Determinants of Bone Density in Healthy Older Men With Low Testosterone Levels

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Background. Osteoporosis is a significant problem in older men; 30% of all hip fractures occur in men and the mortality rate following hip fracture exceeds that of women. Testosterone is thought to be important in the development of peak bone mass but its role in age-related bone loss is not established. The purpose of this study was to define the predictors of bone mass in healthy older men with low testosterone levels but without symptomatic osteoporosis.

Methods. Eighty-three community-dwelling white men, aged more than 65 years old, selected for low bioavailable testosterone levels (≤4.44 nmol/l) participated in a cross-sectional study located at a university general clinical research center. Sex hormone concentrations and markers of bone turnover were assayed in serum and urine. Risk factors for osteoporosis and physical activity were ascertained by physical examination and questionnaire, including the Physical Activity Scale in the Elderly (PASE) questionnaire. Bone mineral densities of the femoral neck (FN BMD), spine, and whole body were measured by dual x-ray absorptiometry. Lower extremity muscle strength (1 repetition maximum) was measured using a leg press machine.

Results. Mean bone mineral density values were 0.93 ± 0.14 g/cm² for femoral neck, 1.31 ± 0.23 g/cm² for spine, and 1.22 ± 0.12 g/cm² for whole body. Thirty-one of the 82 subjects (37%) had T-scores <−1 and 12 of 82 subjects (15%) had T-scores <−2.5 at the femoral neck. Multiple linear regression analysis demonstrated that bioavailable testosterone, body mass index (BMI), and PASE scores were positively correlated with, and significant predictors of, femoral neck BMD, accounting for 34.4% of the variance in FN BMD (F = 10.10, p = .001). Examining each variable independently, bioavailable testosterone accounted for 20.7%, physical activity score for 9.0%, and BMI for 6.5% of FN BMD. Using analysis of variance, mean values for FN BMD were significantly different between men grouped by tertile of bioavailable testosterone (F = 6.192, p = .003). FN BMD mean values were 0.86 ± 0.14 g/cm² for the lowest tertile, 0.94 ± 0.16 for the middle tertile, and 0.99 ± 0.14 for the highest tertile. Markers of bone turnover were inversely correlated, and strength directly correlated with BMD, but did not contribute to the multiple regression model.

Conclusions. Fifty-two percent of older men with low bioavailable testosterone levels had BMD levels below the young adult normal range and are likely at an increased risk of fracture. Bioavailable testosterone, BMI, and physical activity score were significant determinants of FN BMD in these men. These variables are potentially modifiable and, therefore, amenable to intervention. Hence, our results suggest the need for testosterone replacement and physical activity intervention trials in men at risk for osteoporotic fractures.

Osteoporosis is a significant problem in men. Thirty percent of hip fractures occur in men and the cost of care for these patients is estimated to be $2.7 billion per year (1). Furthermore, mortality rates following hip fracture are higher in men than in women (2). Yet, to date, little attention has focused on the diagnosis and prevention of osteoporotic fractures in men. It is well recognized that testosterone levels decline with advancing age (3), and as many as 50% of men over age 65 have bioavailable testosterone levels below the reference range for young adults (4–6). However, the relationship of this gradual decline of testosterone levels in aging men to bone loss and fracture is not known. In studies of men presenting to osteoporosis clinics, marked testosterone deficiency is commonly associated with low bone mass and fracture (7–9). Several studies found bioavailable, or free, testosterone to be an important contributor to bone mass in men (10–12), but these findings are not consistent. (13–15). Others have found that testosterone levels are low in men with a history of hip fracture (16–19). The relationship between bone mineral density (BMD) and testosterone in older men with modest testosterone insufficiency has not been specifically addressed. Therefore, the purpose of this study was to define the predictors of bone mass in older healthy men with low testosterone levels, but without symptomatic osteoporosis.

Methods

Men were recruited using the following strategies: letters sent to those aged more than 65 years whose names were provided by the department of motor vehicles for the area surrounding the University of Connecticut Health Center; community outreach lectures at men’s groups and senior centers; mailings sent to men’s groups; and flyers distributed at senior centers and housing sites. The informative talks were about general health, and the flyers solicited men to be involved in a research proposal, not specifically to study bone health. Inclusion criteria were (i) community-dwelling men over the age of 65 years in relatively good health and (ii) bioavailable testosterone levels below 4.44 nmol/l (normal range 4.44–14.91 nmol/l). The men were not seeking medical treatment for symptoms of osteoporosis or low testosterone. Exclusion criteria were as follows: (i) sys-
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Participants in the study underwent medical history, physical exam, and measurement of fasting serum calcium (Ca), Cr, alkaline phosphatase, thyroid function tests, total and bioavailable testosterone, sex hormone-binding globulin (SHBG), estrone, estradiol, and urinary Ca and Cr. In addition, the group was divided into tertiles based on bioavailable testosterone score for comparison of femoral neck bone mineral density (FN BMD) scores. Ca and vitamin D intake (including supplements) were estimated by a 3-day food record. Activity was estimated by the Physical Activity Scale for the Elderly (PASE) questionnaire (20). BMD (Lunar DPX-L, Lunar Corp, Madison, WI) of the proximal femur, lumbar spine, and total body was obtained at baseline. The coefficient of variation of BMD measurement at the proximal femur, spine, and total body was <1%, 1.5%, and 2%, respectively. Leg extension strength [1 repetition maximum (21); intra- and intertester variability <10%] was measured on the Keiser sitting leg press (Keiser Co, Fresno, CA).

