Effects of Aromatase Inhibition on Bone Mineral Density and Bone Turnover in Older Men with Low Testosterone Levels

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Context: Aging is associated with declining gonadal steroid production, low bone mineral density (BMD), and fragility fractures. The efficacy and safety of testosterone replacement in older men remains uncertain.

Objective: The objective of the study was to assess the effects of aromatase inhibition on BMD in older men with low testosterone levels.

Design and Setting: This was a 1-yr, double-blind, randomized, placebo-controlled trial that was conducted at a tertiary care academic center in Boston, MA.

Participants: Participants included 69 men aged 60+ yr with borderline or low testosterone levels and hypogonadal symptoms.

Intervention: Intervention included 1 mg anastrozole daily or placebo.

Main Outcome Measures: Changes in gonadal steroid hormone levels, BMD, and bone turnover markers were measured.

Results: Mean serum testosterone increased from 319 ± 93 ng/dl at baseline to 524±139 ng/dl at month 3 ($P < 0.0001$) and declined slightly to 474 ± 145 ng/dl by 1 yr. Estradiol levels decreased from 15 ± 4 pg/ml at baseline to 12 ± 4 pg/ml at month 3 and then remained stable ($P < 0.0001$). Posterior-anterior (PA) spine BMD decreased in the anastrozole group as compared with placebo ($P = 0.0014$). In the anastrozole group, PA spine BMD decreased from 1.121 ± 0.141 g/cm^2 to 1.102 ± 0.138 g/cm^2, whereas in the placebo group, PA spine BMD increased from 1.180 ± 0.145 g/cm^2 to 1.189 ± 0.146 g/cm^2. Qualitatively similar, but not statistically significant, changes occurred at the other sites. Bone turnover markers were not affected by anastrozole therapy.

Conclusions: In older men, aromatase inhibition increases testosterone levels, decreases estradiol levels, and appears to decrease BMD. Aromatase inhibition does not improve skeletal health in aging men with low or low normal testosterone levels. **(*J Clin Endocrinol Metab* 94: 4785–4792, 2009)**

Testosterone and estradiol are critical for normal bone development and maintenance in men (1–3). Male aging is associated with declines in both androgen and estrogen levels (4). These age-related decreases in gonadal steroids are associated with a number of health problems, including low bone mineral density (BMD) and an increased incidence of fragility fractures (5). Testosterone administration is a potentially beneficial therapy in aging

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Abbreviations: BMD, Bone mineral density; bone ALP, bone-specific alkaline phosphatase; BTM, bone turnover marker; CTX, C-terminal telopeptide of type 1 collagen; CV, coefficient of variation; DHT, dihydrotestosterone; DXA, dual x-ray absorptiometry; NTX, N-telopeptide of type 1 collagen; OC, osteocalcin; OPG, osteoprotegerin; PINP, N-terminal propeptide of type I procollagen; PSA, prostate-specific antigen; QCT, quantitative computerized tomography.

hypogonadal men, although questions remain as to its safety and efficacy. Furthermore, some of the risks and adverse effects of testosterone administration may be mediated by its conversion to estradiol (6–10). Aromatase inhibitor therapy lowers circulating estradiol by blocking its conversion from testosterone. In so doing, negative feedback signals at the hypothalamus and pituitary are reduced, the production of GnRH and LH increase and testosterone production increases (11). Thus, an aromatase inhibitor may be a safer (given the lowering of estrogen levels) and more convenient (given the oral formulation) way to replace testosterone in hypogonadal men. We have previously shown that anastrozole (Arimidex; AstraZeneca Pharmaceuticals, Wilmington, DE), a potent orally administered aromatase inhibitor used in breast cancer treatment, increases testosterone production and normalizes serum testosterone in older men with low testosterone levels (12, 13). Given the importance of both testosterone and estradiol in male skeletal health, it is possible that anastrozole therapy could improve bone health (by increasing testosterone) or worsen bone health (by lowering estradiol), as has been demonstrated in women with breast cancer (14). To investigate the effects of aromatase inhibition on bone metabolism, we assessed the effects of daily anastrozole administration on BMD and bone turnover markers (BTMs) in men aged 60 yr or older with low or low normal testosterone levels, who participated in a 1-yr, double-blind, randomized, placebo-controlled trial (13).

