ORIGINAL ARTICLE

Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study

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Abstract

Summary Sclerostin is a key regulator of bone formation. In a population of 572 postmenopausal women (mean age, 67 years) followed prospectively for a median of 6 years, there was no significant association between baseline levels of serum sclerostin and incidence of all fractures which occurred in 64 subjects.

Introduction Sclerostin, an osteocyte soluble factor, is a major negative regulator of osteoblastic activity. Circulating sclerostin levels were reported to increase with age and to be modestly associated with bone mineral density (BMD) and bone turnover, but there are no data on the association with fracture risk.

Methods We investigated 572 postmenopausal women (mean age, 67±8.5 years) from the OFELY population-based cohort. The associations of serum sclerostin measured with a new two-site ELISA and spine and hip BMD by DXA, serum β-isomerized C-terminal crosslinking of type I collagen (CTX), intact N-terminal propeptide of type I collagen (PINP), intact PTH, 25-hydroxyvitamin D [25 (OH)D], estradiol, testosterone, and fracture risk were analyzed. At the time of sclerostin measurements, 98 postmenopausal women had prevalent fractures. After a median of 6 years (interquartile range, 5–7 years) follow-up, 64 postmenopausal sustained an incident fracture.

Results Serum sclerostin correlated positively with spine ($r=0.35$, $p<0.0001$) and total hip ($r=0.25$, <0.0001) BMD. Conversely, serum sclerostin was weakly negatively associated with the bone markers PINP ($r=-0.10$, $p=0.014$) and CTX ($r=-0.13$, $p=0.0026$) and with intact PTH ($r=-0.13$, $p=0.0064$). There was no significant association of serum sclerostin with 25(OH)D, estradiol, free estradiol index, or testosterone. Serum sclerostin considered as a continuous variable or in quartiles was not significantly associated with the risk of prevalent or incident fracture.

Conclusion Serum sclerostin is weakly correlated with BMD, bone turnover, and PTH in postmenopausal women. It was not significantly associated with the risk of all fractures, although the number of incident fractures recorded may not allow detecting a modest association.

Keywords Bone markers · Fracture · Osteoporosis · PTH · Sclerostin

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Introduction

Osteocytes which are terminally differentiated cells embedded within the mineralized matrix play important roles in bone metabolism [1]. These cells which comprise an extensive network of canaliculae are believed to participate in the regulation of the targeted remodeling process by sensing microfractures [1]. This mechanism is crucial for replacing altered bone tissue by a new collagen matrix with optimal biochemical competence. Osteocytes are also important in the termination of the remodeling cycle by secreting factors such as sclerostin which inhibits osteoblast activity and promotes their apoptosis [1].

Sclerostin which is coded by the SOST gene is a member of the DAN (differential screening–selected gene aberrant in neuroblastoma) family of glycoproteins. This family consists of a group of secreted proteins that share the ability to antagonize bone morphogenetic protein (BMP) activity, although sclerostin is not a classical BMP antagonist [2]. Sclerostin binds to the lipoprotein receptor 5 [3], an essential membrane-bound cofactor of the canonical Wnt signaling and, thereby, inhibits this pathway leading to decreased osteoblast activity [4].

The mRNA expression of sclerostin is inhibited by PTH both in vitro [5, 6] and in vivo in mice and rats [7, 8]. Recent studies showed that administration of a monoclonal antibody blocking the activity of sclerostin results in increased of bone mineral density (BMD) and bone strength in ovariectomized rats [9] and primate [10] and an increase of markers of bone formation while decreasing markers of bone resorption in healthy men and women [11].

Because sclerostin is a secreted soluble factor, different immunoassays have been recently developed to measure its concentration in serum, and three are commercially available [12, 13]. Clinical studies performed in healthy individuals showed that serum sclerostin levels increase with age in both women and men, are modestly and inconsistently associated with bone turnover markers and BMD and decrease in postmenopausal women receiving intermittent PTH or 17 β estradiol, whereas bisphosphonate therapy has no effect [14–21]. However, in women with corticosteroid-induced osteoporosis, teriparatide did not decrease sclerostin levels [22]. However, there is a no published study that has investigated the relationships between serum sclerostin and fracture risk assessed prospectively.

The primary objective of this study was to analyze the association of serum sclerostin levels measured by a novel commercially available immunoassay and fracture risk assessed prospectively in a large population of postmenopausal women. We also investigated the association of serum sclerostin with BMD, bone turnover markers, PTH, and steroid hormones.

