

Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men

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Sinha-Hikim, Indrani, Stephen M. Roth, Martin I. Lee, and Shalender Bhasin. Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. *Am J Physiol Endocrinol Metab* 285: E197–E205, 2003. First published April 1, 2003; 10.1152/ajpendo.00370.2002.—Testosterone (T) supplementation in men induces muscle fiber hypertrophy. We hypothesized that T-induced increase in muscle fiber size is associated with a dose-dependent increase in satellite cell number. We quantitated satellite cell and myonuclear number by using direct counting and spatial orientation methods in biopsies of vastus lateralis obtained at baseline and after 20 wk of treatment with a gonadotropin-releasing hormone agonist and a 125-, 300-, or 600-mg weekly dose of T enanthate. T administration was associated with a significant increase in myonuclear number in men receiving 300- and 600-mg doses. The posttreatment percent satellite cell number, obtained by direct counting, differed significantly among the three groups (ANCOVA $P < 0.000001$); the mean posttreatment values (5.0 and 15.0%) in men treated with 300- and 600-mg doses were greater than baseline (2.5 and 2.5%, respectively, $P < 0.05$ vs. baseline). The absolute satellite cell number measured by spatial orientation at 20 wk (1.5 and 4.0/mm) was significantly greater than baseline (0.3 and 0.6/mm) in men receiving the 300- and 600-mg doses ($P < 0.05$). The change in percent satellite cell number correlated with changes in total ($r = 0.548$) and free T concentrations ($r = 0.468$). Satellite cell and mitochondrial areas were significantly higher and the nuclear-to-cytoplasmic ratio lower after treatment with 300- and 600-mg doses. We conclude that T-induced muscle fiber hypertrophy is associated with an increase in satellite cell number, a proportionate increase in myonuclear number, and changes in satellite cell ultrastructure.

anabolic steroids; anabolic effects of androgens; mechanisms of androgen action; testosterone effects on muscle stem cells

TESTOSTERONE SUPPLEMENTATION increases muscle mass in healthy hypogonadal men (7, 11, 25, 43, 48, 49), older men with low testosterone levels (26, 44, 45), and men with chronic illnesses and low testosterone levels (5, 8, 14). The anabolic effects of testosterone on muscle mass are dose and concentration dependent (9). Tes-

tosterone-induced increase in muscle mass is associated with a dose-dependent increase in cross-sectional areas of both type I and type II muscle fibers but is not attended by a change in muscle fiber number (40). However, the mechanisms by which testosterone increases muscle mass are not well understood (4). The prevalent dogma for the past 50 years has been that testosterone increases muscle mass by stimulating fractional muscle protein synthesis (11, 13, 28, 29, 37, 47, 52). Indeed, lowering of circulating testosterone concentrations by administration of a gonadotropin-releasing hormone (GnRH) agonist is associated with a reduction in fractional muscle protein synthesis (29); conversely, testosterone replacement in healthy hypogonadal men increases muscle protein synthesis (11). However, in a recent study (40), we noted that testosterone-induced muscle fiber hypertrophy is associated with a dose-dependent increase in myonuclear number. It would be difficult to explain this increase in myonuclear number if the sole effect of testosterone were on muscle protein synthesis. Muscle growth during postnatal development or hypertrophy is dependent on the addition of myonuclei to muscle fibers (1, 2, 30, 35, 36). Because the nuclei within the muscle fibers are postmitotic, new myonuclei must be contributed by the satellite cells that are outside the muscle fiber. Inhibition of satellite cell proliferation by gamma irradiation at doses that do not produce overt cellular damage prevents the muscle growth and increase in myonuclear number that follow muscle atrophy due to hindlimb suspension (1). Muscle remodeling and repair following injury often involve satellite cell replication and recruitment of new stem cells into the myogenic cell lineage (35, 36). Similarly, the hypertrophy of levator ani muscle in the female rat induced by exogenous testosterone administration is associated with satellite cell entry into the cell cycle and proliferation (20–22, 31, 32, 46). Taken together, these animal data suggest that an increase in satellite cell number is an important antecedent of an increase in myonuclear number and muscle fiber adaptation leading to hypertrophy. However, we do not know whether similar changes in satellite cell number are also observed in

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testosterone-treated humans. Furthermore, previous reports studied satellite cells in the levator ani muscle of rodents, which differs significantly from skeletal muscle in the magnitude of response to castration and testosterone supplementation. Therefore, in this study, we tested the hypothesis that testosterone-induced muscle fiber hypertrophy would be associated with an increase in satellite cell number in the skeletal muscle of testosterone-treated men. Because the gains in muscle mass during testosterone supplementation are correlated with testosterone dose and concentrations (9), we hypothesized that the change in satellite cell number would also be correlated with testosterone dose and concentrations.

