Circulating Sclerostin in Children and Young Adults with Heritable Bone Disorders

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Context: Sclerostin is an inhibitor of bone formation and is an important determinant of bone mass. The role of sclerostin in heritable metabolic bone disorders has not been studied in detail.

Objective: We evaluated serum sclerostin levels in patients with X-linked hypophosphatemic rickets (XLH) and osteogenesis imperfecta (OI) and analyzed the relationship of circulating sclerostin concentrations with lumbar spine areal bone mineral density (LS-aBMD).

Setting: The study was conducted in the metabolic bone clinic of a pediatric orthopedic hospital.

Patients: Participants were 128 individuals, including 30 patients with XLH, 76 patients with OI types I, III, and IV, and 22 healthy subjects.

Main Outcome Measures: Sclerostin was quantified in serum samples.

Results: Patients with XLH had higher circulating sclerostin concentrations (mean \[SD\]: 30.2 [16.7] pmol/L) than healthy control subjects (21.4 [9.2] ng/mL) (P < .02), as well as relatively high LS-aBMD Z-scores (1.1 [1.7]). In the XLH cohort, serum sclerostin levels were positively associated with the LS-aBMD Z-score (r = 0.56; P < .002) and with alkaline phosphatase (r = 0.45; P < .01). In patients with OI, sclerostin serum levels were similar to those of healthy control subjects despite low LS-aBMD.

Conclusions: The elevated sclerostin serum levels in XLH and the normal concentrations in OI suggest that the bone mass abnormalities in these disorders are not caused by primary sclerostin dysregulation.

Sclerostin is a glycoprotein secreted by osteocytes that plays an important role in the control of bone formation (1). It is thought to exert its function by interacting with low-density lipoprotein receptor–related proteins 5 and 6 on the surface of osteoblasts, thus decreasing the activity of WNT signaling in these cells. Because WNT signaling stimulates osteoblast activity, the effect of sclerostin is to inhibit bone formation (2). Genetic defects that cause a lack of sclerostin therefore lead to high bone mass, whereas overexpression of sclerostin in mice results in low bone mass (1). Sclerostin seems to act mainly in bone tissue, but it is also released into the systemic circulation. Although the relationship between sclerostin serum levels and bone marrow concentrations is not entirely clear (3, 4), serum sclerostin has been investigated in a wide range of situations in adults (1). For example, high levels are found in disuse and in primary hypoparathyroidism (5), whereas low sclerostin serum concentrations have been reported in primary hyperparathyroidism (6).

Less is known about sclerostin serum levels in the pediatric context. One study on healthy individuals from birth to 21 years of age showed that sclerostin levels did not vary with age and were similar between the sexes (7), whereas another study reported a decrease in sclerostin concentrations after puberty and higher levels in boys than in girls (8). The behavior of sclerostin in pediatric meta-

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Abbreviations: FGF, fibroblast growth factor; LS-aBMD, lumbar spine areal bone mineral density; NTX, cross-linked N-telopeptides of type I collagen; OI, osteogenesis imperfecta; PTH, parathyroid hormone; XLH, X-linked hypophosphatemic rickets.
bolic bone disorders has not been investigated in any detail.

X-linked hypophosphatemic rickets (XLH) and osteogenesis imperfecta (OI) are 2 of the most common heritable metabolic bone disorders in children and adolescents. XLH is a phosphate-wasting disorder that is caused by mutations in PHEX (9, 10). The current treatment for XLH consists of oral phosphate supplementation and calcitriol (9, 10). Individuals with XLH typically have high bone mass, especially when they are receiving treatment with phosphate and calcitriol (11). OI is characterized by increased bone fragility and low bone mass and usually is caused by mutations in COL1A1 or COL1A2, the genes coding for the 2 α-chains of collagen type I (12). Bone density is usually very low in OI. Intravenous bisphosphonates are the current mainstay of treatment for patients with more severe forms of bone fragility (13).

