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Long-Term Effect of Testosterone Therapy on Bone Mineral Density in Hypogonadal Men

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ABSTRACT

In both men and women, a decrease in bone mineral density (BMD) is a major symptom of hypogonadism. Although the effects of estrogens on osteoporosis in women are well documented, comparatively little is known about the effects of long term testosterone substitution on BMD in hypogonadal men. Therefore, we studied BMD in 72 hypogonadal patients (37 men with primary and 35 men with secondary hypogonadism) under testosterone substitution therapy that continued for up to 16 yr. Thirty-two of these men were also seen before initiation of therapy. At annual intervals, trabecular BMD of the lumbar spine was measured by quantitative computed tomography, a true volumetric and reproducible method for long term serial BMD measurements. Serum levels of testosterone increased to the normal range in all androgen-treated hypogonadal men. The most significant increase in BMD was seen during the first year of testosterone treatment in previously untreated patients, when BMD increased from 95.2 ± 5.9 to 120.0 ± 6.1 mg/cm^3 hydroxyapatite (mean ± SE). Long term testosterone treatment maintained BMD in the age-dependent reference range in all 72 hypogonadal men, independent of the type of hypogonadism. Transdermal testosterone patches applied to the scrotum were as effective in normalizing BMD as im testosterone enanthate injections. In summary, testosterone therapy increases BMD in hypogonadal men regardless of age. The greatest increase is seen during the first year of treatment in previously untreated patients with low initial BMD. In hypogonadal men, BMD can be normalized and maintained in the normal range by continuous, long term testosterone substitution. (*J Clin Endocrinol Metab* **82:** 2386–2390, 1997)

ONE OF THE prominent clinical symptoms of testosterone deficiency in men is a significant decrease in bone mineral density (BMD) (1, 2). Case-controlled studies have demonstrated that in hypogonadal men this reduced BMD is associated with a significant increase in bone fractures (3). The biological action of testosterone on bone is of high clinical and socioeconomic relevance, as the prevalence of hypogonadism increases significantly in elderly men and exceeds 20% in men over 60 (4, 5). Along with lower testosterone levels a clear decrease in BMD (6, 7) and an increase in hip and spine fractures (8) have been demonstrated in aging males, similar to the well-described changes in postmenopausal women.

Large multicenter clinical trials have shown that estrogen replacement therapy prevents loss of BMD and decreases the incidence of bone fractures, which in untreated women are caused by the decrease in endogenous estrogens after menopause (9–11). As comparatively little is known about the beneficial effects of long term testosterone substitution therapy on bone in men (2), we investigated BMD changes in hypogonadal men treated with testosterone preparations for up to 16 yr. In addition, we compared the effects of standard im testosterone injection therapy to those of modern transdermal testosterone application.

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Subjects and Methods

Patients

Adult male hypogonadal patients, aged 18–74 yr, who received effective androgen substitution therapy and underwent serial measurements of lumbar spine BMD by quantitative computed tomography (QCT) after giving informed consent were included in the evaluation. Patients with constitutional delay of puberty, other endocrine diseases causing lowered BMD (*e.g.* Cushing's disease), dietary calcium or vitamin D deficiency, or heritable disorders of connective tissue were excluded from the analysis. Thirty-seven patients with primary hypogonadism (mean ± SD age, 35.0 ± 12.5 yr; body mass index, 24.6 ± 4.5 kg/m^2; 21 patients with Klinefelter's syndrome and 16 patients with primary hypogonadism because of anorchia, previous orchitis or unknown reasons) and 35 patients with secondary hypogonadism (age, 35.4 ± 11.3 yr; body mass index, 25.3 ± 4.7 kg/m^2; 14 patients with idiopathic hypogonadotropic hypogonadism or Kallmann's syndrome and 21 patients with pituitary tumor including macroprolactinoma or panhypopituitarism with effective substitution therapy of the other endocrine axes) qualified for the study analysis. In 32 of these patients initial QCT measurement was carried out before initiation of androgen substitution therapy. The other 40 patients had received effective androgen therapy for 3.4 yr (range, 1–11 yr) when QCT was introduced into our diagnostic work-up 10 yr ago.