Accordingly, we measured the number of satellite cells in muscle biopsies obtained from healthy men in whom endogenous testosterone production was suppressed by administration of a GnRH agonist, and different levels of serum testosterone concentrations were created by weekly intramuscular injections of graded doses of testosterone enanthate. The design and the main findings of this study have been published (9, 40). Because significant increases in type I and type II muscle fiber cross-sectional areas were observed only in men receiving the 600-mg weekly doses of testosterone enanthate (40), and to a lesser extent in those receiving the 300-mg dose, we focused our analysis of satellite cells on these two groups. In addition, we included the 125-mg dose group as a control, because men in this group did not experience demonstrable muscle fiber hypertrophy or an increase in myonuclear number.

MATERIALS AND METHODS

Subjects. This was a double-blind, randomized study. The details of the study design have been previously published (9, 40). Briefly, the participants were 61 healthy, eugonadal men, 18–35 yr of age. Each participant provided informed consent, and the study was approved by the institutional review boards of Charles R. Drew University and Harbor-UCLA Research and Education Institute. Participants were randomly assigned to one of five experimental groups to receive monthly injections of a long-acting GnRH agonist (decapetyl; Debio Pharm, Geneva, Switzerland) to suppress endogenous testosterone production and weekly injections of 25, 50, 125, 300, or 600 mg of testosterone enanthate for 20 wk. In our previous study, we had noted muscle fiber hypertrophy in men receiving the 600-mg dose and to a lesser extent in men in the 300-mg dose group (40); therefore, we focused our analysis on these groups. We included as a control group one additional group that had not demonstrated a significant increase in muscle fiber cross-sectional area. In this investigation, muscle biopsies were available for electron microscopy before and after testosterone treatment in six men receiving 125 mg, eight men receiving 300 mg, and five men receiving 600 mg of testosterone enanthate weekly.

Nutritional intake and exercise stimulus were controlled at the outset of the study, as previously described (9, 40). Two weeks before the initiation of the study, subjects were prescribed a diet standardized for energy intake at 150 kJ·kg⁻¹·day⁻¹ and protein intake at 1.3 g·kg⁻¹·day⁻¹. These instructions were reinforced every 4 wk during a

meeting with the dietitian in which each subject's actual nutrient intake was verified by analysis of 3-day food records.

Hormone assays. Serum total testosterone was measured by a previously reported radioimmunoassay (5–7, 9, 10). Free testosterone was separated by an equilibrium dialysis procedure and measured in the dialysate by radioimmunoassay (41). The sensitivity of total testosterone assay was 0.6 ng/dl; intra-assay coefficient of variation was 8.2%, and interassay coefficient of variation was 13.2%. For the free testosterone assay, the sensitivity was 0.22 pg/ml, and intra- and inter-assay coefficients of variation were 4.2 and 12.3%, respectively.

Muscle biopsy and tissue fixation. Percutaneous needle muscle biopsies were obtained from the midbelly of the vastus lateralis muscle in the quadriceps muscle group before and within 5 days after the final testosterone enanthate injection. Biopsy specimens were immediately fixed in 5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) overnight and processed for epoxy embedding (38). After fixation, the tissues were washed in cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in graded series of ethanol, and embedded in Epon. During embedding, the tissue blocks were oriented longitudinally. Embedded tissue blocks were sectioned with an LKB ultramicrotome. Thin sections showing pale gold interference color were stained with uranyl acetate and lead citrate and examined with a Hitachi 600 electron microscope.

Spatial distribution of myonuclei and satellite cells. All samples were processed and micrographed by an observer who was unaware of the treatment assignment. Parts of longitudinally oriented, healthy muscle fibers were selected randomly and photographed using an electron microscope at ×3,500 magnification. The electron micrographs were further magnified three times to obtain a total magnification of ×10,500. Montages of the fibers were constructed, and total numbers of myonuclei and satellite cells were counted and expressed per unit length (mm) of muscle fiber. Six montages were constructed for each subject before and after treatment (averaging 0.9–1.2 mm of muscle fiber in each sample).

Identification of satellite cells. The satellite cells were identified on the basis of their characteristic ultrastructural morphology (34, 42) by using the following criteria (Fig. 1). 1) The satellite cells are mononucleated cells with an independent cytoplasm that are inside the basal lamina of the muscle fibers. 2) The satellite cells are located outside the sarcolemma of the muscle fiber.

Determination of satellite cell proportion by direct counting and by spatial distribution. We used two separate counting procedures to determine the number of satellite cells and myonuclear number in muscle fibers. In the direct-counting procedure, the numbers of myonuclei and satellite cells were counted in randomly selected photomicrographs from each subject. A total of 620–1,189 myonuclei were photographed in each of the three groups (97–210 myonuclei per biopsy) at each time point, and the number of satellite cells was expressed as a percentage of myonuclear number.

In addition, all myonuclei and satellite cells were counted in the reconstructed fiber montages (6 per biopsy) to obtain the satellite cell percentage by spatial distribution. Percent satellite cells equaled the number of satellite cells divided by the sum of myonuclear number and the number of satellite cells and was expressed as a percentage (19, 34).

