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Sclerostin and Bone Strength in Women in their 10th Decade of Life

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Abstract

Sclerostin is a potent inhibitor of bone formation but has been shown to correlate positively with areal bone mineral density (aBMD). Little is known about its relationship to parameters of bone strength and volumentric BMD (vBMD) as measured by peripheral quantitative computed tomography (pQCT). We measured both serum sclerostin and parameters of tibial bone size and strength by pQCT to characterize this relationship. Our study population consisted of 223 Caucasian and 35 African American women (mean age 87) from the Study of Osteoporotic Fractures (SOF) cohort, who had usable pQCT scans of the tibia at sites 4% (T4%), 33% (T33%), and 66% (T66%) from the ankle. Analysis of covariance was used to test for differences in age-adjusted means of aBMD, pQCT variables, and serum biomarkers across sclerostin quartiles. Black women had significantly lower median sclerostin (34.3 pmol/L) than white women (48.5 pmol/L) (p=0.05). Women in the highest sclerostin quartile had 7-14.5% higher hip aBMD and pQCT parameters of vBMD and bone size than those in the lowest quartile in multivariate models adjusting for age, race, weight, height and diabetes. The association of sclerostin with parameters of bone strength differed dramatically between T33% and T66% sites. At T66%, women in the highest sclerostin quartile had pQCT strength parameters 9.4-15.3% greater than the lowest quartile, whereas no trend was found for the T33% site. Our results suggest paradoxical associations between circulating sclerostin and bone size, density and strength.

Introduction

Sclerostin is a protein produced by osteocytes that decreases osteoblastic bone formation through inhibition of canonical Wnt/β-catenin signaling[(1)]. People who lack sclerostin develop sclerosteosis, a disease of extremely high bone mass[(2)] and treatment with a sclerostin antibody has been shown to increase bone formation, mass and strength in both

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human [3] and animal models[4,5]. Although sclerostin is an inhibitor of bone formation, several studies have found serum sclerostin to be *positively* correlated to lumbar spine bone mineral density (BMD) [6], [7] and markers of bone formation[8], although the latter is inconsistent [9]. Studies have reported higher serum sclerostin levels in post- versus pre-menopausal women[10], men versus women[10], [11], bed-ridden [12], immobilized or 'mechanically unloaded' patients[13], and those with acute spinal cord injury[14]. Individuals with certain diseases have both higher circulating sclerostin and increased risk of bone fracture, including type 2 –but not type 1- diabetes mellitus[9], [15], Paget's disease, and prostate cancer with metastases to bone[8]. With regards to osteoporosis, we previously reported that despite being positively associated with areal BMD (aBMD), women with higher serum sclerostin had an increased risk of hip fracture risk[16], although a smaller French study found no association with fracture e[17]. A study from Saudi Arabia recently reported >7-fold increased fracture risk for each standard deviation increase in sclerostin [18]. This study was unique in its finding of a negative association between sclerostin and aBMD.

It is unknown if sclerostin is related to measures of bone geometry and strength. Peripheral quantitative computed tomography (pQCT) assesses volumetric BMD (vBMD) and geometric parameters of trabecular and cortical bone separately and can be used to estimate mechanical properties of bone. In older men, every SD decrease in many pQCT parameters was significantly associated with increased fracture risk (hazard ratio, (HR) 1.4-2.2) independent of age, BMI, and femoral neck aBMD[19]. Other studies in women have found similar links between pQCT parameters and fracture risk[20], [21], [22].

The aim of the current study is to characterize the relationship between serum sclerostin and parameters of bone strength, architecture and bone turnover in elderly women and to test whether these associations are in part mediated by sex steroid hormones.

Methods

Sclerostin and pQCT

Participants—Between 1986 and 1987, 9704 Caucasian women ages 65 and older were recruited for the Study of Osteoporotic Fractures at four metropolitan areas in the United States, including 2401 women from the Monongahela valley near Pittsburgh PA. Age-eligible participants were contacted by mail using community-based listings such as HMO membership and voter registration. In 1997, 662 African American women were added to the national study, including 177 at the Pittsburgh site. The current analysis was limited to the 258 surviving members of the Pittsburgh cohort (ages 79-96, mean 87.0 years) who attended a clinic visit in 2006-2008, a median of 20 years after their baseline examination, Figure 1.

Measurements at the follow-up visit—Serum samples were collected in a fasting state and after processing were stored at −80 C until assay. Seclerostin assays were carried out at the Heinz Nutrition Laboratory at the Graduate School of Public Health, University of Pittsburgh. Sclerostin levels were measured by ELISA sandwich assay (Biomedica Medizinprodukte GmbH & Co. KG, Wien, Austria), which has a sensitivity ranging from 50-50,000 pg/ml and CV between 4.1 - 9.8%[23]. Serum sclerostin measured with this assay has been shown to correlate with levels in bone marrow [24]. CTX (Serum-b-Crosslaps) was measured on Elecsys 2010 (Roche GmbH) by ECLIA (ElectroChemiLuminscence ImmunoAssay), a sandwich assay measuring ranges of 0.010 - 2.880 ng/mL with intra- and interassay variation of <4.7% and 4.8%, respectively. Serum P1NP was also measured on Elecsys 2010 (Roche GmbH) by ECLIA, measuring ranges of 5.0 – 437.2 ng/mL with intra- and interassay variations of <2.1% and 2.1%. Testosterone and estradiol were quantified in