Material and methods

Subjects

We investigated 572 postmenopausal women (mean age, 67 ±8.5 years) from the Os des FEmmes de LYon (OFELY) cohort at the ninth annual follow-up. OFELY is an ongoing prospective study of the determinants of bone loss in 1,039 volunteer women, 31 to 89 years of age, recruited between February 1992 and December 1993, randomly selected from the affiliates of a large health insurance company (Mutuelle

Générale de l' Education Nationale) from the Rhône district with an annual follow-up. The OFELY cohort has been described elsewhere [23, 24].

Fracture assessment

Nonvertebral and clinical vertebral fractures were reported during each annual follow-up. For women who did not come to the clinical center, a letter was sent every year to identify the occurrence of fractures. All fractures were confirmed by radiographs or by a surgical report. Only low-trauma fractures (i.e., those occurring as a result of falls from standing height or less) were taken into account, and we excluded fractures of fingers, toes, skull, and face. Vertebral fractures were assessed on lateral X-ray films of the thoracic and lumbar spine obtained every 4 years, including the ninth annual follow-up, in women aged 50 years and over at the inclusion in the study. The semiquantitative method of Genant [25] was used by a trained physician. For the present analysis, prevalent fragility fractures were all those that occurred between the inclusion visit and the ninth annual follow-up, in addition to the fragility fractures of the wrist, humerus, vertebral, or hip that occurred before the inclusion and after the age of 40 years. Incident fragility fractures were all those that occurred between the 9th and the 16th annual follow-up.

Bone mineral densitometry

Areal bone mineral density (BMD) was measured at ninth annual follow-up by dual-energy X-ray absorptiometry (DXA), at the lumbar spine and total hip (QDR 4500A, Hologic®, Waltham, MA, USA). The in vivo precision error of DXA, expressed as the coefficient of variation, was 0.9 and 1 % for lumbar spine and total hip BMD, respectively. A control phantom was scanned every day, and all DXA measurements were performed by the same experienced operator.

Biochemistry

Blood samples were collected between 8:00 and 9:30 a.m. after an overnight fast at the ninth visit of the OFELY which is considered as baseline for this analysis. Serum samples were stored frozen at −70°C until assayed. Serum intact N-terminal propeptide of type I collagen (PINP), and β-isomerized C-terminal crosslinking of type I collagen (CTX) were measured using an automatic test (Elecsys P1NP, Elecsys β–Crosslaps; Roche Diagnostics). Intra- and interassay variations were <8 % for all markers. Serum total 17 β-estradiol was measured by radioimmunoassay without extraction with a limit of detection of 11 pM/L. Bioavailable estradiol was calculated by multiplying the

fraction of estradiol not bound to sex hormone-binding globulin with total estradiol. Serum total testosterone was measured by radioimmunoassay after extraction with a limit of detection of 0.04 nM/L. Intra- and interassay variations were <10 % for both dosages. Serum intact PTH was measured by an immunoradiometric assay using two monoclonal antibodies (ELSA-PTH, Cisbio) with a limit of detection of 0.7 pg/mL. Intra- and interassay variations were <8 %. Serum 25-hydroxyvitamin D [25(OH)D] was measured by an automatic competitive two-step chemiluminescence assay (LIAISON 25OH Vitamin D TOTAL, DiaSorin). The lower limit of detection was 4 ng/mL.

Serum sclerostin was measured by a novel two-site ELISA (TECO® Sclerostin EIA Kit) [12]. This assay uses two antibodies raised against human recombinant sclerostin. Briefly, serum samples or standard (human recombinant sclerostin) are incubated with a biotinylated polyclonal antibody as well as with a horseradish peroxidase (HRP)-labeled secondary monoclonal antibody that specifically recognizes human sclerostin in streptavidin-coated wells. After incubation overnight at 2–8°C, the unbound material is washed away. After this washing step, the substrate is added to the well which reacts with the HRP, and color is formed. After a 15-min incubation, the reaction is stopped with HCl, and the plate is read using a plate reader at 450 nm. The amount of color generated is directly proportional to the amount of sclerostin in the sample. In our laboratory, the intra- and interassay coefficients of variation were lower than 10 %. The detection limit defined as the concentration of sclerostin corresponding to the OD value of standard 0+3 standard deviations was 0.13 ng/ml. The recovery of spiked standards which was tested by adding three different concentrations of human recombinant sclerostin (0.5, 1, and 2 ng/ml) into eight different serum human samples presenting with various levels of endogenous sclerostin ranged from 88 to 111 %.