Morphometry of the satellite cells. The electron micrographs of the satellite cells were evaluated to identify possible differences in morphology before and after treatment in each group. The point counting method was used to obtain

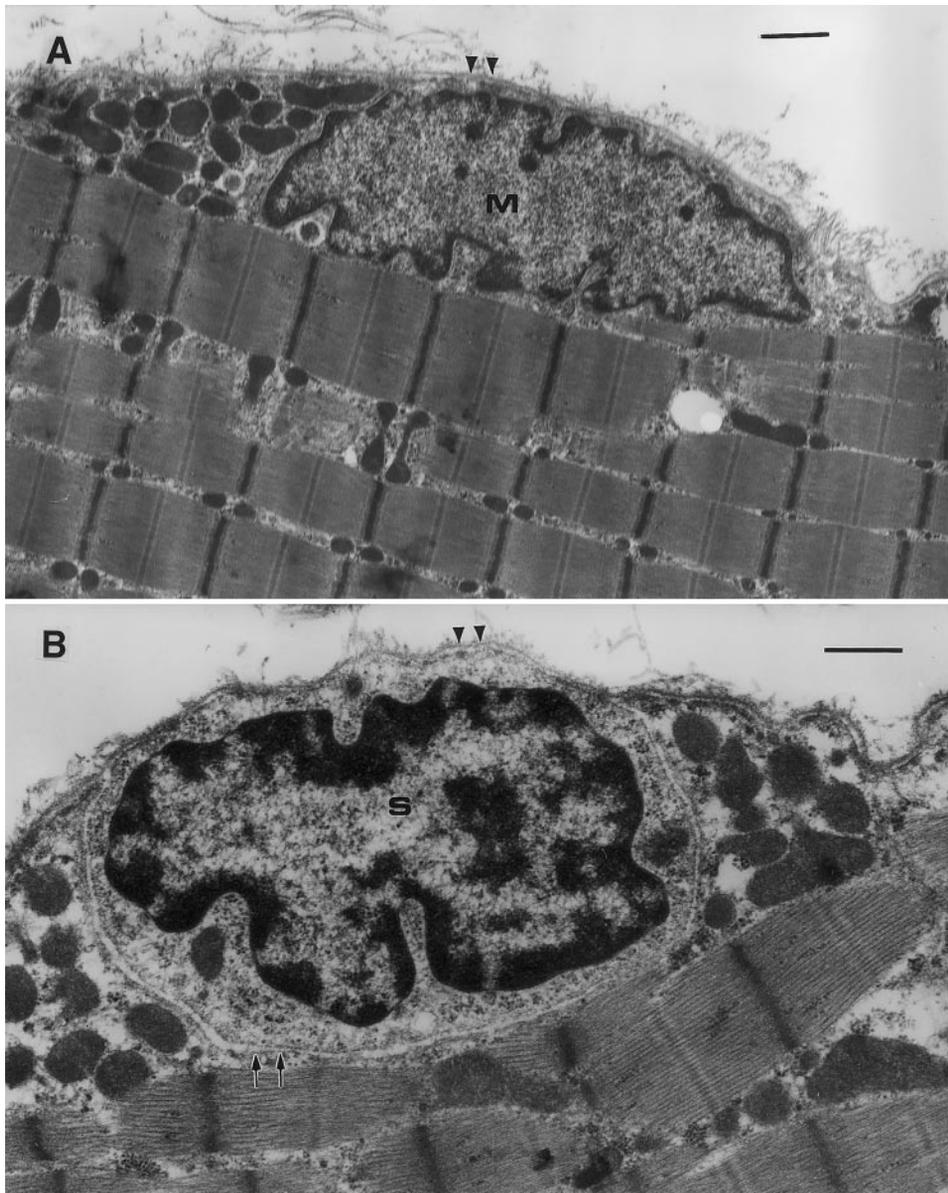


Fig. 1. Electron micrograph of a typical myonucleus (A) and a muscle satellite cell (B). Muscle satellite cells (S) were identified by their location inside the basal lamina (arrowheads) and outside the sarcolemma (arrows) and an independent cytoplasm. In contrast, a myonucleus (M) is located inside the sarcolemma of the muscle fiber and does not contain an independent cytoplasm. Bar, 1 μ m.

the volume densities [volume of a given component per unit volume of the cell (V_v)] of the satellite cell components (3, 18, 50).

The total cellular, nuclear, and mitochondrial areas were calculated by the equation: $A = P \cdot U^2$ (15, 16, 38), where A denotes area, P denotes the number of points falling on the satellite cell or its nucleus, and U represents the distance between two neighboring points in the grid, taking into account the magnification used. Because no significant changes were observed in either muscle volume or satellite cell number in men in the 125-mg dose group, we performed the ultrastructural analysis only in the 300- and 600-mg dose groups. Also, because the number of satellite cells in baseline biopsies was very small, we pooled the baseline data on ultrastructural analysis.