serum (0.5 ml) by previously described radio-immunoassay (RIA) methods [25-27]. Prior to the RIAs, steroids are extracted with hexane:ethyl acetate (3:2) and then testosterone and estradiol are separated from each other and their metabolites by Celite column partition chromatography. Appropriate tritiated internal standards are added to each serum sample before the extraction step in order to follow and correct for procedural losses. The assay sensitivities for the testosterone and estradiol RIAs are 1.5 ng/dL and 2 pg/ml, respectively, and the interassay coefficients of variation (CVs) for these assays are 8%, 12%, and 12% at 13, 30 and 96 ng/dL, and 11%, 13% and 12% at 15, 36 and 101 pg/ml, respectively.

Analysis of SHBG is carried out by chemiluminescent immunometric assay on the Immulite analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). The assay utilizes monoclonal murine anti-SHBG attached to a bead and a polyclonal rabbit anti-SHBG conjugated to alkaline phosphatase. The assay sensitivity is 1 nmol/L, and the interassay CV is 9.1% at 69 nmol/L.

Free and bioavailable (non-SHBG-bound) testosterone and estradiol are calculated using the measured total testosterone and estradiol levels, respectively, and SHBG concentrations as well as an average assumed concentration for albumin [28,29]. This method has been found to have high validity [30]. Blind duplicates from 26 randomly selected women showed excellent correlations for all biomarkers ($r=0.87$-0.99, $p<0.05$). Procollagen type I N-terminal propeptide (P1NP) and CTX were measured at Synarc.

Measurements obtained at the year 20 visit included anthropometry, vital signs, physical function, and hip and whole body areal (aBMD). Body mass index (BMI) was calculated as weight in kilograms (measured on a balance beam scale) divided by height (measured by Harpenden stadiometer) squared. Vital signs were obtained using a digital ear thermometer (Omron MC-514) and automatic blood pressure monitor (Omron HEM-780). Physical function evaluation utilized the short physical performance battery (SPPB) developed by Guralnik and colleagues, which included grip strength (measured by Preston Grip or TEC dynamometer), gait speed, single and multiple chair stands, and tandem stands [31]. BMD was measured by dual energy x-ray absorptiometry (Hologic QDR 4500W, Bedford, MA) for the hip and whole body.

Self-reported functional status was obtained by in-clinic interview. Participants were asked to bring into the clinic all their prescription and over-the-counter medications for review by study staff. Information on medical history, lifestyle and tobacco use was collected by a self-administered questionnaire.

Peripheral quantitative computed tomography—The Stratec Three XCT-2000 pQCT (Stratec Medizintechnik, Pforzheim, Germany) was used to perform scans on different sites: 4% (T4%), 33% (T33%) and 66% (T66%) of the total length of the tibia. Phantom scans were performed on a daily basis as a quality control measure. Trained technicians followed a standardized protocol for scanning, using anatomical landmarks to optimize patient positioning in the machine. Tibia length was measured from medial malleolus to medial epicondyle. Scans at 4% of tibial site represent predominantly trabecular bone, whereas scans at the 33% and 66% sites consisted mainly of cortical bone. A single axial slice of 2.5 mm thickness with a voxel size of 0.5 mm and a speed of 20 mm/s was taken. Image processing was performed by a single investigator using the Stratec software package (Version 5.5E).

pQCT Bone parameters—Of 36 available pQCT parameters, we analyzed three that reflect bone density and size as well as five strength parameters shown to be predictive of fracture risk[19]. All scanning sites were measured for total volumetric bone mineral density

(vBMD, mg/cm^3). At T33% and T66%, size parameters of cortical area (mm^2) and thickness (mm) were measured, in addition to strength parameters such as cross sectional moment of inertia (CSMI, mm^4), polar moment of inertia (PMI, mm^4), section modulus (SM, mm^3), and polar (SSIp) and axial (SSIx) stress strain indices. Formulas for calculating CSMI, SM and SSI have been described in a previous publication [32]. CSMI and PMI reflect estimations of bone resistance to bending and torsion. SM derives from the CSMI and is an estimator of torsional strength. SSI estimates bending strength by accounting for material properties of bone and multiplying SM by the quotient of the measured cortical density and normal physiologic cortical density (1200 mg/cm^3). SSIp accounts for torsional load, where SSIx does not, and has proven to be a precise indicator of architectural strength in bone-bending tests. In terms of breaking force, however, it is SSIx that correlates stronger than areal BMD, CSMI or cortical vBMD[20].

Statistical Analysis

We compared participant characteristics across quartiles of sclerostin. For normally distributed variables, a test of linear trend was performed by treating sclerostin quartile cutoffs as category integer valued steps. For non-normally distributed or skewed variables, the Jonckheere-Terpstra test for trend was performed. The Cochran-Armitage test for trend was used for dichotomous variables.

