CLINICAL STUDY

Serum sclerostin levels in men with idiopathic osteoporosis

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Abstract

Objective: Sclerostin inhibits osteoblast differentiation and bone formation. If aberrant sclerostin action is involved in less efficient bone acquisition in men with idiopathic low bone mass, this might be reflected in higher serum sclerostin levels.

Methods: In 116 men with idiopathic osteoporosis (≤ 65 years old), 40 of their sons and healthy controls, areal bone parameters were measured using dual-energy X-ray absorptiometry, and volumetric and geometric bone parameters were measured using peripheral quantitative computed tomography. Serum analytes were measured using immunoassays and estradiol (E_2) levels using liquid chromatography–tandem mass spectrometry.

Results: Men with idiopathic low bone mass had lower levels of sclerostin than the controls (0.54 ± 0.17 vs 0.66 ± 0.23 ng/ml; $P < 0.001$). In both groups, sclerostin levels were strongly associated with age; when adjusting for age, no associations with anthropometrics were observed ($P > 0.14$). In multivariate analyses, sclerostin levels displayed a positive association with whole-body bone mineral content (BMC) and areal BMD (aBMD), as well as with trabecular and cortical volumetric bone mineral density (vBMD) at the tibia in the probands. No clear associations were observed in the control group, neither were sclerostin levels associated with BMC at the radius or lumbar spine (all $P > 0.11$). Testosterone, but not E_2 , was inversely related to sclerostin levels in the probands. No difference in sclerostin levels was found in their sons when compared with their controls.

Conclusion: Lower rather than higher serum sclerostin levels in the probands with idiopathic low bone mass suggest that aberrant sclerostin secretion is not involved in the pathogenesis of low bone mass in these subjects.

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Introduction

Osteoporosis and related fractures are prevalent and lead to increased morbidity and mortality in both sexes (1). In about half of men with osteoporosis, no clear pathogenic cause can be established (2). Our previous findings in a family-based study of men with idiopathic osteoporosis concurred to indicate the existence of a familial defect in the acquisition of bone mass and size, involving both trabecular and cortical bone.

Sclerostin, a glycoprotein secreted by osteocytes partially in response to mechanical loading, is a strong negative regulator of osteoblast differentiation and bone formation through antagonizing effects on the Wnt/ β -catenin signaling pathway (3, 4). Serum levels of sclerostin increase with aging, have been reported to be positively related to overall bone mass, and sex differences have been described (5, 6, 7, 8). Furthermore, observational and interventional data suggest that circulating sclerostin levels are modulated by estrogen exposure (6, 7, 8, 9). In our family-based study, we reported lower serum estradiol (E_2) levels in

men with idiopathic low bone mass, suggesting an estrogen-related factor in the pathogenesis of male idiopathic osteoporosis. In addition, these men displayed lower serum pro-collagen 1 amino-terminal propeptide (PINP) levels, reflecting less bone formation (10, 11). We therefore hypothesized that lower bone mass in these men might result from constitutionally higher sclerostin levels and/or action, possibly linked to lower E_2 exposition at the tissue level.

Materials and methods

Subjects

Male study subjects ($n=116$) were diagnosed with idiopathic low bone mass using the following inclusion criteria: age ≤ 65 years at presentation and areal BMD (aBMD) Z-score ≤ -2.0 at the lumbar spine or proximal femur. Extensive clinical and laboratory investigations excluded secondary causes of low bone mass. All sons were invited to participate without any

DXA-based selection. Subjects with a history of hyperthyroidism, metabolic bone disease, delayed puberty, current or past alcohol consumption of ≥ 5 units/day more than once weekly, malabsorption, hemochromatosis, renal or gonadal dysfunction, malignancy or chronically treated with glucocorticoids, levothyroxin, or (anti)androgens were excluded. For each proband and son, a gender- and age-matched (± 2 years) control was recruited among healthy volunteers without a family history of osteoporotic fractures. None had a history of disease or medication use interfering with bone metabolism, or were heavy smokers (≥ 40 pack-years) or drinkers (≥ 5 units/day more than once weekly). Physical activity was assessed by recording the weekly frequency of recreational and/or professional activities and scored as proposed by Baecke *et al.* (12). All participants gave written informed consent for participation in this study, approved by the ethics review board of the Ghent University Hospital.

