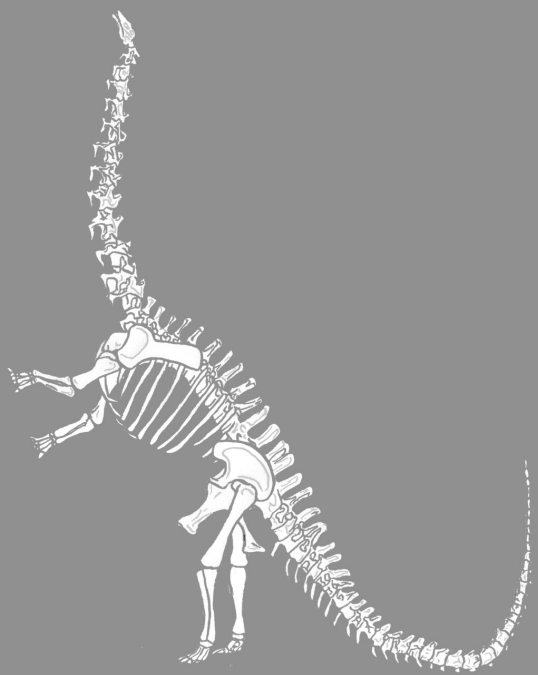
Sclerostin : a key regulator of bone metabolism

Lierop, A.H.J.M. van

Chapter 7

Patients with primary hyperparathyroidism have lower circulating sclerostin levels than euparathyroid controls

AH van Lierop
JE Witteveen
NAT Hamdy
SE Papapoulos



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Abstract

Objective

In vitro and in vivo studies in animal models have shown that parathyroid hormone (PTH) inhibits the expression of the *Sost* gene which encodes sclerostin, an osteocyte-derived negative regulator of bone formation. We tested the hypothesis that chronic PTH excess decreases circulating sclerostin in humans.

Design

We studied 25 patients with elevated serum PTH concentrations due to primary hyperparathyroidism (PHPT) and 49 patients cured from PHPT after successful parathyroidectomy (EuPTH).

Methods

We measured plasma PTH and serum sclerostin levels and the serum markers of bone turnover alkaline phosphatase, P1NP, and β-CTX.

Results

As expected by the design of the study, mean plasma PTH was significantly higher ($p < 0.001$) in PHPT patients (15.3 pmol/l; 95%CI: 11.1-19.5) compared to that of EuPTH controls (4.1 pmol/l; 95%CI: 3.6- 4.5). PHPT patients had significantly lower serum sclerostin values compared to EuPTH subjects (30.5 pg/ml; 95%CI: 26.0-35.1 vs 45.4 pg/ml; 95%CI: 40.5-50.2; $p<0.001$) and to healthy controls (40.0 pg/ml; 95%CI: 37.1-42.9; $p=0.01$). Plasma PTH concentrations were negatively correlated with serum sclerostin values ($r= -0.44$; $p<0.001$). Bone turnover markers were significantly correlated with PTH, but not with sclerostin.

Conclusion

Patients with primary hyperparathyroidism have significantly lower serum sclerostin values compared to PTx controls with normal PTH concentrations. The negative correlation between PTH and sclerostin strongly suggests that *SOST* is downregulated by PTH in humans.

Introduction

Parathyroid hormone (PTH) exerts its calciotropic action by acting directly on bone and kidney and indirectly on the intestine to increase the transport of calcium to the circulation. The skeletal effect of PTH is to increase the rate of remodeling (1), and it is generally believed that this effect is achieved by the binding of PTH to its specific receptor (PTHR1) on stromal/osteoblastic cells of the bone marrow. This in turn stimulates the production of RANK-ligand (RANKL) and decreases that of its decoy receptor osteoprotegerin (OPG) (2-5). Recent in vitro and animal studies suggest, however, that at least some of the effects of PTH on bone are also exerted by specific binding of the hormone to PTHR1 in osteocytes, resulting in inhibition of the expression of the *Sost* gene (6-9). This gene encodes sclerostin, a protein exclusively expressed in osteocytes in the skeleton (10), which decreases bone formation by binding to LRP5/6, resulting in inhibition of the Wnt signaling pathway in osteoblasts (11, 12).

Whether chronic PTH excess has similar effects on sclerostin secretion in humans as in animal models has not so far been investigated. In the present study, we tested the hypothesis that chronic hypersecretion of PTH, as seen in patients with hyperparathyroidism, may decrease sclerostin secretion, and that PTH may thus represent a potential regulator of sclerostin production in humans. To this effect, we measured sclerostin in serum of patients with untreated primary hyperparathyroidism (PHPT) and in a control group of patients with PHPT after establishment of cure following parathyroidectomy (PTx).