Analysis of covariance was used to test for differences across sclerostin quartiles in age-adjusted and multivariable-adjusted means of continuous variables (DXA BMD, pQCT tibia bone strength and geometry, and bone turnover markers, P1NP and beta CTX). The multivariable-adjusted model included age, race, weight, BMI, and diabetes status. To explore whether the results are modified by 25-hydroxyvitamin D, sex hormone binding globulin, or bioavailable estradiol and testosterone, we subsequently individually adjusted for each marker in the multivariable models for 25(OH)D. All analyses were conducted with Statistical Analysis System (SAS; version 9.3; SAS Institute, Cary, NC).

Results

Descriptive characteristics of study participants across quartiles of serum sclerostin are shown in Table 1. With increasing levels of sclerostin, women tended to be older, have lower grip strength and higher total 25-hydroxyvitamin D levels ($p < .05$). Other markers of physical function/activity including gait speed, time to complete 5 chair stands, using arms during chair stand and self-reported ‘walks for exercise’ showed no significant relationship to serum sclerostin. A greater proportion of white women had the highest sclerostin levels. The median serum sclerostin concentration was higher in whites (48.45 pmol/L) compared to blacks (34.30 pmol/L) ($p < .05$). There was no association between sclerostin and smoking, body weight, height, BMI or sex steroid hormones. There was no significant difference in the prevalence of stroke or health status across sclerostin quartiles. There was some suggestion that a higher proportion of diabetics was observed among the highest sclerostin quartiles but there was no significant difference in serum sclerostin level by diabetes status: diabetes yes, median=51.0 pmol/L; no, median=45.8, pmol/L, p-0.16.

Table 2 shows unadjusted trends of hip aBMD and pQCT tibial measurements at T4%, T33% and T66% across sclerostin quartiles. Total hip BMD had the strongest positive association with sclerostin and was 11.6% higher in the top sclerostin quartile compared to the lowest, followed by trabecular vBMD at T4%, which was almost 10% higher in the top sclerostin quartile compared to the lowest. Cortical vBMD at T33% and T66% showed a more modest difference of 1.3% and 1.5%, comparing the fourth and first quartiles, respectively. Parameters of bone size also showed strong positive associations with

sclerostin. For the T33% site, cortical area and thickness were 7.6% and 10.8% higher in the top sclerostin quartile compared to the lowest. The same parameters at the T66% site showed a similar difference of 12.2% and 12.7%, respectively. Interestingly, the association between sclerostin and parameters of bone strength differed dramatically between T33% and T66% sites. At T33%, no parameters of bone strength were associated with sclerostin, whereas all T66% parameters showed a strong positive trend. Comparing women with the lowest sclerostin, women with the highest sclerostin had 10.1-13.4% higher cross-sectional and polar moments of inertia, section modulus, and both polar and axial stress-strain indices at the T66% site.

Multivariate adjustment strengthened the positive trend between sclerostin and parameters of BMD at all three sites, as well as parameters of bone size at T33%, Table 3. Additional adjustment for serum 25-hydroxyvitamin D did not influence these findings, nor did adjustment for serum hormone binding globulin, bioavailable estradiol or bioavailable testosterone (data not shown). Multivariate adjustment did not affect the positive relationship between sclerostin and cortical vBMD at T66%, but it tended to strengthen the positive association between sclerostin and pQCT parameters of bone size. Similarly, positive trends between sclerostin and parameters of strength at T66% persisted after adjustment, increasing 9.4-15.3% from the lowest to highest sclerostin quartiles. Additional adjustments for 25-hydroxyvitamin D, serum hormone binding globulin, bioavailable estradiol, or bioavailable testosterone had no effect on our findings (data not shown).

Both PINP and beta CTX tended to decrease with increasing sclerostin, Table 5. However, in the full multivariable model and in models adding 25-hydroxyvitamin D, there was no association between sclerostin and markers of bone turnover.

Discussion

Women with the highest sclerostin levels had significantly higher aBMD, vBMD and larger bones than women with the lowest sclerostin levels. After adjusting for age, race, weight, height and diabetes, we found that women with the highest serum sclerostin levels had pQCT parameters of bone strength 9.4 to 15.3% higher than those with the lowest, but only at the 66% site of the tibia. No association was found at the T33% site for reasons that remain unclear, even though additional analyses showed measurements at T33% and T66% to correlate with each other strongly ($r > 0.75$, $p < .0001$). To our knowledge, this is the first study to examine the relationship between circulating sclerostin and pQCT parameters of bone strength and differences by race/ethnicity. The lower sclerostin levels among the African American women are consistent with their lower risk of fracture.

Sclerostin and Bone Strength

These results were contrary to our initial hypothesis that higher sclerostin would confer decreased bone strength and lower vBMD, guided by previous reports that higher sclerostin was associated with increased risk of fracture[16]. Assuming that fracture risk is directly related to bone strength and that pQCT acts as a reliable proxy for bone strength, we would have expected lower pQCT values in the highest sclerostin quartile, when in fact just the opposite was true, especially, for the T66% site. However, our results are consistent with previous observations showing positive cross-sectional associations between sclerostin and aBMD[6], [9], [33]. Measurements of bone strength by pQCT may also reflect osteocyte number which may be why we see a positive association between sclerostin and pQCT parameters.

