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Effect of supplemental vitamin D and calcium on serum sclerostin levels

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Abstract

Objective—Serum sclerostin has been inversely associated with serum 25OHD concentration, but the effect of supplementation with vitamin D and calcium on serum sclerostin is unknown. This study was done to determine whether supplementation altered serum sclerostin levels in healthy older adults.

Design—We measured serum sclerostin at baseline and after two years in 279 men and women who participated in a placebo-controlled vitamin D (700 IU per day) and calcium (500 mg per day) intervention trial in men and women age 65 years and older.

Method—Serum sclerostin levels were measured by MesoScale Discovery chemiluminescence assay.

Results—In the men, sclerostin levels increased over 2 years by 4.11 ± 1.81 ng/L (13.1%) in the supplemented group and decreased by 3.16 ± 1.78 ng/L (10.9%) in the placebo group (P = 0.005 for difference in change). Adjustment for season, baseline physical activity, baseline serum sclerostin and total body bone mineral content (BMC) did not substantially alter the changes. In the women, there was no significant group difference in change in serum sclerostin either before or after the above adjustments. In both sexes, supplementation significantly increased serum ionized calcium and decreased parathyroid hormone (PTH) levels.

Conclusion—In conclusion, men and women appear to have different serum sclerostin responses to supplementation with vitamin D and calcium. The reason for this difference remains to be determined.

Keywords

serum sclerostin; calcium; vitamin D; 25-hydroxyvitamin D; total body bone mineral content

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DECLARATION OF INTEREST

The authors have no conflict of interest. The authors have full control of all primary data and agree to allow the journal to review the data if requested.

AUTHOR CONTRIBUTIONS

Roles of authors: BD-H and SH – designed and implemented the trial; BD-H, SH and LC designed and implemented the sclerostin component, SH and NP performed data management and data analyses, and all authors contributed to preparation of the manuscript.

INTRODUCTION

Sclerostin is a glycoprotein produced by osteocytes that is being evaluated as a potential clinical marker of bone turnover. A recent report has identified an inverse association of serum 25OHD and sclerostin levels in healthy postmenopausal women [1]. Vitamin D and calcium supplementation may influence circulating sclerostin levels for several reasons. Supplementation with these nutrients decreases serum PTH levels [2], and PTH is a negative regulator of sclerostin expression [3, 4]. Hence the PTH decline should increase serum sclerostin levels. Sclerostin is known as an inhibitor of bone formation and several cross-sectional studies have identified inverse associations of serum sclerostin with a variety of biochemical markers of bone turnover [5–7], although this has not been a consistent finding in men and women [6]. Supplementation with vitamin D and calcium lowers other bone turnover marker levels by up to 10% but whether it alters serum sclerostin levels has not been examined.

It is important to understand the determinants of the serum sclerostin level to gain insight into the regulation of osteocyte function. Toward this end, we examined whether treatment with calcium and vitamin D_3 for two years, when compared with placebo, altered serum sclerostin levels in healthy men and women age 65 years and older. We also examined, at baseline, the associations of serum sclerostin with bone mineral density (BMD), total body BMC, and the biochemical marker of bone formation, serum osteocalcin. The subjects of this study participated in STOP/IT, a randomized trial in which supplementation with calcium 500 mg plus vitamin D_3 700 IU per day, when compared with placebo, lowered serum PTH, improved BMD, and lowered fracture rates [2].

SUBJECTS AND METHODS

This study was carried out using existing data and new sclerostin measurements in serum archived at baseline and 2 years from healthy men and women age 65 years and older who participated in our STOP/IT calcium and vitamin D intervention trial (Clinical Trial: NCT00357643) [2]. Of 389 who completed the trial, we excluded 21 with diabetes because they have been reported to have higher sclerostin levels [8, 9], 14 non-white subjects because they have different PTH homeostasis [10], and subjects with no stored serum (n = 40). The remaining 314 subjects had sclerostin measurements at baseline and, of these, 279 had samples available and were measured at 2 years.

Subjects were enrolled in February, 1992 through February, 1993. The study was approved by the Tufts Medical Center Human Investigation Review Committee and all participants gave written informed consent. Criteria for exclusion included use of calcium or vitamin D supplements for 2 months prior to enrollment, bone-altering conditions or medications, kidney or liver disease, and current cancer (see [2] for detailed list).

