The Relation between Renal Function and Serum Sclerostin in Adult Patients with CKD

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Summary
Background and objectives Sclerostin, a bone antianabolic peptide involved in osteoporosis, is elevated in patients undergoing maintenance dialysis. However, there are no data for patients with early CKD.

Design, setting, participants, & measurements Between January and July 2010, serum sclerostin and GFR (calculated by inulin clearance) were measured in 90 patients with CKD. Fasting blood samples were also drawn for determination of calcium, phosphorus, parathyroid hormone, bone alkaline phosphatase, and 25-OH vitamin D.

Results Median GFR was 66.5 (interquartile range, 40.0–88.3) ml/min per 1.73 m². Median sclerostin level was 53.5 (interquartile range, 37.5–77.2) pmol/L, was higher in patients with a GFR < 60 ml/min per 1.73 m², and was highest in those with ESRD. Sclerostin levels were significantly more elevated in men than women (P < 0.05). An inverse relationship was found between sclerostin and GFR (r = –0.58; P < 0.001), and a positive correlation was seen with age (r = 0.34; P < 0.01) and serum phosphate (r = 0.26; P = 0.02). In multiple regression analyses, GFR, sex, and serum phosphate were the only variables associated with serum sclerostin (P < 0.001). Age lost its relationship with sclerostin level.

Conclusions This is the first study reporting higher serum sclerostin levels starting at CKD stage III. GFR, sex, and serum phosphate were the only measures associated with sclerostin level, suggesting that the effect of age reported in the literature might instead be attributable to the altered renal function in the elderly. Correcting the serum phosphorus level may be associated with lower sclerostin levels.


Introduction
Sclerostin is a recently identified osteocyte-derived bone morphogenetic protein antagonist (1). Sclerostin is the product of the SOST gene, which was discovered in 2001 (2). Inactivating mutations of the SOST gene have been associated with sclerosteosis, a high bone mass phenotype. Sclerostin is a Wnt signaling pathway antagonist that results in negative regulation of bone formation by repressing differentiation and proliferation of osteoblasts (3,4). It also promotes osteoblast apoptosis. Increased sclerostin action is thought to be involved in osteoporosis. In transgenic mice overexpressing a normal human SOST gene, the bone phenotype observed is an osteopenia; experimentally, blocking sclerostin action by a specific antibody induces more rapid tibial defect repair (5) and an osteoanabolic effect (6). Sclerostin may affect bone metabolism during CKD (7). In patients undergoing maintenance dialysis, sclerostin has been reported to be increased and associated with bone quality impairment (8). To our knowledge, the reasons for this increase have not been evaluated. We therefore designed a cross-sectional study to assess, in patients with a wide range of renal function who were not undergoing dialysis, the relation of serum sclerostin with GFR and its potential causes of accumulation.

Materials and Methods
Patients
The study involved 90 patients with a known kidney disease who were referred for renal function assessment in the Service d’Explorations Fonctionnelles Rénale et Métaboliqes at Hôpital Edouard Herriot from January to July 2010. These outpatients were attending a planned appointment and therefore did not have acute disease that could have dramatically modified their physical activity. Twenty volunteers with no known kidney disease and a negative result on urinary dipstick analysis (mean age ± SD, 43.7 ± 11.1 years; estimated GFR, 93.5 ± 12.5 ml/min per 1.73m² [calculated according to the Modification of Diet in Renal Disease formula]) served as controls.

The study protocol was described to all patients, and each participant provided informed consent.