Sclerostin was related to bone strength only at the T66% site. One explanation could be that the production of sclerostin differs across the skeleton and possibly local levels differ at

T66% vs. T33%. If this is true, then our findings present another paradox in that the associations were found at the T66% site instead of the part of the bone bearing the greatest load, that is, T33%. Other studies report that sclerostin is upregulated in mechanically unloaded animal models and immobilized human subjects[1), (13)], suggesting that sclerostin levels should actually be *higher* at T66% because it is relatively 'unloaded' compared to T33%. One possible explanation is that bone strength and microstructure reflects mechanical forces other than just gravitational load and that surrounding musculature plays a role. The T66% site differs to that of T33%, with larger muscle mass and origins of several muscles, including the tibialis anterior and posterior. These may produce multidirectional forces that downregulate local sclerostin production, thereby improving bone architecture and strength. In addition, we found an inverse association between grip strength and sclerostin suggesting that sclerostin is also regulated by mechanical forces caused by gravity. An inverse association between sclerostin and physical activity has also been recently reported showing that minor changes in physical activity had effects on sclerostin levels [(34)].

Sclerostin and Bone Size/Density

Higher sclerostin was associated with greater parameters of bone size for all three tibial sites, with pQCT size parameters 9.6-15.2% higher in women with the highest sclerostin compared to women with the lowest sclerostin. These results were consistent with the current model of serum sclerostin as a partial reflection of osteocyte number. Women in the highest sclerostin quartile had 11.6% higher total hip aBMD, 12.1% higher trabecular vBMD at T4% and slightly higher cortical volumetric BMD at T33% and T66% than those in the lowest quartile. Our study included measurement of volumetric BMD, expanding upon previous studies that have only used areal BMD to characterize associations with sclerostin.

Sclerostin and Other Biomarkers

Negative associations between bioavailable estradiol and circulating sclerostin have been reported [(35)]. Our study showed no association between sex hormones and sclerostin, which may reflect the very old age and low estrogen levels of participants involved. With regards to bone turnover markers, the literature is inconsistent, although a weak negative association with sclerostin is most commonly reported. Our univariate analysis of P1NP and CTX revealed a weak negative relationship, which was attenuated after adjustment. Sclerostin was positively correlated with 25-hydroxyvitamin D levels, but adjusting for 25(OH)D had no effect on results. The jury is still out regarding the relationship between these two variables: some studies have found a similar positive correlation [(36)] while others have found no relationship at all [(37)], even among similar post-menopausal female populations [(38)].

Strengths of our study include a well-characterized, unique population of very old women, at higher risk of fracture. Other studies have focused on much younger individuals. Our inclusion of African American participants allowed us to describe racial differences in sclerostin for the first time. Measuring volumetric BMD and pQCT parameters of bone strength extended knowledge on sclerostin's relationship to bone microstructure and strength. We demonstrated excellent reproducibility for sclerostin. Because of our cross-sectional study design, limitations include lack of longitudinal data and lack of generalizability to other ages. We were unable to examine associations with fracture because only 24 women reported a fracture after this clinic visit and statistical power was low. Our sample of African American participants was small (n=35) and we had no power to test whether the relationship differed in Whites and Blacks. Other known factors such as PTH that influence sclerostin levels were not included, which may have yielded insight into the relationship we found with sclerostin and 25-hydroxyvitamin D.

In conclusion, higher serum sclerostin levels are associated with higher vBMD and bone size in elderly women, as well as higher pQCT parameters of bone strength at the T66% site. Further research should address why sclerostin is positively associated with measures of bone size, BMD and strength yet is a potent inhibitor of bone formation in vivo.