Because sclerostin is such an important determinant of bone mass, and bone mass is affected in both XLH and OI, it is reasonable to hypothesize that sclerostin plays a role in these 2 conditions. However, studies in XLH mouse models have yielded contradictory results, because normal, low, and elevated sclerostin expression levels have been described (14–16). Sclerostin expression does not seem to have been reported in OI bone, but one study found that injections of anti-sclerostin antibody into an OI mouse model led to higher bone mass and fewer fractures (17). OI is therefore a potential application of anti-sclerostin therapy (2), which makes it important to evaluate the role of sclerostin in this disorder in more detail.

In the present study, we therefore assessed serum sclerostin in young patients with XLH and with OI and analyzed the relationship of circulating sclerostin concentrations with bone mass, bone turnover, and therapeutic interventions.

Subjects and Methods

Study population

The study population comprised 128 individuals (68 male and 60 female; age range, 0.9–26 years) assessed at the Shriners Hospital for Children (Montreal, Quebec, Canada). The diagnostic groups were XLH (aged 1.6–25 years), n = 30; OI types I, III, and IV (aged 0.9–21 years), n = 76; and healthy subjects (aged 1.2–26 years), n = 22. Individuals with XLH or OI were followed long term at the Shriners Hospital for medical care, whereas control subjects were assessed only once.

The diagnosis of XLH was based on the presence of rickets in the clinical and radiological evaluation and low serum levels of inorganic phosphorus but normal serum concentrations of calcium, parathyroid hormone (PTH), and 25-hydroxyvitamin D. Sequence analysis of PHEX had been performed in 26 patients, of whom 16 were positive for disease-causing PHEX mutations. In 10 patients, sequence analysis of all exons of PHEX did not show abnormalities, and in 4 patients, analysis of PHEX had not been performed. At the time of the present study, 15 patients were receiving treatment with phosphate supplementation and calcitriol, 11 had discontinued therapy because they had reached final height, and 4 had never received any treatment.

The group of patients with OI was subdivided into 2 groups, namely patients who had previously received iv treatment with pamidronate or zoledronic acid (n = 45; aged 2.6–21 years) and patients who had not received bisphosphonate treatment. The bisphosphonate-treated group had the following clinical diagnoses: OI type I, n = 27; OI type III, n = 5; and OI type IV, n = 13. The group of patients without a history of bisphosphonate treatment included 31 individuals with OI type I (aged 0.9–21 years).

The results of DNA sequence analysis of COL1A1 and COL1A2 were available for 68 of the 76 patients with OI and revealed disease-causing mutations in 55 patients. These mutations were grouped together as haploinsufficiency mutations (stop or frameshift mutations in COL1A1, which lead to a quantitative loss of protein expression from the affected allele; n = 21 patients), triple helical mutations (affecting a glycin residue in the triple helical domain of either the α 1 or the α 2 chain of collagen type I; n = 19 patients), or other mutations (eg, splice site or complex mutations; n = 15 patients). Among the 13 patients with negative COL1A1 and COL1A2 sequence analyses, 1 patient was positive for a homozygous mutation in LEPRE1. In the 12 patients with negative sequencing results and in the 8 patients without DNA sequence analysis results, the diagnosis of OI was clinically asserted by the association of frequent fractures, low bone mass, blue or gray sclera, or dentinogenesis imperfecta.

The control group included 22 individuals previously assessed at the Shriners Hospital because they had a family member with a metabolic or genetic bone disorder caused by a known mutation. All control subjects were healthy and had no clinically significant abnormalities in laboratory values, and the presence of the mutation found in their family member had been ruled out.

The study was approved by the institutional review board of McGill University, and informed consent was obtained from the participants or, for minors, from their legal guardians. Assent was obtained from children and adolescents aged 7 to 17 years.

Anthropometric measurements

Height was measured using a Harpenden stadiometer (Holtain). Weight was determined using digital electronic scales for infants and mechanical scales for older children and adults (Heathrometer). Height, weight, and body mass index were converted to age- and sex-specific Z-scores on the basis of reference data published by the Centers for Disease Control and Prevention (18).

