Anabolic effects of testosterone are preserved during inhibition of 5α-reductase

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Borst SE, Conover CF, Carter CS, Gregory CM, Marzetti E, Leeuwenburgh C, Vandenborne K, Wronski TJ. Anabolic effects of testosterone are preserved during inhibition of 5α-reductase. Am J Physiol Endocrinol Metab 293: E507–E514, 2007. First published May 8, 2007; doi:10.1152/ajpendo.00130.2007.—At replacement doses, testosterone produces only modest increases in muscle strength and bone mineral density in older hypogonadal men. Although higher doses of testosterone are more anabolic, there is concern over increased adverse effects, notably prostate enlargement. We tested a novel strategy for obtaining robust anabolic effects without prostate enlargement. Orchiectomized (ORX) male rats were treated for 56 days with 1.0 mg testosterone/day, with and without 0.75 mg/day of the 5α-reductase inhibitor MK-434. Testosterone administration elevated the prostate dihydrotestosterone concentration and caused prostate enlargement. Both effects were inhibited by MK-434. ORX produced a catabolic state manifested in reduced food intake, blunted weight gain, reduced hemoglobin concentration, decreased kidney mass, and increased bone resorption, and in the proximal tibia there was both decreased cancellous bone volume and a decreased number of trabeculae. In soleus and extensor digitorum longus muscles, ORX reduced both the percentage of type I muscle fibers and the cross-sectional area of type 1 and 2 fibers. Testosterone administration caused a number of anabolic effects, including increases in food intake, hemoglobin concentration, and grip strength, and reversed the catabolic effects of ORX on bone. Testosterone administration also partially reversed ORX-induced changes in muscle fibers. In contrast to the prostate effects of testosterone, the effects on muscle, bone, and hemoglobin concentration were not blocked by MK-434. Our study demonstrates that the effects of testosterone on muscle and bone can be separated from the prostate effects and provides a testable strategy for combating sarcopenia and osteopenia in older hypogonadal men.

dihydrotestosterone; prostate; body composition; bone resorption

APPROXIMATELY 40% OF MEN aged 60 or older are hypogonadal, as defined by a serum total testosterone concentration of 300 ng/dl (10.4 nM) or less (32). In older men, a low circulating testosterone concentration is associated with a decreased sense of well-being, with loss of muscle mass and strength and with loss of bone mineral density and increased risk of fractures (41). In recent years there have been many trials of testosterone replacement as a strategy for combating sarcopenia in older men. When administered to hypogonadal men at replacement doses of 35–100 mg/wk, testosterone improves mood and cognition and increases the hematocrit (32) but produces little increase in bone mineral density (38). Although some studies report that testosterone replacement produces a substantial increase in lower body strength (20), most have found either no change in strength or increases that are small compared with what is obtainable through resistance exercise training (8, 15, 27, 36). Recently, Bhasin et al. (5) have shown that treating older men with very high doses of testosterone, up to 600 mg/wk, produces a remarkable increase in strength. However, in this same study, 25% of those receiving the highest doses dropped out due to serious adverse events such as polycythemia, edema, and urinary retention. In addition to concerns over short-term adverse effects, there are unanswered concerns that higher doses of testosterone might accelerate underlying early-stage prostate cancer (25). To date, studies of testosterone replacement in older men have not reported an increased incidence of prostate cancer (25). However, the number of subjects studied and the duration of treatment are not sufficient to rule out the possibility. Such concerns have been rekindled by publication of a 40-yr longitudinal study from the Baltimore Longitudinal Study on Aging showing a positive correlation between life-long blood testosterone levels and the risk of prostate cancer (29). In addition, autopsy data from men over 60 who died of all causes revealed that 42% carry early-stage prostate cancer (28).