Subjects and Methods

Study subjects

The details of subject recruitment and enrollment have been previously described (13). Men 60 yr or older were recruited through advertisements and mass mailings. Eligible subjects had serum testosterone between 150 and 300 ng/dl on a single measure or between 300 and 350 ng/dl on two consecutive measures that were obtained on different days; normal serum LH and prolactin; and a positive response to the St. Louis University Androgen Deficiency in Aging Males questionnaire. The Androgen Deficiency in Aging Males questionnaire defines a positive response as either: 1) yes to question 1 ("do you have a decrease in libido?") or question 7 ("are your erections less strong?") or 2) yes to three of the remaining eight questions (15). Subjects with a history of acute urinary retention, prostate nodules on digital rectal examination, or prostate-specific antigen (PSA) greater than 2.5 μg/liter were excluded. Additionally, subjects with known testicular or pituitary disease, obstructive sleep apnea, malignancy, hypercoagulable syndrome, thromboembolic disease, or use of drugs known to affect steroid hormone or SHBG levels were excluded from participation. The Human Research Committee of Partners HealthCare System approved the study and subjects provided written informed consent.

Of the 2470 men screened by telephone, 632 presented for screening and 114 were found to be eligible by the above criteria. Of these, 88 enrolled in the study and 69 subjects completed the first 12 months on study medication and form the basis of this report (Fig. 1).

Study protocol

Using a randomly varying blocking scheme, subjects were randomized by computer-generated assignment in a blinded 1:1 ratio to receive either anastrozole 1 mg daily or matching placebo for 12 months. Subjects were seen at baseline, 3, 6, and 12 months. At each visit, which occurred in the morning, a fasting blood sample was collected for measurement of hormones and BTM. Dual x-ray absorptiometry (DXA) and quantitative computerized tomography (QCT) were performed at baseline and month 12. Medication compliance was assessed through the use of diaries and returned pill counts.

Laboratory methods

Total testosterone was measured by double-antibody RIA (Diagnostic Products, Los Angeles CA) with sensitivity of 4 ng/dl and intra- and interassay coefficients of variation (CVs) of 6.9 and 7.5%, respectively. Bioavailable testosterone was measured by differential precipitation of testosterone bound to globulins with 50% ammonium sulfate after equilibration of the serum sample with [^{3}H]T. Sensitivity of the BT assay was 5 ng/dl with intra- and interassay CVs of 7.9 and 8.6%. Total serum estradiol was measured using an ultrasensitive competitive RIA after extraction and chromatographic purification (Diagnostic System Laboratory Inc., Webster, TX) with sensitivity of 2.2 pg/ml and intra- and interassay CVs of 6.5 and 9.7%. Serum dihydrotestosterone (DHT) was measured by double-antibody nonextraction RIA (I-125) using a commercial kit (Diagnostic System Laboratory) with sensitivity 4 ng/dl and intra- and interassay CVs of 7.9 and 8.4%.

Bone-specific alkaline phosphatase (bone ALP) was measured by ELISA (Quidel Inc., San Diego, CA) with sensitivity 0.7 U/liter and intra- and interassay CVs of 4–6 and 5–8%, respectively. N-terminal propeptide of type I procollagen (PINP) was measured by quantitative RIA (Immunodiagnostic Systems, Inc., Fountain Hills, AZ) with sensitivity of 2 μg/liter and intra- and interassay CVs of 6.5–10.2 and 6.0–9.8%, respectively. Osteocalcin (OC) was measured by solid-phase enzyme-amplified sensitivity immunoassay (American Laboratory Products Com-

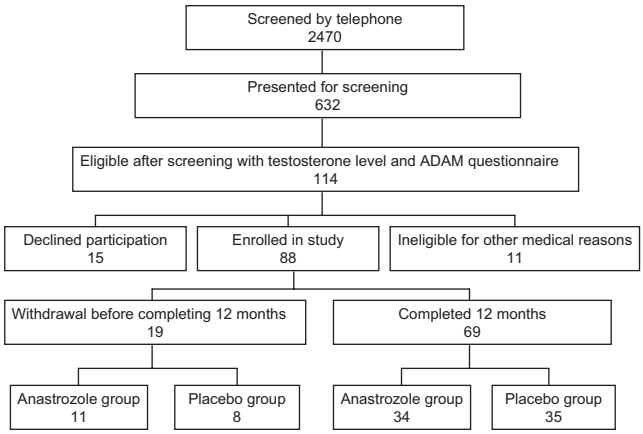

FIG. 1. Flow of patients through the trial. ADAM, Androgen Deficiency in Aging Males.