Statistical analyses. All data are presented as means \pm SE for each of the three treatment groups. The main outcome measures were absolute and percent satellite cell number and myonuclear number. We used analysis of covariance (ANCOVA) to compare posttreatment values, adjusting for

baseline values for each outcome measure. If the overall ANCOVA revealed a significant effect, the statistical significance of the between-group differences was tested by the Tukey-Kramer multiple comparison procedure. We also compared the change from baseline in each outcome measure by one-way ANOVA. The significance of the change from baseline to posttreatment within a dose group was evaluated using the paired t -test. For all statistical analyses, P values < 0.05 were considered significant. The NCSS2001 (version 5/02, Kaysville, UT) and Sigma-Stat programs (SPSS, Chicago, IL) were used for all statistical analyses. The correlations between serum testosterone concentrations and various outcomes were evaluated using the Pearson correlation coefficient and were tested for significance from zero with an appropriate t -test.

RESULTS

Of the 61 men who enrolled in the main study, 54 completed the treatment (9). Of these, sufficient tissue

was available for evaluation of satellite cells by electron microscopy in 19 men: six in the 125-mg group, eight in the 300-mg group, and five in the 600-mg group. We selected the 300- and 600-mg groups for evaluation of satellite cells because men in these groups had experienced significant increases in muscle fiber area and myonuclear number (40); in addition, we selected the 125-mg group as a reference for comparison because men in this group had not demonstrated significant changes in muscle fiber cross-sectional area.

A detailed description of the hormonal changes in all 61 treated men has been published (7). Among the 19 men who were evaluated by electron microscopy, the three groups did not significantly differ in their baseline characteristics, including age, body weight, and height, or in serum total and free testosterone concentrations (Table 1). Combined administration of GnRH agonist and graded doses of testosterone enanthate resulted in dose-dependent changes in serum total and free testosterone concentrations and muscle volumes (change in thigh muscle volumes by MRI, +21, +43, and +68 cm³, overall $P = 0.001$), as previously reported (9). Consistent with our previous report (40), testosterone administration was associated with dose- and concentration-dependent increases in cross-sectional area of type I and type II muscle fibers (data not shown).

Myonuclear number. When we used the spatial distribution method, the myonuclear number per millimeter of muscle fiber length did not differ significantly among the three groups at baseline (Table 2). However, posttreatment myonuclear number after combined administration of a GnRH agonist and graded doses of testosterone was significantly different among the three groups, after control for baseline values (ANCOVA $P = 0.003$). The change from baseline in myonuclear number in the three groups was significantly different (ANOVA, $P < 0.001$; Fig. 2). The change in myonuclear number was greater in men receiving the 600-mg dose than in those receiving the 125- or the 300-mg dose of testosterone enanthate ($P < 0.05$ for each of the two comparisons based on the global significance level of the Tukey-Kramer procedure). The number of myonuclei was significantly greater after treatment compared with baseline in men receiving the 300-mg ($P < 0.05$ vs. baseline) and 600-mg ($P < 0.05$ vs. baseline) doses of testosterone enanthate. The

Table 1. Baseline characteristics of subjects in the 3 treatment groups

	Testosterone Dose, mg/wk		
	125	300	600
Age, yr	29 ± 1	23 ± 1	26 ± 2
Body weight, kg	79.6 ± 4.4	80.1 ± 3.3	80.1 ± 7.4
Height, cm	177.9 ± 3.1	175.6 ± 2.3	173.7 ± 2.9
Serum testosterone, ng/dl	536 ± 44	638 ± 79	663 ± 119
Free testosterone, pg/ml	52 ± 8	74 ± 12	69 ± 9

Values are means ± SE.

Table 2. Myonuclear number per millimeter of muscle fiber assessed by spatial orientation method before and after treatment with GnRH agonist and graded doses of testosterone

	Testosterone Dose, mg/wk		
	125	300	600
Baseline	8 ± 1	9 ± 2	7 ± 1
Week 20	9 ± 1	13 ± 2	19 ± 3
N	6	8	5
P vs. baseline	0.45	<0.05	<0.05

Myonuclear no./mm muscle fiber was measured by spatial orientation by using electron microscopy. Baseline and week 20 values are means ± SE. GnRH, gonadotropin-releasing hormone; N, no. of subjects per group. Overall ANOVA $P < 0.013$.

change in myonuclear number was significantly correlated with serum total ($r = 0.53$, $P = 0.02$) testosterone concentrations.