5α-Reductase is the enzyme responsible for the conversion of testosterone to dihydrotestosterone (DHT). A review of the literature by Gormley (22) indicates that significant expression of 5α-reductase occurs in prostate but not in muscle or bone. This finding led us to hypothesize that administering the combination of a high dose of testosterone and a 5α-reductase inhibitor might produce anabolic effects on muscle and bone without producing prostate enlargement. There are two isoforms of 5α-reductase, type I and type II. In humans, benign prostatic hypertrophy (BPH) requires 5α-reductase type II, as BPH is inhibited both by finasteride, an inhibitor of type I, and by dutasteride, an inhibitor of type I and II (40). Because we were not sure which isoform mediates prostate enlargement in rats, we selected an inhibitor capable of blocking both isoforms. In the rat, both finasteride and MK-434 block both isoforms of 5α-reductase. MK-434 was chosen for the present study because it has a relatively high potency in the rat (16, 17, 31, 34, 35).

We have published a pilot study in rats (9) demonstrating that MK-434 blocks prostate enlargement caused by administration of a supraphysiological dose of testosterone for 28 days

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without blocking testosterone-induced muscle hypertrophy or suppression of a urine maker of bone resorption. The present study was undertaken to determine whether MK-434 could prevent testosterone-induced prostate enlargement over a longer period and to determine the effects of testosterone and MK-434 on bone morphology, muscle fiber type and size, strength, and muscle apoptosis.

MATERIALS AND METHODS

Animals and experimental design. Barrier-raised and viral pathogen-free Fischer 344 male rats aged 3 mo were obtained from Charles River Laboratories (Wilmington, MA). Experimental procedures conformed to the Institute of Laboratory Animal Resources Guide to the Care and Use of Experimental Animals and were approved by the Institutional Animal Care and Use Committee at the Gainesville Veterans Affairs Medical Center. Rats were fed a diet of Purina Rodent Chow, no. 5001 (Purina Mills, St. Louis, MO), containing 3.3 kcal/g and distributed at 58.9% carbohydrate, 12.4% fat, and 28.7% protein. Closed orchectomy (ORX) was performed under isoflurane anesthesia on 32 rats and involved removal of testes, epididymis, and epididymal fat. Sham surgery was performed on eight rats. In the same procedure, 30-day drug pellets (Innovative Research of America, Sarasota, FL) were implanted subcutaneously to deliver 1.0 mg testosterone/day vs. placebo and 0.75 mg MK-434/day vs. placebo. This dose of testosterone is supraphysiological in rats, causing marked prostate enlargement and elevation of the prostate DHT concentration (9). Similar results were obtained in our previous study (9). Similarly, we have shown that this dose of MK-434 is sufficient to markedly inhibit testosterone-induced increases in prostate mass and DHT concentration (9). Rats were treated for 56 days under the following experimental conditions: Sham, ORX, ORX + T (testosterone), ORX + MK, and ORX + T + MK. For 2 days after surgery, rats received a nutritional supplement of Jello-O with added fat (STAT VME; J. A. Webster, Alachua, FL), added protein (Challenge Isolated Soy Protein 95 powder; General Nutrition Centers), and Pediasure (Abbott Laboratories). Instructions for preparation are available online at http://www.researchtraining.org/moduletext.asp?intModuleID=602. After 28 days, a second surgery was performed to replace drug pellets. MK-434 was a gift from Merck (Rahway, NJ).

Forelimb grip strength was measured, as we have previously reported (13, 39), using an automated grip strength meter (Columbus Instruments, Columbus, OH). The experimenter grasped the rat by the tail and suspended it above a grip ring. After ~3 s, the animal was gently lowered toward the grip ring and allowed to grasp the ring with its forepaws. The experimenter then quickly lowered the body to a horizontal position and tugged the tail until its grasp of the ring was broken. The mean force in grams was determined with a computerized electronic pull strain gauge fitted directly to the grasping ring and was divided by body mass. Average measurements from three successful trials were taken as the final outcome. Successful trials were defined as those in which the animal grasped the ring with both forepaws and pulled the ring without jerking. The experimenter was blinded as to which treatment group each rat belonged to.