Anthropometrics, soft tissue composition, and areal bone parameters

Participants' body weight was measured to the nearest 0.5 kg in light indoor clothing without shoes. Standing height was measured to the nearest 0.1 cm using a wall-mounted Harpenden stadiometer. Areal bone parameters at the lumbar spine, proximal femur (total hip and neck), radius (non-dominant side), and whole-body composition were measured using DXA: QDR-2000 (first 68 matched couples; software version 7.20) or QDR-4500A (all subsequent couples; software version 11.2.1; Hologic, Inc., Bedford, MA, USA); all matched couples were measured on the same device and devices were cross-calibrated. Vertebrae with apparent fractures were excluded from the analysis; coefficients of variation (CV) were $<1\%$ for daily spine and weekly whole-body phantom measurements.

pQCT measurements

In this ongoing study, peripheral quantitative computed tomography (pQCT) measurements were added and extended during the study course and lack for some of the early recruited probands and their sons. Cross-sectional slices (2.0 mm thick; voxel size 0.4 mm) at the metaphysis (at 4% of bone length from the distal end of the radius) of the non-dominant forearm (all participants) and the lower leg (probands only, 66 couples) were taken to determine trabecular volumetric bone mineral density (vBMD) by pQCT (XCT2000; Stratec GmbH, Pforzheim, Germany). Cortical bone parameters were determined at the midshaft of the non-dominant forearm (probands, 70 couples and sons, 23 couples) and the lower leg (probands, 66 couples) at 33 and 38% of bone length from the distal end of the radius and tibia respectively. Analyses were performed using the manufacturer's software (version 5.4). The cross-sectional

area of the radius/tibia was determined after detecting the outer bone contour at a threshold of 280 mg/cm^3 . The threshold was set at 180 mg/cm^3 for determining trabecular vBMD and at 710 mg/cm^3 for cortical bone. Cortical thickness and the endo- and periosteal circumference were estimated using the circular ring model in which the shaft of a long bone is assumed to be an ideal cylinder.

Laboratory measurements

Venous blood was obtained between 0800 and 1000 h after overnight fasting and serum stored at -80°C until analysis. Samples from the probands, their sons, and respective controls were measured in a single assay run. Commercial immunoassays were used to measure serum leptin (Linco Research, Inc., MO, USA), P1NP, total testosterone, SHBG (Orion Diagnostica, Espoo, Finland), C-terminal telopeptides of type 1 collagen (CTX; Biomedica Medizinprodukte GmbH, Vienna, Austria), bone-specific alkaline phosphatase (bAP; Beckman Coulter, Inc., Fullerton, CA, USA), osteocalcin (Nordic Bioscience Diagnostics A/S, Herlev, Denmark), and sclerostin (TECOmedical AG, Sissach, Switzerland). For sclerostin, the limit of detection was 0.12 ng/ml and the limit of quantification 0.20 ng/ml ; intra-assay CV was $<15\%$ for levels between 0.20 and 0.50 ng/ml and $<6\%$ for levels $>1.00 \text{ ng/ml}$ and inter-assay CV was $<6\%$ for levels $>0.50 \text{ ng/ml}$. Serum E_2 was determined by liquid chromatography–tandem mass spectrometry as described previously (13). Free testosterone and E_2 were calculated from testosterone, E_2 , SHBG, and albumin concentrations using equations based on the mass action law (14, 15). For all measurements, intra- and inter-assay CV were below 10 and 15% respectively. Assays were performed at the Laboratory for Hormonology at the Ghent University Hospital except for serum sclerostin, which was assayed at the Bone and Cartilage Markers Laboratory, Unilab Ig, CHU Sart Tilman, University of Liège. Kidney function was determined by the calculation of estimated glomerular filtration rate (eGFR; $\text{ml/min per } 1.73 \text{ m}^2$) according to the MDRD formula.

Statistical analysis

If normally distributed, variables were described as mean $\pm$ s.d., and as median (1st–3rd quartiles) otherwise. If necessary, variables were transformed (natural logarithmic) to meet the required model assumptions. Comparisons between groups were performed using independent sample *t*-tests and ANCOVAs to account for potential confounding by age, body weight, or height. Univariate and age-, weight-, and height-adjusted associations between sclerostin and different patients' characteristics were explored using multiple linear regression models, with effect estimates reported as standardized regression coefficients (β). The level indicating

Table 1 General and bone parameters. Data presented as mean $\pm$ s.d.