Satellite cell number. By use of the spatial distribution method, the number of satellite cells at baseline in all three groups varied from 0 to 2 satellite cells/mm of muscle fiber and did not differ significantly among the three groups (Table 3). By ANCOVA, the mean post-treatment values for satellite cell number per millime-

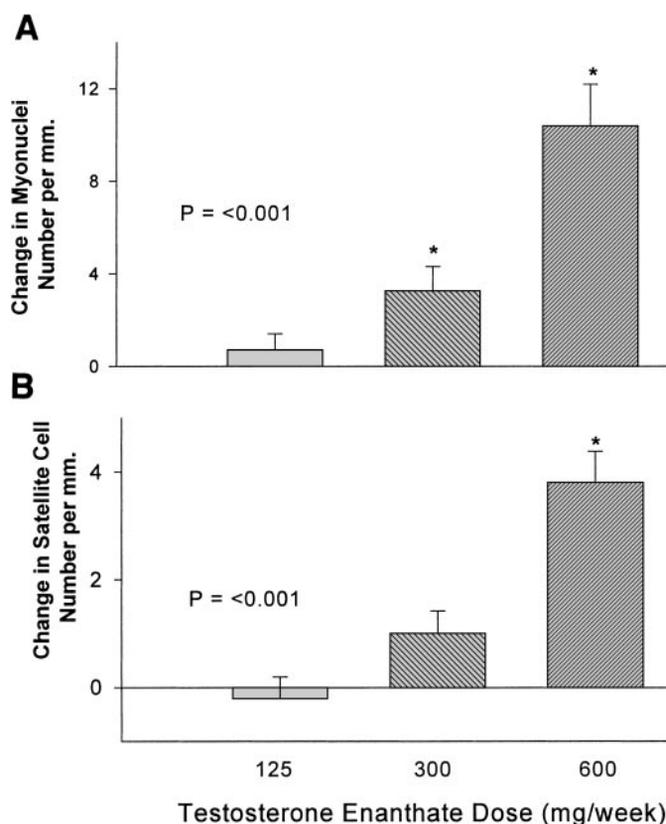


Fig. 2. Effect of testosterone administration on myonuclear number (A) and absolute satellite cell number (B). Number of myonuclei and satellite cells per mm of muscle fiber length were computed by spatial distribution. Change was calculated as the difference between posttreatment and baseline values. *Values significantly different from 0. X-axis: weekly dose of testosterone enanthate is shown. * $P < 0.05$ vs. 0 change.

Table 3. Satellite cell number before and after treatment with GnRH agonist and graded doses of testosterone

	Testosterone Dose		
	125	300	600
<i>Satellite cell no./mm muscle fiber by spatial orientation</i> (Overall ANOVA $P < 0.007$)			
Baseline	0.7 ± 0.5	0.3 ± 0.3	0.0 ± 0.0
Week 20	0.5 ± 0.5	1.4 ± 0.8	7.0 ± 1.0
N	6	8	5
P vs. baseline	0.82	<0.05	<0.05
<i>Satellite cell no. as % myonuclear no. by direct counting</i>			
Baseline	1.9 ± 0.7	2.5 ± 0.8	2.5 ± 0.5
Week 20	1.6 ± 0.4	5.0 ± 0.8	15.0 ± 1.6
N	6	8	5
P vs. baseline	1.0	<0.05	<0.001

ter of muscle fiber measured by spatial orientation were significantly different among the three groups, after control for the baseline values ($P = 0.0002$). The posttreatment satellite cell number was greater in men receiving the 600-mg dose than in those receiving the 125- or the 300-mg doses ($P < 0.05$ for each comparison by use of the global significance level of the Tukey-Kramer procedure). The increase in the number of satellite cells per millimeter of muscle fiber from baseline was statistically significant in men receiving the 300- and 600-mg testosterone doses ($P < 0.05$ vs. baseline for each comparison; Fig. 2). The change in satellite cell number was significantly correlated with the change in total ($r = 0.548$, $P = 0.015$) and free testosterone concentrations ($r = 0.468$, $P = 0.043$; Fig. 3A).

The mean posttreatment values for satellite cell number, measured by direct counting and expressed as a percentage of myonuclear number (Table 3), were also significantly different by ANCOVA, after control for the baseline values ($P < 0.000001$). The change from baseline in satellite cell number was greater in the 600-mg dose compared with the 125- and 300-mg dose groups ($P < 0.05$ for each comparison). The satellite cell number after treatment was significantly greater compared with baseline in men treated with the 300- and 600-mg doses of testosterone ($P < 0.05$ for each comparison).