Blood was collected between 7:00 and 9:00 am after the subjects had fasted for at least 8 hours. During the trial, serum osteocalcin was measured by immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA), PTH by immunometric assay (Nichols Institute), and serum 25OHD was measured by the method of Preece [11] with coefficients of

variation (CVs) of 5.6 to 7.7%. Serum estradiol was measured by radioimmunoassay following solvent extraction and celite chromatography, with CVs of 7.0 and 13.2%. Total testosterone was measured with use of radioimmunoassay kits from Diagnostic Products Corp (Los Angeles, CA) with CVs of 5.9 and 8.7%. Serum creatinine was measured by colorimetry with use of the Cobas Fara centrifugal analyzer (Roche Instruments, Belleville, NJ). Urinary creatinine was measured by direct-current plasma emission spectroscopy with a Spectrascan 6 (Beckman Instruments, Palo Alto, CA) and serum ionized calcium with the Nova 7 analyzer (Nova Beomedical, Neton, MA). Serum sclerostin was batch analyzed in 2013 in serum archived at −80°C and not previously thawed. The samples were assayed on a MesoScale Discovery (Rockville, MD), utilizing a proprietary combination of electrochemiluminescence detection and patterned arrays. This assay detects only intact sclerostin in the serum [5]. The reference range for this assay is 18–156 ng/L, the mean CV of this assay is 4% and the lower level of detection (defined as 2.5 SD above the background) is 1.1 ng/L.

BMD of the spine, femoral neck, and total body and BMC of the total body were measured by dual-energy x-ray absorptiometry with use of a DPX-L scanner (Lunar Radiation, Madison, WI with coefficients of variation of 1.0 percent (spine), 1.7 percent (femoral neck) and 0.7 percent (total body BMD) and 1.2% (total body BMC) [12].

Leisure, household, and occupational activity was estimated with use of the Physical Activity Scale for the Elderly questionnaire [13].

Analyses were conducted with SPSS version 21.0 (IBM Corp., Armonk, NY). Preliminary analyses indicated that sex modified the effect of treatment on changes in sclerostin (test for interaction, $P = 0.003$). For this reason, final analyses were conducted separately in men and women. Baseline characteristics were compared across treatment groups with t-tests for two independent samples. Mean sclerostin values at baseline, adjusted for season of measurement, were computed with the LSMeans option in the General Linear Models procedure and compared across sex-specific tertiles of related variables. The same method was used to compute mean changes in sclerostin values, adjusted for covariates, and compare them across treatment groups. P values less than 0.05 were considered to indicate statistical significance.

RESULTS

At baseline, mean serum sclerostin levels were 30.1 ± 18.2 (SD) ng/L in the men and 28.1 ± 14.3 ng/L in the women (P for difference = 0.290); mean PASE scores were 129 ± 57 in the men and 106 ± 50 in the women ($P < 0.001$). Clinical characteristics of the 314 participants, by gender and treatment group, are shown in Table 1. There were no significant treatment group differences in serum sclerostin levels in either the men or the women.

Over two years of treatment, in the men, serum sclerostin increased by 4.11 ± 1.81 (SE) ng/L (13.1%) in the vitamin D and calcium group and decreased by 3.16 ± 1.78 ng/L (10.9%) in the placebo group (P for group difference < 0.005, Table 2). Adjustment for baseline sclerostin, season, and PASE score, all significant predictors of change in

sclerostin, did not substantially alter the effect of supplementation on change in serum sclerostin (Table 2). Additional adjustment for total body BMC similarly did not change the finding (Table 2). In the women, serum sclerostin decreased by 3.55 ± 1.50 ng/L (12.5%) in the vitamin D and calcium group and decreased by 1.02 ± 1.40 ng/L (3.7%) in the placebo group, but the group difference was not statistically significant either before or after the same adjustments (Table 2). The gonadal hormones, estradiol and testosterone, were not significantly correlated with serum sclerostin at baseline and they were not significant predictors of change in serum sclerostin and they did not modify the effect of supplementation on changes in serum sclerostin in the men or the women. As expected, supplementation with vitamin D and calcium significantly increased serum ionized calcium and lowered serum PTH and osteocalcin levels in the men and the women (Table 2).