pany, Ltd., Salem, NH) with sensitivity of 0.4 ng/ml and intra- and interassay CVs of 0.8–1.1 and 4.0–6.6%, respectively. C-terminal telopeptide of type 1 collagen (CTX) was measured by ELISA (Immunodiagnostic System, Inc., Fountain Hills, AZ) with sensitivity of 0.02 ng/ml and intra- and interassay CVs of 1.7–3.0 and 2.5–10.9%, respectively. Serum N-telopeptide of type 1 collagen (NTX) was measured by ELISA (Wampole Laboratories, Inc., Princeton, NJ) with sensitivity of 3.2 nM bone collagen equivalent and intra- and interassay CVs of 4.6 and 6.9%, respectively. Osteoprotegerin (OPG) was measured by enzyme immunoassay (American Laboratory Products Company, Ltd., Salem, NH) with a sensitivity of 0.14 pM and intra- and interassay CVs of 4–10 and 7–8%, respectively.

BMD of the posterior-anterior spine, total hip, femoral neck, and total body were determined from DXA total-body scan (QDR 4500A; Hologic, Bedford, MA) using software version 11.1. The SD for *in vivo* measurements of the posterior-anterior spine, total hip, femoral neck, and total body were 0.005, 0.006, 0.007, and 0.012 g/cm^2, respectively. Trabecular BMD of the midbody of the first four lumbar vertebrae was assessed by QCT with a GE model 9800 scanner (General Electric Medical Systems, Milwaukee, WI) (16). The precision error for this technique is 3–5 mg/cm^3.

Study end points

The primary study end point was the change in posterior-anterior spine BMD by DXA with anastrozole, compared with placebo. Secondary end points included the change in total body, femoral neck, and total hip BMD by DXA, the change in trabecular BMD by QCT and the change in markers of bone formation (bone ALP, OC, and PINP), markers of bone resorption (CTX and NTX), and OPG.

Statistical analysis

All subjects who received a BMD measurement at months 0 and 12 were included in this analysis. Data are summarized as mean ± SD. Differences in the baseline characteristics between the anastrozole and placebo groups were examined using *t* test. Data from our laboratory were used to calculate the power to detect a clinically significant change in posterior-anterior spine BMD based on a study in which elderly men were treated with alendronate (17). In that study, the SD of the change in posterior-anterior spine BMD was 2.5%. Given the present study's sample size of 69, there was 90% probability of detecting a treatment effect of anastrozole on posterior-anterior spine BMD of 1.7% or more ($\alpha = 0.05$). Between-group differences in primary and secondary end points were assessed by repeated-measure ANOVA. The ANOVA model included group, time, and (group) (time) interaction; the model was adjusted for the baseline level of each variable. A secondary analysis was performed to compare the between-group differences at each time point using *t* test as well as the within-group changes in these outcomes between time points using repeated-measures ANOVA. An additional secondary analysis was performed to assess the relationship between baseline testosterone and estradiol levels (measured in tertiles) and the change in BMD at each site and relationship between the change in testosterone and estradiol (measured in tertiles) and the change in BMD at each site. $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics and gonadal steroid levels

We previously reported the baseline characteristics and the effects of aromatase inhibition on gonadal steroid levels (13). In summary, there were no significant differences at baseline in the two groups, except for small differences in estradiol levels (Table 1). Additionally, there were no significant differences between the 69 subjects who completed the study and the 19 who did not complete all study visits (data not shown). Anastrozole therapy increased mean serum testosterone at all time points ($P < 0.0001$ *vs.* placebo for repeated measures ANOVA) (Fig. 2A). Specifically, mean serum testosterone increased from 319 ± 93 ng/dl at baseline to 524 ± 139 ng/dl at month 3 ($P < 0.0001$ *vs.* baseline). From months 3 to 12, however, whereas testosterone levels remained significantly higher than baseline and placebo, there was a notable decline. Specifically, mean serum testosterone decreased to 505 ± 143 ng/dl at month 6 and to 474 ± 145 ng/dl at month 12 ($P = 0.03$ compared with month 3). Anastrozole therapy