Urine collection and analysis of deoxypyridinoline and creatinine clearance. Deoxypyridinoline (Dpd) is a degradation product of type I collagen and a specific marker for bone resorption. Rats were housed in metabolic cages for 24-h urine collection. Dpd was measured using a Pyrlinks-D EIA kit with a sensitivity of 1.1 nmol/l and an interassay coefficient of variation (CV) of 4% (Quidel, Santa Clara, CA). Because the concentration of urine solutes is altered by water excretion, Dpd was normalized to urine creatinine and reported as nanomolars Dpd per millimolars creatinine. Creatinine was measured using a colorimetric assay kit with an interassay CV of 2% (Sigma Chemical). After collection of 24-h urine, blood was collected by tail tip amputation for analysis of serum creatinine. Creatinine clearance was calculated from the serum and urine creatinine values and expressed as milliliters per minute.

Death and hemoglobin determination. Rats were euthanized under pentobarbital anesthesia. Blood was sampled by tail tip amputation and the hemoglobin concentration measured using a Hemopoint H2 hemoglobin analyzer (Stanbio Laboratory, Boerne, TX). Soleus (SOL) and extensor digitorum longus (EDL) muscles were mounted in embedding medium and frozen in isopentane that was cooled by liquid N2. Muscles and other tissues were stored frozen at ~80°C.

Bone morphology. Tibiae were stripped of musculature and placed in 10% phosphate-buffered formalin (pH 7.4) for 24 h before transfer to 70% ethanol and dehydration in increasing concentrations of ethanol. Bones were then embedded undecalcified in modified methyl methacrylate (3) and sectioned longitudinally at 4-μm thickness with Leica/Jung 2050 or 2165 microtomes. The bone sections were stained by the Von Kossa method with a tetrachrome counterstain. Cancellous bone structural variables were measured in these sections with the Osteometrics (Atlanta, GA) system. The sample area within the proximal tibial metaphysis began 1 mm distal to the growth plate to exclude the primary spongiosa. The following variables were measured or calculated (21): cancellous bone volume (%), trabecular width (μm), trabecular number (no./mm), and trabecular separation (μm).

Muscle fiber type. The right SOL and EDL muscles were stored at ~80°C prior to analysis. Serial sections (10 μm) were cut from the central portion of each muscle, and immunohistochemical reactions were performed against anti-myosin heavy chain antibodies (BA-D5, SC-71, BF-F3, and BF-35) and incubated at 4°C overnight. Sections were subsequently incubated in rhodamine-conjugated anti-rabbit IgG and FITC-conjugated anti-mouse IgG (Nordic Immunological Laboratories) and were classified as either type I, IIa, IIx, or IIb. Additional sections were stained with hematoxylin and eosin and matched to those used to identify fiber type. Stained cross sections were photographed at ×10 with a Leica fluorescence microscope and a digital camera. The relative proportion of fibers of each type as well as measures of individual fiber cross-sectional area (CSA) were determined in each sample. Fiber CSA values were determined on a Macintosh computer using the public domain National Institutes of Health (NIH) Image (version 1.62) program (available at NIH and available on the Internet at http://rsb.info.nih.gov/nih-image/). Fiber type and fiber CSA analyses were performed on an average of 488 and 266 fibers in the SOL and EDL, respectively.

Prostate DHT was extracted by the method of Theobald et al. (42). Briefly, homogenates were extracted twice with 10 volumes of anhydrous ethyl ether. Organic phases were dried under N2 and reconstituted in assay buffer for the commercial DHT radioimmunoassay kit obtained from Diagnostic Systems Laboratories (Webster, TX). The assay has a sensitivity of 4 pg/ml and an interassay CV of 4.5%.