Androgen therapy

Hypogonadism was treated by exogenous testosterone or, in seven patients with secondary hypogonadism, with hCG or pulsatile GnRH. Testosterone preparations were administered im, transdermally, or orally. Intramuscular substitution therapy was applied to 52 patients by 250 mg testosterone enanthate (Testoviron Depot 250, Schering, Berlin, Germany), injected in most cases every 3 weeks (range, 2–4 weeks). Eleven patients were given transdermal therapy with scrotal testosterone patches (Testoderm, Alza, Palo Alto) at a daily dose of 4–6 mg/day. Two patients received oral substitution therapy with 3 doses of 40 mg testosterone undecanoate (Andriol, Organon, Oberschleißheim, Germany)/day. Patients with secondary hypogonadism desiring paternity received combined treatment with human menopausal gonadotropin

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(hMG; Pergonal, Serono, Unterschleißheim, Germany; 150 IU, three times per week) and hCG (Pregnesin, Serono, Unterschleisheim, Germany; 1000–2500 IU, twice per week; 6 patients) or pulsatile GnRH (Lutrelef, Ferring, Kiel, Germany; 1 patient) for a period of 12–24 months.

Hormone measurements

Morning blood samples for testosterone measurements were drawn between 0800–1200 h. To test for effective testosterone substitution, blood was taken at time points indicating average testosterone serum levels during substitution therapy (12). In patients treated with transdermal testosterone patch or oral testosterone undecanoate, blood was taken 3–6 h after administration (12, 13). In patients treated with im testosterone, blood was collected preferentially during the second week, and in hCG/hMG-treated patients, blood was obtained 2 days after injection (12). Serum testosterone levels were measured before initiation of therapy and then at yearly intervals. Testosterone levels before initiation of therapy, after 1 yr of treatment, and at the last QCT measurement of all individual patients were included in the statistical analysis. Serum testosterone levels were measured by RIA as described previously (14). The detection limit for testosterone was 0.7 nmol/L. The intra- and interassay coefficients of variation were 5.8% and 8.5%, respectively. Estradiol was measured by RIA (Sorin Biomedica, Saluggia, Italy). The detection limit for estradiol was 37 pmol/L. The intra- and interassay coefficients of variation for estradiol were 6.6% and 8.1%, respectively. The upper normal limit for estradiol is 250 pmol/L.

Measurement of BMD

In most cases BMD was obtained by QCT measurements at yearly intervals. The examinations were performed with a Tomoscan 350 (Philips, Best, The Netherlands; January 1985 to March 1993) and a Tomoscan LX (Philips; since April 1993). A calibration phantom was used in all studies, initially a liquid Cann-Genant-calibration standard (15) (January 1985 to March 1993) and afterward a solid calibration standard (Image Analysis, Columbia, KS; since April 1993). To achieve comparable results, data were adjusted by cross-calibration analysis as previously described (16). Ten-millimeter thick midvertebral slices of L2, L3, and L4 were obtained with the gantry angled parallel to the vertebral end plate using a low dose technique. Trabecular BMD was measured in an oval and a round region of interest, respectively.

Single energy QCT was used to improve the long term precision of BMD measurements and to reduce total radiation exposure of the patients during longitudinal follow-up (17). The known bone marrow fat error of single energy compared to dual energy QCT has been shown to be of limited impact on follow-up BMD measurements (17, 18). A total of 249 BMD measurements (32 before testosterone therapy and 217 during therapy) were performed during the study period in 72 hypogonadal patients. Age-adjusted reference ranges for trabecular BMD (19) and reference ranges for fracture risk (20, 21) were applied for estimation of the therapeutic efficacy of testosterone substitution therapy.

Statistics

Comparisons between BMD and testosterone, respectively, at the first examination, after 1 yr of therapy, and at the last examination were performed by ANOVA for repeated measures. Multiple linear regression and partial correlation analysis were applied for evaluation of the association of testosterone, estradiol, age, and body mass index with BMD and increase in BMD after therapy, respectively. Independent data at the first QCT measurement in the individual patients were used for the analysis. Comparisons between different androgen treatment groups were made by multifactor ANOVA. To adjust for different age, etiology of hypogonadism, different androgen pretreatment, and duration of testosterone substitution therapy in the individual patients, these variables were included in the ANOVA as covariates. Because of the small group number, patients treated with oral testosterone (n = 2), hCG/hMG (n = 6), and pulsatile GnRH (n = 1) were excluded from this comparison. When necessary, analysis was performed on logarithmically or, for percentage data, arcsine-transformed data. Two-sided $P < 0.05$ was considered significant. Computations were performed using the statistical software package SPSS, version 6.1.3 (SPSS, Chicago, IL). Unless otherwise stated, results are given as the mean ± SE.

