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Comparison of Tamoxifen and Testosterone Propionate in Male Rats: Differential Prevention of Orchidectomy Effects on Sex Organs, Bone Mass, Growth, and the Growth Hormone–IGF-I Axis

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ABSTRACT: Testis dysfunction can weaken bone and reduce muscle mass as well as impair sexual function. Testosterone (T) therapy has useful effects on sex organs, bone, and muscle in T-deficient males, but prostate concerns can preclude T use in some men. Although estrogens or other drugs can protect bone in men, gynecomastia makes estrogens unappealing, and other drugs may also be undesirable in some cases. Selective estrogen receptor modulators (SERMs) inhibit estrogen-evoked sex organ growth but mimic estrogen effects on bone and cholesterol and are advantageous for some women. SERMs may also be useful in men who must avoid androgens. As a preclinical test of this idea, tamoxifen (a SERM) and testosterone propionate (TP, a classic androgen) were compared for their efficacy in preventing varied effects of orchidectomy (ORX) in adult male rats. ORX led to ventral prostate and seminal vesicle atrophy and decreases in somatic growth, proximal tibia bone mineral density (BMD), and serum growth hormone (GH) and insulin-like growth factor I (IGF-I). ORX also increased anterior pituitary glandular kallikrein, serum cholesterol, and body temperature. Pituitary prolactin (PRL) content was unaltered. ORX effects on sex organs, somatic growth, IGF-I, cholesterol, body temperature, and pituitary kallikrein were prevented by TP at 1 mg/kg (3 doses per week), but BMD and GH were unresponsive. ORX effects on BMD and GH were prevented by TP at 10 mg/kg, but this dose evoked supraphysiologic increases in sex organs and PRL, failed to restore somatic growth, and further reduced IGF-I. Tamoxifen (1 mg/kg daily) prevented ORX effects on BMD, GH, and cholesterol without altering basal or TP-induced sex organ growth and further reduced IGF-I and somatic growth. Tamoxifen did not alter basal PRL but blocked increases caused by TP at 10 mg/kg. In summary, tamoxifen prevented ORX effects on bone and cholesterol in male rats without affecting sex organs or PRL and might be useful for men who must avoid androgens. Unexpectedly, a TP dose that replicated testis effects on sex organs and other targets had no effect on BMD or GH, and a larger TP dose that restored BMD and GH was worse at replicating normal male physiology. In addition, correlation/regression results suggested that the GH–IGF-I axis contributes to changes in BMD.

Key words: Testis, androgens, estrogens, aromatase, osteoporosis, prostate, seminal vesicle.

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In male rats and men, estrogen production appears to arise mostly in nongonadal tissues that express aromatase (the estrogen synthetase complex) and use testosterone (T) derived from the testis as substrate (Sharpe, 1998; Simpson et al, 2000). Such estrogen synthesis may have biological significance. Men lacking functional estrogen receptors (ERs) or aromatase exhibit decreases in bone mass and other abnormalities (Faustini-Fustini et al, 1999; Rochira et al, 2001), and bone mass in male rodents can be reduced by aromatase inhibitors or inactivation of the aromatase gene (Vanderschueren et al, 1996, 1997; Oz et al, 2000). In addition, declines in T may contribute to decreases in libido, sexual function, muscle mass, and physical strength in aging men, while related estrogen reductions may foster the development of osteoporosis (Orwoll, 1998; Lombardi et al, 2001). Indeed, androgen-deprivation therapy achieved with orchidectomy (ORX) or drug treatment is routinely used in men with disseminated prostate cancer, and osteoporosis has emerged as a prevalent and potentially serious complication of such therapy (Daniell et al, 2000; Oeflein et al, 2001).

In postmenopausal women, hormone replacement regimens that include estrogens are widely used to treat common menopausal complaints and have proven effective in the prevention or treatment of osteoporosis. Similarly, in hypogonadal men, therapy with aromatizable androgens would be expected to relieve problems due to both androgen deficiency and estrogen deficiency. Indeed, T replacement has beneficial effects on bone metabolism in orchidectomized rats (Wakley et al, 1991; Vanderschueren et al, 1992, 2000), and studies of hypogonadal men describe similar actions (Anderson et al, 1997; Behre et al, 1997). Nonetheless, androgen use can be worrisome in men with prostate health concerns and is inadvisable in

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men with a history of prostate cancer or urethral obstruction due to benign prostatic hypertrophy (Bhasin and Tenover, 1997; Advisory Panel on Testosterone Replacement in Men, 2001; Vermeulen, 2001).

Given the concerns about androgen effects on prostate health in older hypogonadal men, it may often prove more practical to manage problems related to estrogen deficiency, such as osteoporosis. However, agents available for such use can be problematic. Modest estrogen doses have beneficial effects on bone in men (Orwoll, 1998; Lombardi et al, 2001), but T deficiency greatly enhances the estrogen sensitivity of breast tissue (Glass, 1994), and the resulting gynecomastia is intolerable for most men. In contrast, oral bisphosphonates (alendronate and risedronate) prevent osteoporosis by nonhormonal actions specifically targeting bone and have been well tolerated in clinical trials. In general medical practice, however, oral bisphosphonates can be troublesome in patients at risk for upper gastrointestinal problems, and patient compliance with therapy has been disappointing (Ettinger et al, 1998; Kherani et al, 2002; Tosteson et al, 2003). Other agents available for osteoporosis prevention in men either require parenteral injections (parathyroid hormone 1-34) or have less efficacy (intranasal sprays of salmon calcitonin). Thus, an effective alternative to estrogens and oral bisphosphonates may be helpful for many men.

Selective ER modulators (SERMs) such as tamoxifen evoke a novel mix of estrogenic and anti-estrogenic actions that might be advantageous for some hypogonadal men. Tamoxifen acts as an estrogen antagonist in its effects on the breast, rat prolactin (PRL), and certain other targets, yet it also acts as an estrogen agonist to lower blood cholesterol and prevent bone loss in estrogen-deficient female animals and women (Sato et al, 1996; Ke et al, 1997; MacGregor and Jordan, 1998). Indeed, the potential utility of the effects of tamoxifen in males was notable in a recent study of estradiol (E2) and tamoxifen interplay with triiodothyronine (T_3) in hypothyroid male rats (Fitts et al, 2001). ORX effects to decrease bone mass and serum growth hormone (GH) were inhibited by tamoxifen—which also lowered serum cholesterol. Moreover, tamoxifen had negligible estrogenic effects on pituitary PRL and did not influence male sex organ growth.