	Probands (n=116)	Controls (n=116)	<i>P</i> ^a	Sons (n=40)	Controls (n=40)	<i>P</i> ^a
Age (years)	45.3 $\pm$ 12.9	46.0 $\pm$ 13.1	0.68	28.6 $\pm$ 6.0	28.8 $\pm$ 6.4	0.91
Weight (kg)	74.4 $\pm$ 9.3	82.3 $\pm$ 11.6	<0.001	73.5 $\pm$ 11.3	79.1 $\pm$ 10.0	0.087
Height (cm)	174.4 $\pm$ 6.3	177.1 $\pm$ 6.5	0.002	180.8 $\pm$ 6.7	180.8 $\pm$ 6.1	0.99
Trabecular vBMD at radius 4% (g/cm ³) ^b	183 $\pm$ 37	226 $\pm$ 43	<0.001	217 $\pm$ 32	242 $\pm$ 38	0.036
Cortical vBMD at radius 66% (g/cm ³)	1191 $\pm$ 50	1207 $\pm$ 22	0.004	1178 $\pm$ 59	1197 $\pm$ 46	0.32
Cortical bone area at radius 66% (cm ²)	94 $\pm$ 13	103 $\pm$ 13	<0.001	97 $\pm$ 10	105 $\pm$ 12	0.072

^aAccording to an independent Student's *t*-test.^bpQCT data only available for 23 of the sons.

statistical significance was <0.05 (two-tailed). Statistical analyses were performed using SPSS (version 19.0; SPSS, Inc.).

Results

General characteristics

As reported previously (11), men with idiopathic osteoporosis had a lower body weight, height, lean, and fat mass than the age-matched control subjects. Their bone mass deficit involved trabecular and cortical bone density as well as cortical bone size (Table 1), and these men presented lower serum levels of E₂ and free E₂, in part due to higher SHBG levels (Table 2). Regarding sclerostin, lower serum levels were observed in men with idiopathic osteoporosis when compared with their controls (Fig. 1), whereas no difference was found in their sons when compared with their respective controls. The results were not essentially different when only considering the affected sons (data not shown). When adjusting for age and whole-body BMC, the observed between-group difference in serum sclerostin levels between the probands and their controls was no longer significant (*P*=0.13).

Relationship with serum sclerostin levels

In both probands and healthy controls, sclerostin levels were strongly and essentially similarly related to age (Fig. 2). In the control group, positive associations

between sclerostin levels and body weight, BMI, and total fat mass were no longer significant after adjusting for age, whereas positive associations with leptin levels persisted even after controlling for weight or body fat mass (probands, β =0.341; *P*=0.062 and controls, β =0.265; *P*=0.037; Table 3).

When adjusting for age, weight, and height, sclerostin levels in the probands were positively related to whole-body aBMD and BMC, as well as to trabecular vBMD and BMC and cortical vBMD at the tibia. In the controls, sclerostin levels were associated with aBMD at the proximal femur and tended to be positively related to cortical vBMD (β =0.228; *P*=0.057). No associations were found with trabecular or cortical bone parameters at the radius, neither were sclerostin levels associated with areal bone parameters at the radius or lumbar spine in either group (all *P*>0.06). Except for the endosteal circumference at the tibia (β =−0.23; *P*=0.032) in the probands, no significant associations between sclerostin levels and bone size parameters were found. The results were essentially unaltered when adjusting for lean and/or fat mass instead of weight (data not shown).

Sclerostin levels were inversely related to the serum levels of P1NP, CTX, bAP, and osteocalcin in the control group and to P1NP levels in the probands; findings, however, were lost after correcting for participant's age (data not shown). There was no difference in serum sclerostin levels between those with and without previous fractures (*P*>0.22) in either group, and no relationship with indices of physical activity was observed (data not shown; all *P*>0.19). Serum

Table 2 Serum sclerostin and sex steroid levels.