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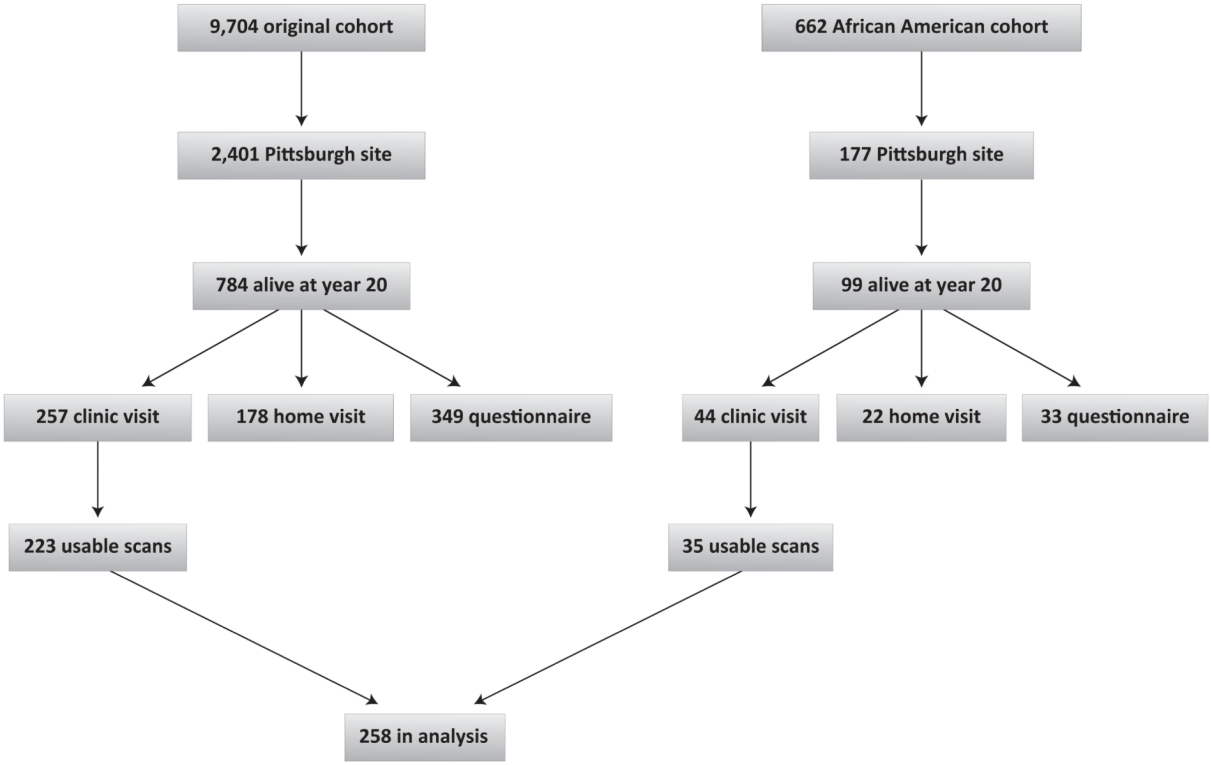

Figure 1.

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Table 1

Descriptive characteristics across Sclerostin quartiles

	Sclerostin Quartiles				
	Quartile 1 (n = 64)	**Quartile 2 (n = 65)**	**Quartile 3 (n = 65)**	**Quartile 4 (n = 64)**	**p-trend**
Age (years), mean ± SD	86.23 ± 3.39	86.85 ± 3.20	87.45 ± 2.99	87.30 ± 2.90	0.0307†
White, N (%)	47 (73.44)	58 (89.23)	59 (90.77)	59 (92.19)	0.0026†
Height (cm), mean ± SD	154.13 ± 5.75	154.73 ± 6.03	154.75 ± 5.47	154.99 ± 6.43	0.4304
Weight (kg) at Visit 9, mean ± SD	63.52 ± 11.92	65.29 ± 12.81	66.42 ± 13.33	61.61 ± 11.04	0.5102
Weight loss since baseline (kg), mean ± SD	−4.95 ± 8.51	−4.72 ± 8.90	−4.20 ± 10.74	−6.26 ± 8.59	0.5072
Body mass index (kg/m^2), mean ± SD	26.66 ± 4.29	27.22 ± 4.69	27.66 ± 4.97	25.60 ± 4.05	0.2826
Grip strength (kg), mean ± SD	15.55 ± 4.46	16.52 ± 4.24	14.51 ± 4.01	14.28 ± 3.43	0.0128†
Walking speed (m/s), mean ± SD	0.84 ± 0.20	0.84 ± 0.23	0.79 ± 0.19	0.79 ± 0.21	0.1049
Time to complete 5 chair stands (s), mean ± SD	12.98 ± 3.96	11.95 ± 3.12	13.69 ± 4.24	13.19 ± 4.05	0.3360
Use arms during chair stand, N (%)	11 (17.46)	15 (23.08)	12 (18.75)	18 (29.03)	0.1984
Fracture since Visit 8, N (%)	4 (6.25)	3 (4.69)	8 (12.31)	4 (6.25)	0.5989
Falls in last 12 months, N (%)	20 (31.25)	18 (27.69)	27 (41.54)	20 (31.25)	0.5928
Self-report of any fracture since age 50, N (%)	34 (53.13)	36 (55.38)	32 (50.00)	40 (62.50)	0.4135
Use Calcium supplement, N (%)	31 (48.44)	24 (36.92)	31 (47.69)	33 (51.56)	0.4664
Use Multivitamin, N (%)	44 (68.75)	40 (61.54)	41 (63.08)	45 (70.31)	0.8137
Stroke, N (%)	7 (10.94)	3 (4.62)	3 (4.62)	5 (7.81)	0.5107
Diabetes, N (%)	6 (9.38)	7 (10.77)	12 (18.46)	11 (17.19)	0.1070
MI, N (%)	14 (21.88)	11 (16.92)	9 (13.85)	10 (15.63)	0.2985
Current smoker, N (%)	3 (4.69)	1 (1.54)	1 (1.54)	1 (1.56)	0.2662
Self-rated good/excellent health compared to others your age, N (%)	54 (84.38)	57 (87.69)	48 (73.85)	50 (78.13)	0.1523
Physical activity – take walks for exercise, N (%)	24 (37.50)	23 (35.94)	21 (32.31)	19 (29.69)	0.3060
Sclerostin (pmol/L), mean ± SD	25.70 ± 5.13	40.58 ± 3.85	56.81 ± 6.40	94.99 ± 32.14	<0.0001†
Sclerostin (pmol/L), median (IQR)	26.45 (21.8 – 30.05)	40.7 (37.4 – 44.0)	56.85 (50.45 – 62.9)	83.75 (74.2 – 103.0)	<0.0001†
P1NP (ng/mL), median (IQR)	48.46 (35.06 – 72.35)	49.28 (37.78 – 70.78)	44.70 (29.94 – 69.40)	39.90 (27.98 – 55.51)	0.0168†

