Inhibition of 5α-reductase blocks prostate effects of testosterone without blocking anabolic effects

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Submitted 12 July 2004; accepted in final form 26 August 2004

Borst, Stephen E., Jun Hak Lee, and Christine F. Conover. Inhibition of 5α-reductase blocks prostate effects of testosterone without blocking anabolic effects. Am J Physiol Endocrinol Metab 288: E222–E227, 2005. First published September 14, 2004; doi:10.1152/ajpendo.00305.2004.—We studied the effect of the 5α-reductase inhibitor MK-434 on responses to testosterone (T) in orchietomized (ORX) male Brown Norway (BN) rats aged 13 mo. At 4 wk after ORX or sham surgery, a second surgery was performed to implant pellets delivering 1 mg T/day or placebo pellets. During the second 4 wk of the study, rats received injections of MK-434 (0.75 mg/day) or vehicle injections. Treatment with T elevated serum T to 75% above that for sham animals (P = 0.002) and did not affect serum dihydrotestosterone (DHT) or serum estradiol. T treatment also caused an elevation of prostate T and a marked elevation of prostate DHT. During the second half of the study, ORX rats lost an average of 18.86 ± 4.62 g body wt. T completely prevented weight loss, and the effect was not inhibited by MK-434 (P < 0.001). ORX produced a nonsignificant trend toward a small (5%) decrease in the mass of the gastrocnemius muscle (P = 0.0819). This trend was also reversed by T, and the effect of T was not blocked by MK-434. T caused a significant 16% decrease in subcutaneous fat that was not blocked by MK-434 (P < 0.05). Finally, T caused a 65% decrease in urine excretion of deoxypyridinoline, a marker of bone resorption, and again the effect was not blocked by MK-434 (P < 0.0001). In contrast, T caused a greater than fivefold increase in prostate mass, and the effect was almost completely blocked by MK-434 (P < 0.0001). This study demonstrates that 5α-reductase inhibitors may block the undesirable effects of T on the prostate, without blocking the desirable anabolic effects of T on muscle, bone, and fat.

dihydrotestosterone; body composition; bone resorption

WE HAVE RECENTLY REVIEWED the many trials evaluating testosterone administration as a strategy for combating loss of muscle mass and strength in elderly men (5). Most studies report no more than a modest increase in lean body mass, and only a few report any increase in strength. These findings do not necessarily indicate that older men are unresponsive to testosterone. Most studies have been conducted with replacement doses of testosterone, and higher doses have not been considered for fear of accelerating underlying prostate cancer (6, 19, 37). Recently, Magliano et al. (22) reported that a very high dose of 600 mg testosterone/wk produced substantial increases in muscle mass and that increases were equal in younger vs. older men.

Testosterone is the principal androgen acting in tissues lacking 5α-reductase. In tissues expressing 5α-reductase, testosterone is converted to dihydrotestosterone (DHT), and, in those tissues, DHT is the principal androgen. 5α-Reductase is highly expressed in the prostate, but not in muscle or bone (17). This observation led us to the hypothesis that inhibitors of 5α-reductase might block the undesirable effects of testosterone on the prostate without blocking the desirable effects of testosterone on muscle, fat, and bone.

We examined the prostate and anabolic effects of a high dose of 1 mg testosterone/day, alone and in combination with a 5α-reductase inhibitor. For the latter, we chose MK-434 (MK) because its reported potency in rats (32) is higher than that of finasteride (12, 30, 33). Our study was conducted with Brown Norway (BN) male rats, an established model of age-induced hypogonadism (1, 23). Orchiectomy (ORX) of BN males causes a marked reduction in prostate mass and high-turnover osteopenia, which was characterized by an elevation in urine deoxypyridinoline (Dpd), a marker on bone resorption (13, 43). Phillip et al. (27) and Prakasam et al. (28) found that testosterone prevents castration-induced losses in the width of the tibial epiphysial growth plate and in femoral bone mineral density in Sprague-Dawley and Wistar rats, respectively. Carson et al. (10) and Wimalawansa et al. (46) reported that testosterone administration prevents loss of muscle mass and bone mineral density in hindlimb-suspended BNxF344 and Sprague-Dawley rats, respectively.