Skeletal muscle apoptosis was assessed by quantifying DNA fragmentation in biceps muscle, using a cell death ELISA kit (Roche Molecular Biochemicals). This assay allows for the quantification of cytosolic mono- and oligonucleosomes (180 base pair nucleotides or multiples). Briefly, samples were minced on ice in 5 volumes of isolation buffer (0.21 M mannitol, 0.07 M sucrose, 0.005 M HEPES, 0.001 M EDTA, and 0.2% fatty acid-free bovine serum albumin, pH 7.4), homogenized on ice in a Potter-Elvehjem glass-to-glass homogenizer, and centrifuged for 10 min at 1,000 g at 4°C. The resulting supernatant was collected and centrifuged again for 20 min at 14,000 g at 4°C. The supernatant (cytosolic fraction) was used for the assay. Results are reported as arbitrary optical density units/milligram protein. Protein concentrations were determined by Bradford assay.

Statistics. Statistical analyses were performed using Prism software (GraphPad Software, San Diego, CA). Unless otherwise noted, data were analyzed by one-way ANOVA with treatment as a factor (Sham, ORX, ORX + T, ORX + MK, ORX + T + MK). Post hoc analyses of differences between individual experimental groups were made using the Newman-Keuls multiple comparison test. Body mass and

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RESULTS

Body mass and food intake. Over the course of 56 days, food intake was reduced by 13.5% in ORX rats compared with Sham (P < 0.001; see Fig. 1). The reduction in food intake was ~60% inhibited in ORX + T rats (P < 0.001) and fully inhibited in ORX + T + MK rats (P < 0.05; see Table 1). Despite this, ORX + T and ORX + T + MK rats did not gain substantially more weight than ORX rats, especially toward the end of the treatment period. In orchietomized rats, the 56-day increase in body mass was inhibited by 32% compared with Sham (P < 0.002 at all time points). In ORX + T and ORX + T + MK rats, the ORX-induced decrease in weight gain was inhibited briefly following initial surgery for ORX and pellet implantation (P < 0.0001 at 3 and 7 days, P = 0.012 at 14 days) and again following surgery at 28 days to replace drug pellets (P = 0.0057).

Body composition and tissue weights. In orchietomized rats we observed a small, 6% decrease in the combined wet weights of five skeletal muscles compared with Sham (plantaris, SOL, tibialis anterior, EDL, and biceps humerus, P < 0.05; see Table 1). Muscle mass in ORX + T and ORX + T + MK rats was not different than ORX. None of the treatment groups showed a change in adiposity. The latter was assessed as the wet weight of three adipose depots removed at death: inguinal subcutaneous fat, retroperitoneal depot of visceral fat, and mesenteric visceral fat. In ORX rats, kidney mass was decreased 22% compared with Sham (P < 0.001; see Table 1). The decrease was prevented in the ORX + T and ORX + T + MK groups (P < 0.001). Changes in kidney mass were not associated with corresponding changes in creatinine clearance, which was 0.801 ± 0.112 ml/min for Sham, 0.592 ± 0.030 for ORX, 0.622 ± 0.026 for ORX + T, 0.621 ± 0.036 for ORX + MK, and 0.937 ± 0.065 for ORX + MK + T.

Hemoglobin. Blood hemoglobin concentration was not significantly different in ORX vs. Sham rats (see Fig. 2). However, hemoglobin was elevated 8.2% in ORX + T rats compared with ORX (P < 0.05), and the effect of testosterone was not blocked by MK-434 (P < 0.05).

Prostate. Prostate mass was decreased 88% in ORX rats compared with Sham (P < 0.001; see Fig. 3) and was increased 12-fold in ORX + T compared with ORX (P < 0.001). MK-434 alone had no effect on prostate mass, but prostate mass was reduced 66% in ORX + T + MK compared with ORX + T (P < 0.001). ORX caused an 81% reduction in the prostate DHT concentration (1,333 ± 822 pg/tissue in Sham vs. 822 pg/tissue in ORX, P < 0.001).}

