ± 0.1	0.1721
300 mg	0.7 ± 0.1	0.9 ± 0.3	0.2 ± 0.2	0.4525
600 mg	0.5 ± 0.1	0.7 ± 0.1	0.1 ± 0.0	0.0010
Plasma HDL cholesterol (mg/dl) (overall ANOVA P = 0.0049)				
25 mg	46 ± 3	51 ± 4	+4.5 ± 2.6	0.1202
50 mg	48 ± 3	47 ± 5	−0.7 ± 4.0	0.8653
125 mg	48 ± 2	43 ± 3	−4.0 ± 1.7	0.0476
300 mg	47 ± 3	41 ± 2	−5.7 ± 2.8	0.0690
600 mg	43 ± 2	34 ± 2	−8.4 ± 1.8	0.0005

Values on each day represent the mean (±SE) of all available values on that day. However, the change from baseline represents the difference between paired values only. PSA, prostate-specific antigen; HDL, high-density lipoprotein.

pendent increases in fat-free mass, muscle size, strength, and power. The relationships between circulating testosterone concentrations and changes in fat-free mass and muscle size conform to a single log-linear dose-response curve. Our data do not support the notion of two separate dose-response curves reflecting two independent mechanisms of testosterone action on the muscle. Forbes et al. (22) predicted 25 years ago that the muscle mass accretion during androgen administration is related to the cumulative androgen dose, the product of daily dose and treatment duration. Our data are consistent with Forbes's hypothesis of a linear relationship between testosterone dose and lean mass accretion; however, we do not know whether increasing the treatment duration would lead to further gains in muscle mass.

In addition, we do not know whether responsiveness to testosterone is attenuated in older men. Testosterone dose-response relationships might be modulated by other muscle growth regulators, such as nutritional status, exercise and activity level, glucocorticoids, thyroid hormones, and endogenous growth hormone secretory status.

Serum PSA levels decrease after androgen withdrawal, and testosterone replacement of hypogonadal men increases PSA levels into the normal range (16, 34). We did not find significant changes in PSA at any dose, indicating that the lowest dose of testosterone maintained PSA levels. We did not measure prostate volume in this study; therefore, we do not know whether prostate volume exhibits the same relationship with testosterone dose as PSA levels.

Hemoglobin levels changed significantly in relation to testosterone dose and concentration. Testosterone regulates erythropoiesis through its effects on erythropoietin and stem cell proliferation (14, 35, 40). Although modest increments in hemoglobin might be beneficial in androgen-deficient men with chronic illness who are anemic, marked increases in hemoglobin levels could increase the risk of cerebrovascular events (25) and hypertension (42).

Although men, on average, perform better on tests of spatial cognition than women, testosterone replacement has not been consistently shown to improve spatial cognition in hypogonadal men (1, 29, 48). We did not find changes in spatial cognition at any dose. The effect size of gender differences in spatial cognition is small; it is possible that our study did not have sufficient power to detect small differences. We cannot exclude the possibility that gender differences in spatial cognition might be due to organizational effects of testosterone and might not respond to changes in testosterone levels in adult men.

Although mean change in fat-free mass and muscle size correlated with testosterone dose and concentration, there was considerable heterogeneity in response to testosterone administration within each group. These individual differences in response to androgen administration might reflect differences in activity level, testosterone metabolism, nutrition, or polymorphisms in androgen receptor, myostatin, 5-α-reductase, or other muscle growth regulators.

Our data demonstrate that different androgen-dependent processes have different testosterone dose-response relationships. Some aspects of sexual function and spatial cognition, and PSA levels, were maintained by relatively low doses of testosterone in GnRH agonist-treated men and did not increase further with administration of higher doses of testosterone. In contrast, graded doses of testosterone were associated with dose and testosterone concentration-dependent changes in fat-free mass, fat mass, muscle volume, leg press strength and power, hemoglobin, IGF-I, and plasma HDL cholesterol. The precise mechanisms for the tissue- and function-specific differences in testosterone dose dependence are not well understood (36). Although only a single androgen receptor protein is expressed in all androgen-responsive tissues, tissue specificity of androgen action might be mediated through combinatorial recruitment of tissue-specific coactivators and corepressors (36).

Testosterone doses associated with significant gains in fat-free mass, muscle size, and strength were associated with significant reductions in plasma HDL concentrations. Further studies are needed to determine whether clinically significant anabolic effects of testosterone can be achieved without adversely affecting cardiovascular risk. Selective androgen receptor modulators that preferentially augment muscle mass and strength, but only minimally affect prostate and cardiovascular risk factors, are desirable (36).

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