The present study used euthyroid male rats as a preclinical model to further assess the possible value of tamoxifen for men and tested the hypothesis that tamoxifen can protect bone mass and lower cholesterol in males without inducing either sex organ growth (a classic androgen response) or pituitary PRL (a classic estrogen response in the rat). Moreover, tamoxifen (a classic SERM) was directly compared to testosterone propionate (TP, a classic aromatizable androgen) with respect to its efficacy in preventing diverse ORX effects. In addition, the experimental data were subjected to correlation/regression analyses to assess a secondary hypothesis that changes in proximal tibia BMD during gonadal manipulations are partly related to changes in serum GH and insulin-like growth factor I (IGF-I).

Materials and Methods

Animals

All protocols followed National Institutes of Health guidelines on the use and care of animals and were approved by the institutional Animal Care and Use Committee. Young adult male Sprague-Dawley rats 79–84 days old were used (Taconic Farms, Germantown, Pa). Like adult humans, young adult rats exhibit marked sex organ atrophy and an accelerated resorption of cancellous bone in response to gonadectomy and have provided a valuable model for the preclinical evaluation of androgens, antiandrogens, and anti-osteoporosis drugs (Wronski et al, 1989; Kalu, 1991; Thompson et al, 1995; Sato et al, 1996; Ke et al, 1997). Young adult rats are also vigorous with respect to gonadal and GH–IGF-I axis functions and are well suited for efforts to assess the physiologic interplay of these hormonal systems.

ORX was performed under halothane-O_2 anesthesia 4 days after arrival, and study treatments were started 4 days after surgery (87–92 days of age; mean starting weight = 335 g). Eight rats received only sham surgery to provide testis-intact controls (mean starting weight = 352 g). In young adult rats of the ages studied, gonadectomy and sex hormone therapy produces changes in cancellous bone mass that are clearly evident in 2 weeks and unequivocal in 4 weeks (Wronski et al, 1989; Kalu, 1991; Wakley et al, 1991; Thompson et al, 1995). Thus, a 6-week treatment period was used in this study. Note that similar preclinical models that use aged rats (older than 1 year) employ much longer treatment periods, since it takes much longer for aged rats to exhibit changes in bone mass in response to sex hormone manipulations (see ''Discussion'').

Experimental Design and Drug Treatments

Six groups of orchidectomized rats received the following drug and hormone treatments: 1) vehicle solution alone (n = 8); 2) testosterone 17β-propionate (TP; Sigma Chemical Co, St Louis, Mo) at 1 mg/kg via subcutaneous (SC) injection 3 times weekly (n = 7); 3) TP at 10 mg/kg via SC injection 3 times weekly (n = 7); 4) tamoxifen (trans isomer, free base; Sigma) at 1.0 mg/kg via SC injection once daily (n = 7); 5) TP at 1 mg/kg plus tamoxifen (n = 7); and 6) TP at 10 mg/kg plus tamoxifen (n = 8). The 8 testis-intact rats received daily injections of vehicle solution. TP and tamoxifen were initially dissolved in benzyl alcohol (a pharmaceutical solvent with bacteriostatic and local anesthetic activity) and then diluted with sesame oil to achieve the desired concentrations in 2% benzyl alcohol: 98% sesame oil (vol/vol). Injection volumes were 50 μL per 100 g of body weight.

The above dosing regimens were tailored to fit drug pharmacokinetics. Tamoxifen is a lipophilic agent with a large volume of distribution (>50 L/kg) and an 8-hour plasma half-life in the rat (Borgna and Rochefort, 1981; Robinson et al, 1991). Tamoxifen and its potent metabolite (4-hydroxytamoxifen) have

20- and 48-hour half-lives, respectively, in uterine nuclei (Borgna and Rochefort, 1981), and tamoxifen at 0.7 mg/kg increases nuclear retention of the ER for more than 2 days (Bowman et al, 1982). These pharmacokinetics indicate that once-daily dosing should produce a ''steady state'' in tamoxifen and 4-hydroxytamoxifen levels in plasma and target tissue nuclei within 4–8 days. A daily tamoxifen dose of 1 mg/kg was used, since it yielded maximal estrogen agonist and antagonist effects in previous dose-response studies (Powers et al, 1989; DiPippo et al, 1995).

T replacement was provided with TP, the first 17-OH ester of T to become widely available for experimental and clinical use and the prototype for such therapeutics (Miescher et al, 1936; Meikle, 1999). TP is more lipophilic than T and is absorbed from oil vehicles much more slowly than T after injection. Once absorbed, TP is rapidly hydrolyzed to T (Fujioka et al, 1986)—which mediates the androgenic effects of TP. The differing disposition of TP dramatically increases its androgenic potency and duration of action compared to T. TP has a large volume of distribution (>70 L/kg) and yields peak levels of plasma T about 24 hours after injection; T levels then decline with roughly a 24-hour half-life (Gerrity, 1979; Fujioka et al, 1986). The pharmacokinetics of TP indicate that 3 SC injections per week will produce a steady state in plasma T in about 6–8 days. TP doses of 1 and 10 mg/kg were used in this study, and androgenic efficacy was assessed relative to maintenance of sex organ weights at levels seen in normal testis-intact males.

During the treatment phase of the study, body weight was measured 3 times per week, and food intake was measured once per week. Rats were housed in gangs of 3–4 rats per cage; thus, total food intake per group was measured during a 24-hour period, and average intake per rat was interpolated. Body temperature was measured weekly with a digital thermometer equipped with a rectal thermocouple probe. This regimen provided an index of treatment effects on thermoregulation.

Tissue Processing

After 6 weeks of treatment, rats were euthanized in random order with sodium pentobarbital at 100 mg/kg (intraperitoneally) between 10:30 AM and 3:30 PM. Blood, anterior pituitaries, and right tibias were collected within 5 minutes of pentobarbital injection as previously described (DiPippo et al, 1995; Fitts et al, 1998). Blood samples were allowed to clot for 5 minutes at room temperature, cooled on ice, and then refrigerated at 5°C; serum was collected the next day, aliquoted, and stored at −80°C until assay. The right tibia was stripped of most muscle and connective tissue and stored in 70% ethanol for subsequent measurements of tibia length (an index of longitudinal growth) and bone mineral density (BMD). Anterior pituitaries were sonicated in 400 μL of homogenization buffer (10 mmol of sodium phosphate per liter, 150 mmol of NaCl per liter, and 0.1% Triton X-100, pH 7.5), separated into aliquots, and stored at −20°C until assay.