Satellite cell morphology and ultrastructure. The mean satellite cell area was significantly greater after treatment with 300- and 600-mg doses compared with baseline area (ANOVA $P < 0.05$; Table 4 and Fig. 4). The mean absolute nuclear area was not significantly different after treatment with the 300- or 600-mg dose compared with baseline ($P = 0.40$); however, the ratio of the nuclear to total cellular area decreased significantly in men receiving the 300- and 600-mg doses ($P < 0.005$ by ANOVA). The increase in cytoplasmic area compared with the nuclear area during treatment was statistically significant ($P < 0.005$ by ANOVA). The ratio of Vv% nucleus to Vv% cytoplasm was significantly different after treatment with the 300- and 600-mg doses ($P < 0.01$). The mean satellite cell mito-

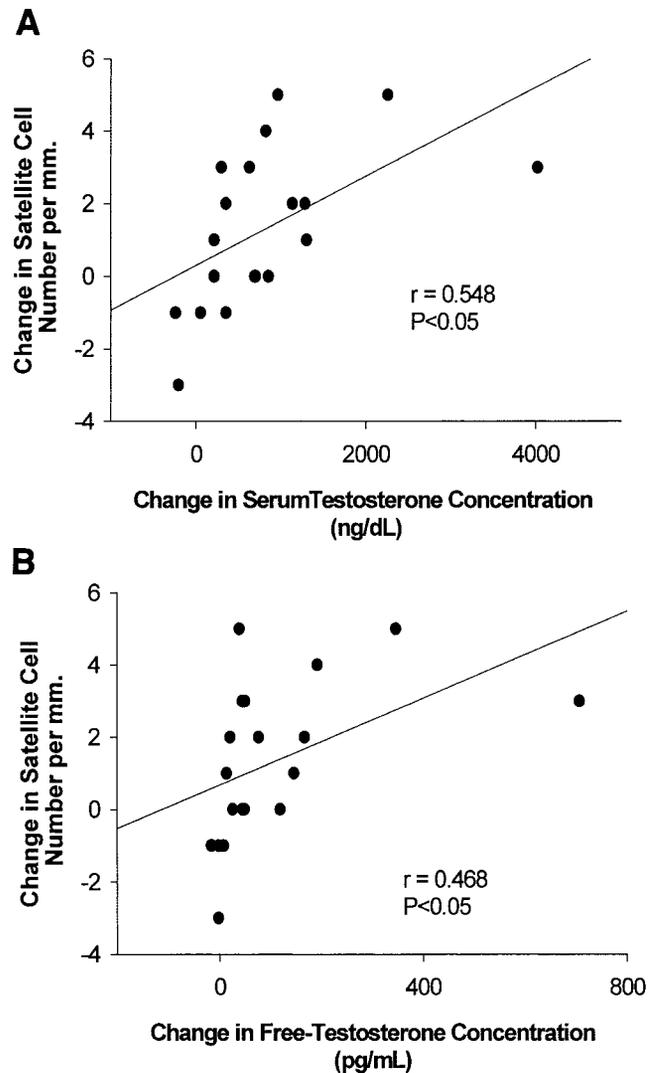


Fig. 3. Correlation of change in satellite cell number with change in total (A) and free (B) testosterone concentrations.

chondrial area increased significantly from baseline in men receiving the 300 and 600 mg of testosterone enanthate weekly ($P < 0.05$). The heterochromatin volume, expressed as a percentage of satellite cell nu-

Table 4. Morphometric analysis of satellite cell ultrastructure

Ultrastructural Feature	Baseline (n = 19)	Testosterone Dose, mg/wk		ANOVA <i>P</i> Value
		300	600	
Satellite cell area, μm^2	18.4 ± 1.4	21.4 ± 1.7	26.4 ± 2.3	<0.05
Nuclear area, μm^2	11.9 ± 1.1	11.3 ± 1.3	14.1 ± 1.2	NS
Ratio of nuclear to cellular area	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.02	<0.005
Ratio of nucleus to Vv% cytoplasm	2.3 ± 0.3	1.3 ± 0.1	1.4 ± 0.1	<0.01
Mitochondrial area, μm^2	0.2 ± 0.1	0.7 ± 0.3	1.2 ± 0.4	<0.05

Values are means \pm SE; n, no. of satellite cells counted; NS, not significant. Vv, volume/volume.