MATERIALS AND METHODS

Animals and experimental design. Barrier-raised and viral pathogen-free BN male rats aged 13 mo were obtained from Zivic-Miller Laboratories (Zellenopple, PA). Experimental procedures conformed to the Institute for Laboratory Animal Research 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. Closed ORX was performed on 32 rats and involved removal of the testes, epididymis, and epididymal fat pads. Sham surgery was performed on eight rats. After surgery, rats received a nutritional supplement (Jello-O plus protein and fat) daily for 2 days. After 25 days, rats were housed overnight in metabolic cages, and urine was collected for analysis of Dpd. At 28 days, a second surgery was performed for subcutaneous implantation of pellets designed to deliver 1 mg testosterone/day for 30 days (Innovative Research of America, Sarasota, FL). At the time of the second surgery, we also began 28 days of treatment with MK (0.75 mg/day in 0.15 ml of 90% DMSO, 10% ethanol sc). MK was a gift from Merck (Rahway, NJ). There were five experimental groups: SHAM, ORX, ORX + testosterone, ORX + MK, and ORX + testosterone + MK. Rats not receiving testosterone pellets received placebo pellets. Rats not receiving MK received vehicle injections. After 25 days of drug treatment,

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rats were again housed in metabolic cages for urine collection. After 28 days of drug treatment, rats were killed and tissues collected.

**Steroid hormone assays.** Steroid hormone assays were performed using kits obtained from Diagnostic Systems Laboratories (Webster, TX). Serum androgens and estradiol (E2) were extracted according to the manufacturer’s instructions. Serum testosterone was measured using an enzyme immunnoassay (EIA) kit with a sensitivity of 3.8 pg/ml and an interassay coefficient of variation (CV) of 11%. Serum DHT was measured using an RIA kit with a sensitivity of 4 pg/ml and an interassay CV of 4.5%. Prostate androgens were extracted according to the protocol of Theobald et al. (40). Serum E2 was measured using an RIA kit with a sensitivity of 2.2 pg/ml and an interassay CV of 7%.

**Urinary Dpd.** Urinary Dpd is a degradation product of type I collagen and a specific marker for bone resorption. Dpd was measured in urine samples with a Pyrilinks-D EIA kit with a sensitivity of 1.1 nmol/l and an interassay CV of 4% (Quidel, Santa Clara, CA). Because the concentration of urine solutes may be altered by water excretion, Dpd was normalized to urine creatine and reported as nanomoles per millimoles creatinine. Creatinine was measured using a colorimetric assay kit with a high sensitivity (since 10-fold dilution of samples with a Pyrilinks-D EIA kit with a sensitivity of 1.1 nmol/l and an interassay CV of 2% (Sigma Chemical, St. Louis, MO).

**Free fatty acid.** Free fatty acid [nonesterified fatty acid (NEFA)] concentration was measured using a colorimetric assay kit that relies on fatty acids as substrate for enzymatic acylation of coenzyme A (Wako Chemicals, Neuss, Germany).

**Statistics.** Two-way and repeated-measures ANOVA were performed using PRISM software (GraphPad Software, San Diego, CA).

**RESULTS**

During ORX surgery, an average of 10.82 ± 0.26 g of tissue was removed (testes, epididymis, and most of epididymal fat pad). In the first 4 wk after surgery, ORX rats gained 5.20 ± 2.71 g over their postsurgical weight, whereas SHAM rats gained 11.68 ± 4.49 g (Fig. 1, left). During the second 4 wk of the study, after surgical pellet implantation, SHAM + vehicle (veh) rats maintained body weight (average loss of 0.56 ± 0.08 g), whereas ORX + veh rats lost an average of 18.86 ± 4.62 g. ORX-induced loss of body weight was prevented by testosterone (P < 0.0001), and the effect of testosterone was not blocked by MK (P = 0.910, Fig. 1, right). No differences in serum NEFA were observed among the experimental groups (0.212 ± 0.020 meq/l for SHAM, 0.218 ± 0.036 meq/l for ORX + veh, 0.171 ± 0.010 meq/l for ORX + testosterone, 0.211 ± 0.024 meq/l for ORX + MK, and 0.218 ± 0.022 meq/l for ORX + MK + testosterone).

Testosterone-induced prostate enlargement was blocked by MK (Fig. 2, left). ORX caused a 51% decrease in prostate mass (P < 0.01). In ORX rats, testosterone caused a 5.4-fold increase in prostate mass, which was almost completely blocked by MK (P < 0.0001). Testosterone caused a nonsignificant trend toward a 5% increase in mass of the gastrocnemius muscle (Fig. 2, center: P = 0.0819, ANOVA), and the effect was not blocked by MK (P = 0.925). Testosterone caused a significant 16% decrease in the inguinal depot of subcutaneous fat (inguinal white adipose tissue, P = 0.0303) and the response was not blocked by MK (Fig. 2, right: P = 0.325 for MK, P = 0.789 for interaction).

