Serum Estradiol Levels Are Inversely Associated With Cortical Porosity in Older Men

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Context: The key role of serum estradiol (E2) for bone health in men is well established. The effect of serum sex steroids on bone microstructure, measured by high-resolution peripheral quantitative computed tomography, remains unknown in elderly men.

Objective: The objective of the study was to examine the associations between serum sex steroids and bone microstructural parameters in older men.

Methods: Trabecular and cortical bone microstructure at the tibia was measured by high-resolution peripheral quantitative computed tomography in 440 men (mean 80 y of age) participating in the population-based Osteoporotic Fractures in Men Sweden cohort. Serum levels of E2 and T were analyzed with mass spectrometry and free E2 and free T levels were calculated using law-of-mass-action equations.

Results: Age-adjusted models demonstrated that E2 and free E2 but not T or free T associated significantly inversely with cortical porosity. The associations between E2 and free E2 and cortical porosity remained significant after further adjustment for height, weight, physical activity, calcium intake, and smoking. Models including both serum E2 and T demonstrated that E2 (standardized \( \beta = -0.12, P < 0.05 \)) but not T associated independently with cortical porosity. A similar independent association was found for free E2 (standardized \( \beta = -0.12, P < 0.05 \)) but not free T. Free E2 associated significantly with trabecular bone volume fraction in the age-adjusted models, but this association did not remain significant after further adjustment.

Conclusions: Serum E2 levels associated inversely with cortical porosity in 80-year-old men. We propose that low serum E2 may reduce cortical bone strength, at least partly, by increasing cortical porosity and thereby increase fracture risk in older men. (J Clin Endocrinol Metab 99: E1322–E1326, 2014)

Both androgens and estrogens affect bone health in men (1). Still, serum levels of estradiol (E2) are more strongly associated with areal bone mineral density (BMD) and BMD loss than serum T levels are (2, 3). Also, E2 levels but not T levels are consistently associated with volumetric BMD both at trabecular and cortical sites (4).

Accumulating evidence shows that a minimum E2 level is required for prevention of bone loss (5) and fractures (6, 7), and this threshold appears to be more apparent at cortical than trabecular sites (4). In addition, genetic evidence from genome-wide association studies recently demonstrated that a common single nucleotide polymorphism in
the estrogen receptor-α gene was significantly associated with cortical but not trabecular volumetric BMD (8). Thus, it is clear that E2 is crucial not only for trabecular but also cortical bone in men.

Bone loss in old age is mainly loss of cortical and not trabecular bone. Moreover, 80% of all fractures occur at sites containing mostly cortical bone. Cortical porosity increases with age (9–12) and has been postulated as an important determinant of bone strength and fracture susceptibility (10). With the advent of high-resolution peripheral quantitative computed tomography (HRpQCT), it has become possible to in vivo assess trabecular and cortical bone microstructure in the appendicular skeleton. This technique is now readily applied in population studies. The importance of serum sex steroids for bone microstructure in elderly men remains, however, unknown. Therefore, the aim of this study was to examine the associations between serum sex steroid levels, assessed by the reference method mass spectrometry, and bone micro-citations between serum sex steroid levels, assessed by the reference method mass spectrometry, and bone micro-

Materials and Methods

Study sample

The study subjects of the present study participated in the 5-year follow-up of the Gothenburg part of the population-based Osteoporotic Fractures in Men Sweden study (13). Detailed information of the study subjects is given in the Supplemental Data. A total of 440 men with a mean age of 80.1 year having both HRpQCT and sex steroid analyses were included in the analysis (Table 1). The study was approved by the Ethics Com-

| Table 1. Characteristics of the Study Subjects | n = 440 |
|-----------------------------------------------|--------|
| Age, y                                        | 80.1 ± 3.5 |
| Height, cm                                    | 175.0 ± 6.5 |
| Weight, kg                                    | 79.5 ± 11.3 |
| BMI, kg/m²                                    | 25.9 ± 3.2 |
| Smoking, %                                    | 5.9 |
| Physical activity, km/d                       | 3.7 ± 3.4 |
| Calcium intake, mg/d                          | 949 ± 437 |
| Serum sex steroids                            |        |
| Estradiol, pg/mL                              | 16.0 ± 6.2 |
| Estrone, pg/mL                                | 24.1 ± 9.0 |
| T, ng/mL                                      | 3.77 ± 1.74 |
| SHBG, nmol/L                                  | 46.3 ± 19.1 |
| Free estradiol, pg/mL                         | 0.34 ± 0.13 |
| Free T, pg/mL                                 | 63.0 ± 26.9 |
| Bone microstructure by HRpQCT                 |        |
| Cortical porosity, %                          | 11.7 ± 4.1 |
| Cortical pore diameter, μm                    | 208 ± 25 |
| Trabecular BV/TV, %                           | 14.9 ± 2.8 |
| Trabecular number, mm⁻¹                       | 1.97 ± 0.30 |
| Trabecular thickness, μm                      | 76.0 ± 11.6 |