Statistical analyses

All data were reported as mean±SD unless otherwise specified. Association of serum sclerostin with bone turnover markers, BMD, and hormone levels was assessed by linear regression analysis after logarithmic transformation of the data for normalization of the distribution. Association of serum sclerostin with incident fracture was analyzed by logistic regression analyses using sclerostin levels as continuous or categorized (per quartile) variables and were adjusted for age. A sample size of 522 individuals (lower than the size of our population) was needed to detect an odds ratio of 1.6 between incident fracture and serum sclerostin with a power of 0.85 and an alpha risk of 0.05 (two sided).

Results

Baseline associations of sclerostin with bone turnover, BMD, and hormones

Serum sclerostin was slightly negatively associated with serum PINP and serum CTX, as bone formation and resorption markers, respectively. Serum sclerostin was modestly associated with PTH, but did not correlate with all other hormones including total and free estradiol index, testosterone, and 25(OH)D (Table 1). Serum sclerostin was positively associated with spine and total hip BMD ($p<0.0001$, Table 1). Women with levels in the highest quartile of serum sclerostin had spine and total hip BMD values which were 15 and 8.5 % higher, respectively, than individuals with levels in the lowest quartile (Fig. 1).

Serum sclerostin and fracture risk

At baseline, 98 postmenopausal women had a prevalent fracture. During a median of 6 years (interquartile range, 5–7 years) follow-up, 64 postmenopausal women sustained an incident vertebral or non-vertebral fracture. There was no significant association between serum sclerostin levels and prevalent fracture, whether serum sclerostin was considered as a continuous variable (p logistic regression analysis, 0.10) or in quartiles (p Chi-square=0.35). The proportion of women who had a prevalent fracture was slightly higher in women with levels in the lowest quartile (19.9 %) of sclerostin values than women with levels in the highest quartile (12.6 %), a difference which was not statistically significant after adjustment for age ($p=0.086$).

There was also no significant association between baseline sclerostin levels and the incidence of fracture. When considered as a continuous variable, logistic regression analyses showed no significant association between serum

Table 1 Associations of serum sclerostin with BMD, bone turnover markers, and hormones in 572 postmenopausal women

Variable	Age-adjusted r value	p
Spine BMD	+0.35	<0.0001
Total hip BMD	+0.25	<0.0001
PINP	−0.10	0.014
S-CTX	−0.13	0.0026
Total estradiol	−0.07	0.13
Free estradiol index	−0.06	0.18
Testosterone	−0.021	0.64
25(OH)D	0.023	0.61
Intact PTH	−0.13	0.0064

The Table shows the Pearson correlation coefficients and associated p values

Fig. 1 Association between serum sclerostin and BMD in 572 postmenopausal women. Women were categorized in quartile of serum sclerostin. *p* values refer to the trend across quartiles
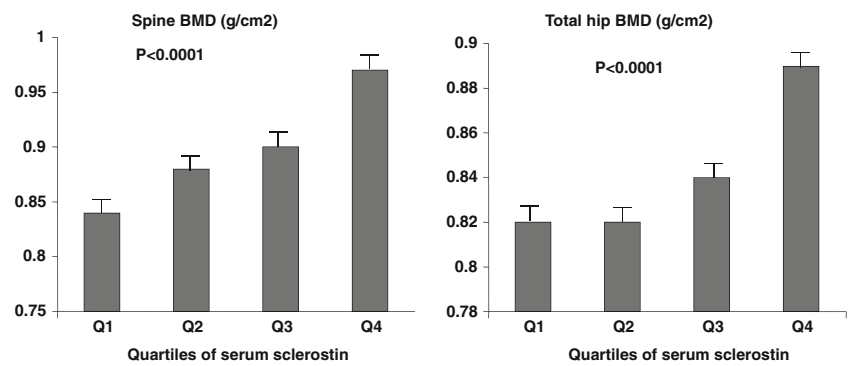
sclerostin and fracture incidence (*p*=0.22). Table 2 shows the number and percentage of women with incident fracture according to quartiles of baseline serum sclerostin. Consistent with the continuous analysis approach, there was no significant association (*p*=0.25) between quartiles of sclerostin and incident fracture risk. Additional analyses showed that serum sclerostin was not associated with prevalent or incident fracture risk when women were classified in tertile or median values. Adjustment for BMD at the spine or hip, prevalent fracture and estrogen use did not have any influence on the association between sclerostin and incident fracture risk.