At baseline, serum sclerostin did not differ across tertiles of serum 25OHD in the men or the women. In the men, serum sclerostin levels were higher in the highest tertile of serum osteocalcin than in the two lower tertiles (P = 0.008 for both comparisons) (Table 3). In the women, serum sclerostin did not differ significantly across tertiles of serum osteocalcin. Baseline serum sclerostin did not differ across tertiles of serum PTH before or after adjustment for total body BMC in the men or the women. Similarly, serum sclerostin did not differ across tertiles of BMD at any skeletal site or BMC of the total body (Table 3).

There were no significant associations between 2-yr change in serum sclerostin and 2-yr change in BMD at any site or change in total body BMC.

DISCUSSION

In this large randomized controlled trial, treatment with vitamin D and calcium, when compared with placebo, increased serum sclerostin levels in the men. This increase was compatible with the observed declines in PTH and osteocalcin that occurred on the supplements. Surprisingly, the same pattern was not observed in the women. On the supplements, serum sclerostin levels in the women did not increase, and in fact declined, although not significantly, despite declines in serum PTH and osteocalcin that were similar to those seen in the men. In contrast to the observed sex difference in sclerostin change, sex did not modify the effect of calcium and vitamin D on change in BMD [2]. The reason for the divergent sclerostin responses to supplementation in the men and the women is not clear. It is possible that their higher basal level of physical activity may have sensitized the men and enhanced their sclerostin response to the supplements. There is precedent for divergent sclerostin findings in different sexes and in populations with different basal levels of physical activity. Fazeli et al. recently observed that among eumenorrheic young women, serum sclerostin was positively associated with spinal BMD in athletic women but inversely associated in the nonathletic women [14]. We have recently observed seasonal variation in serum sclerostin levels [15], but our findings were adjusted for season; they were also adjusted for physical activity.

In the analyses of baseline data, we identified no association of serum sclerostin with BMD at any site or with total body BMC. This is in contrast to Durosier et al. [5], who, using the same sclerostin assay, reported a positive association of sclerostin with BMD at several sites

in a similar older population. It is also in contrast to findings of Szulc et al who reported that higher serum sclerostin levels are associated with lower bone turnover rate, higher BMD, and lower risk of fracture [16]. Modder, using the Biomedica assay, identified a positive correlation of serum sclerostin with total body BMC and also found positive correlations of serum sclerostin with BMD of the spine, hip and total body in a random sample of healthy older men and women [6]. This finding has been confirmed in healthy postmenopausal women [17] and in a small group of adults with type 2 diabetes [8].

In the men, sclerostin levels at baseline were positively associated with serum osteocalcin levels, but the association was not linear. Higher sclerostin levels were seen in the top tertile of osteocalcin values but not in the lower two tertiles. No association was identified in the women. Modder did not find significant associations of serum sclerostin with osteocalcin in older men or women [6], but did observe significant inverse associations of sclerostin with several biochemical markers of bone resorption in men, although not in women. Durosier observed a significant inverse association of serum sclerostin with serum P1NP and CTX in a combined group of older men and women [5], as did Garnero in postmenopausal women [17]. Thus findings linking serum sclerostin to biochemical markers of bone turnover in older adults are somewhat variable across study populations for reasons that are unclear.

A factor that is undoubtedly adding to divergence and inconsistency in findings related to serum sclerostin is that available assays are measuring different components of the sclerostin molecule. As elegantly described by Durosier [5], the MesoScale Discovery, the assay used in this current study, appears to detect only the intact sclerostin molecule, whereas the other commonly used assays detect various circulating fragments as well as intact sclerostin. The reference range for the MesoScale assay is 30-fold lower than that of other assays [5]. Our sclerostin values were similar to those measured in samples collected recently from older men and women and analyzed by the same method [5]. Samples from the Study of Osteoporotic Fractures that had been stored for about 20 years also gave sclerostin values in the expected range [18]. These findings suggest that serum sclerostin is stable for an extended period when stored at −80°C and not exposed to thawing and refreezing.