Table 1. Effects of ORX, T, and MK-434 on food intake and body composition

<table>
<thead>
<tr>
<th></th>
<th>Food intake, g/day</th>
<th>Muscle, g</th>
<th>IWAT, g</th>
<th>RPWAT, g</th>
<th>MWAT, g</th>
<th>Kidney, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>17.50 ± 0.20</td>
<td>1.291 ± 0.022</td>
<td>3.14 ± 0.11</td>
<td>1.80 ± 0.10</td>
<td>4.32 ± 0.20</td>
<td>1.013 ± 0.024</td>
</tr>
<tr>
<td>ORX</td>
<td>15.13 ± 0.20*</td>
<td>1.214 ± 0.017*</td>
<td>3.80 ± 0.18</td>
<td>2.11 ± 0.16</td>
<td>4.83 ± 0.26</td>
<td>0.785 ± 0.018*</td>
</tr>
<tr>
<td>ORX + T</td>
<td>16.61 ± 0.34#</td>
<td>1.131 ± 0.025</td>
<td>3.25 ± 0.12</td>
<td>1.74 ± 0.10</td>
<td>4.90 ± 0.22</td>
<td>1.088 ± 0.028#</td>
</tr>
<tr>
<td>ORX + MK</td>
<td>14.98 ± 0.17</td>
<td>1.210 ± 0.025</td>
<td>3.69 ± 0.24</td>
<td>2.09 ± 0.10</td>
<td>4.63 ± 0.25</td>
<td>0.807 ± 0.013*</td>
</tr>
<tr>
<td>ORX + MK + T</td>
<td>17.69 ± 0.27#</td>
<td>1.186 ± 0.022</td>
<td>3.68 ± 0.20</td>
<td>1.91 ± 0.10</td>
<td>5.78 ± 0.32</td>
<td>1.075 ± 0.017#</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 40. ORX, orchietomized; T, testosterone; IWAT, inguinal subcutaneous depot of white adipose tissue; RPWAT, retroperitoneal depot of visceral white adipose tissue; MWAT, mesenteric depot of white adipose tissue. At death, we measured the combined wet weights of 5 skeletal muscles (muscle), the IWAT, the RPWAT, and the MWAT. *P < 0.05 vs. Sham; #P < 0.05 vs. ORX.
The prostate DHT concentration was 4.2-fold higher in ORX rats (1,077.1 ± 273 pg/tissue, \( P = 0.001 \)) compared with ORX, and that increase was reduced by 44% in ORX + MK rats (611 ± 117 pg/tissue, \( P = 0.05 \)). The prostate DHT concentration was low in ORX rats and was only 19% lower in ORX + MK rats (209.4 ± 13.4, \( P = 0.05 \)).

**Physical performance.** Grip strength was not significantly changed in ORX rats compared with Sham (\( P = 0.084 \); see Fig. 4). Compared with ORX, grip strength was significantly elevated in ORX + T, ORX + MK, and ORX + T + MK rats (\( P < 0.05 \)).

**Bone.** Sham animals, aged 3 mo at the beginning of the experiment, displayed a 50% decrease in bone resorption over 56 days (\( P < 0.0001 \); see Fig. 5). Compared with sham, ORX rats displayed a 25% increase in Dpd/creatinine at 28 days (\( P < 0.05 \)) and an 80% increase at 56 days (\( P < 0.05 \)). Compared with ORX, Dpd/creatinine was reduced in ORX + T and ORX + T + MK rats (\( P < 0.05 \)) and was not different from Sham. In proximal tibiae, ORX rats had a 28.4% decrease in cancellous bone volume compared with Sham (\( P < 0.05 \); see table 2). Compared with ORX, cancellous bone volume was substantially increased in ORX + T and ORX + T + MK rats (\( P < 0.05 \)). Compared with Sham, ORX rats displayed a 33% decrease in the number of bone trabeculae (\( P < 0.001 \)). This decrease was prevented in ORX + T and ORX + T + MK rats (\( P < 0.05 \)). Finally, ORX rats displayed a 61% increase in trabecular separation compared with Sham (\( P < 0.05 \)). This increase was also prevented in ORX + T and ORX + T + MK rats (\( P < 0.05 \)). The effect of ORX on bone resorption was robust enough to cause a 6% decrease in the wet weight of tibiae (0.620 ± 0.007 g for Sham vs. 0.586 ± 0.006 g for ORX, \( P = 0.05 \)). This decrease was significantly pre-