	Sclerostin Quartiles				
	Quartile 1 (n = 64)	**Quartile 2 (n = 65)**	**Quartile 3 (n = 65)**	**Quartile 4 (n = 64)**	**p-trend**
Beta CTx (ng/mL), median (IQR)	0.37 (0.24 – 0.44)	0.34 (0.25 – 0.46)	0.28 (0.17 – 0.39)	0.26 (0.18 – 0.38)	0.0110 †
Total Estradiol (pg/mL), median (IQR)	10.20 (7.95 – 12.78)	10.73 (7.04 – 14.54)	12.32 (10.04 – 15.33)	9.28 (7.45 – 11.72)	0.4687
Bioavailable Estradiol (pg/mL), median (IQR)	5.29 (4.0 – 7.09)	5.81 (3.85 – 8.37)	6.44 (5.22 – 8.62)	5.08 (3.77 – 7.02)	0.9637
Total Testosterone (ng/dL), median (IQR)	21.91 (16.02 – 34.48)	22.72 (16.59 – 39.94)	26.08 (17.22 – 31.11)	20.31 (11.05 – 30.66)	0.2413
Bioavailable Testosterone (ng/dL), median (IQR)	8.43 (5.61 – 11.57)	9.19 (6.03 – 13.42)	9.32 (6.55 – 12.85)	7.60 (4.46 – 12.25)	0.5938
SHBG (pmol/L), median (IQR)	77.35 (51.05 – 102.00)	66.40 (55.70 – 78.80)	62.90 (48.90 – 90.40)	62.95 (42.35 – 89.30)	0.0524
25 - Hydroxy D total (ng/mL), median (IQR)	27.5 (19.0 – 35.5)	28.0 (20.0 – 36.0)	30.0 (21.0 – 37.0)	32.0 (23.0 – 38.0)	0.0331 †

* Sclerostin quartile cut-points are 33.3, 46.4 and 66.3 pmol/L. IQR: Inter-quartile range.

† p-value for trend < 0.05

Table 2

Means (SD) of DXA BMD and pQCT tibia measurements across sclerostin quartiles

	Sclerostin Quartiles			
	Quartile 1 (n = 64)	**Quartile 2 (n = 65)**	**Quartile 3 (n = 65)**	**Quartile 4 (n = 64)**
Total hip BMD (g/cm^2)	0.69 ± 0.12	0.71 ± 0.12	0.75 ± 0.15	0.77 ± 0.10
Trabecular vBMD T4% (mg/ccm)	177.10 ± 47.84	181.81 ± 42.45	189.61 ± 44.13	194.63 ± 41.75
Cortical vBMD T33% (mg/ccm)	1083.78 ± 48.61	1090.87 ± 44.60	1114.90 ± 49.08	1097.64 ± 44.57
Cortical area T33% (mm^2)	182.72 ± 42.82	192.16 ± 33.83	202.66 ± 37.59	196.60 ± 39.95
Cortical thickness T33% (mm)	3.35 ± 0.94	3.54 ± 0.75	3.88 ± 0.87	3.71 ± 0.82
Cross sectional moment of inertia T33% (mm^4)	9008.22 ± 2462.51	9093.80 ± 2063.58	9192.93 ± 2369.24	9207.84 ± 2810.06
Polar moment of inertia T33% (mm^4)	16019.43 ± 4014.49	16649.66 ± 3317.47	16514.12 ± 3763.46	16311.14 ± 4710.95
Section modulus T33% (mm^3)	1171.49 ± 260.86	1222.49 ± 200.89	1228.31 ± 238.63	1203.17 ± 273.86
Stress-strain index (axial) T33%	756.55 ± 156.81	768.83 ± 134.11	779.27 ± 150.88	762.36 ± 167.47
Stress-strain index (polar) T33%	1185.30 ± 240.06	1237.19 ± 193.55	1253.92 ± 229.09	1219.90 ± 279.46
Cortical vBMD T66% (mg/ccm)	1003.87 ± 44.86	1006.43 ± 40.70	1028.09 ± 50.73	1016.39 ± 42.88
Cortical area T66% (mm^2)	168.98 ± 53.08	175.85 ± 44.17	193.19 ± 54.52	189.59 ± 44.71
Cortical thickness T66% (mm)	2.21 ± 0.79	2.25 ± 0.66	2.57 ± 0.88	2.49 ± 0.70
Cross sectional moment of inertia T66% (mm^4)	18790.65 ± 6301.58	19972.67 ± 5856.83	21273.29 ± 5719.66	21310.31 ± 6781.61
Polar moment of inertia T66% (mm^4)	28803.24 ± 8813.75	31167.58 ± 8531.41	32285.92 ± 8132.19	32315.04 ± 9394.04
Section modulus T66% (mm^3)	1557.43 ± 468.80	1685.22 ± 432.53	1780.58 ± 449.75	1756.76 ± 431.35
Stress-strain index (axial) T66%	1224.56 ± 308.85	1301.57 ± 264.93	1364.69 ± 257.74	1347.71 ± 299.06
Stress-strain index (polar) T66%	1745.22 ± 417.59	1899.80 ± 364.39	1945.71 ± 374.13	1927.75 ± 420.37