Results

Androgen substitution therapy up to 16 yr resulted in normalization of testosterone serum levels in all 72 patients (mean ± SE, 5.7 ± 0.6 nmol/L in untreated hypogonadal patients; 19.1 ± 1.3 nmol/L in all patients during testosterone substitution therapy). Of the 32 patients whose initial QCT measurement preceded initiation of androgen therapy, the 15 patients with secondary hypogonadism had lower pretreatment serum levels of testosterone (3.3 ± 0.8 nmol/L) and estradiol (61.7 ± 11.5 pmol/L), and lower BMD (84.1 ± 6.5 mg/cm^3 hydroxyapatite) compared to the 17 patients with primary hypogonadism (7.8 ± 0.6 nmol/L, 83.5 ± 7.4 pmol/L, and 105.0 ± 9.1 mg/cm^3, respectively). In the 32 hypogonadal men with QCT measurements before treatment, multiple regression analysis revealed a significant association of BMD with serum levels of testosterone (partial correlation coefficient = 0.50; $P = 0.005$), and with age (partial correlation coefficient = −0.65; $P = 0.001$), whereas estradiol ($P = 0.53$) levels showed no additional independent association with BMD.

Effective testosterone substitution therapy resulted in a uniform increase in BMD from 95.2 ± 5.9 to 120.0 ± 6.1 mg/cm^3 after the first year and to 125.8 ± 6.0 mg/cm^3 after 2.7 ± 0.3 yr of therapy (range, 1–7 yr) in patients who were still untreated at the first QCT ($P < 0.0001$; Fig. 1). In patients who had been effectively treated for at least 1 yr before their first QCT measurement, BMD increased significantly from 131.6 ± 4.8 to 150.9 ± 5.4 mg/cm^3 during an average treatment period of 4.0 ± 0.4 yr (range, 1–16 yr; $P < 0.001$). However, this increase of 19.3 ± 2.7 mg/cm^3 (15.4 ± 2.1%) was significantly smaller than the increase in patients untreated at the time of first BMD measurement (increase of 30.6 ± 3.1 mg/cm^3 or 39.3 ± 6.0%; $P < 0.001$, different duration of treatment as covariate in the analysis). No significant association could be detected between the age of the hypogonadal patients at initiation of therapy and the in-

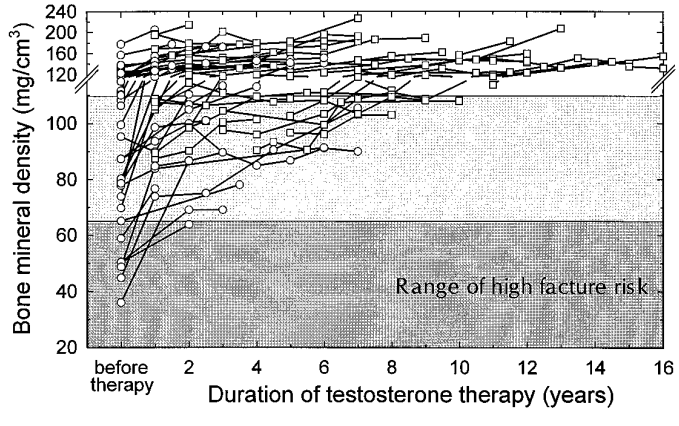

FIG. 1. Increase in BMD during long term testosterone substitution therapy up to 16 yr in 72 hypogonadal patients. *Circles* indicate hypogonadal patients with first QCT measurement before initiation of testosterone substitution therapy, *squares* show those patients already receiving testosterone therapy at the first QCT. The *dark shaded area* indicates the range of high fracture risk, the *unshaded area* shows the range without significant fracture risk, and the *light shaded area* indicates the intermediate range where fractures may occur (20, 21).

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crease in BMD during effective testosterone substitution ($P = 0.81$).

In all 72 patients, multiple regression analysis revealed a significant association between serum levels of testosterone and BMD at the first QCT measurement (partial correlation coefficient = 0.58; $P < 0.0001$) and a weaker one between age and BMD (partial correlation coefficient = −0.49; $P < 0.0001$), whereas estradiol had no additional significant association ($P = 0.96$). Regression analysis revealed a strong negative association between the initial BMD and the relative increase in BMD during androgen substitution therapy ($r = -0.59$; $P < 0.0001$), indicating that the patients with low BMD at the initial QCT measurement had the largest increase in BMD achieved by testosterone therapy (Fig. 2). No significant association could be detected between duration of androgen therapy longer than 1 yr and BMD ($P = 0.54$). This indicates that, on the average, long term androgen therapy maintains the BMD achieved during initial therapy, but does not further significantly increase BMD.