Seminal vesicles and ventral prostate lobes were dissected, drained of their luminal contents, and weighed. The weights of these male sex accessory organs provided a reliable index of circulating bioavailable androgenic activity. Unlike radioimmunoassays (RIAs) for total T, measures of androgen bioactivity are sensitive to differences in free T arising from changes in sex hormone–binding globulin or differences in dihydrotestosterone formation in target tissues.

Analysis of BMD

Tibia BMD was measured by dual-energy x-ray absorptiometry with a Hologic QDR1000 (Waltham, Mass) as previously described (DiPippo et al, 1995; Fitts et al, 1998). Image analysis software (Hologic) calculated bone mineral content (in grams), cross-sectional area (square centimeters), and BMD (grams per square centimeter) in 2 regions: the proximal tibia and the tibia diaphysis. The proximal tibia (upper one third of tibia length) is relatively enriched in cancellous bone and has a higher ratio of cancellous to cortical bone than the diaphysis (middle one third of tibia length) (Kalu, 1991; Shen et al, 1993).

Cholesterol and Glucose Analyses

Total cholesterol was measured using a colorimetric kit (Sigma). Serum glucose was measured as an index of carbohydrate metabolism using a colorimetric kit (Stanbio Laboratories, San Antonio, Tex).

Assay of Serum GH and IGF-I

Serum GH and IGF-I levels were used to assess changes in the GH–IGF-I axis. Total serum levels of IGF-I were determined using an RIA kit (Nichols Diagnostics, San Juan Capistrano, Calif) after extraction of IGF-I from serum. IGF-I in rat serum was separated from IGF-binding proteins (IGFBPs) using the acid-ethanol extraction method described by Crawford et al (1992). Serum samples (100 μL) were mixed with 900 μL of acid-ethanol solution (12.5% 2 M HCl:87.5% ethanol [vol/vol]), incubated for 30 minutes at room temperature, and then centrifuged at 1500 × *g* for 30 minutes at 5°C (protein/IGFBP precipitation 1). A 200-μL sample of the supernate was then mixed with 100 μL 0.855 M Tris base (pH 11), incubated 30 minutes at room temperature, and centrifuged at 1500 × *g* for 30 minutes at 5°C (protein/IGFBP precipitation 2). A 100-μL sample of the final supernate was mixed with 1.4 mL of phosphate buffer (pH 7.5) and used for RIA. This acid-ethanol extraction method has previously been validated for RIA of total IGF-I in male and female rat serum by comparison with results using high-pressure liquid chromatography (HPLC) methodology (Crawford et al, 1992). The sensitivity of the IGF-I RIA was 57.5 ng/mL with the serum extraction protocol used, and the assay precision (the intra-assay coefficient of variation) was 2.13%.

Serum GH was determined using a rat GH RIA kit (Amersham Pharmacia Biotech, Piscataway, NJ). The sensitivity of the GH RIA was 3.2 ng/mL (with 50-μL serum aliquots per RIA tube), and the intra-assay coefficient of variation was 1.97%.

Anterior Pituitary Analyses

PRL and glandular kallikrein are estrogen-induced proteins in the anterior pituitary with differing sensitivities to E2 (Hatala and Powers, 1988a; Powers et al, 1989); their measurement provided a sensitive index of estrogenic bioactivity. PRL levels were determined using a rat PRL RIA kit (Amersham). The sensitivity of the PRL RIA was 2.0 μg per pituitary with the sample

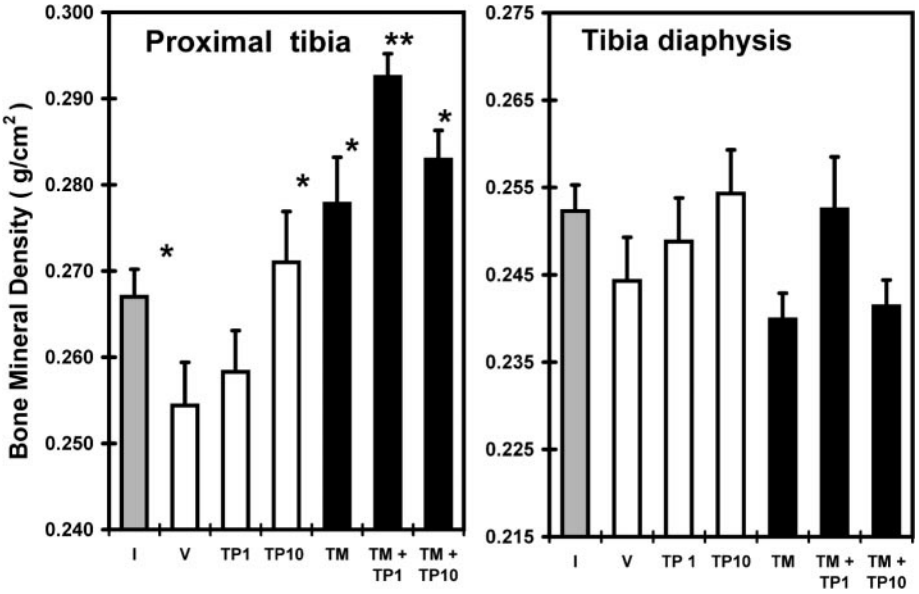

Figure 1. Effect of testosterone propionate (TP) and tamoxifen (TM) on bone mineral density in the proximal tibia and tibia diaphysis. This figure and all other figures and tables show results from orchidectomized rats treated for 6 weeks with the following: vehicle (V; n = 8); TP at 1 mg/kg 3 times weekly (TP1; n = 7); TP at 10 mg/kg 3 times weekly (TP10; n = 7); TM at 1 mg/kg daily (TM; n = 7); TM + TP1 (n = 7); or TM + TP10 (n = 8). Testis-intact male rats (I; n = 8) were also studied. Values represent mean ± SE. * $P < .05$ vs V; ** $P < .05$ vs TM.

dilution used, and the intra-assay coefficient of variation was 1.45%.

Levels of glandular kallikrein were measured enzymatically using D-Val-Leu-Arg-p-nitroanilide as previously described (Hatala and Powers, 1988a). Total pituitary protein provided an index of estrogenic effects to induce anterior pituitary hyperplasia (Hatala and Powers, 1988a).

Statistical Analysis of Grouped Data

Data were subjected to an analysis of variance and then to a multiple comparisons analysis with the Fisher least significant difference test; $P < .05$ was the criterion of significance. Serum GH values in all statistical tests were log transformed to normalize variances. Data in the 1 mg/kg TP group were also transformed to express the percentage of prevention of the various ORX effects [% prevention = 100 × (observed value − orchidectomized group mean)/(intact group mean − orchidectomized group mean)]. This format enabled a formal statistical comparison of the responsiveness of different parameters to TP at 1 mg/kg.