Another potential source of inconsistency of observations may be that not all studies have adjusted for a surrogate of osteocyte pool size such as total body BMC. It has been proposed that sclerostin in the circulation may be influenced by the osteocyte pool size [5, 6, 17], which should be proportional to total body BMC. Durosier estimated that 17% of the variability in circulating sclerostin is determined by the osteocyte pool size, as measured by DXA total body BMC [5]. However, unlike Durosier, we identified no association of serum sclerostin with total body BMC. Finally, associations of sclerostin with bone turnover may vary with age, sex, circulating testosterone or estrogen levels, and usual level of physical activity. Until the influence of these and other factors on sclerostin is better understood, findings related to this measure should be interpreted with caution.

In conclusion, we observed that treatment with vitamin D and calcium increases serum sclerostin levels in healthy older men but not in women. Similarly, baseline sclerostin and osteocalcin levels were positively associated in the men but not the women. These observations contribute to scattered findings reported in other data sets and, in themselves,

do not indicate much clinical utility of serum sclerostin measurements as indicators of bone turnover at this time.

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Table 1

Characteristics of 314 subjects at baseline by gender and treatment group ± S.D.

	Men			Women		
	Placebo	**Calcium+D**	**P**	**Placebo**	**Calcium+D**	**P**
N	67	66		95	86	
Age, yrs	71.2 ± 5.1	70.6 ± 4.5	0.475	71.9 ± 4.6	70.9 ± 4.3	0.143
Body Mass Index, kg/m^2	27.0 ± 3.2	26.9 ± 3.0	0.760	26.5 ± 4.7	26.3 ± 3.9	0.742
PASE score	125.9 ± 56.9 (66)	132.3 ± 56.6 (65)	0.519	108.5 ± 53.0	104.8 ± 45.9	0.535
Calcium intake, mg/d	700.6 ± 382.0	750.0 ± 411.1	0.474	801.0 ± 349.0	678.1 ± 281.4	0.010
eGFR, ml/min/1.73m^2	74.8 ± 14.8	79.9 ± 15.1	0.050	73.8 ± 11.9	76.3 ± 14.1	0.202
Serum measures						
Sclerostin, ng/ml	28.9 ± 11.4	31.3 ± 23.2	0.450	27.8 ± 14.1	28.5 ± 14.5	0.726
25-hydroxyvitamin D, nmol/L	88.9 ± 30.0	84.9 ± 40.8	0.516	63.3 ± 7.5	73.3 ± 33.7	0.030
Total calcium, ng/L	2.51 ± 0.07 (66)	2.51 ± 0.08	0.526	2.52 ± 0.08	2.55 ± 0.07	0.034
Parathyroid hormone, pmol/L	3.61 ± 1.37	4.09 ± 2.06	0.122	4.45 ± 1.96	3.88 ± 1.40	0.027
Osteocalcin, nmol/L	1.01 ± 0.34	0.93 ± 0.23	0.131	1.25 ± 0.41	1.19 ± 0.38	0.309
Estradiol, pmol/L	99.4 ± 38.8 (63)	106.5 ± 44.5 (64)	0.336	93.0 ± 54.1 (90)	94.4 ± 68.0 (83)	0.878
Testosterone, nmol/L	16.6 ± 4.8 (62)	17.5 ± 4.8 (63)	0.302	1.3 ± 0.9 (89)	1.4 ± 1.1 (83)	0.782
BMD measures						
Femoral neck BMD, g/cm^2	0.94 ± 0.11	0.98 ± 0.14	0.033	0.80 ± 0.10	0.80 ± 0.11	0.609
L2-4 BMD, g/cm^2	1.26 ± 0.19 (66)	1.33 ± 0.20 (65)	0.053	1.03 ± 0.20 (91)	1.03 ± 0.19 (79)	0.913
Total body BMD, g/cm^2	1.19 ± 0.08 (66)	1.21 ± 0.09	0.159	1.01 ± 0.09	1.01 ± 0.09	0.921
Total body BMC, g	2937 ± 404 (66)	2990 ± 346	0.426	1910 ± 292	1894 ± 286	0.709

Table 2

Mean (±SE) 2-yr changes in serum sclerostin by gender and treatment group in 279 subjects