Biochemical Measurements

Blood and urine samples were collected between 7:00 am and 9:00 am after a 10- to 12-hour fast. Urine and serum were divided into 0.5 ml aliquots and stored at −70°C. Ionized Ca was measured within 2 hours of collection. All bone marker assays were performed on batched serum or urine in the Core Laboratory of the General Clinical Research Center (GCRC) at the University of Connecticut Health Center.

Markers of bone formation included bone-specific alkaline phosphatase (BAP) and N-terminal type I procollagen peptide (PINP), measured by enzyme-linked immunosorbent assay, or ELISA (Metra Biosystems, Inc, Palo Alto, CA). Average intra-assay variability was <5% for both measures of bone formation. Markers of bone resorption were cross-linked N-telopeptide (NTX) and C-telopeptide (CTX) of type I collagen measured by ELISA (Osteometry International, Inc, Seattle, WA, and Osteometer A/S, Copenhagen, Denmark, respectively). Intra-assay variability was <10% for measures of bone resorption.

Total and bioavailable testosterone and SHBG measurements were performed at Endocrine Sciences, Calabasas Hills, California. Testosterone levels were measured by radioimmunoassay, SHBG by competitive binding assay, and bioavailable testosterone by competitive binding of the non-SHBG-bound portion of testosterone following ammonium sulfate precipitation of the SHBG-bound steroid as described by Nankin (4). Intra-assay variability of the testosterone assay is less than 7%, bioavailable testosterone less than 4%, and SHBG less than 10%. Subsequently, 25-hydroxyvitamin (OH) vitamin D levels were measured by competitive protein binding with an intra-assay coefficient of variance of less than 10%. Samples for offsite assay were shipped on dry ice by overnight mail. Estradiol and estrone measurements were performed in the GCRC core lab using radioimmunoassay (Diagnostic Systems Lab, Inc, Webster, TX); intra-assay variability was less than 10%. The detection limit of the estradiol assay is 2 pg/ml.

Statistical Analysis

Pearson correlation coefficients were calculated to determine the relationships among BMD, sex hormone levels, bone turnover markers, physical activity scores, and strength. Multiple linear regression was employed to evaluate the contribution of sex hormones, strength, physical activity, social habits, and bone turnover markers to variance in BMD. Analysis of variance (ANOVA) was used to compare the means of FN BMD, based on tertiles of bioavailable testosterone. The SPSS statistical software package, version 10.0 (SPSS Inc, Chicago, IL) was used for all analyses.

Results

The characteristics of the 83 men are presented in Table 1. None of the men were active smokers; 33% had never smoked and 66% reported a prior history of smoking. Sixty-six percent (55 of 83) of the men reported the use of alcohol; mean alcohol consumption was 11 drinks per week (range, 1–21 drinks/wk), using the standard definition of 2 oz of ethanol per drink (22). Medical conditions most commonly reported included coronary artery disease (n = 40), hypertension (n = 43), and osteoarthritis (n = 41). No man was seeking treatment for medical conditions that might
cause hypogonadism, such as liver disease, alcoholism, hemochromatosis, or Klinefelter’s disease. Commonly used medications included antihypertensives (n = 39), cholesterol-lowering agents (n = 14), thiazide diuretics (n = 16), and histamine blockers (n = 12). No one in the study had ever received long-term corticosteroid for previous medical conditions. When the baseline characteristics were evaluated in the men and divided into tertiles of bioavailable testosterone scores, there were no differences in body mass index (BMI), dietary calcium intake, physical activity score, illness, or medication use. There was a significant difference between tertiles in age, with the lowest tertile having a mean age of 77 ± 5 years compared with a mean of 74 ± 4 years in the other two tertiles. Thirty-one men had a history of a previous fracture; all but two were traumatic and occurred prior to the age of 50 years. During review of bone density readings with two men, one reported a prolonged back pain at age 70, 7 years prior to our study and consistent with a spinal fracture, and another reported a wrist fracture due to a fall.