TABLE 1. Baseline characteristics

Clinical or biochemical end points	Anastrozole group (n = 34)	Placebo group (n = 35)	*P*
Age (yr)	66 ± 4	65 ± 4	ns
BMI (kg/m^2)	30 ± 5	32 ± 5	ns
Testosterone (ng/dl)	319 ± 93	337 ± 96	ns
Bioavailable testosterone (ng/dl)	77 ± 23	89 ± 34	ns
Estradiol (pg/ml)	15 ± 4	19 ± 5	0.001
DHT (ng/dl)	53 ± 21	55 ± 20	ns
SHBG (nmol/liter)	32 ± 10	31 ± 12	ns
Posterior-anterior spine BMD (g/cm^2)	1.121 ± 0.141	1.180 ± 0.145	ns
Femoral neck BMD (g/cm^2)	0.850 ± 0.119	0.882 ± 0.127	ns
Total hip BMD (g/cm^2)	1.052 ± 0.145	1.104 ± 0.135	ns
Total body BMD (g/cm^2)	1.249 ± 0.088	1.295 ± 0.110	ns
Posterior-anterior spine by QCT (mg/cm^3)	101 ± 22	107 ± 28	ns
Bone ALP (U/liter)	23 ± 7	25 ± 8	ns
PINP (ng/ml)	32 ± 14	33 ± 10	ns
OC (ng/ml)	11 ± 4	10 ± 4	ns
NTX (nM BCE)	13 ± 5	11 ± 4	ns
CTX (ng/ml)	0.50 ± 0.25	0.44 ± 0.22	ns
OPG (p/M)	4.8 ± 1.5	4.3 ± 1.3	ns

All values are presented as mean ± SD. Systeme international conversion factors: testosterone and bioavailable testosterone (nanomoles per liter), 0.0347; estradiol (picomoles per liter), 3.671; DHT (nanomoles per liter), 0.0344. BMI, Body mass index; BCE, bone collagen equivalent; ns, not significant ($P \geq 0.05$).