	Men			Women		
	Placebo	**Calcium+D**	**P**	**Placebo**	**Calcium+D**	**P**
N	62	60		84	73	
Change in sclerostin, ng/L						
unadjusted	−3.16 ± 1.78	4.11 ± 1.81	0.005	−1.02 ± 1.40	−3.55 ± 1.50	0.217
adjusted[a]	−3.85 ± 1.64	3.99 ± 1.70	0.001	−0.99 ± 1.17	−3.23 ± 1.25	0.186
adjusted[b]	−3.78 ± 1.67	3.98 ± 1.71	0.001	−0.97 ± 1.18	−3.23 ± 1.25	0.184
Change in ionized calcium, mmol/L						
unadjusted	0.04 ± 0.01[c]	0.09 ± 0.01	0.001	0.03 ± 0.01	0.09 ± 0.01	<0.001
Change in PTH, pmol/L						
unadjusted	0.65 ± 0.16	−0.88 ± 0.21	<0.001	0.88 ± 0.22	−0.81 ± 0.14	<0.001
Change in osteocalcin, nmol/L						
unadjusted	0.03 ± 0.03	−0.14 ± 0.02	<0.001	−0.06 ± 0.03	−0.25 ± 0.03	<0.001

[a] Adjusted for season, baseline PASE score, and baseline serum sclerostin (ng/L).

[b] Adjusted also for total body BMC

[c] N=61

Table 3

Mean (±SEM) baseline serum sclerostin, adjusted for season, by tertile of selected variables in 314 subjects

	Men			Women		
	Tertile boundaries	**Sclerostin, ng/L**	**P**	**Tertile boundaries**	**Sclerostin, ng/L**	**P**
25-hydroxyvitamin D, nmol/L	< 69.9	26.8 ± 2.9	0.420	<52.4	25.8 ± 1.8	0.690
	69.9 – 97.3	31.6 ± 3.0		52.4 – 74.8	27.7 ± 1.9	
	≥ 97.4	31.4 ± 2.7		≥ 74.9	27.7 ± 1.7	
PTH, pmol/L	< 2.9	31.7 ± 2.7	0.445	< 3.3	25.4 ± 1.8	0.556
	2.9 – 4.2	31.0 ± 2.8		3.3 – 4.4	27.7 ± 1.7	
	≥ 4.3	27.2 ± 2.7		≥ 4.5	27.9 ± 1.7	
Serum osteocalcin, nmol/L	< 0.8	26.9 ± 2.6	0.008	< 1.0	25.4 ± 1.7	0.249
	0.8 – 1.0	26.3 ± 2.7		1.0 – 1.3	29.3 ± 1.7	
	≥ 1.1	36.9 ± 2.7[a]		≥ 1.4	26.5 ± 1.7	
Bone mineral density (BMD), g/cm^2						
Femoral neck	< 0.90	28.2 ± 2.9	0.712	< 0.75	29.4 ± 1.8	0.108
	0.90 – 1.00	29.9 ± 2.6		0.75 – 0.83	24.4 ± 1.7	
	≥ 1.01	31.5 ± 2.7		≥ 0.84	27.7 ± 1.7	
L2-L4 spine	< 1.20	29.3 ± 2.8	0.673	< 0.93	28.5 ± 1.8	0.667
	1.20 – 1.36	28.7 ± 2.8		0.93 – 1.08	26.2 ± 1.8	
	≥ 1.37	32.1 ± 2.9		≥ 1.09	27.7 ± 1.8	
Total body	< 1.16	26.6 ± 2.7	0.231	< 0.97	27.5 ± 1.8	0.939
	1.16 – 1.23	30.3 ± 2.7		0.97 – 1.05	27.2 ± 1.7	
	≥ 1.24	33.2 ± 2.8		≥ 1.06	26.6 ± 1.7	
Bone mineral content (BMC), g						
Total body	< 2774	28.6 ± 2.7	0.437	< 1770	27.1 ± 1.8	0.749
	2774 – 3116	28.5 ± 2.8		1770 – 2041	28.0 ± 1.7	
	≥ 3117	32.9 ±2.8		≥ 2042	±1.7	

[a] Differs from other two tertiles at P = 0.007.