Subjects and Methods

a. Patients

Thirty-four consecutive patients with primary hyperparathyroidism (PHPT), which was untreated, persistent, or recurrent following parathyroidectomy (PTx), and 54 patients cured after successful PTx (EuPTH) were studied. Inclusion criteria included willingness to participate in the study, no impairment in renal function (serum creatinine levels <120 µmol/l), adequate vitamine D status (25-hydroxy vitamin D levels >50nmol/l) and no use of bone and mineral metabolism modifying agents such as bisphosphonates, calcimimetics or glucocorticoids.

We defined PHPT as plasma PTH concentrations above the upper limit of the normal laboratory reference range (>8pmol/l) in the presence of increased serum calcium concentrations (>2.55 mmol/l).
Patients with EuPTH were included when cure was confirmed by post-operative normalization of serum PTH and calcium concentrations, which was sustained for at least 6 months after PTx.
As per inclusion criteria, 9 patients were excluded from the PHPT group, 3 because of impaired renal function and 6 because of use of bisphosphonates, and 6 were excluded from the EuPTH group because of use of bisphosphonates.

The study was approved by the Medical Ethics Committee of the Leiden University Medical Center, and informed consent was obtained from all patients.

b. Methods.

Serum biochemistry

Serum calcium adjusted for albumin binding, phosphate, and creatinine were measured by semi-automated techniques. Serum alkaline phosphatase activity (ALP) was measured using a fully automated P800 modulator system (Roche BV, Woerden, Holland). P1NP (a marker of bone formation) and β-CTX (a marker of bone resorption) were determined using the E-170 system (Roche BV, Woerden, Holland). Plasma PTH was measured using the Immulite 2500 (Siemens diagnostics, Breda, Holland) and serum 25-hydroxyvitamin D (25-OHD) was measured using the LIAISON® 25-OH Vitamin D TOTAL assay (DiaSorin S.A./N.V., Bruxelles, Belgium)

Sclerostin measurement

Sclerostin was measured in serum by an electrochemiluminescence assay (MSD® 96-well MULTI-ARRAY® Human Sclerostin Assay, Gaithersburg, Maryland, USA) which uses two polyclonal antibodies raised against the whole sclerostin molecule. The sclerostin standard for the assay is produced in a NS0 derived myeloma cell line, and the purity is checked by SDS-PAGE gel with silver stain. In our hands, the precision and reproducibility of the assay were $< 6\%$ and $< 15\%$, respectively, the detection limit was ± 1 pg/ml, and the detection range was 1 to 10,000 pg/ml.
Sclerostin was measured in serum of 77 healthy subjects (30 male and 47 female,

aged 20 to 77 years). All had normal serum calcium concentrations, renal function and bone turnover and none were using bisphosphonates, the calcimimetic cinacalcet or glucocorticoids. Sclerostin was detected in serum of all healthy subjects; mean 40.0 pg/ml (95%CI = 37.1 - 42.9 pg/ml), range 12.4 to 68.19 pg/ml, while it was undetectable in serum of 3 patients with sclerosteosis .

c. Statistical analysis

Data was analysed using SPSS 16.0 (SPSS Inc. Chicago, USA). Between groups differences in baseline characteristics and serum biochemistry were assessed by student's t-test. Pearson correlation coefficients were calculated to assess correlations between PTH (after logarithmic transformation), sclerostin and biochemical markers of bone turnover. A probability level of random difference of 0.05 was considered significant.

Results

Baseline characteristics

There were no differences in age, gender, weight or BMI between patients with PHPT and EuPTH controls (table 1).

As expected by inclusion criteria, mean serum calcium and PTH concentrations were significantly higher and those of phosphate significantly lower in the PHPT group compared to the EuPTH group. There were no differences in serum 25-OHD or creatinine concentrations between the two groups.

Table 1. Subject characteristics.

	PHPT	EuPTH	p-value*
Male : Female	10:15	13:36	0.41
Age (years)	59.6 ± 16.7	62.4 ± 10.9	0.44
Weight (kg)	80.0 ± 18.2	81.1 ± 15.3	0.78
BMI (kg/m^2)	27.0 ± 6.0	28.1 ± 4.6	0.42

Values are given as mean ± standard deviation. BMI = body mass index. * PHPT vs EuPTH

Patients with PHPT had significantly higher levels of biochemical markers of bone formation (P1NP) and bone resorption (β-CTX) compared to EuPTH controls. Combining all patients, there was a significant positive correlation between plasma PTH concentrations and the concentrations of all three measured biochemical markers of bone turnover (ALP: r=0.23, p=0.047; P1NP: r=0.45, p<0.001; β-CTX: r=0.54, p<0.001). There was also a significant correlation between PTH and P1NP (r=0.51, p=0.009) in the PHPT group, but not between PTH and ALP (r=0.35, p=0.085), or PTH and β-CTX (r=0.31, p=0.13). In the EuPTH group alone PTH was not correlated with any of the biochemical markers of bone turnover (Tabel 2).