	Probands (n=116)	Controls (n=116)	<i>P</i> [*]	Sons (n=40)	Controls (n=40)	<i>P</i> [*]
Sclerostin (ng/ml)	0.54 (0.41–0.66)	0.61 (0.51–0.78)	<0.001	0.52 (0.41–0.62)	0.54 (0.45–0.66)	0.31
SHBG (nmol/l) ^a	29.6 (22.9–37.4)	25.8 (20.2–37.1)	0.027	29.4 (24.3–36.9)	24.9 (20.4–32.5)	0.14
Testosterone (ng/dl)	537 $\pm$ 163	554 $\pm$ 157	0.42	585 $\pm$ 179	584 $\pm$ 1690	0.99
Free testosterone (ng/dl)	11.5 $\pm$ 3.8	12.9 $\pm$ 4.2	0.009	12.8 $\pm$ 4.3	13.6 $\pm$ 3.8	0.39
Estradiol (E ₂ ; pg/ml)	17.4 $\pm$ 5.4	21.9 $\pm$ 6.2	<0.001	19.3 $\pm$ 5.1	20.2 $\pm$ 5.3	0.47
Free E ₂ (pg/ml)	0.32 $\pm$ 0.12	0.43 $\pm$ 0.15	<0.001	0.37 $\pm$ 0.11	0.40 $\pm$ 0.11	0.26

Data presented as mean $\pm$ s.d., unless ^a: presented as median (1st–3rd quartiles) in the case of a non-Gaussian distribution. ^{*}According to an independent Student's *t*-test or ^a Wilcoxon signed-rank test, where appropriate. Conversion factor for testosterone from ng/dl to nmol/l is 0.0347, for estradiol from pg/ml to pmol/l is 3.671.

Figure 1 Dot plots of serum sclerostin levels in men with idiopathic osteoporosis and age-matched controls. The full line represents the mean.

sclerostin levels were higher in the probands taking vitamin D supplements vs those who did not (0.57 ± 0.16 vs 0.48 ± 0.16 ; $P=0.007$), and sclerostin levels were inversely related to the eGFR in both groups ($r=-0.26$; $P=0.007$ and $r=-0.26$; $P=0.010$ respectively). These latter findings lost statistical significance when adjusting for participant's age (all $P>0.18$).

In the probands, and in the univariate analyses in the control group, negative associations between serum sclerostin and both total ($r=-0.18$; $P=0.047$) and free ($r=-0.20$; $P=0.031$) testosterone levels were found. No associations with (free) E_2 or SHBG levels were found, but when controlling for testosterone levels, total E_2 levels were found to be positively related to sclerostin levels ($\beta=0.20$; $P=0.039$) in the probands only.

In the sons and their respective controls, sclerostin levels tended to be positively related to total body weight (age-adjusted $\beta=0.379$; $P=0.098$ and $\beta=0.447$; $P=0.028$ respectively), whereas no significant associations with age, bone parameters, or sex steroids were found (data not shown).

Discussion

Contrary to the initial working hypothesis, lower rather than higher serum sclerostin levels were observed in men with idiopathic low bone mass when compared with age-matched control subjects. Given that sclerostin is acknowledged as having anti-proliferative and anti-differentiating effects on osteoblasts (3, 4), this finding pleads against the involvement of aberrant sclerostin secretion in the pathogenesis of low bone mass in these subjects.

This between-group difference in sclerostin levels was no longer significant after adjusting for whole-body BMC, and sclerostin levels were positively related to parameters reflecting bone strength in the probands and

– albeit to a lesser extent – in the controls. Although not unequivocally (7, 16), earlier studies have also reported positive associations between serum sclerostin levels and bone mass in both men and women (5, 7, 8). These positive associations between bone mass, other parameters of bone strength, and serum sclerostin levels may seem counterintuitive since sclerostin is considered to inhibit bone formation. However, they could be explained by the fact that for the same forces applied, lower bone mass will lead to higher mechanical strain, thereby decreasing sclerostin secretion by osteocytes. Taken together, this suggests that lower serum sclerostin levels in men with idiopathic osteoporosis reflect their lower bone mass and osteocyte number or cell mass.

Corroborating previous findings (5, 7, 8, 9, 16), we also found a strong positive association between serum sclerostin levels and age. Several *in vivo* studies have shown that mechanical loading decreases, whereas unloading increases local sclerostin production (17, 18, 19). Since aging is associated with less physical activity and consequently mechanical loading (20, 21), this might at least partially explain the positive association between age and sclerostin levels.

Previous studies reported an inverse association between serum sclerostin and E_2 levels in pre- and postmenopausal women (16), lower sclerostin levels in postmenopausal women under hormonal replacement therapy (7, 9), and an interventional study by Mödder *et al.* (9) demonstrated that E_2 withdrawal increases serum sclerostin levels in men in whom endogenous sex steroid production was eliminated and then substituted at physiological levels. Contrary to what could be expected from these findings, total E_2 levels were positively related to sclerostin levels when controlling for testosterone levels in our probands. Further, a consistent negative association was observed between sclerostin and both total and free testosterone levels in our probands, as well as in the univariate analyses in

Figure 2 Scatterplots of both groups' age and serum sclerostin levels.