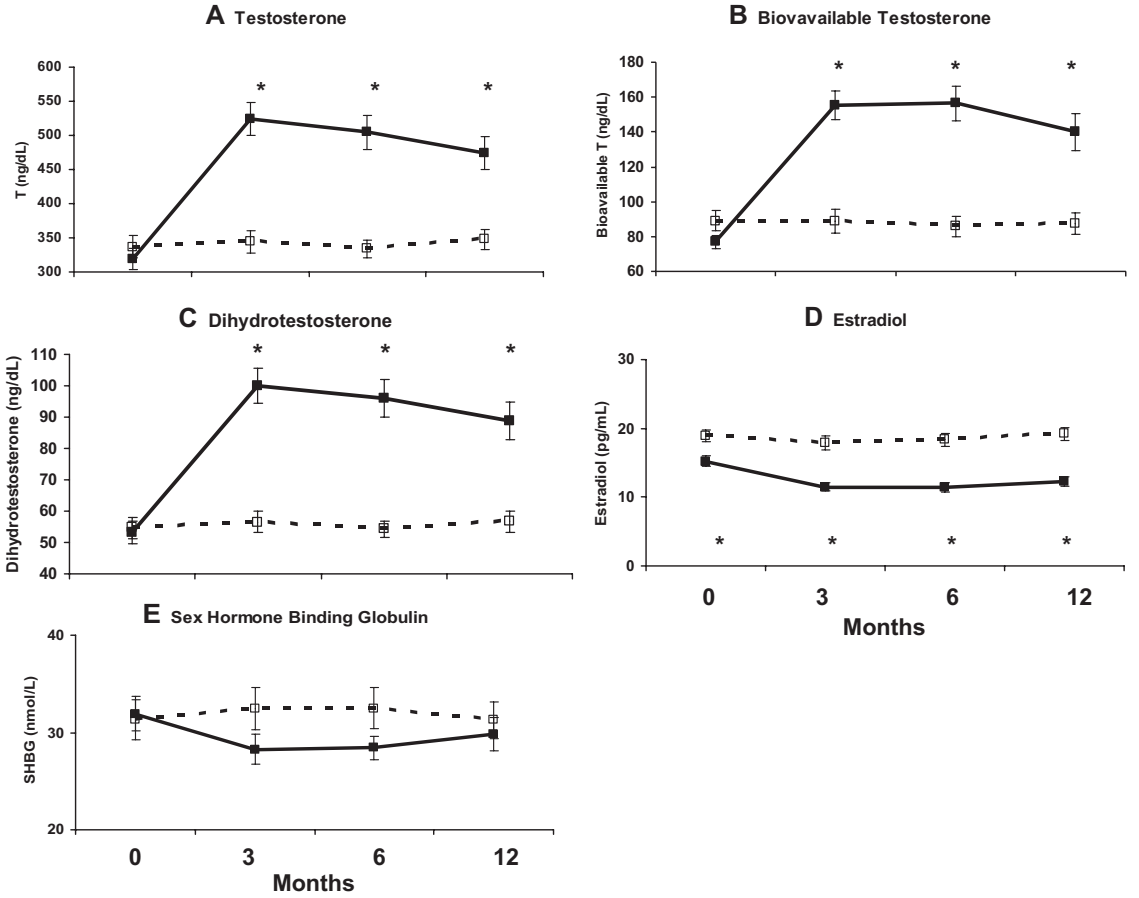

FIG. 2. Mean (± SE) percent change in testosterone (A), bioavailable testosterone (B), DHT(C), estradiol (D), and SHBG (E) with anastrozole (*solid line*) *vs.* placebo (*dashed line*) for 12 months. *, $P < 0.05$ compared with placebo for each time point.

also increased mean bioavailable testosterone (Fig. 2B) and DHT levels (Fig. 2C) ($P < 0.0001$ *vs.* placebo for repeated measures ANOVA). Similar to testosterone, bioavailable testosterone and DHT levels declined between months 3 and 12 but remained significantly higher than baseline and placebo ($P < 0.0001$). The effects of anastrozole on estradiol were more modest than its effects on androgens (Fig. 2D). Estradiol levels decreased from 15 ± 4 pg/ml at baseline to 12 ± 4 pg/ml at month 3 and remained stable thereafter ($P = 0.0004$ *vs.* placebo for repeated measures ANOVA). Anastrozole therapy lowered SHBG levels ($P = 0.0013$ *vs.* placebo for repeated measures ANOVA) (Fig. 2E). Mean testosterone, bioavailable testosterone, DHT, estradiol, and SHBG were unchanged in the placebo group.

BMD and BTMs

The changes in BMD with anastrozole therapy are shown in Fig. 3. After 12 months, posterior-anterior spine BMD by DXA decreased in the treatment group (from 1.121 ± 0.141 to 1.102 ± 0.138 g/cm^2), whereas it increased in the placebo group (from 1.180 ± 0.145 to 1.189 ± 0.146 g/cm^2) ($P = 0.0014$ for the between group difference) (Fig. 3A). Similarly trabecular BMD by QCT decreased slightly in the treatment group (from 101 ± 22 to 99 ± 23 mg/cm^3) and increased slightly in the placebo group (from 107 ± 28 to 109 ± 27 mg/cm^3), but this was not statistically significant ($P = 0.187$ for the between group comparison) (Fig. 3B). Qualitatively similar between-group changes in BMD were observed at the femoral neck, total hip, and total body (Fig. 3, C–E); however, none of these changes were statistically significant.

The changes in bone formation (bone ALP, PINP, and OC), bone resorption (CTX and NTX), and OPG are shown in Fig. 4. Anastrozole therapy did not affect bone turnover.

As a secondary analysis, we investigated whether the BMD response to anastrozole therapy was influenced by either the baseline testosterone or estradiol level. Furthermore, we wanted to investigate whether the magnitude of the change in testosterone or estradiol levels with anastrozole therapy affected the BMD response. Specifically, we tested whether those subjects in the lowest or highest tertile of each variable (baseline testosterone, baseline estradiol, change in testosterone, or change in estradiol) had differential BMD response. We did not detect any significant differences in response based on the baseline or change in gonadal steroid levels (data not shown).