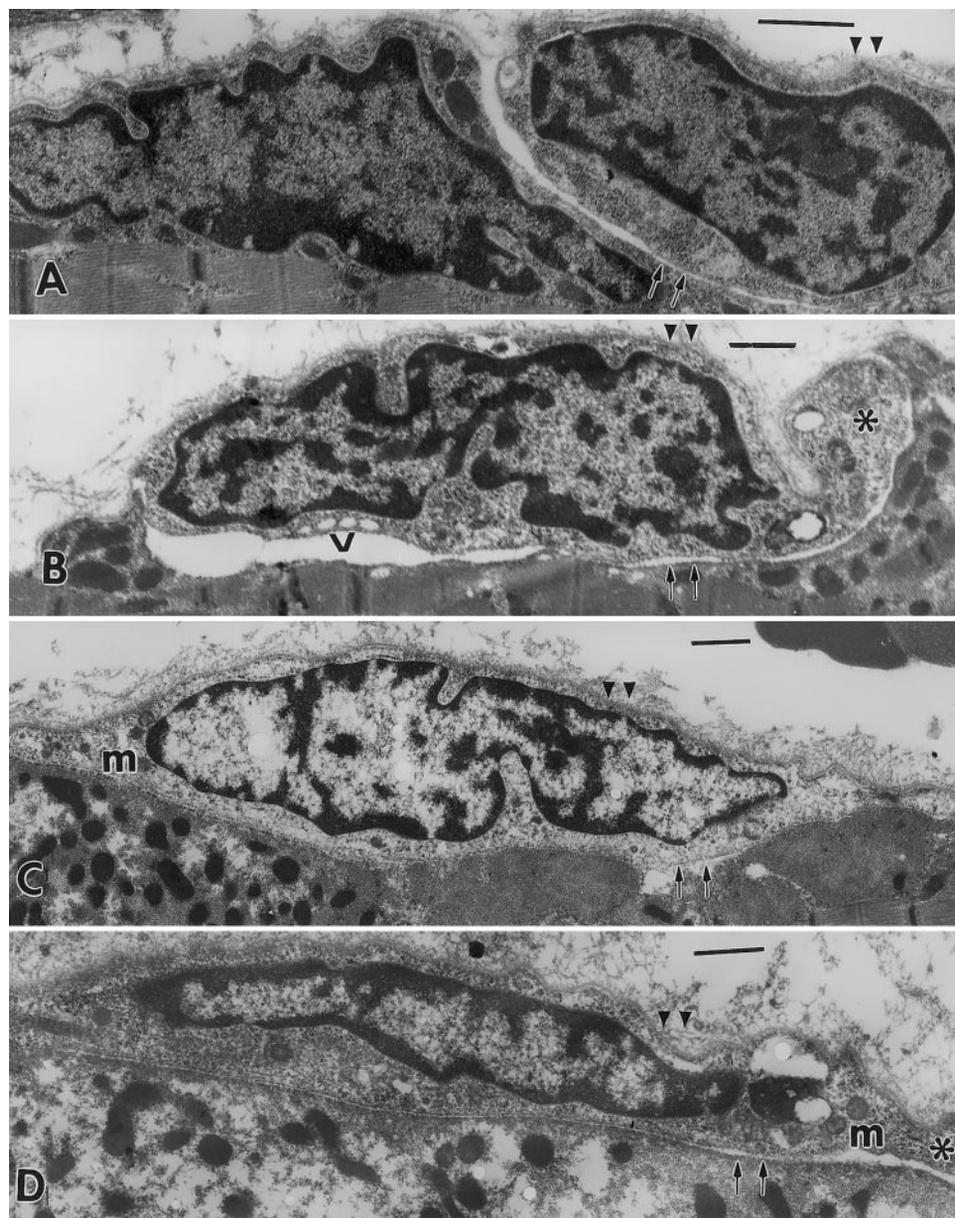


Fig. 4. Satellite cell ultrastructure in 1 subject at baseline and after 20 wk of treatment with gonadotropin-releasing hormone (GnRH) agonist and 600 mg of testosterone enanthate weekly. *A*: electron micrograph of a muscle biopsy obtained at baseline. *B*, *C*, and *D*: electron micrographs of biopsies obtained after 20 wk of treatment with 600 mg of testosterone enanthate and GnRH agonist. Arrowheads, basilar lamina; upright thin arrows, sarcolemma; *, lamellopodia; V, pinocytotic vesicles; m, mitochondria. Bar, 1 μ m.

clear volume ($P = 0.45$), did not differ significantly among the groups.

We noted additional qualitative changes in satellite cell morphology after testosterone treatment, including the presence of lamellopodia in one-half of the posttreatment biopsies in the 300- and 600-mg groups, a greater amount of endoplasmic reticulum, and a greater number of pinocytotic vesicles compared with the baseline biopsies (Fig. 4).

DISCUSSION

Our data demonstrate that testosterone-induced muscle fiber hypertrophy is associated with increases in the numbers of myonuclei and satellite cells. The satellite cell number, measured by spatial orientation as well as by direct counting methods, increased significantly in proportion to the testosterone dose. The

increase in satellite cell number during testosterone administration was dose dependent: higher doses of testosterone were associated with a greater increase in satellite cell number than lower doses. Testosterone supplementation was also associated with ultrastructural changes in satellite cells, including increased cellular and mitochondrial areas, a decrease in the nuclear-to-cytoplasmic ratio, and an increased number of lamellopodia compared with baseline.

In this study, significant increases in satellite cell number were observed only in men who were treated with supraphysiological doses of testosterone enanthate. We do not know whether physiological replacement doses of testosterone when administered to older men with low testosterone concentrations or to men with chronic illness would produce similar anabolic effects.