Kidney Function Measurement
GFR was measured by urinary inulin clearance (ml/min per 1.73 m²) (9). Briefly, inulin (Polyfructosan [inutest], Laevosan, Linz, Austria) was infused continuously for 3 hours after a priming dose, and urine was collected every 30 minutes by spontaneous voiding. Inulin was measured by the enzymatic method (10).
Blood Measurements

Blood was collected from all participants for determination of various chemistries. Blood was drawn at 07:45 (before breakfast and after an overnight fast). Samples were immediately chilled on ice and centrifuged at 3000 g; serum or plasma was then separated, and samples were kept at −80°C until measurements. The following biologic data were recorded in patients with CKD: serum levels of calcium, phosphorus, bicarbonate, and intact parathyroid hormone (measured with a second-generation assay: Elecsys, Roche Diagnostics, Mannheim, Germany; normal values, 15–65 pg/ml) and 25-OH vitamin D (radioimmunologic assay: Diasorin Diagnosis, Saluggia, Italy; range of desirable values, 30–80 ng/ml). Serum bone alkaline phosphatase was measured with an Ostase assay (Beckman Coulter; normal values, 4–21 μg/L). Serum sclerostin was measured with a quantitative sandwich ELISA (Biomedica Gruppe, Vienna, Austria). Intra-assay and interassay coefficients of variation were 4% and 5.5%, respectively.

Statistical Analyses

Data were analyzed using SPSS software, version 18.0 (SPSS Inc., Chicago, IL). Data are presented as mean ± SD or as median (interquartile range [IQR]) when variables were not normally distributed. Correlation between two variables was assessed by simple regression. A multiple regression model was used to define the variables most predictive of circulating sclerostin concentration after selection of the measures found to be associated with sclerostin by simple regression or known to be important in the physiology of sclerostin: sex, body mass index, age, serum calcium, serum phosphate, and GFR. A multiple regression model was used to define the variables most predictive of circulating sclerostin concentration after selection of the measures found to be associated with sclerostin by simple regression or known to be important in the physiology of sclerostin: sex, body mass index, age, serum calcium, serum phosphate, and GFR. A P value <0.05 was considered to represent a statistically significant difference.

Results

Patients Characteristics

A cohort of 90 patients (46 women) with a median age of 52.0 years (IQR, 35.5–66.0 years) was recruited. The main clinical and anthropometric characteristics of the study population are summarized in Table 1.

Biochemical Measures

The median serum sclerostin level in patients was 53.5 (IQR, 37.5–77.2) pmol/L and was significantly greater than that in controls (38.2 [IQR, 27.1–57.8] pmol/L). Median serum calcium level was 9.3 (IQR, 9.0–9.6) mg/dl, and median phosphate level was 3.4 (IQR, 3.0–3.8) mg/dl. Mean serum bicarbonate level was 25.2 ± 3.2 mmol/L. Median serum bone alkaline phosphatase and median serum parathyroid hormone levels were in the normal range: 11.9 (IQR, 9.1–16.2) μg/L and 54.5 (IQR, 36.7–97.3) pg/ml, respectively. Mean 25-OH vitamin D level was low (20.6 ± 8.7 ng/ml). Median proteinuria was mild at 0.22 (IQR, 0.11–0.43) g/d.

GFR

The median GFR was 66.5 (IQR, 40.0–88.3) ml/min per 1.73 m². Twenty patients had stage 1 CKD, 30 had stage II, 25 had stage III, 12 had stage IV, and 3 had stage V (Figure 1).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>52.0 (35.5–66.0)</td>
</tr>
<tr>
<td>Men/women (n/n)</td>
<td>44/46</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.3 (22.0–29.2)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>71.0 (60.0–80.0)</td>
</tr>
<tr>
<td>GFR (mL/min per 1.73 m²)</td>
<td>66.5 (40.0–88.3)</td>
</tr>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>9.3 (9.0–9.6)</td>
</tr>
<tr>
<td>Serum phosphorus (mg/dl)</td>
<td>3.4 (3.0–3.8)</td>
</tr>
<tr>
<td>Serum parathyroid hormone (pg/ml)</td>
<td>54.5 (36.7–97.3)</td>
</tr>
<tr>
<td>Serum 25-OH vitamin D (ng/ml)</td>
<td>20.6 ± 8.7</td>
</tr>
<tr>
<td>Serum sclerostin (pmol/L)</td>
<td>53.5 (37.5–77.2)</td>
</tr>
<tr>
<td>Serum bone alkaline phosphatase (μg/L)</td>
<td>11.9 (9.1–16.2)</td>
</tr>
<tr>
<td>Serum bicarbonate (mmol/L)</td>
<td>25.2 ± 3.2</td>
</tr>
<tr>
<td>Proteinuria (g/d)</td>
<td>0.22 (0.11–0.43)</td>
</tr>
</tbody>
</table>

Unless otherwise noted, data are presented as mean ±SD or as median (interquartile range) when variables were not normally distributed (n=90).