Serum sclerostin

Mean serum sclerostin level of patients with PHPT (30.5 pg/ml, 95%CI: 26.0-35.1) was significantly lower than that of patients with EuPTH and healthy controls (45.4 pg/ml, 95%CI: 40.5-50.2; p<0.001, and 40.0 pg/ml, 95%CI: 37.1-42.9; p=0.01, respectively) (Figure 1). There was no significant difference in mean sclerostin values between EuPTH and healthy subjects (p=0.13).

There was no significant correlation between PTH and sclerostin concentrations within each individual group of patients but there was a significant negative correlation between sclerostin and PTH when all patients were pooled together (r=-0.44, p<0.001) (Figure 2).

There was no significant relationship between serum sclerostin and biochemical markers of bone turnover in patients with PHPT or in all patients combined.

Table 2. Biochemical measurements

	PHPT	EuPTH	Reference range	p-value*
Calcium homeostasis				
PTH (pmol/l)	15.3 ± 10.7	4.1 ± 1.6	1.5 - 8.0	<0.001
Calcium (mmol/l)	2.61 ± 0.13	2.26 ± 0.12	2.15 - 2.55	<0.001
Phosphate (mmol/l)	0.92 ± 0.14	1.14 ± 0.24	0.90 - 1.50	<0.001
25 (OH) D (nmol/l)	53.1 ± 34.3	53.9 ± 20.2	30 -120	0.90
Creatinine (µmol/l)	80.2 ± 18.1	76.8 ± 14.7	44 - 80	0.39
Bone turnover				
ALP (U/l)	87.0 ± 23.0	75.7 ± 23.6	40 - 120	0.055
P1NP (ng/ml)	45.9 ± 16.9	34.0 ± 15.5	16 - 80	0.004
β-CTX (ng/ml)	0.32 ± 0.15	0.17 ± 0.11	0.01– 0.66	<0.001

Values are given as mean ± standard deviation.

PHPT = primary hyperparathyroidism; EuPTH= euparathyroid controls.

* PHPT vs EuPTH

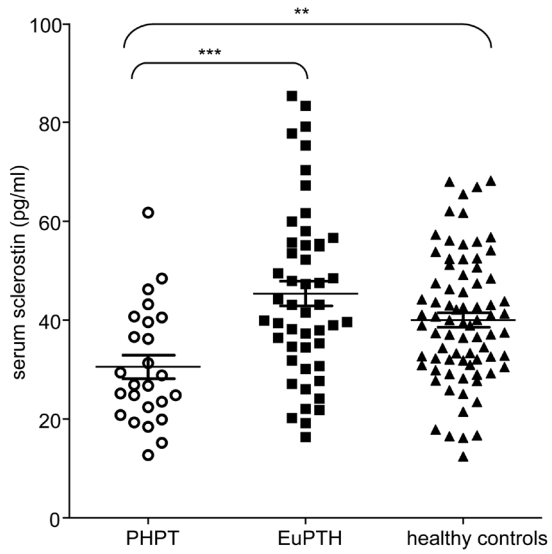

Figure 1. Serum sclerostin levels in PHPT, EuPTH and healthy subjects.
PHPT = primary hyperparathyroidism; EuPTH= euparathyroid controls.
*** p <0.001 ** p =0.01 (student's t-test).

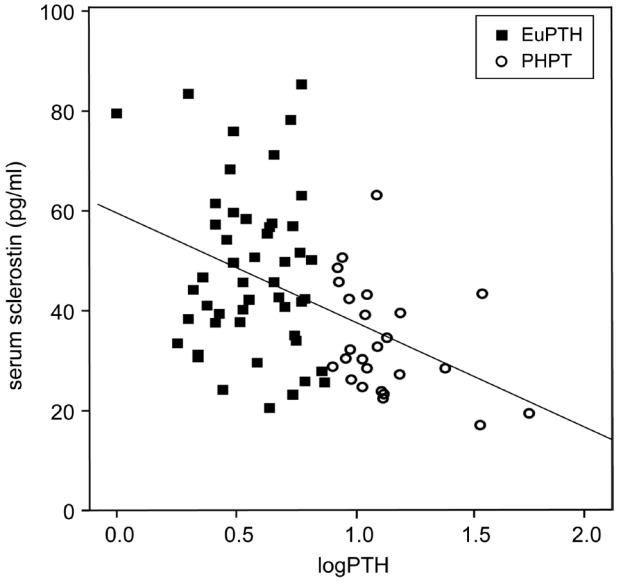

Figure 2. Relationship between circulating sclerostin and PTH levels.
r=-0.44 , p<0.001 (pearson's correlation). PTH levels were log transformed because of skewness.
PHPT = primary hyperparathyroidism; EuPTH = euparathyroid controls.