### Table 2. Structural properties of tibial cancellous bone

<table>
<thead>
<tr>
<th>Bone Volume, %</th>
<th>Trabecular Width, μm</th>
<th>Trabecular No., no./mm</th>
<th>Trabecular Separation, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>14.8 ± 1.61</td>
<td>43.6 ± 2.42</td>
<td>4.00 ± 0.28</td>
</tr>
<tr>
<td>ORX</td>
<td>10.6 ± 0.834*</td>
<td>43.3 ± 2.09</td>
<td>2.67 ± 0.24</td>
</tr>
<tr>
<td>ORX + T</td>
<td>14.7 ± 1.36#</td>
<td>45.2 ± 2.05</td>
<td>3.90 ± 0.3618</td>
</tr>
<tr>
<td>ORX + MK</td>
<td>10.3 ± 1.36</td>
<td>48.1 ± 2.63</td>
<td>2.50 ± 0.252</td>
</tr>
<tr>
<td>ORX + T + MK</td>
<td>20.1 ± 1.85#</td>
<td>50.9 ± 2.62</td>
<td>4.70 ± 0.3158</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 40 \). Effects of ORX, T, and MK-434 on morphology of cancellous bone in proximal tibia. *Significant effect of ORX; #significant effect of T.
vented in ORX + T rats ($P < 0.05$). Tibia weights were 0.593 ± 0.011 g for ORX + T, 0.575 ± 0.016 g for ORX + MK, and 0.575 ± 0.005 g for ORX + MK + T.

Muscle fiber type. In SOL muscle, the mean CSA of all myofibers was reduced by 16.1% in ORX rats compared with Sham (see Fig. 6, $P < 0.001$), with reductions of 12.1 and 23.6% in the CSA of type I and type IIA fibers, respectively. The change in mean CSA was prevented in ORX + T and in ORX + T + MK rats ($P < 0.01$). Also in SOL muscle, the fraction of type I fibers was reduced by 8% in ORX rats compared with Sham ($P < 0.001$; see Fig. 7). This change was prevented in ORX + T and in ORX + T + MK rats ($P < 0.001$).

In EDL muscle, the CSA of all muscle fibers was reduced by 17.7% in ORX rats compared with Sham ($P < 0.001$; see Fig. 6), with reductions of 26.6 and 20.6% in the CSA of type I and IIB fibers, respectively. These changes were partially prevented in ORX + T and in ORX + T + MK rats ($P < 0.05$). Also in EDL muscle, the percentage of type I fibers was reduced by 57% in ORX rats compared with Sham ($P < 0.01$; see Fig. 7).

Muscle apoptosis. Assessment of apoptosis in biceps humerus muscle yielded variable results (see Fig. 8), and because the variance was not homogeneous, Kruskal-Wallis statistical analysis was performed. Apoptosis may have been higher in ORX rats compared with control, but the change was not statistically significant. Compared with ORX, apoptosis may have been lower in ORX + T and ORX + MK rats, but again the change was not statistically significant.

DISCUSSION

The present study confirms our previous report (9) and the report of Brown et al. (11) demonstrating an ORX-induced catabolic state in rats. Our main new findings are 1) that administration of a high dose of testosterone prevented those...
catabolic changes and caused prostate enlargement and 2) that coadministration of the 5α-reductase inhibitor MK-434 substantially inhibited prostate enlargement while allowing most of the anabolic effects of testosterone to occur. To produce consistent prostate enlargement, we selected a supraphysiological dose of 1.0 mg testosterone/day. This dose is the equivalent of 1,200 mg/wk for a person weighing 70 kg. In comparison, human replacement doses are in the range of 35–100 mg/wk (10, 15, 27, 36), and the highest dose administered to humans is 600 mg/wk (5).