Values for bioavailable testosterone and free androgen index (testosterone/SHBG) were closely correlated (r = 0.87, p < 0.001), and data for bioavailable testosterone will be reported. No significant correlation was found among total testosterone, SHBG, estradiol, estrone, free estrogen index (estradiol/SHBG), or 25 (OH) D and BMD at any site. One marker of resorption (CTX) and one marker of formation (PINP) correlated inversely with BMD of the whole body (r = –0.34 for both, p < 0.001). FN (r = –0.29 and –0.32, respectively; p < 0.001), and spine (r = –0.27 and –0.30, respectively; p < 0.05), although the other markers, BAP and NTX, did not demonstrate significant correlations (data not shown). Strength and physical activity scores correlated with BMD of the FN (r = 0.29, p < 0.05, and r = 0.31, p < 0.001) and total body (r = 0.36, p < 0.001, and r = 0.25, p < 0.05), but not at the spine. BMD correlated at all sites; spine (r = 0.31, p < 0.001), FN (r = 0.26, p < 0.05), and total body (r = 0.23, p < 0.05). In addition, all four markers of bone formation and resorption correlated inversely with sex hormones (r = –0.20 to –0.36, p < 0.05), but not with BMI, strength, or physical activity scores. Bioavailable testosterone levels did not correlate with physical activity scores (r = 0.13, p = 0.23).

Several variables contributed to femoral neck BMD in linear regression analysis, including bioavailable testosterone, BMI, physical activity, estradiol, strength, lean body mass, and C-terminal telopeptide. When these same variables were included in a multiple linear regression analysis, bioavailable testosterone, BMI, and PASE scores were the only remaining predictors of femoral neck BMD, accounting for 34.4% of the variance in FN BMD (F = 10.10, p = 0.001; Table 3). Examining each variable independently, bioavailable testosterone accounted for 20% of the variance in FN BMD values. When the group was evaluated based on tertiles of bioavailable testosterone,

### Table 2. Correlation of Variables With Bone Mineral Density

<table>
<thead>
<tr>
<th>Variable</th>
<th>Femoral Neck</th>
<th>Lumbar Spine</th>
<th>Total Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailable testosterone</td>
<td>0.46**</td>
<td>0.22*</td>
<td>0.30**</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>0.18</td>
<td>0.16</td>
<td>0.18</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.11</td>
<td>0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.10</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>Sex hormone binding globulin</td>
<td>–0.22*</td>
<td>–0.03</td>
<td>–0.10</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.26*</td>
<td>0.31**</td>
<td>0.23*</td>
</tr>
<tr>
<td>C-terminal telopeptide (CTX)</td>
<td>–0.29**</td>
<td>–0.27*</td>
<td>–0.34**</td>
</tr>
<tr>
<td>Type I procollagen peptide (PINP)</td>
<td>–0.32**</td>
<td>–0.30**</td>
<td>–0.34**</td>
</tr>
<tr>
<td>Lean mass</td>
<td>0.36**</td>
<td>0.34*</td>
<td>0.40**</td>
</tr>
<tr>
<td>Strength</td>
<td>0.29*</td>
<td>0.24</td>
<td>0.36**</td>
</tr>
<tr>
<td>Physical activity score (PASE)</td>
<td>0.31**</td>
<td>0.11</td>
<td>0.25*</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01.

**DISCUSSION**

Bioavailable testosterone, BMI, and physical activity are the most important determinants of FN BMD in this group of healthy older men selected on the basis of a low bioavailable testosterone level. Bioavailable testosterone accounted for 20% of the variance in FN BMD values. When the group was evaluated based on tertiles of bioavailable testosterone,
there was a significant difference in the mean FN BMD between each group, and, strikingly, two thirds of the men in both the low and the middle tertile (bioavailable testosterone levels 0–3.10 and 3.11–3.80 nmol/l) had BMD scores consistent with a diagnosis of osteopenia or osteoporosis. Past studies have not specifically evaluated the BMD in men with low testosterone levels, but two case control studies have found 59% to 70% of men who had recently suffered a femoral fracture had low testosterone levels, compared with 18% to 30% of controls (16,17). In addition, a
retrospective analysis of nursing home residents with a history of hip fracture found 66% of the men had testosterone levels below 300 ng/dl (19).

While severe testosterone deficiency has long been recognized as a risk factor for osteoporosis (23), our data suggest that the moderate testosterone deficiency that is frequently found in older men may play a significant role in osteoporosis risk in older men. Studies of testosterone replacement in older men with testosterone levels in the low range have found either beneficial changes in bone turnover markers (24,25) or improvement in bone mineral density (26). Although there was a significant inverse correlation between bone turnover markers and BMD in our study, the relationship was no longer significant by multiple linear regression, perhaps because of the similar inverse relationship between sex hormones and BMD, suggesting sex hormones are the major determinant for changes in bone turnover. The decline in bioavailable or free testosterone level common in older men has been attributed to several factors including increasing SHBG levels with increasing age and body fat, medical illnesses and medications, and a blunted response to decreasing leutening hormone levels (27).