Correlation/Regression Analysis

Linear regression analysis and the Pearson correlation coefficient (*r*) were used to identify significant associations between GH or IGF-I and other parameters. These analyses were performed on individual rat data sets (single units of association) from 41 of the 44 orchidectomized rats in the study. Serum GH and IGF-I data were not obtained for 3 rats that did not yield enough serum for RIAs (1 in the tamoxifen group; 2 in the 10 mg/kg TP + tamoxifen group).

Results

Effects on Tibia BMD

Orchidectomized rats exhibited a 5% decrease in proximal tibia BMD relative to testis-intact rats (Figure 1). Treatment with TP at 1 mg/kg did not significantly alter BMD in orchidectomized rats, but TP at 10 mg/kg maintained proximal tibia BMD at the levels of testis-intact rats—presumably due to estrogenic activity arising with this dose (see below). Tamoxifen evoked even greater increases in proximal tibia BMD, yielding levels 4% higher than testis-intact controls. Proximal tibia BMD was highest in rats given both tamoxifen and TP (Figure 1), but only the combination with TP at 1 mg/kg yielded increases that were significantly greater than those achieved with tamoxifen alone. Tibia diaphysis BMD was not significantly affected by ORX, tamoxifen, or TP (Figure 1). This is consistent with the much lower ratio of cancellous to cortical bone in the tibia diaphysis compared to the proximal tibia and the greater sensitivity of cancellous bone to gonadal hormones (Kalu, 1991; Shen et al, 1993; Thompson et al, 1995).

Effects on Serum Cholesterol

Serum cholesterol was increased in orchidectomized rats relative to testis-intact controls (Figure 2). TP treatment blocked this increase, with doses of 1 and 10 mg/kg yielding equivalent effects. Tamoxifen also decreased cholesterol in orchidectomized rats, but unlike TP, tamoxifen

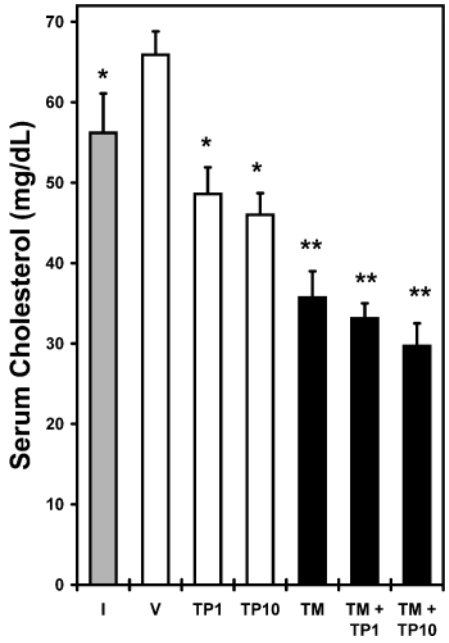

Figure 2. Effect of testosterone propionate (TP) and tamoxifen (TM) on serum cholesterol. Values represent mean ± SE. Testis-intact = I; vehicle-treated = V. * $P < .05$ vs V; ** $P < .05$ vs I.

yielded levels significantly lower than in intact controls. TP had little effect on serum cholesterol in orchidectomized rats receiving tamoxifen (Figure 2).

Effects on Prostate and Seminal Vesicle Weight

Growth of male sex accessory organs is androgen-dependent, and seminal vesicle and ventral prostate wet weights provided an index of endogenous androgenic bioactivity. Orchidectomized rats displayed a marked atrophy of the seminal vesicle and prostate relative to testis-intact rats (Figure 3), and treatment with TP at 1 mg/kg maintained sex organ weights at intact control levels. Sex organ weights in rats given TP at 10 mg/kg were almost twice that of intact controls, indicating supraphysiologic stimulation. Tamoxifen had no effect on sex organ weight and neither inhibited nor enhanced TP actions.

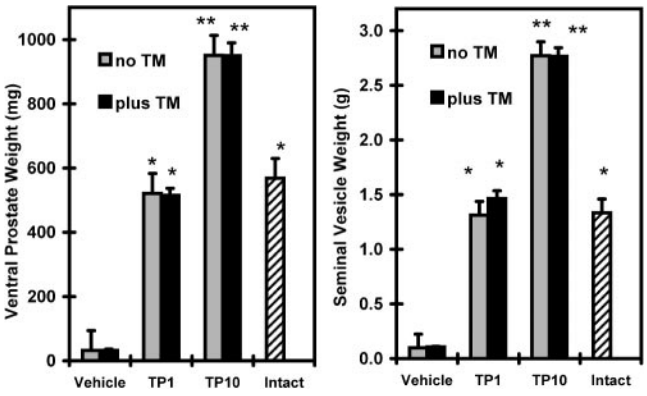

Figure 3. Effect of testosterone propionate (TP) and tamoxifen (TM) on seminal vesicle and ventral prostate weights. Values represent mean ± SE. Intact = normal male rats. Vehicle = orchidectomized rats given sesame oil/2% benzyl alcohol. TP1 = orchidectomized rats treated with TP at 1 mg/kg. TP10 = orchidectomized rats treated with TP at 10 mg/kg. * $P < .01$ vs vehicle and TP10. ** $P < .01$ vs vehicle, TP1, and intact.

Effects on Somatic Growth

Compared to testis-intact rats, orchidectomized rats gained 30% less body weight, and this was coupled with a retardation of longitudinal growth (ie, a 1.1-mm decrease in tibia length) (Table 1). Androgen replacement with TP at 1 mg/kg prevented 84% and 64% of ORX effects on weight gain and tibia length, respectively, but TP at 10 mg/kg did not—probably because of dose-related estrogenic activity (see below).

Tamoxifen totally inhibited body weight gain and decreased tibia length a further 1.4 mm relative to orchidectomized controls. TP modestly increased weight gain and tibia length in rats given tamoxifen but did not reverse the bulk of tamoxifen effects (Table 1).