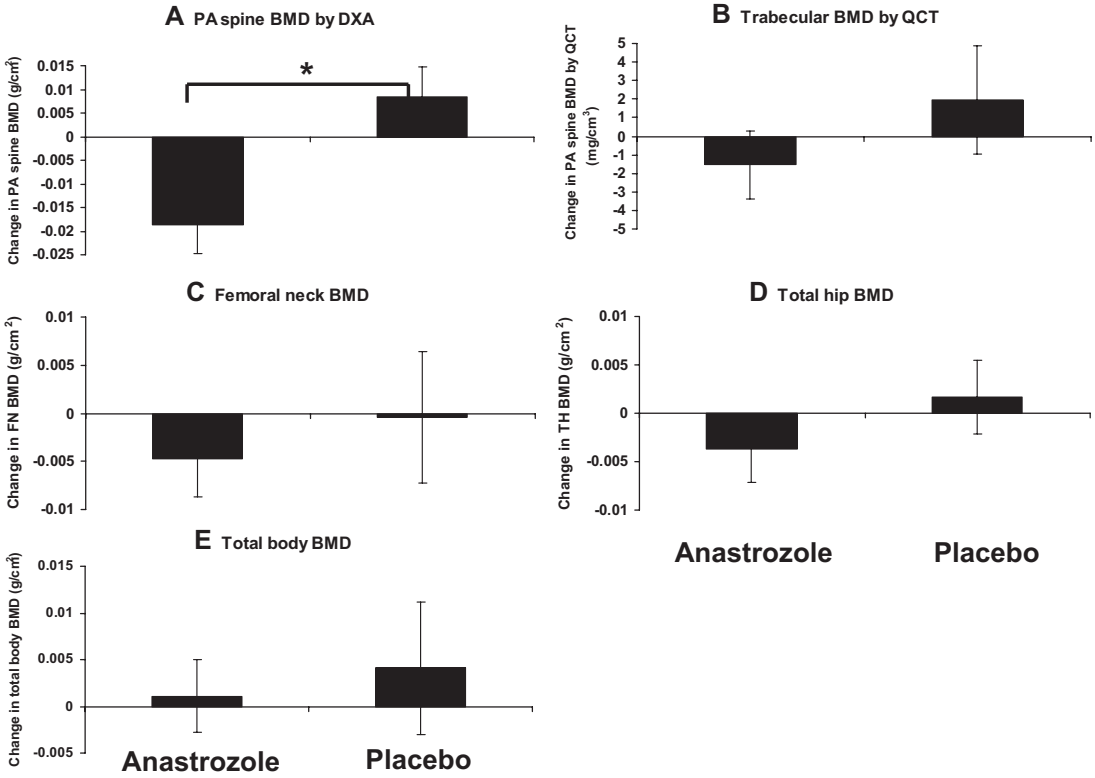

FIG. 3. Mean (± SE) change in posterior-anterior spine BMD by DXA (A), trabecular (lumbar spine) BMD by QCT (B), femoral neck BMD by DXA (C), total hip BMD by DXA (D), and total body BMD by DXA (E) with anastrozole or placebo for 12 months. *, $P < 0.05$ compared with placebo.

Subject withdrawal and compliance

Subjects were withdrawn for the following safety criteria: hematocrit greater than 50%; PSA increase of greater than 0.4 μg/liter at month 3; PSA increase of greater than 0.8 μg/liter at month 6; or other laboratory or physical examination abnormalities at the discretion of the study physician. No subject was withdrawn for an increase in hematocrit. Prostate-related withdrawals are shown in Table 2. As previously described (13), study subject withdrawal was balanced between the anastrozole (n = 11) and placebo (n = 8) groups. Additionally, compliance was excellent as assessed by medication diaries and pill counts (13). With one exception, subjects took more than 95% of the study medication.