Biochemical measurements

Serum sclerostin levels were measured using an ELISA (Biomedica). The detection limit of the assay is 3.2 pmol/L. Intra- and interassay precision are <7% and <10%, respectively (8, 19–21).

Serum total calcium, phosphate, alkaline phosphatase, creatinine, and liver enzymes were measured using standard laboratory methods. Serum active intact PTH (fragment 1–84) was determined by RIA (Diasorin). The 25-hydroxyvitamin D \( \text{H}_{21} \) RIA kit (Diasorin) was used to determine 25-hydroxyvitamin D.
Results
Clinical characteristics of the study groups are shown in Table 1. Serum sclerostin concentrations varied widely between study participants (Figure 1A), ranging from 10.2 to 41.1 pmol/L in control subjects, 6.5 to 80.3 pmol/L in the XLH group, and 5.3 to 77.1 pmol/L in patients with OI types I, III, or IV.

The mean serum sclerostin concentration was significantly higher in the XLH group than in control subjects or in patients with OI (Figure 1B). On average, sclerostin levels were 39% higher in the XLH cohort than in control subjects; Within the XLH group, sclerostin levels were higher in males than in females (mean [SD], 47.6 [18.6] pmol/L vs 23.8 [10.7] pmol/L; P < .001), and serum sclerostin concentrations were positively associated with the LS-aBMD Z-score (r = 0.56; P = .002) (Figure 2), as well as with serum activity of alkaline phosphatase (r = 0.45; P = .01). With adjustment for age and sex, the positive association between sclerostin levels and LS-aBMD Z-score and alkaline phosphatase remained significant. Sclerostin serum levels were similar among patients who were currently receiving treatment with phosphate and calcitriol and patients who had discontinued or who had never received this treatment (P = .70). No difference in sclerostin concentrations was found between patients with XLH with a positive finding on PHEX sequence analysis and patients with a negative result (P = .93).

In the OI cohort, no significant differences in sclerostin concentrations were found between sexes (P = .23), between genotypic groups (haploinsufficiency mutations, triple helical mutations, and other mutations; P = .52) and

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Abbreviations: BMI, body mass index; NA, Not available; OI + BP, patients with OI treated with bisphosphonates; OI – BP, patients with OI not exposed to bisphosphonates.

b Significant different from the control group.

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between bisphosphonate-treated and untreated patients (mean [SD], 23.2 [12.7] vs 21.2 [12.5] pmol/L, \(P = .91\)). Within the group of bisphosphonate-treated patients with OI, serum sclerostin was positively associated with the serum bone resorption marker NTX (\(r = 0.43; P = .03\)), serum alkaline phosphatase (\(r = 0.46; P = .01\)), 25-hydroxyvitamin D (\(r = 0.30; P = .045\)), and LS-aBMD Z-score (\(r = 0.32; P = .03\)).

In none of the groups was there a significant correlation between sclerostin and serum concentrations of inorganic phosphorus, calcium, creatinine, and PTH. Serum 25-hydroxyvitamin D was only correlated with serum sclerostin in the group of bisphosphonate-treated patients with OI.

**Discussion**

In this study we found that young patients with XLH on average had 39% higher serum sclerostin levels than healthy control subjects. In contrast, patients with OI had sclerostin concentrations that were similar to those of healthy control subjects, regardless of bisphosphonate treatment history and type of disease-causing mutation. In the XLH cohort and in the group of bisphosphonate-treated patients with OI, serum sclerostin levels correlated significantly and positively with LS-aBMD Z-scores.

The mechanisms behind the elevated sclerostin level in XLH are not clear at present. Even though PTH is an important determinant of sclerostin levels in other situations (1), we did not find a correlation between PTH and sclerostin concentrations in the present study. In any case, our XLH cohort had significantly higher mean PTH levels than control subjects, and higher PTH should lead to lower serum sclerostin (1).