Relationships between Plasma Sclerostin and Clinical and Biochemical Measures

The strongest relationship was observed between GFR and serum sclerostin, and the correlation was negative (r=−0.58; P<0.001). Also important was age, which was significantly and positively correlated with serum sclerostin (r=0.34; P<0.01) (Figure 2). A positive relationship was seen between serum phosphate and sclerostin (r=0.26; P=0.02) (Figure 3). Body mass index was positively associated with serum sclerostin (r=0.22; P<0.05). There was no further significant relation between sclerostin and parathyroid hormone, serum calcium, serum bicarbonate, bone alkaline phosphatase, 25-OH vitamin D, or proteinuria.

Regarding the expression of sclerostin by sex, we found that sclerostin was more elevated in men than in women (65.5 [IQR, 41.7–95.6] pmol/L versus 45.0 [IQR, 33.3–68.8] pmol/L; P<0.05), as reported in healthy adults by Gennari et al. (11) and Amrein et al. (12).

Serum sclerostin levels were also higher in male controls than in female controls (40.5 [IQR, 36.2–66.2] pmol/L versus 35.7 [IQR, 26.0–49.8] pmol/L).

In men with CKD, we observed a significant correlation between sclerostin and age (r=0.56; P=0.001) and sclerostin and body mass index (r=0.30; P=0.05). However, in contrast to Gennari and colleagues’ findings, these relationships were less marked and were not significant in women, even though our study had twice the number of observations as that study.

Multiple regression analysis that included sex, GFR, age, body mass index, serum calcium, and phosphorus indicated that GFR (P=0.001), sex (P=0.01), and serum phosphate (P=0.02) were associated with serum sclerostin (Table 2). Of note, in multiple regression analysis, age was unrelated to sclerostin, possibly because of the major effect that the decreased renal function seen with aging has on sclerostin regulation. Sex, GFR, age, body mass index,
Figure 1. Serum sclerostin as a function of CKD stage based on GFR measured by inulin clearance. \( n=90 \); results are expressed as median (interquartile range).

Figure 2. Relationship between serum sclerostin and age. \( n=90; \ r=0.34; \ P=0.001 \).

Figure 3. Relationship between serum sclerostin and serum phosphorus. \( n=90; \ r=0.26; \ P=0.016 \).
serum calcium, and serum phosphorus explained 38% of the variance of serum sclerostin in this study (Table 2). In a separate, three-factor model, GFR, sex, and serum phosphate explained 32% of the variance of serum sclerostin.

Discussion
To our knowledge, this is the first study to show that sclerostin, an antianabolic bone factor released by the osteocyte, accumulates in patients with CKD who are not undergoing dialysis. It is important to characterize this abnormality because patients with CKD may be prone to early adynamic bone disease, which may further induce secondary hyperparathyroidism (13). Although sclerostin is influenced by age and other bone metabolic factors, such as parathyroid hormone, 25-OH vitamin D, and sex steroids (12,14–17), we found by multiple regression that GFR, sex, and serum phosphorus were the only measures associated with sclerostin concentration in patients with CKD.

Patients with CKD Show High Sclerostin Levels Compared with Healthy Volunteers
The median sclerostin level was 53.5 (IQR, 37.5–77.2) pmol/L, was higher in patients with GFR <60 ml/min per 1.73 m², and was highest in patients with ESRD. The sclerostin values we report here at stage V before ESRD (Figure 1) agree with those observed in patients undergoing maintenance hemodialysis. Indeed, Čejka et al. recently showed in two different cohorts of such patients that sclerostin values were two to four times greater than those in post- and premenopausal women without CKD (18) or healthy volunteers (8). In this study, we found a fourfold higher serum sclerostin level in predialysis patients with stage V CKD than in participants with normal renal function. Thus, the abnormal sclerostin status observed during maintenance dialysis (8,18) may just be the continuation of the predialysis stage alteration.