Discussion

In this study, we examined the effects of 12 months of daily anastrozole therapy on BMD and markers of bone formation and resorption in older men with low normal serum testosterone. As previously described, aromatase inhibition increased testosterone levels by about 50%, resulting in levels that were generally in the mideugonadal range for young men (13). Aromatase inhibition also modestly decreased estradiol (~20%), although mean levels remained in the normal range. Additionally, anastrozole therapy increased bioavailable testosterone and DHT compared with placebo. These increases in androgen levels and the associated mild decrease in estradiol were associated with a statistically significant decrease in posterior-anterior spine BMD *vs.* placebo as measured by DXA and qualitatively similar, although nonsignificant, changes at the other bone sites. In contrast, bone turnover was not affected by aromatase inhibition. The observed changes in BMD were not affected by baseline testosterone or estradiol levels or the magnitude of change in testosterone or estradiol with anastrozole administration.

Testosterone and estrogen are important for the maintenance of skeletal health in adult men. In some prior cross-sectional or longitudinal analyses of the relationship between gonadal steroids and BMD, both testosterone and estradiol have been independently associated with BMD in aging men (18–20). However, in some studies there was greater association between estradiol and BMD in older men (21–23), whereas in others, there was greater association between testosterone and BMD (24–26). Interventional studies in which gonadal steroid levels are manipulated have been insightful. Specifically, studies rendering men either selectively estrogen deficient, selectively androgen deficient or deficient in both hormones have demonstrated that both testosterone and estrogen have independent roles in maintaining normal

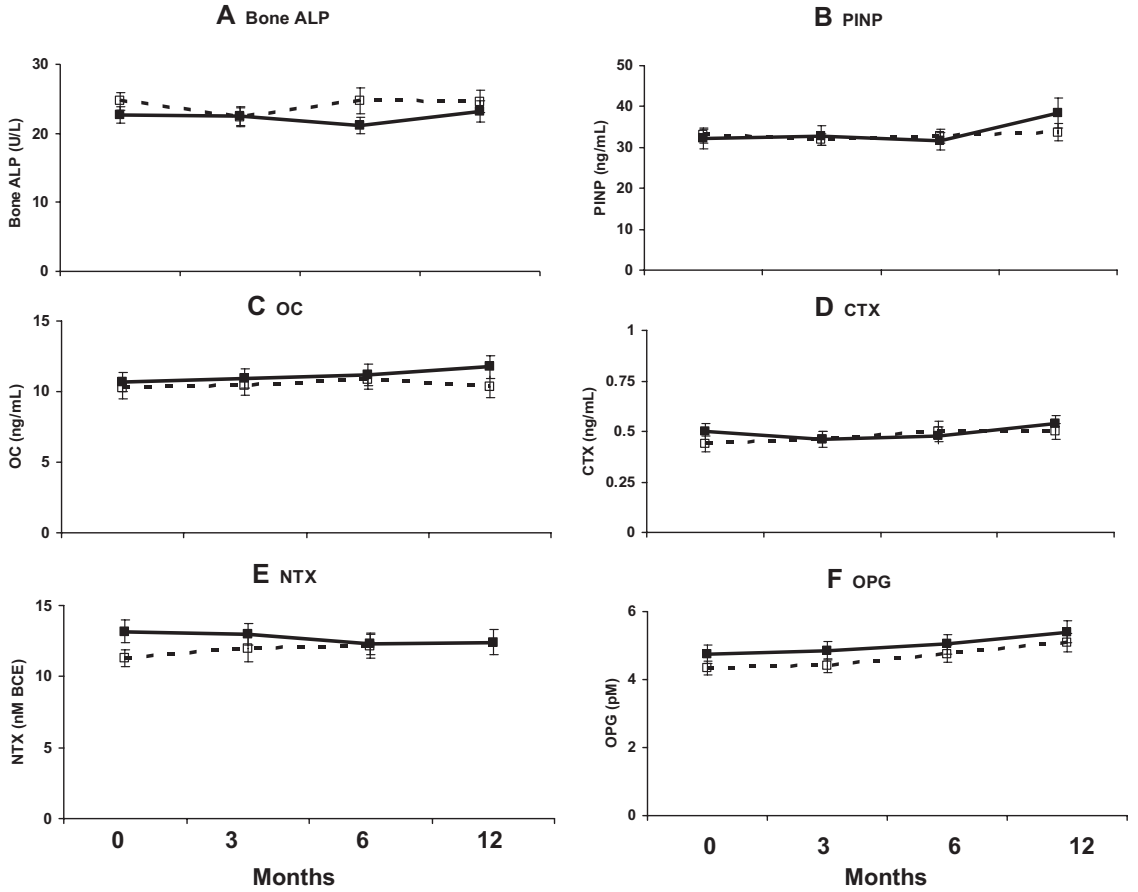

FIG. 4. Mean (± SE) change in bone ALP (A), PINP (B), OC (C), CTX (D), (E) NTX, and OPG (F) with anastrozole (*solid line*) *vs.* placebo (*dashed line*) for 12 months. There were no statistically significant differences between the treatment and placebo groups.