Urine Dpd was measured at 25 and 55 days (Fig. 3). Dpd was normalized to urine creatinine and was expressed as nanomoles per millimoles creatinine. At 25 days, ORX caused a 75% increase in Dpd/creatinine (P < 0.001). At 55 days, testosterone caused a 65% decrease in urine Dpd/creatinine (P < 0.0001), and the effect was not blocked by MK (P = 0.670).

The effects of treatment on serum androgens are shown in Fig. 4. ORX reduced serum testosterone by 76% (P < 0.002). In ORX rats, testosterone administration elevated serum testosterone to a level that was somewhat higher that what was observed for SHAM rats (P < 0.0024). Serum DHT was not significantly affected by treatment. Serum E2 concentrations were very low and were not significantly affected by treatment.

![Fig. 1](https://ajpendo.physiology.org/doi/10.220.336)
(1.67 ± 1.675 pg/ml for SHAM, 16.53 ± 7.22 pg/ml for ORX + veh, 30.68 ± 14.96 pg/ml for ORX + testosterone, 5.09 ± 1.56 pg/ml for ORX + MK, and 15.40 ± 11.62 pg/ml for ORX + MK + testosterone). The effects of treatment on prostate androgens are shown in Fig. 5. ORX caused a 40% decrease in prostate testosterone ($P < 0.0002$) and a 90% decrease in prostate DHT ($P < 0.0001$). MK alone had no effect on prostate androgens. Testosterone administration to ORX animals elevated prostate testosterone by three- to fourfold, producing higher concentrations than were observed in SHAM animals ($P < 0.0002$). Testosterone administration to ORX animals increased prostate DHT 26-fold ($P = 0.0055$), an effect that was almost completely blocked by MK ($P = 0.0096$).

DISCUSSION

The most important findings of this study were as follows. 1) ORX resulted in a loss of body weight, a very small loss of muscle mass, a significant increase in bone resorption, and a decrease in prostate mass. 2) In ORX rats, testosterone administration elevated serum testosterone and prostate testosterone and DHT to above that observed in SHAM rats. Testosterone administration prevented the ORX-associated losses of body weight and muscle mass and caused a small reduction in subcutaneous fat. Testosterone also suppressed bone resorption to a level below that of SHAM animals and caused marked prostate enlargement. 3) MK almost entirely blocked testosterone-induced prostate enlargement but did not block any of the anabolic effects of testosterone on body weight, muscle mass, fat mass, or bone resorption. Inhibition of testosterone-induced prostate enlargement by MK was probably the result of inhibition of 5α-reductase, since MK caused small changes in serum and prostate testosterone, but a marked decrease in prostate DHT. Taken together, these findings indicate that coadministration of testosterone and MK produces desirable anabolic effects on muscle bone and fat, without undesirable prostate enlargement.

Although testosterone and DHT mediate separate effects in different tissues, there is strong evidence that they act through the same receptors. Conversion to DHT amplifies the effects of testosterone in several ways. First, DHT has a higher affinity for the androgen receptor than does testosterone (16). Second,
binding of DHT stabilizes the androgen receptor, slowing the rate of receptor degradation (47). Third, conversion of testosterone to DHT prevents its conversion to androsteindione, a much weaker androgen (15). Virilization of external genitalia during development depends on DHT (2). However, in adult men, the effects of DHT appear to be generally undesirable. The latter include increased body hair, acne, male pattern baldness, and prostate enlargement. Suppression of DHT with the 5α-reductase inhibitor finasteride does not appear to have major undesirable effects. Finasteride does not have catabolic effects on muscle or bone (23). In contrast, the effects of testosterone that are not mediated by DHT are generally considered to be desirable. These include increased muscle mass and bone mineral density, deepening of the voice, increased libido, spermatogenesis, and increased hematocrit (desirable in the absence of polycythemia). Thus obtaining only the effects of testosterone that are not mediated by DHT is an attractive therapeutic target.

Recent evidence suggests that some of the anabolic effects of administered testosterone may depend on aromatase activity and tissue conversion of testosterone to E2. The essential role of E2 in regulating bone mass in males was confirmed, in part, by E2. We did not observe any elevation of serum E2 after testosterone administration. However, we cannot rule out the possibility that bone E2 was elevated and thus the possibility that testosterone-induced suppression of bone resorption is mediated by local aromatase activity.