ORX-induced catabolic effects included reduced food intake and body weight, reduced prostate and kidney mass, decreased cross-sectional area of type I and type IIb muscle fibers, increased bone resorption, and loss of cancellous bone. Testosterone caused marked prostate enlargement as well as the following anabolic effects: increased food intake, increased kidney mass, increased blood concentration of hemoglobin, increased strength and partial prevention of muscle fiber changes, and decreased bone resorption and prevention of bone loss. In general, MK-434 substantially blocked testosterone-induced prostate enlargement but did not block the anabolic effects of testosterone.

5α-Reductase type II is expressed in androgenic tissues (e.g., prostate, seminal vesicles) and also in other tissues, where it may play a role in undesirable effects of testosterone (22). 5α-Reductase type II is also expressed in hair follicles, where it plays a role in male pattern baldness and increased body hair, and in sebaceous glands, where it plays a role in acne. In tissues expressing 5α-reductase, DHT is the primary androgen. However, 5α-reductase is not expressed in appreciable quantities in muscle or bone (22). Although to our knowledge sex steroid concentrations have not been measured in these tissues, testosterone is presumably the primary androgen. These considerations led us to the hypothesis that a 5α-reductase inhibitor might block testosterone-induced prostate enlargement without blocking anabolic effects on muscle or bone.

Brown et al. (11) reported that ORX in Sprague-Dawley rats caused a decrease in the peak tetanic tension of several muscles without any loss of muscle mass. Our findings were for the most part consistent with theirs. We found that ORX reduced the percentage and size of type I fibers in SOL and EDL muscles. Reductions were also noted in the size of type IIa fibers in SOL and in the size of type IIb fibers in EDL. ORX may have reduced grip strength, although the effect was not statistically significant. We found that ORX caused only a very small decrease in muscle mass. It is well established (1, 43) that, over the long term, low serum testosterone is associated with both reduced muscle mass and strength. Taken together, these findings suggest that loss of testosterone produces a loss of muscle quality that precedes a measurable loss in muscle mass.

As expected, testosterone caused an increase in grip strength, and the effect was not inhibited by MK-434. The ORX-induced changes in the number and size of muscle fibers were at least partially prevented by testosterone. Notably, the effects of testosterone were not inhibited by MK-434. The effects of testosterone on fiber type composition and fiber size are consistent with the known effects of testosterone on strength and power (6, 46). We (23) have previously reported that testosterone attenuates the loss of type I fibers following spinal cord injury, suggesting that testosterone may increase muscle endurance as well as strength. In further support of this concept is the report of Van Zyl et al. (44), who found that treatment with testosterone increases exercise treadmill performance in rats.

Phillips and Leeuwenburgh (30) and Dirks and Leeuwenburgh (18) have shown that apoptosis is markedly increased in type II muscle fibers with aging male rats. We hypothesized, since aging is associated with a decrease in sex steroid hormones, that ORX might result in an increase in DNA fragmentation and that testosterone might reverse that increase. In the present study, the level of DNA fragmentation observed was far below what we have previously observed in older animals. Our method may not be sensitive enough to detect a small increase in apoptosis occurring in young animals following ORX. Although Sinha-Hikim et al. (37) have shown that significant activation of muscle satellite cells occurs mainly at higher doses of testosterone, inhibition of apoptosis may be another mechanism by which long-term testosterone replacement might prevent progression of sarcopenia.

As a marker of bone resorption, we measured urine excretion of Dpd, a covalent cross-linker of bone collagen fibers. We observed a progressive decrease in bone resorption in intact, sham-operated animals that were aged 3 mo at the beginning of the study and 5 mo at the end. This finding is in agreement with previous reports (33) showing that skeletal maturation is associated with a decrease in bone turnover. At 28 and 56 days following surgery, Dpd increased progressively in ORX rats compared with sham. Our findings are in agreement with those of Erben et al. (19), who reported that ORX of rats results in a high-turnover osteopenia, with a large increase in bone resorption and a small reflex increase in bone formation. In ORX rats, increased bone resorption over the course of 56 days resulted in dramatic decreases in the volume of cancellous bone and in the number of trabeculae, with no change in trabecular width and an increase in trabecular separation. Probably the most robust findings of this study are that testosterone administration completely prevented these changes and that MK-434 did not inhibit the effect of testosterone.