In all individual hypogonadal patients, effective testosterone substitution therapy of longer than 3-yr duration increased BMD to the previously described age-dependent reference range (19). No significant difference in BMD at the last QCT examination was seen between patients treated with im testosterone enanthate (n = 52) and those given transdermal testosterone (n = 11; $P = 0.82$; Table 1). The difference in BMD was still insignificant when different age, different pretreatment at entry into the study, and different duration of testosterone substitution therapy of the two treatment groups were included as covariates in multifactor ANOVA ($P = 0.90$).

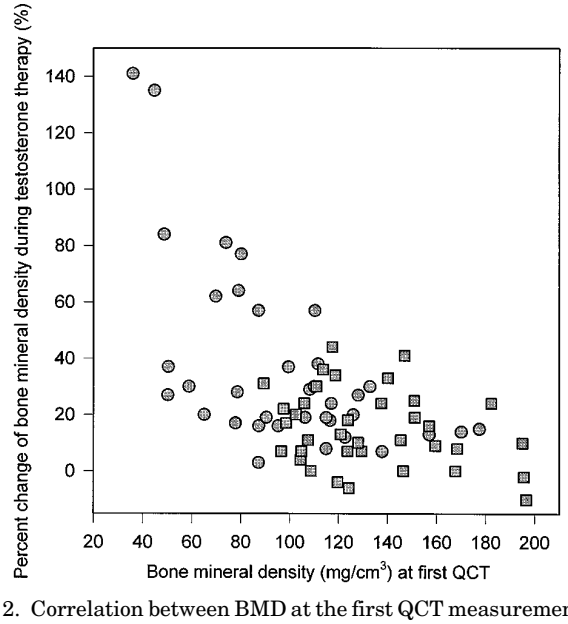

FIG. 2. Correlation between BMD at the first QCT measurement and percent change in BMD during testosterone substitution therapy. *Circles* indicate hypogonadal patients with first QCT measurement before initiation of testosterone substitution therapy; *squares* show those patients already receiving testosterone therapy at the first QCT.

Discussion

Previous studies in hypogonadal men, which in most publications included only a few patients, showed discrepant effects of testosterone therapy on BMD. Of 21 hypogonadal men with GnRH deficiency, only 6 patients, aged 19–26 yr, with open epiphyses experienced an increase in trabecular BMD, as measured by QCT after treatment with testosterone enanthate, hCG, or GnRH for 12–25 months (22). No change in trabecular BMD was seen in the older patients, aged 24–52 yr, with closed epiphyses even after treatment of up to 31 months. In 8 patients with hyperprolactinemic hypogonadism, normalization of testosterone due to different therapies only caused an increase in cortical BMD, whereas no change in trabecular BMD was detected during an observation period of 6–48 months (23). Treatment of 6 hypogonadal patients with 250 mg of a testosterone ester mixture (Sustanon) every 3 weeks for 24 months increased trabecular BMD of the spine by 13.1% (24). Treatment of 14 hypogonadal men with 250 mg Sustanon every month caused an increase in cortical BMD of 5.9%/yr during 55 ± 9 months of therapy, as measured by single photon absorptiometry at the distal radius (25). Treatment of 6 hypogonadal patients with 250 mg Sustanon every week for 3 months caused a small increase in BMD at the lumbar spine by 7% (median), as measured by dual photon absorptiometry (26). In a recent study treatment with sublingual testosterone cyclodextrin at a dose of 5 mg, 3 times daily, in 34 hypogonadal men for 6 months did not change the BMD of total body, hip, or spine when measured by dual energy x-ray absorptiometry (27).

In part, the varying effects of androgen replacement on BMD in hypogonadal men seen in previous studies can be explained by different techniques of BMD measurements, different durations of treatment, and different testosterone preparations applied. In our study we included only patients receiving long term testosterone substitution therapy for at least 1 yr who were treated effectively with established and well described androgen substitution therapies (12, 28). In addition, we applied a true volumetric method for BMD measurement at the same site in all patients and used the single energy QCT technique to improve long term precision during serial measurements (15–17).