Effects on Serum GH and Serum IGF-I

ORX markedly decreased serum GH relative to testis-intact rats (Table 1); TP at 1 mg/kg had no effect on GH, but TP at 10 mg/kg prevented about 70% of the decrease. Tamoxifen also partly restored serum GH in orchidectomized rats and interacted with TP to yield GH levels match-

Table 1. *Effect of testosterone propionate and tamoxifen on parameters related to growth and metabolism in orchidectomized male rats†*

Groups	Δ Body Weight (g)	Tibia Length (mm)	Serum GH (ng/mL)	Serum IGF-I (ng/mL)	Serum Glucose (mg/dL)	Body Temperature (°C)
Intact	132 ± 14*	43.1 ± 0.1	152 ± 56	988 ± 25*	197 ± 6	35.8 ± 0.1*
Vehicle	99 ± 9	42.0 ± 0.2***	38 ± 12***	858 ± 24	197 ± 5	36.7 ± 0.1
TP1	127 ± 6*	42.7 ± 0.3	24 ± 6***	965 ± 43*	180 ± 11	36.0 ± 0.1*
TP10	96 ± 14	42.0 ± 0.1***	117 ± 36	771 ± 24*	181 ± 7	35.7 ± 0.2*
TM	−1 ± 2**	40.6 ± 0.3**	93 ± 37	691 ± 32**	162 ± 8*	36.4 ± 0.1
TP1 + TM	26 ± 3**	41.2 ± 0.3**	168 ± 24	683 ± 29**	162 ± 6*	35.7 ± 0.2*
TP10 + TM	24 ± 8**	40.8 ± 0.3**	253 ± 65	648 ± 34**	165 ± 6*	35.5 ± 0.1*

† All rats were orchidectomized except for those in the intact group. GH indicates growth hormone; IGF-I, insulin-like growth factor I; TP1 and TP10, 1 and 10 mg of testosterone propionate per kilogram 3 times weekly for 6 weeks; and TM, 1 mg of tamoxifen per kilogram daily for 6 weeks. Values represent mean ± SEM.

* $P < .05$ vs vehicle-treated group; ** $P < .05$ vs matching group without tamoxifen; *** $P < .05$ vs intact group.

Table 2. *Effect of testosterone propionate and tamoxifen on anterior pituitary parameters in orchidectomized male rats†*

Groups	Prolactin (μg)		Kallikrein (nmol/min)		
	Total	Per mg Protein	Total	Per mg Protein	Protein (total μg)
Testis intact	57.5 ± 3.5	37.6 ± 2.2	0.88 ± 0.06*	0.58 ± 0.03*	1588 ± 97
Vehicle	56.9 ± 4.3	35.4 ± 1.5	1.20 ± 0.04	0.75 ± 0.03	1603 ± 59
TP1	59.7 ± 5.6	39.9 ± 3.5	0.94 ± 0.04*	0.63 ± 0.02*	1500 ± 67
TP10	96.2 ± 10.0*	65.9 ± 5.9*	0.77 ± 0.04*	0.53 ± 0.03*	1447 ± 36
TM	49.5 ± 2.8	41.9 ± 1.5	0.91 ± 0.07*	0.78 ± 0.04	1181 ± 61*
TP1 + TM	49.8 ± 2.4	47.9 ± 2.6	0.57 ± 0.04**	0.55 ± 0.03*	1048 ± 42*
TP10 + TM	46.4 ± 3.6	44.2 ± 3.5	0.47 ± 0.03**	0.44 ± 0.02*	1053 ± 32*

† All rats were orchidectomized except for those in the intact group. TP1 and TP10 indicate 1 and 10 mg of testosterone propionate 3 times weekly for 6 weeks; TM, 1 mg of tamoxifen per kilogram daily for 6 weeks. Values represent mean ± SEM.
* $P < .05$ vs vehicle-treated group; ** $P < .05$ vs TM or matching group without TM.

ing those in testis-intact rats. Indeed, TP alone at 1 mg/kg lacked effect on GH but enhanced tamoxifen effects to increase GH (Table 1).

Orchidectomized rats also had lower serum IGF-I levels than testis-intact rats (−130 ng/mL) (Table 1). TP doses of 1 mg/kg prevented this effect of ORX. However, TP doses of 10 mg/kg had the opposite effect and lowered IGF-I a further 87 ng/mL, an action consistent with the emergence of estrogenic activity at high doses. Tamoxifen caused even larger decreases in serum IGF-I (−167 ng/mL), and TP had little effect on IGF-I in rats given tamoxifen.

Effects on Food Intake, Serum Glucose, and Body Temperature

There were no significant group differences in food consumption per kilogram of body weight (data not shown). Serum glucose was unaffected by ORX or TP treatment, whereas tamoxifen caused small decreases (Table 1). Body temperature was 0.9° higher in orchidectomized rats than in testis-intact rats, and TP prevented this increase (Table 1); body temperature was unaffected by tamoxifen. These data match prior results from this laboratory and indicate that changes in food intake, glucose metabolism, or thermogenesis are unlikely to explain changes in BMD, cholesterol, or growth.

Effects on Anterior Pituitary Levels of PRL, Glandular Kallikrein, and Total Protein

Anterior pituitary PRL and glandular kallikrein levels are higher in female than in male rats due to induction by ovarian estrogens, and tamoxifen acts as an antagonist in these estrogen responses (Hatala and Powers, 1987, 1988b; Powers et al, 1989). These measures provided indices of endogenous estrogenic bioactivity. ORX did not alter PRL levels (Table 2). PRL also was unaffected by TP or tamoxifen at 1 mg/kg, but TP at 10 mg/kg induced an increase that was blocked by tamoxifen.

Unlike PRL, glandular kallikrein activity in the anterior pituitary (total or per milligram of protein) increased with ORX, and TP at 1 mg/kg prevented the increase (Table 2). Tamoxifen also reduced total pituitary kallikrein, but its effects were additive with TP. Moreover, unlike TP, tamoxifen did not lower kallikrein activity per milligram of protein. Thus, the tamoxifen effect on total kallikrein was secondary to a nonspecific decrease in total pituitary protein (Table 2). Neither ORX nor TP notably altered total pituitary protein or tamoxifen effects on this parameter.

Overall, the PRL data provided evidence for the presence of a low level of estrogenic bioactivity in orchidectomized rats given TP at 10 mg/kg but not in rats given TP at 1 mg/kg or in testis-intact rats.

Differential Prevention of ORX Effects by TP at 1 mg/kg

Informal comparisons of the TP responsiveness of various parameters indicated that BMD and serum GH were much less responsive to TP at 1 mg/kg than other parameters. To statistically confirm this observation, data for each parameter in the TP group given 1 mg/kg were transformed to express the percentage of prevention of the ORX effect (see ''Materials and Methods''). This transformation enabled a formal statistical comparison of the responsiveness of the different parameters to TP at 1 mg/kg (Figure 4). The greatest responsiveness to TP at 1 mg/kg was displayed by cholesterol (109% prevention) and seminal vesicle weight (98.2% prevention). BMD (31% prevention) and serum GH (−8.5% prevention) had the lowest responsiveness and were the only parameters whose responsiveness to TP at 1 mg/kg significantly differed from the seminal vesicles.