Because of the difficulties inherent in obtaining multiple muscle biopsies from volunteers and the labor-intensive nature of electron microscopic analysis, the sample size was relatively small. The number of satellite cells in the adult muscle is relatively small and constitutes a very small fraction of the myonuclear number. Not surprisingly, in baseline samples, we were able to locate only one or two, and in some subjects no satellite cells. Although this increases the imprecision in quantitation of satellite cell number in baseline biopsies, the remarkable increases in satellite cell numbers following testosterone treatment observed in the present study are particularly striking. Similarly, the ultrastructural details were evaluated in a small number of satellite cells that were identified and should be viewed cautiously. The satellite cells were identified by electron microscopy using previously established histological criteria (19, 34); the satellite cells recognized by these criteria might not represent a monomorphic cell population. There is growing evidence that these cells represent myogenic cells at more than one stage of differentiation (17).

Although testosterone's effects on muscle have generated enormous controversy for over five decades (10, 51), a number of recent studies are in agreement that testosterone supplementation increases muscle mass in healthy hypogonadal men (7, 11, 25, 43, 48, 49), older men with low or low normal testosterone concentrations (13, 26, 44, 45), and men with chronic illnesses (5, 8, 14). Our dose-response study demonstrated that testosterone's anabolic effects on fat-free mass and muscle size are dose and concentration dependent (9). The mechanisms by which testosterone induces muscle mass accretion are not well understood. The prevalent hypothesis that testosterone stimulates muscle protein synthesis (11, 13, 47) emerged from observations made 50 years ago that testosterone promotes nitrogen retention in castrated males of many mammalian species (26), in prepubertal boys, and in women (27). Lowering of testosterone concentrations by administration of a GnRH agonist is associated with decreased muscle protein synthesis (29). Testosterone supplementation in healthy hypogonadal men (11, 29) and older men with low normal or low testosterone levels (47) increases fractional muscle protein synthesis. Indeed, muscle fiber hypertrophy could not possibly occur without an increase in muscle protein synthesis or a decrease in muscle protein degradation. However, this hypothesis cannot explain our observations that testosterone administration is associated with increases in satellite cell and myonuclear number. Our data lead us to conclude that an increase in satellite cell number and the subsequent fusion of satellite cells with muscle fibers resulting in an increase in myonuclear number and muscle fiber hypertrophy are likely involved in mediating androgen-induced muscle hypertrophy. However, our data cannot exclude the important role that changes in muscle protein synthesis and degradation might play in the process of muscle hypertrophy

associated with testosterone supplementation. We do not know whether the increase in satellite cell number is the primary event that is subsequently associated with increased muscle protein synthesis or whether the increases in myonuclear and satellite cell numbers occur secondarily to maintain the myonuclear domain (2).

Substantial evidence supports a role for the modulation of myonuclear number during muscle remodeling in response to injury or disease (1, 2, 19–23, 33, 35, 36). Muscle hypertrophy involves the addition of newly formed myonuclei via the fusion of myogenic cells to the adult myofibers (2). Therefore, we speculate that muscle fiber hypertrophy and the increase in myonuclear number observed here were preceded by testosterone-induced increases in satellite cell number and fusion of satellite cells with muscle fibers. The hypertrophy of levator ani muscle in the female rat induced by exogenous testosterone administration is associated with satellite cell proliferation (20–22, 31, 32, 46). Muscle remodeling and repair following injury or hormone and growth factor stimulation often involve satellite cell replication and recruitment of new stem cells into the myogenic cell lineage (2, 20, 22–24, 30–33, 35, 36, 42, 46).

The mechanisms by which testosterone increases satellite cell number are not known. An increase in satellite cell number could occur by an increase in satellite cell replication, inhibition of satellite cell apoptosis, and/or increased differentiation of stem cells into the myogenic lineage. We do not know which of these processes is the site of regulation by testosterone. On the basis of the observations that testosterone administration induces a rapid proliferative response in muscle satellite cells of prepubertal rats, Jubert and Tobin (22) inferred that androgen receptors might be present on the satellite cells. Doumit et al. (12) demonstrated the presence of androgen receptors in cultured porcine satellite cell nuclei and myotubes and reported that testosterone exposure increased androgen receptor immunostaining in satellite cells. The authors further demonstrated that the cultured satellite cells exhibited lower differentiation in response to testosterone exposure without an effect on proliferation. Taken together, these data point to a direct role for testosterone in satellite cell regulation.

Our observations that testosterone promotes muscle fiber hypertrophy by increasing the number of satellite cells might have clinical and commercial applications. Testosterone effects on satellite cells could serve as the platform for the development of *in vitro* assays for anabolic agents and have implications for the discovery of selective androgen receptor modulators. Because of the constraints inherent in obtaining multiple biopsy specimens in humans, the effects of testosterone on satellite cell replication and stem cell recruitment into the myogenic lineage would be more easily studied in an animal model that demonstrates androgen responsiveness and sexual dimorphism in skeletal muscle mass similar to what is observed in humans. It is possible that testosterone might promote entry of sat-

ellite cells into the cell cycle (22); however, it is equally plausible that androgens might promote myogenesis by stimulating stem cell differentiation into the myogenic lineage. The molecular mechanisms by which testosterone increases muscle satellite cell number should be examined.

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