We found that testosterone also has a robust trophic effect on the kidney, one that was not blocked by MK-434. The kidney expresses 5α-reductase type I (22), and MK-434 inhibits both type I and II 5α-reductase in the rat. However, our data show that the effects of testosterone on the kidney are direct and do not require conversion to DHT. We found that changes in kidney mass were not associated with changes in creatinine clearance, a measure of the glomerular filtration rate (GFR). However, this result is expected because GFR is not maximal in healthy young animals (14, 24). Future studies will aim to determine whether testosterone supports maximal kidney filtration rates and maintains GFR in aging.

In the present study, we found that 56 days of testosterone treatment in young ORX Fisher 344 rats caused a 12-fold prostate enlargement that was 66% inhibited by MK-434. Previously, we (9) reported that 28 days of testosterone treatment in mature ORX Brown Norway rats caused a fivefold prostate enlargement that was nearly completely blocked by MK-434. Several factors may contribute to the lesser degree to which MK-434 blocked testosterone-induced prostate in the present study. Those factors include strain and age differences
as well as the consistency of MK-434 delivery from implanted pellets (MK-434 was injected in the previous study). Another intriguing possibility relates to the duration of testosterone treatment. Although DHT is considered the chief mediator of prostate enlargement, Winter et al. (47) have shown that prostate enlargement in dogs is caused by administration of DHT plus a small dose of 17β-estradiol, but not by DHT alone, indicating that a small amount of estrogen is required for prostate enlargement. Because administration of androgens inhibits endogenous testosterone secretion and because estrogen can be synthesized from testosterone, but not DHT, it is expected that prostate estrogen concentration would be increased by testosterone administration and decreased by DHT administration (45). In the present study, over the course of 56 days there may have been sufficient estrogen in the prostate to allow for a small amount of growth even in the continued presence of MK-434.

Ferrando et al. (20) administered a replacement dose of testosterone to older hypogonadal men for 6 mo without observing prostate enlargement. However, most studies report an average of 20% prostate enlargement per year of testosterone replacement (4, 26, 48). There is a growing consensus that higher-than-replacement doses of testosterone will be required to produce substantial anabolic effects in older men. The 2003 report of the Institute of Medicine (7) questions the efficacy of replacement doses in producing increases in strength. In addition, the question of whether testosterone treatment may accelerate underlying early-stage prostate cancer has not been settled. For all of these reasons, protection of the prostate will be an important consideration for the future of testosterone therapy. Our study suggests that such protection may be afforded with a 5α-reductase type II inhibitor without compromising the anabolic effects of testosterone.

Loss of testosterone in older men is associated with loss of bone mineral density (43). However, administration of testosterone at replacement doses to older, hypogonadal men has produced only marginal increases in bone mineral density, even in studies lasting as long as 36 mo (38). Studies of the impact of high-dose testosterone on bone have not been performed. Our study demonstrates that substantial changes in bone mineral can occur with high doses of testosterone. Our finding that the effects of high-dose testosterone are not blocked by a 5α-reductase inhibitor is consistent with the hypothesis that anabolic effects of testosterone in bone are mediated by local conversion to estrogen rather than DHT. This hypothesis was generated following the identification of several boys who lacked functional P-450 aromatase activity (12). These patients have an osteopenia that responds to estrogen treatment but not to testosterone.

We conclude that high-dose testosterone produces anabolic effects in ORX rats, including effects on hemoglobin, muscle fiber type and strength, kidney mass, and powerful effects on bone resorption and bone morphology and also produces marked prostate enlargement. We also conclude that coadministration of an inhibitor of 5α-reductase can inhibit prostate effects of testosterone without inhibiting anabolic effects. This study provides, in an animal model, proof of principle and a rationale for testing the effects of higher-dose testosterone and finasteride in older hypogonadal men.

GRANTS
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REFERENCES


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