Correlation/Regression Analyses

Sex hormone effects on longitudinal growth in the rat are mediated by alterations in the GH–IGF-I axis, and similar mechanisms may contribute to sex hormone effects on bone remodeling and BMD (see ''Discussion''). The relation of serum GH and IGF-I to BMD and somatic growth was studied using the individual data sets of or-

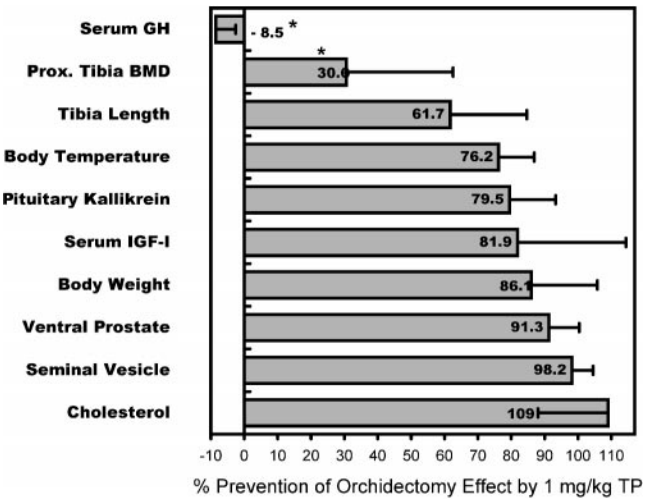

Figure 4. Differential responsiveness of orchidectomy (ORX) effects when compared with testosterone propionate (TP) at 1 mg/kg. Data from the group receiving TP at 1 mg/kg were transformed to express the percentage of prevention of the ORX effect on each of the indicated parameters (see ''Methods''). * $P < .05$ vs seminal vesicle.

chidectomized rats (n = 41); these data sets capture changes associated with TP and tamoxifen treatments.

Serum GH and IGF-I levels were inversely related to each other (Table 3). This is a distinctive feature of the GH–IGF-I axis during estrogenic manipulations in the presence of T_3 (Fitts et al, 2001). Proximal tibia BMD also exhibited a significant positive correlation with serum GH but displayed a significant negative correlation with serum IGF-I (Table 3; Figure 5). Moreover, GH and IGF-I were more related to BMD than to each other, suggesting that GH and IGF-I were each independently related to BMD. This was supported by multiple linear regression analysis. Regression predictions [BMD $\times 10^3$ = 300.9 + (12.02 $\times$ log GH) − (0.06425 $\times$ IGF-I)] were well correlated with observed BMD ($r = 0.692$, $P < .001$), and the GH and IGF-I coefficients each made significant contributions to the prediction of BMD ($P < .01$).

Serum IGF-I also exhibited strong positive correlations with body weight gain and tibia length, while GH exhibited negative correlations with these measures (Table 3). Significant coefficients for IGF-I ($P < .0001$) as well as GH ($P < .05$) were obtained in the multiple regression predicting body weight gain. For the multiple regression predicting tibia length, however, only the IGF-I coefficient was significant ($P < .01$).

Discussion

T is the principal testicular steroid hormone of rats and men and is responsible for the masculinizing effects of the testis. However, certain metabolic and endocrine effects of the testis are postulated to reflect the actions of E2 arising from T aromatization in nongonadal organs. TP is a T ester that is rapidly hydrolyzed to T after absorption, and modest TP doses were expected to readily prevent ORX effects due to a loss of T or its extragonadal estrogen metabolites. However, TP doses of 1 mg/kg that maintained sex organ growth and several other parameters at physiologic levels failed to prevent decreases in proximal tibia BMD and serum GH. Moreover, a TP dose of 10 mg/kg that prevented ORX effects on BMD and GH caused supraphysiologic sex organ growth and other abnormalities. Such data imply that T doses that yield useful effects on bone mass subject the prostate and other tissues to a more intense androgenic stimulation than normal for males. In contrast, tamoxifen blocked ORX effects on BMD and GH without affecting sex organ growth, and tamoxifen effects on BMD and serum GH were selectively enhanced by TP doses of 1 mg/kg. Overall, the data suggest that tamoxifen might be useful for bone protection in T-deficient men when androgens or other therapeutics (eg, bisphosphonates) are problematic. In this regard, it is notable that tamoxifen has been widely used for almost 30 years and is generally considered a reasonably safe and well-tolerated drug (MacGregor and Jordan, 1998). Although tamoxifen and other SERMs increase the risk of stroke and thromboembolism to the same degree as low estrogen doses, such vascular events are very rare either with or without tamoxifen use, and tamoxifen does not increase risk of ischemic heart disease (see Fisher et al, 1998). Nonetheless, clinical trials will be needed to determine the ultimate value of tamoxifen for T-deficient men at risk of osteoporosis.

The present data resemble findings in male rats castrated at 49 days and treated with pellets containing T for 3 weeks; T pellets that prevented cancellous bone loss

Table 3. *Correlation of serum GH and IGF-I with proximal tibia BMD and measures of somatic growth in orchidectomized male rats*†*

Comparison	Serum IGF-I	Δ Body Weight	Tibia Length	Proximal Tibia BMD
Serum GH‡	$-0.380^{0.015}$	$-0.480^{0.002}$	$-0.408^{0.008}$	$0.523^{0.001}$
Serum IGF-I	. . .	$0.754^{0.001}$	$0.503^{0.001}$	$-0.618^{0.001}$

* The Pearson correlation coefficient (r) was calculated using individual data sets from all orchidectomized rats (n = 41). Superscripts indicate the P value for each correlation.

† BMD indicates bone mineral density; GH, growth hormone; and IGF-I, insulin-like growth factor I.

‡ Serum GH values were log transformed to achieve a normal (Gaussian) distribution.