Discussion

This is the first prospective and large population-based cohort study analyzing the association between serum sclerostin levels and the risk of incident fracture in postmenopausal women. There was no significant association between baseline sclerostin and prevalent or incident fracture risk suggesting that the measurement of this osteocyte factor in the peripheral blood may not have clinical value to predict the risk of all fractures in postmenopausal women with mean age of 67 years.

Most previous studies, but the recent one from Saudi Arabia [20], analyzed the association with BMD and bone turnover markers in small populations. In our large sample, we found that serum sclerostin slightly negatively correlated with the bone turnover markers PINP and CTX, but was

positively associated with spine and total hip BMD, which is consistent with a smaller previous study [16]. The counterintuitive positive association of sclerostin with BMD suggests that serum sclerostin may reflect mostly the number of osteocytes—assuming that this number is proportional to level of bone mass—and not the activity of individual cells or at individual bone remodeling units. After adjustment for age, we found that among the various bone-regulating hormones, only intact PTH was significantly negatively associated with serum sclerostin. Such a negative association has been consistently reported in previous studies [15, 20], is in agreement with intervention studies in animal models [5, 7, 26, 27] and in humans [18, 28], and suggests that PTH is probably one of the most important regulators of sclerostin secretion in postmenopausal women. In our large sample of postmenopausal women, we found that serum sclerostin was slightly but not significantly associated with total estradiol levels and the derived free estradiol index. This absence of significant correlation contrasts with previous observational and interventional studies that reported significant negative associations [15, 17, 20]. These discrepancies may be related to differences in population characteristics and/or estradiol assay sensitivity.

Our study has strengths and some limitations. This is the first published study that reports the association between serum sclerostin and the risk of all incident fractures in a large population-based study. This is also one of the largest studies that explores the association of serum sclerostin with two important determinants of bone strength, i.e., BMD and bone turnover markers and several steroid and calcium-regulating hormones. This study has also some limitations. We measured serum sclerostin with a new two-site ELISA that uses antibodies raised against human recombinant protein and which demonstrates adequate precision and accuracy. An internal cross-calibration study performed in a subset of 100 individuals from the present study showed a high correlation (*r*=0.90, *p*<0.0001) between the assay we used and the other commercial ELISA from Biomedica which has been used in several studies [16, 20, 28] (data not shown). Thus, we believe that the findings would not have been different with the use of the other commercially

Table 2 Number (percentage) of women with incident fracture according to quartiles of serum sclerostin at baseline

Incident fracture	Quartile of baseline sclerostin levels			
	Q1	Q2	Q3	Q4
Yes (*n*=64)	11 (8.4 %)	22 (15.4 %)	17 (11.5 %)	14 (9.3 %)
No (*n*=508)	120	121	131	136

p across quartiles=0.25

available test. We analyzed circulating sclerostin levels, whereas this factor is thought to exert primarily its effects locally. Although circulating levels of sclerostin have been shown to be strongly correlated with marrow plasma levels in postmenopausal women [18], it is possible that peripheral levels are not sensitive enough to detect changes occurring locally at the level of the bone. We measured serum sclerostin at a single time point in fasting morning samples. Because there is a diurnal variation of serum sclerostin [29], we cannot preclude that multiple measurements during the day or over several days would have allowed us to detect significant association with fracture risk. Finally, because the number of women with incident fracture was limited, we cannot exclude that we may have found a significant modest association with a larger sample. Indeed, we calculated that our study had at least a 0.85 power (with an alpha of 0.05) to detect an odds ratio of 1.6 of incident fracture among women with increased or decreased serum sclerostin levels. Thus, we were not able to detect a lower association (smaller odds ratio) with a high level of confidence, but probably, a smaller odds ratio would not represent a sufficiently strong association to be of meaningful clinical significance. The relatively low number of fracture also did not allow us to analyze separately vertebral, hip, and non-spine and non-hip fractures. It is thus possible that serum sclerostin may be associated more specifically with one type of fracture as suggested by a recent study reported in an abstract form that found a significant relationship with hip fracture in older women [30].

In summary, this population-based study indicates that although serum sclerostin is modestly correlated with BMD and bone turnover in postmenopausal women, a single measurement of its circulating levels did not predict the risk of all fractures, although our study may lack statistical power to detect a modest association. Circulating sclerostin may however be useful to investigate changes in osteocyte number and/or activity and the effects of agents including bone-forming therapies.

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