In the present evaluation we demonstrated that decreased BMD due to hypogonadism can be restored to the age-dependent reference range by effective, long term androgen substitution therapy. The largest increase was seen in patients with initial low BMD during the first year of treatment. Our results are similar to those obtained in postmenopausal women receiving estrogen replacement therapy, where therapy is most effective during the first year of treatment, and the magnitude of the BMD increase is greatest in those women with low initial BMD (29). In our study we did not assess the incidence of fractures in hypogonadal patients. However, it was shown previously that testosterone deficiency is an important risk factor for fractures in men (3, 21, 30), and in women it has been clearly demonstrated that long term, continuous estrogen hormone replacement therapy that increases BMD reduces the individual risk of bone fractures (9).

In contrast to previous studies we demonstrated that ef-

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TABLE 1. BMD, age, duration of therapy, sex hormones, and BMI in patients treated with im or transdermal testosterone

Androgen substitution therapy	BMD (mg/cm^3)	Age (yr)	Duration of therapy (yr)	Testosterone (nmol/L)	Estradiol (pmol/L)	BMI (kg/m^2)
Im testosterone enanthate (n = 52)	136.6 ± 5.3	38.8 ± 1.9	5.4 ± 0.6	19.7 ± 1.6	104.5 ± 7.0	25.8 ± 0.7
Scrotal testosterone patches (n = 11)	133.8 ± 8.8	42.3 ± 3.4	8.6 ± 1.0	14.7 ± 1.9	76.4 ± 4.8	27.5 ± 1.1

Values are given as the mean ± SE.

fective androgen therapy increases BMD in hypogonadal patients independent of age. The oldest patient in our series was 74 yr old when he was first, remarkably late, diagnosed to have Klinefelter's syndrome. His trabecular BMD increased by im testosterone injections from 36 to 87 mg/cm^3 within 2 yr. Another patient with idiopathic hypogonadotropic hypogonadism was first diagnosed at the age of 61 yr. During treatment with im testosterone enanthate, his trabecular BMD increased from 49 to 85 mg/cm^3 within 12 months. Statistical analysis in all 72 patients of our study revealed that testosterone therapy is an age-independent, highly significant factor influencing BMD. Hypogonadal patients with reduced BMD will benefit from long term, effective androgen substitution therapy, and thus, age *per se* should not preclude patients from appropriate treatment.

It might be argued that relatively high or even supraphysiological serum testosterone levels are necessary to increase BMD in hypogonadal patients. Most previous clinical studies used im testosterone preparations that result in supraphysiological testosterone levels shortly after injection, as demonstrated by detailed pharmacokinetic analysis (12). For this reason we compared the efficacy of im testosterone with transdermal scrotal testosterone systems that lead to physiological serum levels of testosterone in the lower normal range and even mimic normal diurnal variation (13). Statistical analysis revealed no significant difference between both testosterone treatments regarding the BMD achieved. Thus, as is the case for other biological effects (31–33), for treatment of reduced BMD, restoration of testosterone to the normal physiological range seems to be sufficient without the need for pharmacologically high testosterone levels.

The exact mechanism by which androgens affect BMD is not yet fully elucidated (34). *In vitro* studies and clinical trials have shown that nonaromatizable androgens increase bone formation (2, 35). In addition, it has been demonstrated *in vitro* (10) and in male patients with estrogen receptor mutations (36) or aromatase deficiency (37) that estrogens also play an important role in bone mineralization in men. Recently, it was demonstrated by histomorphometry that testosterone treatment of female to male transsexuals after bilateral ovariectomy maintains BMD (38). Available data indicate that both androgens and estrogens have important direct effects on bone. It is well accepted that testosterone substitution therapy should be performed with androgen preparations releasing natural testosterone into the general circulation, which can be converted to the active metabolites dihydrotestosterone and estradiol (28). In our evaluation we cannot discriminate between pure androgenic and estrogenic effects of testosterone therapy on BMD. However, statistical analysis revealed that serum levels of testosterone show the strongest correlation with BMD, and that serum estradiol that is converted from testosterone has no further testosterone-independent association.

In summary, our study demonstrates that long term, effective androgen therapy significantly increases BMD in hypogonadal men. Intramuscular testosterone injections and transdermal testosterone are equally effective. Independent of age, the highest increase in BMD is seen during the first year of treatment and in those patients with initial low values of BMD.

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