*Sclerostin quartile cut-points are 33.3, 46.4 and 66.3 pmol/L

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Table 3

Adjusted means (SE) of DXA BMD and pQCT Tibia 4%, 33% measurements across Sclerostin quartiles

	Sclerostin quartiles				
	Quartile 1 N = 64	**Quartile 2 N = 65**	**Quartile 3 N = 65**	**Quartile 4 N = 64**	**p-trend**
Total Hip BMD					
Model 1: Age-adjusted	0.69 (0.016)	0.71 (0.016)	0.76 (0.016)	0.78 (0.016)	<0.0001 †
Model 2: Base MV model	0.70 (0.015)	0.71 (0.014)	0.74 (0.014)	0.79 (0.014)	<0.0001 †
Model 2 plus 25(OH)D	0.70 (0.015)	0.71 (0.014)	0.74 (0.014)	0.78 (0.014)	<0.0001 †
Trabecular vBMD – T4%					
Model 1: Age-adjusted	174.98 (5.83)	181.92 (5.50)	190.90 (5.66)	195.23 (5.60)	0.0068 †
Model 2: Base MV model	175.31 (5.95)	181.87 (5.51)	189.50 (5.69)	196.34 (5.65)	0.0070 †
Model 2 plus 25(OH)D	175.89 (5.95)	182.39 (5.50)	189.08 (5.68)	195.68 (5.65)	0.0121 †
Cortical vBMD – T33%					
Model 1: Age-adjusted	1082.51 (6.18)	1091.03 (5.97)	1115.66 (5.99)	1097.94 (6.13)	0.0117 †
Model 2: Base MV model	1084.35 (6.25)	1090.37 (5.95)	1113.54 (5.99)	1099.02 (6.13)	0.0181 †
Model 2 plus 25(OH)D	1084.50 (6.27)	1090.56 (5.97)	1113.41 (6.00)	1098.81 (6.16)	0.0221 †
Cortical Area – T33%					
Model 1: Age-adjusted	179.95 (4.94)	192.52 (4.78)	204.31 (4.79)	197.25 (4.90)	0.0046 †
Model 2: Base MV model	181.46 (4.73)	191.68 (4.50)	202.45 (4.53)	198.58 (4.64)	0.0039 †
Model 2 plus 25(OH)D	181.99 (4.70)	192.37 (4.47)	201.99 (4.50)	197.80 (4.61)	0.0079 †
Cortical Thickness – T33%					
Model 1: Age-adjusted	3.29 (0.108)	3.54 (0.105)	3.92 (0.105)	3.72 (0.107)	0.0008 †
Model 2: Base MV model	3.33 (0.109)	3.52 (0.103)	3.88 (0.104)	3.74 (0.107)	0.0012 †
Model 2 plus 25(OH)D	3.34 (0.108)	3.54 (0.103)	3.87 (0.103)	3.73 (0.106)	0.0026 †
Model 2: Base MV model	8881.54 (289.56)	9137.49 (275.32)	9229.15 (277.19)	9250.48 (283.99)	0.3587
Model 2 plus 25(OH)D	8912.20 (287.84)	9176.91 (273.99)	9202.80 (275.48)	9206.07 (282.74)	0.4892

	Sclerostin quartiles				
	Quartile 1 N = 64	**Quartile 2 N = 65**	**Quartile 3 N = 65**	**Quartile 4 N = 64**	**p-trend**
Section Modulus – T33%					
Model 1: Age-adjusted	1157.38 (31.70)	1224.30 (30.65)	1236.74 (30.74)	1206.49 (31.44)	0.2684
Model 2: Base MV model	1161.14 (28.46)	1222.28 (27.06)	1227.27 (27.25)	1214.82 (27.91)	0.2066
Model 2 plus 25(OH)D	1163.37 (28.42)	1225.14 (27.05)	1225.36 (27.20)	1211.59 (27.91)	0.2729
Stress-strain Index (Polar) – T33%					
Model 1: Age-adjusted	1172.12 (30.77)	1238.88 (29.74)	1261.81 (29.83)	1223.01 (30.51)	0.2095
Model 2: Base MV model	1177.04 (26.97)	1236.60 (25.64)	1251.12 (25.82)	1231.73 (26.45)	0.1507
Model 2 plus 25(OH)D	1179.01 (26.94)	1239.14 (25.64)	1249.42 (25.78)	1228.87 (26.46)	0.2006

*Sclerostin quartile cut-points are 33.3, 46.4 and 66.3 pmol/L. Base MV model: Adjusted for age, race, height, weight, and diabetes.