BMI accounted for 9% of the variance in BMD in the present study, similar to previous studies in men and women (8,28). The mechanism by which BMI contributes to BMD is not established, but hypotheses include effects of increased mechanical loading of bone directly or indirectly due to increased muscle mass and increased estrogen levels due to peripheral conversion of testosterone to estrogen. Although we did not find estradiol, estrone, or free estrogen to be significant determinants of BMD in this group of men with low bioavailable testosterone levels, others have found free estrogen to be more predictive of BMD than testosterone (10,13). The main difference between our study and previous reports is that we selected older men with low bioavailable testosterone levels. The studies that found estrogen to be more predictive examined younger men with testosterone levels in the normal range (10) or evaluated only total testosterone rather than bioavailable testosterone (13).

Physical activity score was also a determinant of FN BMD, a finding consistent with the work of several other groups (29,30). The mechanism by which physical activity benefits bone is uncertain and the relationship may not be causal. Although both strength and physical activity correlated with FN BMD in this group of nonfrail individuals, strength did not remain an independent contributor to bone mass in multiple regression analysis.

The World Health Organization has established criteria for osteopenia and osteoporosis based on BMD in women to assist with diagnosis, prevention, and treatment of osteoporosis in women (31). Although similar guidelines are not yet established for men, BMD is a predictor of hip fracture in men (32). Nyquist prospectively assessed fracture risk based on radial BMD in men and found the relative risk for all fractures was 1.75, and for hip fracture, 3.88, for each SD decrease in BMD, increases similar to those described in women (33). Other studies have reported 1.5–3 times higher fracture risk for each SD change in BMD, based on BMD measurement at various sites (34–38). Melton and colleagues suggest that 20% of men over age 50 are at risk for osteoporotic fracture based on BMD findings, and, in their sample, 6.5% of men over age 50 had BMD values 2.5 SD below the mean for men 20–49 years (39). In our study of men with low testosterone levels, 15% had BMD 2.5 SD below the young adult mean. We, accordingly, estimate a 5–10 times increased risk for femoral neck fracture over the general population of men over age 50. Another 37% had values at least 1 SD below the young adult mean, and a 1.5–4 times higher risk for femoral neck fracture.

**Conclusion**

We have found that bioavailable testosterone, BMI, and physical activity scores are significant determinants of FN BMD in older men with low bioavailable testosterone levels. Fifty-two percent of these men have BMD levels more than 1 SD below the normal range and are likely at an increased risk of fracture. Testosterone, body mass, and physical activity are variables that are potentially modifiable and, therefore, amenable to intervention. This possibility can be tested by trials of testosterone replacement and physical activity intervention in men at risk for osteoporotic fractures.

**Acknowledgments**

This work has been supported by the National Osteoporosis Foundation, General Clinical Research Center (MO1-RR06192), the Claude Pepper Older Americans Independence Center (5P60-AI3631), and Dr. Kenny has been supported with fellowships from the Brookdale Foundation and the Paul Beeson Faculty Scholar Program. In addition, we thank Pamela Fall and Christine Abreu for assistance in running the biochemical assays and Julie Fenster for assistance with data management.

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**References**


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**Table 3. Multiple Regression Analysis of Selected Variables With Femoral Neck Bone Mineral Density**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta</th>
<th>CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailable testosterone</td>
<td>0.00201</td>
<td>0.001–0.003</td>
<td>.001</td>
</tr>
<tr>
<td>Estradiol</td>
<td>−0.00328</td>
<td>−0.009–0.002</td>
<td>.24</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.00204</td>
<td>−0.003–0.007</td>
<td>.46</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.01105</td>
<td>0.002–0.020</td>
<td>.014</td>
</tr>
<tr>
<td>Physical activity score</td>
<td>0.00077</td>
<td>0.000–0.001</td>
<td>.003</td>
</tr>
<tr>
<td>C-terminal telopeptide (CTX)</td>
<td>−0.00188</td>
<td>−0.003–0.001</td>
<td>.16</td>
</tr>
<tr>
<td>Age</td>
<td>−0.00120</td>
<td>−0.174–1.066</td>
<td>.16</td>
</tr>
<tr>
<td>Strength</td>
<td>0.000001</td>
<td>−0.001–0.002</td>
<td>.98</td>
</tr>
<tr>
<td>Lean mass</td>
<td>0.004497</td>
<td>−0.003–0.012</td>
<td>.22</td>
</tr>
<tr>
<td>Type I procollagen peptide (PINP)</td>
<td>−0.00089</td>
<td>−0.003–0.001</td>
<td>.42</td>
</tr>
</tbody>
</table>

*Note: CI = confidence interval.*