Table 3 Unadjusted/age-, weight- and height-adjusted standardized regression coefficients (β) between serum sclerostin levels and general, bone and sex steroid parameters.

Characteristics	Probands	Controls
Age	0.389 [§] /0.475 ^{§,a}	0.485 [§] /0.472 ^{§,a}
Weight	0.084/0.038 ^b	0.197 [*] /0.143 ^b
Height	−0.148/0.071 ^c	−0.168/−0.005 ^c
BMI	0.142/0.003 ^d	0.292 [†] /0.132 ^d
Lean mass	−0.030/0.00 ^b	0.027/0.127 ^b
Fat mass	0.158/0.080 ^b	0.336 [†] /0.156 ^b
eGFR	−0.176/0.001	−0.201 [†] /0.007
Leptin	0.280 [†] /0.259 [*]	0.356 [§] /0.189
Whole body BMC	0.037/0.288 [†]	0.076/0.202
Whole body aBMD	0.013/0.267 [†]	0.066/0.194
Lumbar spine BMC	−0.081/0.165	0.018/0.114
Lumbar spine aBMD	−0.152/0.088	0.014/0.049
Total hip BMC	−0.140/−0.166	0.068/0.133
Total hip aBMD	−0.150/−0.072	0.064/0.247 [*]
Radius BMC	0.048/0.044	0.012/−0.008
Radius aBMD	−0.007/0.116	0.023/0.047
Radius trab. BMC	0.103/0.111	0.039/0.038
Radius trab. vBMD	0.012/0.165	0.042/0.079
Radius cort. BMC	0.010/0.070	0.065/0.093
Radius cort. vBMD	−0.113/0.078	−0.079/0.171
Tibia trab. BMC	0.088/0.222 [*]	0.067/0.136
Tibia trab. vBMD	0.024/0.276 [†]	0.055/0.129
Tibia cort. BMC	0.016/0.100	0.066/−0.001
Tibia cort. vBMD	0.049/0.227 [*]	−0.112/0.228
Estradiol	0.071/0.110	−0.046/0.106
Free estradiol	−0.036/0.008	0.003/0.086
Testosterone	−0.186 [*] /−0.184 [*]	−0.208 [*] /−0.018
Free testosterone	−0.311 [†] /−0.202 [*]	−0.183/0.010
SHBG	0.066/−0.145	−0.096/−0.167

* $P < 0.05$, $^{\dagger}P < 0.01$ and $^{\S}P < 0.001$. E₂, estradiol.^aAdjusted for weight and height.^bAdjusted for age and height.^cAdjusted for age and weight.^dAdjusted for age.

the control group. These findings are in line with cross-sectional studies reporting a positive association between serum E₂ and sclerostin levels in older men (7, 8) and a negative association between bioavailable testosterone and sclerostin levels in a middle-aged subgroup (40–59 years) (7). These inconsistent findings are difficult to explain but might result from differences in participants' conditions, leading to different sex hormone levels and/or increased bone remodeling.

The strengths of our study are the well-described patient population of men with idiopathic osteoporosis, together with their well-characterized phenotype. Limitations are the limited sample size and the fact that male idiopathic osteoporosis might represent a spectrum of low bone mass due to heterogeneous mechanisms and different causes. Given the age of our probands and controls, we cannot extrapolate the role of sclerostin during peak bone mass acquisition. However, given that no differences in sclerostin levels were observed between their sons (whether they were affected or not) and age-matched controls, a primary role for sclerostin in the deficient bone acquisition process in men with idiopathic osteoporosis seems unlikely.

In conclusion, this study shows that serum sclerostin levels in men with idiopathic osteoporosis are lower than those in healthy control subjects, possibly reflecting differences in overall bone mass. Although further research is needed, aberrant sclerostin secretion seems unlikely to be causally involved in the pathogenesis of idiopathic osteoporosis in men.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

All authors participated in the writing of the manuscript; S Goemaere, H Zmierzak, and J M Kaufman designed the study; S Goemaere, H Zmierzak, J M Kaufman, S Vandewalle, Y Taes, and B Lapauw recruited the subjects; Y Taes and J M Kaufman were responsible for all laboratory measurements except for sclerostin which was measured in the laboratory of J Collette.

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