In rats, ORX is known to reduce prostate mass (13, 43) and to increase bone resorption (13, 43). Whether ORX reduces muscle mass depends on the strain of rat. Vandenput et al. (41) showed that ORX of 12-mo-old Wistar rats reduced lean body mass, as assessed by DEXA. Brown et al. (8) found that ORX of 7-mo-old Sprague-Dawley rats caused no change in the weight of several muscles. Administration of testosterone increases prostate mass in both ORX and intact rats (10). Several groups have shown that testosterone prevents ORX-induced loss of bone (27, 28, 42). In male rats, the short-term effects of ORX on muscle include loss of glycogen stores (29) and reduced protein synthesis (21), and both of these effects are prevented by testosterone administration. Rogozkin (31) reported that several anabolic steroids stimulate muscle protein synthesis in intact rats. Van Zyl et al. (44) has reported that testosterone treatment significantly increased treadmill exercise performance in intact rats. Our choice of MK as a 5α-reductase inhibitor was based on the fact that it has greater potency in the rats than does finasteride. Although finasteride is effective in the range of 5–40 mg/day (12, 30, 33), Seo et al. (32) have shown that 1 mg MK/day suppresses DHT. This is the first report showing that a 5α-reductase inhibitor blocks the effect of testosterone on the prostate without blocking the anabolic effects of testosterone.

In the present study, rats lost weight after ORX surgery and recovered their initial body weight by 4 wk. In contrast, after the second surgery for implantation of pellets, rats not treated with testosterone had a progressive loss of body weight, whereas those treated with testosterone maintained body weight. It is not clear why weight loss was greater after the second surgery, which was probably less traumatic than the first. We did not measure food intake, and weight loss may have had a central component. Weight loss may be attributable to the use of DMSO as a vehicle for injections, although all five experimental groups received DMSO. It is also not clear which body compartment accounted for the weight loss. ORX rats that were not treated with testosterone had only slightly lower muscle mass, whereas visceral fat was unchanged and subcutaneous fat was increased. It is possible that the prevention of weight loss by testosterone was due to an increase in skin, internal organs, muscles that we did not measure, or fluid retention.

Although testosterone produces substantial anabolic effects in young and middle-aged hypogonadal men (3, 7, 38, 45), the anabolic effects of testosterone replacement therapy in older men have been harder to demonstrate. Most studies of testosterone replacement in older men have reported small increases in lean mass (4, 6, 19, 37, 39). Brill et al. (6), Kenny et al. (19), Clague et al. (11), and Snyder et al. (37) have all reported that replacement doses of testosterone fail to increase strength in elderly hypogonadal men. In contrast, two groups have reported increased strength in elderly men after testosterone replacement. Ferrando et al. (14) administered replacement doses of testosterone enanthate for 6 mo to men aged >60 yr with low circulating testosterone. Treatment was designed to raise nadir testosterone levels into the range of 17–28 nM. At 6 mo there were significant increases in muscle strength and no increase in prostate mass. Sih et al. (34) found that testosterone replacement caused a 5-kg increase in grip strength, amounting...
roughly to a 10% improvement. Although it is still not clear whether testosterone replacement increases strength in older men, a recent abstract by Magliano et al. (22) reported that elderly men have a strong anabolic response to high doses of testosterone. In this randomized, controlled trial, both younger (aged 18–36 yr) and older (aged 60–75 yr) men were treated for 20 wk with testosterone at doses of 125, 300, and 600 mg/wk. In the two age groups, significant and equal increases in muscle mass were observed. Strength and safety were not assessed in the study, and, in general, higher doses of testosterone have not been considered in older men because of the risk of accelerating underlying prostate cancer.

Initial fears that testosterone replacement would promote prostate cancer were lessened by the findings of Hajjar et al. (18), who treated 45 elderly men with a replacement dose of testosterone and found no increase in prostate cancer during a 2-yr follow-up. However, concerns for the effect of testosterone on the prostate have been rekindled by the recent release of 40-yr data from the Baltimore Longitudinal Study on Aging (26) showing a positive correlation between blood testosterone levels and the risk of prostate cancer. Concerns are increased by the high prevalence of early-stage prostate cancer in elderly men. Approximately 10% of men will develop clinically manifested prostate cancer in their lifetime, and ~3% will die of the disease (24). However, autopsy data show that 42% of men over the age of 60 have early-stage prostate cancer (24). In addition to prostate effects, other risks are associated with testosterone replacement in older men, including fluid retention, gynecomastia, worsening of sleep apnea, and polycythemia (39). It is not clear whether these other effects are mediated by DHT.

We conclude that 5α-reductase inhibitors block at least some of the undesirable effects of testosterone, while allowing desirable anabolic effects to occur. Our results suggest that administration of higher doses of testosterone in combination with a 5α-reductase inhibitor might be a safe and effective strategy for combating sarcopenia in elderly men.

ACKNOWLEDGMENTS

We thank Drs. Richard E. Peterson and H. Michael Theobald for consultation regarding measurement of prostate androgens.

GRANTS

This research was supported by a Department Veterans Affairs Merit Award to S. E. Borst.

REFERENCES