bone turnover (27–29). The apparent loss of BMD at the posterior-anterior spine in this study suggests that the observed increase in testosterone with aromatase inhibition was insufficient to overcome the effects of selective estrogen deficiency on the skeleton. Alternately, it is possible that if aromatase inhibition had produced higher serum testosterone levels then BMD would have been maintained.

Administering aromatizable testosterone to hypogonadal older men increases testosterone, estradiol, and BMD. In most studies, this increase in BMD is associated with decreased bone resorption, although increased bone formation has also been reported (30–35), the latter possibly related to the pharmacological dose or parenteral route of administration. Notably, in a prior study in which aromatizable testosterone was administered to hypogonadal older men, similar to our cohort, BMD changed without concomitant changes in BTM (36). It is unclear why we observed significant decreases in posterior-anterior spine BMD but no accompanying change in BTM with anastrozole therapy. Given the discordance between BMD and BTM changes, it remains possible that the decrease in posterior-anterior spine BMD was a chance finding. However, the qualitatively similar BMD changes at the other sites make this somewhat less likely. Furthermore, it is important to note that BMD is only one parameter contributing to bone strength. Bone strength is impacted by bone quality, bone mass, and bone geometry. Specifically, periosteal apposition may offset endosteal or endocortical bone resorption, thus creating bone that is of wider diameter (37). Interestingly, gonadal steroids may have differential effects on periosteal apposition, wherein testosterone stimulates periosteal apposition and estradiol inhibits it (38). Thus, it is possible that aromatase inhibi-

TABLE 2. Prostate-related study withdrawal

Reason for withdrawal	Subjects (n)	Group assignment (n)
Prostate cancer	1	Placebo
Benign prostate nodule (calcium deposit)	1	Anastrozole
Increase in PSA	5	Anastrozole (3), placebo (2)
Increase in BPH symptoms	1	Placebo

BPH, Benign prostatic hypertrophy.

tion therapy could induce beneficial changes in bone geometry and hence skeletal health (39). Conversely, however, recent observational data suggest that estradiol has a more dominant effect on bone geometry than testosterone; thus, aromatase inhibition, by lowering estradiol, may negatively impact bone geometry (40).

In a prior 3-month, double-blind, placebo-controlled study of 37 elderly men with low testosterone levels treated with anastrozole, we demonstrated similar increases in mean testosterone levels to those observed in this study (41). Similar increases in testosterone were also reported in a short-term study in which eugonadal older men were given anastrozole 2 mg daily (42). In that study, Taxel *et al.* (42) observed changes in BTM with anastrozole therapy; however, the men were eugonadal, received a higher dose of anastrozole, and were not compared with a placebo group. Importantly, in our earlier short-term study (41), we also did not observe any change in BTM, but this study was not long enough to provide meaningful insight regarding the effect of aromatase inhibition on BMD.

Limitations of this study deserve mention. Given the small sample size, whereas we had sufficient power to detect small changes in posterior-anterior spine BMD, we did not have adequate power to detect small changes at other sites. Additionally, the observed 20% dropout rate had the potential to introduce bias. Reassuringly, the dropout rate was balanced between the treatment and placebo groups and thus should not have affected our results. Furthermore, at baseline there were no differences in the subjects who completed the study *vs.* those who did not complete all study visits.

In summary, anastrozole therapy given over 12 months increased serum testosterone and modestly reduced estradiol levels in men aged 60 yr and older with low or low normal testosterone levels. Whereas we restored testosterone levels into the midnormal range for healthy young men, BMD at the spine decreased compared with the placebo group. Coupled with its lack of benefit in improving body composition (13), aromatase inhibition does not appear to be an optimal therapy for hypogonadism in aging.

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