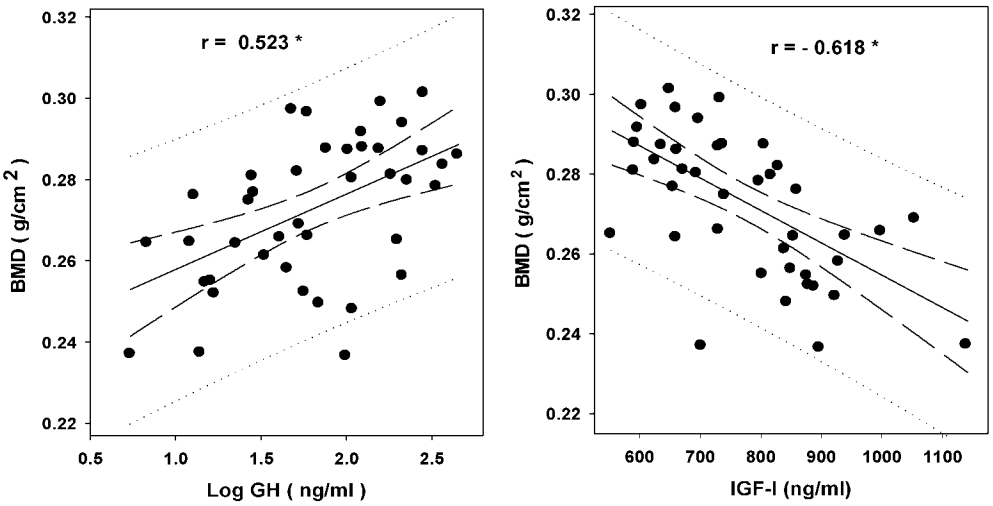

Figure 5. Scatter and line plots of serum growth hormone (GH) and insulin-like growth factor I (IGF-I) relative to proximal tibia bone mineral density (BMD). **(Left panel)** GH and BMD plots. **(Right panel)** IGF-I and BMD plots. The solid line shows the regression plotted from the individual rat data points (n = 41); dashed and dotted lines indicate the 95% confidence intervals for the regression and the predictions, respectively. * $P < .001$.

also suppressed growth and evoked supraphysiologic sex organ weights (Wakley et al, 1991). However, the opposite was reported in aged rats orchidectomized at 12 months and treated with T-filled Silastic capsules for 15 weeks (Vanderschueren et al, 2000). Cancellous bone loss was fully prevented by T capsules that preserved only 50% of seminal vesicle and prostate weights; thus, bone seemed more sensitive to low T doses than male sex accessory organs renowned for their T sensitivity.

The report of Vanderschueren et al (2000) was notable, since it suggested that low T doses might preserve bone and muscle mass in aging men without jeopardizing prostate health (see Vermeulen, 2001). However, further study of this issue seems warranted. Indeed, the influence of different methods of T dosing merits consideration. Classic pharmacokinetic principles dictate that regular TP injections will rapidly yield a steady state that will maintain plasma T within a fairly narrow range for as long as dosing is continued (Meikle, 1999). Steroid-filled Silastic capsules, however, have much different pharmacokinetics (Kincl and Rudel, 1971; Longer and Robinson, 1985). In vivo steroid release by Silastic capsules rapidly peaks and then undergoes an exponential decay featuring a large and rapid decline in its initial phase, which is followed by a longer phase of slowly declining release. This is well documented with Norplant, the only Silastic capsule system approved for systemic clinical therapy. Norplant releases contraceptive doses of levonorgestrel for more than 5 years, but steroid release and plasma levels peak 24 hours after implantation, decline 60%–80% by 18 months, and then decline 4%–5% per year (Drug Facts and Comparisons, 2001). Correspondingly, the sex organ weights of castrated male rats peak 4–6 weeks after T-capsule implantation and then exhibit progressive and sizable declines (Schenck et al, 1977). Such pharmacokinetics may confound efforts to compare the T sensitivity of tissues that differ in the dynamics of their response to T. In particular, male sex organs respond to T manipulations within days (Parkes, 1936), but 12–16 weeks are required for significant changes in bone mass to evolve in aged rats (Wronski et al, 1989; Kalu, 1991; Vanderschueren et al, 1992). Thus, in aged rats bearing T capsules for 15 weeks, sex organ weights may reflect the T-release rate of the 15th week, but bone mass may still show the influence of the much higher rates of T release 8–12 weeks earlier. This scenario highlights the dosing limitations of steroid-filled Silastic capsules.

In normal male rats and men, the miniscule amounts of estrogen made by the testis and other organs appear inadequate for a role as a circulating endocrine hormone. However, such estrogen production has been postulated to yield localized autocrine and paracrine effects that are physiologically important in males (Sharpe, 1998; Simpson et al, 2000). In the present study, TP at 1 mg/kg provided a physiologic level of T replacement for sex organ growth (an androgenic response), somatic growth (an anabolic response), serum IGF-I, cholesterol, pituitary kallikrein, and body temperature. Nonetheless, TP at 1 mg/kg had little effect on BMD or serum GH, and neither ORX nor TP at 1 mg/kg altered pituitary PRL, a sensitive index of estrogenic activity (Powers et al, 1989; DiPippo et al, 1995). Such results are difficult to reconcile with the hypothesis that physiologic levels of T and nongonadal aromatase yield sufficient levels of localized estrogenic activity to explain testis effects on bone mass and serum GH. Nonetheless, the data are consistent with the core concept that a substance other than T mediates testis effects on such targets.

The effects of TP at 10 mg/kg on sex organ growth, cholesterol, body temperature, and pituitary kallikrein were consistent with a modest progression of the androgenic effects evoked by TP at 1 mg/kg. However, the actions of TP at 10 mg/kg on BMD, somatic growth, GH, IGF-I, and PRL were distinct and were consistent with the emergence of effects produced by an estrogen such as E2. Indeed, tamoxifen antagonizes PRL induction by E2 (Powers et al, 1989; MacGregor and Jordan, 1998), and the tamoxifen blockade of PRL increases evoked by TP at 10 mg/kg linked ERs to this response. Nonetheless, it should be emphasized that ORX did not affect pituitary PRL, a sensitive E2 target in the rat. Moreover, TP at 10 mg/kg just barely restored BMD and only partially restored serum GH but yielded PRL levels well above those of normal male rats, failed to reverse ORX effects on somatic growth, and further decreased serum IGF-I. Such data seem incongruous with the idea that nongonadal production of an E2-like estrogen from T can explain testis effects on BMD and serum GH. Rather, the data seem to suggest that a substance other than T or its estrogenic metabolites is involved in such testis effects.

The GH–IGF-I axis powerfully stimulates longitudinal bone growth, which involves a cyclic interplay of chondrocyte growth, endochondral ossification, and bone modeling coordinated by multiple hormone and cytokine signaling systems (Ohlsson et al, 1993; Williams et al, 1998; Spelsberg et al, 1999). The GH–IGF-I axis is also required for the maintenance of bone mass in adults (Holmes and Shalet, 1996; Ohlsson et al, 1998). In rats and men, adult-onset GH deficiency accelerates bone loss, and GH stimulates bone remodeling in a way that enhances bone formation more than bone resorption to eventually yield gains in BMD (Holmes and Shalet, 1996; Ohlsson et al, 1998). The role of IGF-I in such GH effects, however, is not fully understood.