†p-value for trend < 0.05

Table 4

Adjusted means (SE) of pQCT Tibia 66% measurements across Sclerostin quartiles

	Sclerostin quartiles				
	Quartile 1 N = 64	**Quartile 2 N = 65**	**Quartile 3 N = 65**	**Quartile 4 N = 64**	**p-trend**
Cortical vBMD – T66%					
Model 1: Age-adjusted	1002.80 (6.03)	1006.49 (5.74)	1028.79 (5.85)	1016.64 (5.89)	0.0188†
Model 2: Base MV model	1004.12 (6.11)	1006.51 (5.71)	1026.93 (5.86)	1017.25 (5.89)	0.0290†
Model 2 plus 25(OH)D	1004.12 (6.13)	1006.51 (5.74)	1026.92 (5.89)	1017.25 (5.92)	0.0306†
Cortical Area – T66%					
Model 1: Age-adjusted	166.22 (6.48)	176.00 (6.16)	195.02 (6.28)	190.23 (6.32)	0.0018†
Model 2: Base MV model	168.02 (6.29)	175.82 (5.87)	192.61 (6.03)	191.14 (6.07)	0.0022†
Model 2 plus 25(OH)D	168.27 (6.27)	176.49 (5.88)	192.14 (6.03)	190.68 (6.06)	0.0033†
Cortical Thickness – T66%					
Model 1: Age-adjusted	2.17 (0.101)	2.25 (0.096)	2.59 (0.098)	2.50 (0.098)	0.0031†
Model 2: Base MV model	2.19 (0.102)	2.25 (0.095)	2.57 (0.097)	2.51 (0.098)	0.0040†
Model 2 plus 25(OH)D	2.19 (0.102)	2.25 (0.095)	2.56 (0.098)	2.51 (0.098)	0.0052†
Cross-sectional moment of inertia – T66%					
Model 1: Age-adjusted	18532.85 (820.92)	19987.04 (780.72)	21444.44 (796.59)	21370.02 (800.97)	0.0067†
Model 2: Base MV model	18875.88 (736.93)	20018.91 (688.35)	21250.75 (707.17)	21202.32 (710.81)	0.0130†
Model 2 plus 25(OH)D	18935.19 (724.18)	20177.02 (678.21)	21139.67 (695.66)	21091.76 (699.21)	0.0230†
Section Modulus – T66%					
Model 1: Age-adjusted	1536.00 (58.98)	1686.41 (56.09)	1794.80 (57.23)	1761.72 (57.54)	0.0033†
Model 2: Base MV model	1558.45 (54.15)	1687.07 (50.58)	1771.67 (51.96)	1762.88 (52.23)	0.0045†
Model 2 plus 25(OH)D	1561.69 (53.70)	1695.70 (50.29)	1765.61 (51.58)	1756.85 (51.85)	0.0074†
Stress-strain Index (polar) – T66%					
Model 1: Age-adjusted	1725.19 (52.07)	1900.92 (49.52)	1959.00 (50.53)	1932.39 (50.81)	0.0043†

	Sclerostin quartiles				
	Quartile 1 N = 64	**Quartile 2 N = 65**	**Quartile 3 N = 65**	**Quartile 4 N = 64**	**p-trend**
Model 2: Base MV model	1749.63 (45.05)	1900.06 (42.08)	1935.29 (43.23)	1933.81 (43.46)	0.0046 †
Model 2 plus 25(OH)D	1752.97 (44.41)	1908.96 (41.59)	1929.04 (42.66)	1927.58 (42.88)	0.0081 †

* Sclerostin quartile cut-points are 33.3, 46.4 and 66.3 pmol/L Base MV model: Adjusted for age, race, height, weight, and diabetes.

† p-value for trend < 0.05

Table 5

Adjusted Means (SE) of Bone turnover markers across Sclerostin quartiles

	Sclerostin quartiles				
	Quartile 1 N = 64	**Quartile 2 N = 65**	**Quartile 3 N = 65**	**Quartile 4 N = 64**	**p-trend**
P1NP					
Unadjusted	55.20 (3.39)	56.94 (3.99)	53.26 (3.84)	44.56 (2.88)	0.0280†
Model 1: Age-adjusted	54.25 (3.56)	56.75 (3.52)	53.91 (3.50)	45.04 (3.58)	0.0586
Model 2: Base MV model	52.37 (3.61)	56.97 (3.49)	54.20 (3.50)	46.42 (3.57)	0.1921
Model 2 plus 25(OH)D	52.20 (3.61)	56.74 (3.49)	54.29 (3.50)	46.72 (3.58)	0.2349
Beta CTX					
Unadjusted	0.36 (0.021)	0.40 (0.028)	0.31 (0.022)	0.33 (0.027)	0.0910
Model 1: Age-adjusted	0.36 (0.025)	0.40 (0.025)	0.31 (0.025)	0.33 (0.025)	0.1062
Model 2: Base MV model	0.35 (0.026)	0.40 (0.025)	0.31 (0.025)	0.32 (0.025)	0.1285
Model 2 plus 25(OH)D	0.35 (0.025)	0.39 (0.024)	0.32 (0.025)	0.33 (0.025)	0.2085

Base MV model: Adjusted for age, race, height, weight, and diabetes.

*Sclerostin quartile cut-points are 33.3, 46.4 and 66.3 pmol/L.

†p-value < 0.05