Discussion

Data from our study demonstrate that in humans, chronic PTH excess as observed in patients with primary hyperparathyroidism, is associated with a significant decrease in circulating sclerostin and that there is a significant negative correlation between PTH and serum sclerostin levels. Taken together these results suggest that, similar to animal models (6-9), PTH has a regulatory role on sclerostin production also in humans.

Sclerostin, a glycoprotein expressed by osteocytes in bone and encoded by the *SOST* gene, has emerged in recent years as an important regulator of bone formation in humans as well as in animals (13, 14). Inactivating mutations of the *SOST* gene leading to sclerostin deficiency have been shown to be associated with the rare skeletal disorder sclerosteosis, which is characterized by a marked increase in bone mass (13). Deletion of the *Sost* gene in mice have also been shown to increase bone formation, bone mass and bone strength (14). Moreover, inhibition of sclerostin secretion by a monoclonal antibody to sclerostin has been shown to increase bone formation and bone mass in rodents, primates and humans (14-16). Conversely, transgenic mice overexpressing *Sost* have low bone mass and impaired biomechanical competence (17). The mechanism of action of sclerostin to decrease bone formation involves inhibition of the Wnt signaling pathway (11, 12), although its precise molecular mechanism and factors controlling its secretion are as yet to be determined. Recent animal studies have shown that mechanical loading and high PTH levels downregulate the expression of *Sost* in osteocytes and decrease the production of sclerostin resulting in stimulation of bone formation (8, 18).

Human studies of sclerostin regulation have lagged behind due to lack of non-invasive techniques to determine sclerostin production. A number of assays have been recently developed for the measurement of sclerostin in blood. In our study, we used a sclerostin assay which proved to have excellent performance characteristics in our hands. Sclerostin was detectable in serum in all healthy subjects studied, suggesting that the protein is secreted and enters the circulation, while it was undetectable in three patients with sclerosteosis in whom it was measured. We chose to study patients with primary hyperparathyroidism in order to mimic as closely as possible the effect of chronic PTH excess on sclerostin, as previously studied in animals (6, 8). In addition, we chose to use as controls patients with primary hyperparathyroidism

cured after parathyroidectomy to exclude potential confounding factors, other than PTH excess.

We show here that serum sclerostin levels are significantly decreased in patients with chronic PTH excess due to PHPT compared to EuPTH and healthy subjects. PTH has been shown to decrease *SOST* transcription in vitro (6, 7), and continous and intermitent chronic administration of PTH to rodents is associated with decreased *Sost* mRNA and sclerostin expression in osteocytes (6, 7, 9). Moreover, transgenic mice expressing a constitutively active PTH receptor in osteocytes exhibit decreased expression of sclerostin and increased Wnt signaling associated with increased bone mass (8). Additional evidence for an interaction between PTH and *Sost*/sclerostin was recently provided by a study showing that the anabolic actions of PTH on bone was blunted in *Sost*-overexpressing mice (19). In keeping with the notion of a regulatory role of PTH for sclerostin production, our data show a significant correlation between circulating PTH and sclerostin. These data extend those of Mirza et al (20) who recently reported a negative relationship between serum PTH and sclerostin in healthy postmenopausal women, and those of Drake et al (21), who showed that intermittent PTH treatment decreased serum sclerostin levels in postmenopausal women.
As expected in the presence of chronic PTH excess, patients with PHPT had increased bone turnover as indicated by increased biochemical parameters of bone formation and resorption. There was a significant relationship between circulating PTH concentrations and serum P1NP and β-CTX. We did not however, find a significant relationship between biochemical parameters of bone turnover and serum sclerostin, either in patients with PHPT or the combined group of PHPT and EuPTH subjects. This lack of correlation between sclerostin and bone turnover markers was previously reported in healthy postmenopausal women (20).

The actions of PTH on bone are complex and involve a variety of signaling pathways in bone marrow stroma cells, osteoblasts and osteocytes (22, 23). Despite the significant progress in our understanding of the actions of PTH on bone it should be appreciated that the cellular and molecular actions of PTH, which determine the action of the hormone on bone remodeling and bone balance have only been partially unraveled and studies are needed to further elucidate these actions.

Acknowledgments

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