Sex hormone actions on the GH–IGF-I axis are largely responsible for sexual dimorphisms in longitudinal bone growth (Jansson et al, 1985; Ohlsson et al, 1993; Gatford et al, 1998), and related mechanisms may contribute to sex hormone effects on bone mass (Holmes and Shalet, 1996; Ohlsson et al, 1998). Indeed, the disparate responses of the GH–IGF-I axis to different TP doses and tamoxifen were suggestive of a link to BMD. Thus, BMD increases evoked by tamoxifen were attended by GH increases and IGF-I decreases, while TP at 1 mg/kg failed to raise GH or lower IGF-I and lacked effect on BMD, and TP at 10 mg/kg produced a BMD increase that was again coupled with GH increases and IGF-I decreases. Moreover, the enhancement of tamoxifen effects on BMD by TP at 1 mg/kg was coupled with potentiation of tamoxifen effects to increase GH. The relation of serum GH and IGF-I to BMD was more rigorously evaluated by correlation/regression analyses of the individual rat data sets. The resulting multiple linear regression statistics indicated that about 50% of the variation in proximal tibia BMD in experimental rats is related to variations in serum GH and IGF-I (ie, $r^2 = 0.479$). Equivalent multiple regression results were reported in an analysis of orchidectomized, hypothyroid rats during estrogenic manipulations in the presence of T_3 replacement ($r^2 = 0.504$) (Fitts et al, 2001); thus, the present findings match prior results. Although such regressions do not prove causation, they satisfy a key prerequisite of the hypothesis that changes in serum GH and IGF-I contribute to sex hormone and tamoxifen effects on BMD. Direct estrogen effects on bone also contribute to changes in BMD (Spelsberg et al, 1999). Thus, gonadal regulation of BMD seems likely to involve a coordinated interplay of direct sex hormone effects on bone and indirect actions secondary to modulation of the GH–IGF-I axis.

Many GH effects reflect its action to elevate serum IGF-I (the somatomedin hypothesis) (Le Roith et al, 2001), and regression analyses examining T_3 effects in hypothyroid, orchidectomized rats exemplify core predictions of this hypothesis (Fitts et al, 2001). In particular, serum GH and IGF-I exhibited a direct relationship to each other as well as measures of somatic growth, and serum IGF-I more closely predicted somatic growth than GH. It was also notable that BMD showed little relation to GH or IGF-I levels during T_3 manipulations, despite large changes in GH and IGF-I (Fitts et al, 2001). A strong direct relationship between serum IGF-I and measures of somatic growth was also present during the sex hormone and tamoxifen manipulations of this study and prior work (Fitts et al, 2001). However, a novel pattern of associations among GH, IGF-I, and BMD emerged when sex hormone and tamoxifen manipulations were performed: serum GH and IGF-I became inversely related (a reversal of the relationship seen during T_3 manipulations), and BMD increased as GH levels rose and IGF-I levels fell. Such findings appear incompatible with classic predictions of the somatomedin hypothesis but suit the core concept of the dual-effector hypothesis of GH actions (Green et al, 1985). A similar conclusion was reached in a recent study of the skeletal structure of IGF-I–deficient mice (Bikle et al, 2001). Thus, effector mechanisms distinct from those affecting somatic growth may mediate BMD responses to GH in the presence of sex hormones. On the whole, the above findings suggest that gonadal hormones can transform the regulation of the GH–IGF-I axis and create a distinct physiologic state in which serum GH increases are coupled with decreases in serum IGF-I. The altered balance of systemic GH to IGF-I signaling seems likely to modulate bone growth and remodeling and might also be relevant to gonadal effects on other physiologic systems.

The positive correlation between serum GH and BMD

is consistent with the role of GH in maintaining bone mass (Ohlsson et al, 1998). However, the inverse relation between IGF-I and BMD may seem paradoxical, since IGF-I is essential for bone growth, and some studies of osteoporotic patients have reported positive correlations between IGF-I and cancellous BMD (Kurland et al, 1997; Langlois et al, 1998; Garnero et al, 2000). Also, a complex gene locus that lowers serum IGF-I and BMD in mice has been reported (Bouxsein et al, 2002). Nonetheless, exogenous IGF-1 has shown little promise in osteoporotic patients, and corresponding rat studies have reported increases (Ammann et al, 1993; Bagi et al, 1994), no effect (Narusawa et al, 1995), and decreases in cancellous bone mass (Ibbotson et al, 1992; Tobias et al, 1992). On the other hand, cancellous bone mass is increased in IGF-I–deficient mice (Bikle et al, 2001), which fits well with the present data. Even more importantly, animal and human studies have consistently shown that estrogen or SERM treatments that reduce bone turnover and prevent bone loss also yield decreases in serum IGF-I (Wiedemann et al, 1976; Clemmons et al, 1980; Kalu et al, 1994; Borski et al, 1996; Goodman-Gruen and Barrett-Conner, 1996; Fitts et al, 1998, 2001). In the rat, this is associated with decreases in hepatic IGF-I synthesis (Murphy and Friesen, 1988; Huynh et al, 1993; Krattenmacher et al, 1994; Borski et al, 1996) and reductions in serum IGFBP-3, a GH-sensitive IGFBP whose regulation sometimes parallels IGF-I (Kalu et al, 1994; Rosen, 1999; Arjmandi et al, 2000). Thus, the inverse relationship between IGF-I and cancellous BMD suitably fits the metabolic and therapeutic actions of estrogens and SERMs.

In conclusion, in this preclinical modeling study using male rats, tamoxifen blocked ORX effects on bone mass and GH without altering sex organ growth. Such features of tamoxifen could be advantageous for osteoporosis prevention in hypogonadal men when androgens or other anti-osteoporosis agents are undesirable. Unexpectedly, a TP dose that prevented ORX-induced sex organ atrophy did not protect bone mass, and supraphysiologic sex organ growth accompanied bone protection achieved with a larger TP dose. Thus, sex organ hyperstimulation might limit the value of androgens for osteoporosis prevention. The results also imply that products other than T or its nongonadal metabolites are involved in testis effects on bone mass and GH in the rat and show that gonadal effects on bone mass are correlated with novel changes in GH–IGF-I axis function.

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