Main Measures Associated with Elevated Sclerostin Level
Renal function was one of the main measures associated with elevated serum sclerostin levels in patients with CKD.

Of note, a study of diabetic patients reported a similar finding in its control group (11); however, those investigators estimated renal function using the Cockcroft-Gault equation, and the range of renal function should have been limited. In a cohort of healthy adults, Amrein et al. (12) showed a persistent effect of age after adjusting for GFR. However, renal function was in the normal range (84±12 ml/min per 1.73 m²), patients were younger, and GFR was estimated (by the MDRD formula) and thus was not a true GFR measurement. Sclerostin is a small peptide of a 29-kD and 213-amino acid protein, and almost no data on its catabolism are available. Sclerostin may be elevated in response to reduced renal clearance, as has been shown for many other peptides. However, increased sclerostin production cannot be ruled out. Indeed, in a genetic model of mice exhibiting progressive CKD, overall repression of Wnt/β-catenin pathway occurred in conjunction with increased expression of Wnt antagonists (e.g., sclerostin) along with increased osteoclast activity and repression of bone formation, particularly in ESRD (19). This model of experimental CKD suggests that repression of the Wnt/β-catenin pathway and its inhibitor sclerostin are involved in the pathogenesis of renal osteodystrophy (19).

The fact that we found a positive relationship between serum phosphate and sclerostin, independent of GFR, may have clinical importance: Indeed, Matthew et al. (20), by controlling serum phosphate through phosphate binders, reversed CKD-induced trabecular osteopenia and thereby increased osteoblast surfaces in the metaphyseal trabeculae of the tibia and femur, osteoid surfaces, and bone formation rates (20). There was no explanation for these histomorphometric bone changes besides the reduction in serum phosphorus, but this experiment did not measure sclerostin. Because sclerostin varies proportionately with serum phosphorus, it might have played a role in these favorable changes. Documenting this observation in future research may be of interest.

### Table 2. Multivariate analysis of factors associated with serum sclerostin ($r^2=0.38$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient for Difference in Sclerostin Level</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>0.18</td>
<td>0.07</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.09</td>
<td>0.36</td>
</tr>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum phosphorus (mg/dl)</td>
<td>0.23</td>
<td>0.02</td>
</tr>
<tr>
<td>Men</td>
<td>0.24</td>
<td>0.01</td>
</tr>
<tr>
<td>GFR (measured by inulin; ml/min per 1.73 m²)</td>
<td>-0.34</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Age or Renal Function?
Another important finding of this study is the predominant effect of GFR and age on the regulation of serum sclerostin observed in multiple regression analysis. Other researchers have reported that serum sclerostin increases with aging (21). In monovariate analysis, we also found this relationship (Figure 2). However, because elderly patients have noticeably reduced renal function (not easily deducible from serum creatinine), the positive association between sclerostin and age could result from patients’ renal impairment, which is not precisely assessed in other diseases. Thus, osteoporosis in the elderly may partly be the consequence of elevated sclerostin values due to an underestimated renal dysfunction.

It is important to note that this study was cross-sectional and cannot determine causality. The clinical significance of the high sclerostin levels in CKD cannot be deduced from the present results.

The sclerostin measurement techniques have been reviewed elsewhere (22). Two ELISA kits are available: One from Biomedica, which we used in this study, and another from TECOmedical (Sissach, Switzerland). In healthy adults, these kits are not in perfect agreement; the former gives values 50% greater than those of the latter. Possible
binding of sclerostin fragments may occur, and this could partly explain differences in recovery. No data are available on potential degradation fragments that could accumulate in CKD, as has been shown for PTH (23).

In conclusion, we showed a strong and inverse relationship between renal function and serum sclerostin levels in a cohort of patients with CKD. In addition, serum phosphate may be involved in the early and important elevation of sclerostin. Because sclerostin is involved in low bone mass, it may be interesting to analyze the effects of reducing sclerostin on bone mineral disorders long before ESRD develops.

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Disclosures
None.

References

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