Values are given as mean ± SD unless indicated otherwise.

Serum analyses

Serum levels of E2, estrone, and T were analyzed with a validated gas chromatography tandem mass spectrometry system at Endoceutics (Québec, Canada). The limit of quantitation (LOQ) for E2 is 1 pg/mL and the intraassay and interassay CVs are 8.4%, 3.3%, and 2.6% and 6.5%, 4.0%, and 5.9%, respectively, at 3.5, 28.5, and 38.5 pg/mL. The LOQ for estrone is 4 pg/mL and the intraassay and interassay CVs are 4.7%, 2.1%, and 1.3% and 6.7%, 4.9%, and 5.4%, respectively, at 17.1, 117, and 157 pg/mL. The LOQ for T is 50 pg/mL and the intraassay and interassay CVs are 7.0%, 0.9%, and 0.6% and 5.2%, 2.9%, and 3.7%, respectively, at 156, 1406, and 1906 pg/mL. Serum was also assayed for SHBG using an immunoradiometric assay (Spectria; Orion Diagnostica) with an intraassay CV of less than 5.5% and an interassay CV of less than 6.9%. More than half of the subjects (259 of 439) had morning samples (drawn before 10:00 AM); the remaining samples were drawn around noon. Free E2 and free T levels were calculated using law-of-mass-action equations using association constants estimated from a systematic review of published binding studies and an iterative numeric method (18).

Statistical analyses

Adjustment of the associations between serum sex steroid levels and cortical and trabecular bone microstructure for age, height, weight, physical activity, calcium intake, and smoking was performed with linear regression models and standardized β-values are presented. The independent predictors of cortical bone microstructure were tested using stepwise multiple regression analysis including age, height, weight, physical activity, calcium intake, and smoking together with serum levels of E2, T,
and SHBG, or free E2 and free T, in the same analysis. Differences in mean cortical porosity according to quintiles of free E2 were assessed by P value for trend. All data were analyzed using SPSS software (version 21.0 for Windows). Data are presented as means ± SD, unless stated otherwise.

**Results**

**Characteristics of the study subjects**

The basic characteristics of the subjects having HRpQCT and serum sex steroids analyzed at the 5-year follow-up are shown in Table 1. These men with a mean age of 80.1 years have an average cortical porosity of 11.7% (Supplemental Figure 1) and a mean trabecular BV/TV of 14.9% at the tibia.

**Associations between serum sex steroids and cortical bone microstructure**

Age-adjusted linear regression analyses showed that serum levels of both E2 and free E2 associated significantly inversely with cortical porosity (standardized β = −.12, \( P < .05 \), and \( β = −.12, P < .05 \), respectively) and cortical pore diameter (β = −.16, \( P < .001 \), and \( β = −.16, P < .001 \), respectively; Table 2). Neither serum T nor free T levels associated significantly with cortical porosity (Table 2). Also, serum estrone levels did not significantly associate with cortical bone microstructural parameters (cortical porosity; \( β = −.08, P = .08 \)). To further investigate the associations between serum sex steroid levels and cortical bone microstructural parameters, linear regression models adjusted for age, height, weight, physical activity, calcium intake, and smoking were performed (Supplemental Table 1). These analyses showed that serum levels of both E2 and free E2 associated inversely with cortical porosity and cortical pore diameter. Neither T nor free T levels associated significantly with cortical porosity in these adjusted models (Supplemental Table 1).