Apart from PTH, bone mass is an important determinant of sclerostin levels. Because sclerostin is an inhibitor of bone formation, a primary increase in sclerostin is expected to slow down bone formation and lead to lower bone mass, as shown in the sclerostin-overexpressing mouse (25). However, bone mass is relatively high in XLH despite elevated sclerostin levels. Our results therefore suggest that the high bone mass in XLH is not primarily driven by sclerostin. Other factors obviously play a role in determining bone mass. The higher sclerostin level in XLH and the positive correlation between serum sclerostin and LS-aBMD Z-scores are in line with the view that elevated sclerostin levels in XLH are a secondary consequence of high bone mass. A positive correlation between serum sclerostin and areal bone mineral density or bone mineral content is also found in healthy adults (19, 26). One interpretation is that bones with higher mass contain more osteocytes, which together secrete more sclerostin than the fewer osteocytes in a skeleton of lower mass (1).
However, other interpretations are certainly possible. One study found that growing Hyp mice (which carry a Phex mutation) have increased Sost (the gene coding for sclerostin) expression in their femoral bones compared with wild-type littermates (16). This finding indicates a higher production of sclerostin per osteocyte. Recent evidence shows that sclerostin expression is influenced by matrix stiffness (27). Because one of the bone histological hallmarks of XLH is the presence of hypomineralized periosteocytic lesions (28, 29), it is likely that the bone surrounding osteocytes in XLH has a lower than normal stiffness, which may have an influence on sclerostin production by these cells.

Our observation that patients with OI had normal sclerostin levels is somewhat surprising, if one assumes that sclerostin regulates bone mass. If such a regulation exists, then the low bone mass of OI should lead to low sclerostin levels as a compensatory mechanism. However, normal sclerostin levels in OI may be explained if one assumes that sclerostin levels are an indicator of the number of osteocytes in the skeleton. OI is associated with low bone mass, and low bone mass is expected to decrease the number of osteocytes. However, OI is also associated with markedly increased osteocyte density (30). It is therefore possible that the contrasting effects of low bone mass and increased osteocyte density on sclerostin production cancel each other out, resulting in normal sclerostin levels.

We did not find a difference between patients with OI who had received iv bisphosphonate treatment and patients who had not been exposed to these drugs. This finding seems to be in contrast to those of previous studies on patients with postmenopausal osteoporosis in which treatment with neridronate or zoledronate increased sclerostin levels (31, 32). However, it must be acknowledged that our study design was not ideal for assessing bisphosphonate-induced changes, because it was a cross-sectional comparison between 2 groups rather than a longitudinal analysis of treatment-associated changes. In addition, the 2 OI groups differed in severity, because the untreated group comprised patients with OI type I, the mildest form of OI, whereas the treated group included a considerable proportion of patients with the more severe OI types III and IV. It is possible that the treated group had lower sclerostin levels at baseline and that serum sclerostin increased with increasing bone mass during bisphosphonate therapy. The positive correlation between sclerostin levels and LS-aBMD Z-scores in the bisphosphonate-treated group provides indirect evidence for this hypothesis, but longitudinal studies will be needed to clarify this point unambiguously.

Among the limitations of the study is that sclerostin was quantified only in serum. Sclerostin is thought to act in a paracrine manner and the correlation of circulating sclerostin and local bone concentration needs further clarification (4). Another limitation is that fibroblast growth factor (FGF) 23 levels were not measured. Both sclerostin and FGF23 are produced in osteocytes, and it is conceivable that sclerostin inhibits PHEX and may directly regulate FGF23 processing.

In conclusion, this study showed elevated sclerostin levels and a positive association between sclerostin levels and LS-aBMD Z-scores in XLH, whereas patients with OI had normal serum sclerostin concentrations. These results indicate that high bone mass in patients with XLH is not a result of a primary sclerostin dysregulation in these disorders.

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