Multivariate adjusted linear regression models including serum E2, T, and SHBG demonstrated that E2 but not T associated independently with both cortical porosity (standardized \( β = −.12, P < .05 \)) and cortical pore diameter (\( β = −.14, P < .01 \)). Similar independent associations were found for free E2 (cortical porosity, \( β = −.12, P < .05 \); cortical pore diameter, \( β = −.13, P < .01 \)) but not free T. The analysis of cortical porosity according to quintiles of free E2 yielded similar results confirming that older men with low serum free E2 have an increased cortical porosity (\( P \) for trend < .05, Supplemental Figure 2).

**Associations between serum sex steroids and trabecular bone microstructure**

Age-adjusted models revealed that free E2 levels associated significantly directly with the trabecular microstructural parameter bone volume fraction (BV/TV; \( β = .12, P < .05 \), Table 2). This association was mainly a result of a significant association with trabecular number, whereas no association was seen for trabecular thickness (Table 2). However, these associations between serum free E2 levels and bone microstructural parameters did not remain significant after additional adjustment for height, weight, physical activity, calcium intake, and smoking (Supplemental Table 1). Serum estrone did not significantly associate with trabecular bone microstructural parameters (BV/TV; \( β = .09, P = .07 \)) and neither did serum T or free T levels in any of the evaluated models (Table 2 and Supplemental Table 1). Adjustment for the time point of serum sampling did not influence any of the described associations (data not shown).

**Discussion**

To our knowledge, our study provides the first evidence that serum E2 levels are inversely associated with cortical porosity and cortical pore diameter in a large sample of 80-year-old men. Serum levels of E2, but not T, associated independently with cortical bone microstructural parameters and this finding is in line with the well-established pivotal role of E2 for male skeletal health. Ample evidence shows that low E2 levels are associated with low BMD, faster rates of bone loss, and increased risk of fractures in older men (1). Several population studies also reported a minimum level of serum E2 below which bone loss occurs and fracture risk increases (19), but such a threshold could not be identified for cortical porosity in the present study.

Only a few cross-sectional studies have investigated the relationship between serum E2 and measures of bone mi-
crostructure and bone strength (20–22). We demonstrate that free E2 levels associate directly with trabecular bone volume fraction at the tibia in elderly Swedish men, corroborating a previous study by Khosla et al (20) describing a positive association between bioavailable E2 and trabecular bone volume fraction at the radius in men. However, the associations between free E2 and trabecular bone microstructural parameters in the present study were not robust as they did not remain significant after additional adjustment for height, weight, physical activity, calcium intake, and smoking. Cross-sectional data from the MINOS study (21) and the BACH study (22) showed that circulating E2 levels associated directly with structural strength indices at the femur neck and distal radius in men. Longitudinal HRpQCT studies are clearly required to carefully elucidate the role of sex steroids in the age-related changes in trabecular and cortical microstructure and related bone strength indices in men. In older men, most of the circulating total estradiol is derived from aromatization of adrenal precursors in peripheral tissues, mainly fat tissue (23).

Our findings might suggest that serum E2 is involved in the regulation of cortical porosity. Cortical porosity has been postulated as an important determinant of bone strength and fracture susceptibility. This is translated into an increasing clinical interest in bone microstructure in order to understand bone fragility and ultimately improve fracture risk prediction. Whether cortical porosity can be used as a diagnostic marker for fracture risk in men beyond dual-energy x-ray absorptiometry-derived BMD remains unclear to date. Furthermore, serum E2 levels are inversely associated with circulating levels of sclerostin, a secreted Wnt antagonist produced by osteocytes (24, 25). This cross talk between Wnt signaling and sex steroids further supports the importance of serum E2 for cortical bone.

This study has several limitations. We acknowledge that accurate segmentation of the trabecular and cortical bone compartments on the HRpQCT images is challenging. With a resolution of 82 μm, only the larger cortical pores are identified, so cortical porosity may be well underestimated. Our findings are based on cross-sectional data, so longitudinal studies are needed to verify our data. The results are also based on single serum measurements and may thus underestimate true associations. In addition, the free sex steroids levels were calculated and not, as preferable, directly measured by equilibrium dialysis-liquid chromatography-tandem mass spectrometry. Finally, our findings are limited to Caucasian men. Notable strengths of the present study include serum analyses by the gold standard mass spectrometry technique and the high number of older men analyzed by the HRpQCT technique.

In conclusion, serum E2 levels are inversely associated with cortical porosity in a large sample of 80-year-old men. We propose that low serum E2 levels may reduce cortical bone strength, at least partly, by increasing cortical porosity and thereby increase fracture risk in older men.

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