Circulating Sclerostin Levels and Bone Turnover in Type 1 and Type 2 Diabetes

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Context: Previous observations showed a condition of low bone turnover and decreased osteoblast activity in both type 1 and 2 diabetes mellitus (DM1 and DM2). Sclerostin is a secreted Wnt antagonist produced by osteocytes that regulates osteoblast activity and thus bone turnover. Its levels increase with age and are regulated by PTH.

Objectives: The aim of the present study was to evaluate circulating sclerostin levels in patients with DM1 or DM2 with normal renal function and to analyze its relationship with PTH, 25-hydroxyvitamin D, and bone turnover markers.

Design, and Setting: This was a cross-sectional study conducted at a clinical research center.

Participants: Forty DM2 and 43 DM1 patients were studied and compared with a reference control group (n = 83).

Results: In the overall cohort, sclerostin levels were higher in males than in females and significantly increased with age in both genders. The positive correlation between sclerostin and age was maintained in DM1 but not in DM2 patients. Moreover, sclerostin levels were higher in DM2 than in controls or DM1 patients, and this difference persisted when adjustments were made for age and body mass index. Consistent with previous clinical and experimental observations, sclerostin was negatively associated with PTH in nondiabetic patients ($r = -0.30$; $P < 0.01$), independently of age and gender. Conversely, an opposite but nonsignificant trend between PTH and sclerostin was observed in both DM1 ($r = 0.26$; $P = 0.09$) and DM2 ($r = 0.32$; $P = 0.07$) cohorts.

Conclusions: These findings suggest that sclerostin is increased in DM2. Moreover, the transcriptional suppression of sclerostin production by PTH might be impaired in both DM1 and DM2. **(*J Clin Endocrinol Metab* 97: 1737–1744, 2012)**

Diabetes and osteoporosis are common and complex disorders with a consistent health burden. These disorders can often be associated especially in middle-age and elderly individuals. In fact, an increase in fracture risk has been specifically described in subjects with both type 1 or 2 diabetes mellitus (DM1 or DM2) (1). Although common age-related conditions (*i.e.* a decrease in sex hormone or vitamin D levels) or similar risk factors (*i.e.* reduced physical activity) may explain at least in part the association between diabetes and osteoporosis, the detrimental skeletal effects of glucose toxicity, insulin resistance or deficiency, adipose tissue-derived hormones, diabetic complications, and pharmacological treatment have also been described (2–4). However, the pathogenetic mechanisms of skeletal fragility in diabetic patients remain to be clarified in detail and are only in part reflected

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Abbreviations: BALP, Bone-specific alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; CT1, control cohort 1; CT2, control cohort 2; CTX, C-telopeptide of type I collagen; CV, coefficient of variation; DM1, type 1 diabetes mellitus; DM2, type 2 diabetes mellitus; GFR, glomerular filtration rate; HbA1c, glycosylated hemoglobin; LRP5, lipoprotein receptor-related protein 5; 25OHD, 25-hydroxyvitamin D.

by variation in bone mineral density (BMD) (1, 4). Of interest, previous experimental and histomorphometry observations showed a condition of low bone turnover and decreased osteoblast activity in both DM1 and DM2 (4–9).

Sclerostin is a secreted Wnt antagonist produced almost exclusively by osteocytes that binds to the low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6) inhibiting the canonical Wnt/β-catenin signaling pathway and thus osteoblast activity (10). Its biological importance is underlined by experimental studies in knockout animals and clinical observations in subjects with sclerosteosis and van Buchem disease, two genetic disorders with impaired sclerostin production and markedly increased bone mass (10). Consistent with these observations and given the restricted expression pattern of the gene encoding for sclerostin (*SOST*), neutralizing monoclonal antibodies against sclerostin have been developed and are under investigation as potential novel anabolic therapy for osteoporosis (11–13). Circulating sclerostin levels can be measured in peripheral blood, increase progressively with age (14, 15), and are negatively regulated by estrogens and PTH in both women and men (16–18). Remarkably, a recent study also demonstrated that changes in circulating sclerostin levels reflect changes of similar magnitude in bone marrow plasma sclerostin (17). Moreover, sclerostin levels are increased in long-term immobilized patients and negatively correlate with bone formation markers (19).

The aims of the present study were 1) to evaluate sclerostin levels in patients with DM1 or DM2 compared with age- and sex-matched control subjects and 2) to analyze the relationship between sclerostin and PTH, 25-hydroxyvitamin D (25OHD), or bone turnover markers in patients with DM1 and DM2.

Patients and Methods

Study population

A total of 43 consecutive patients with DM1 (age range 24–77 yr, time since diagnosis 1–52 yr, mean disease duration 18.5 ± 12.6 yr) and 40 consecutive patients with DM2 (age range 48–79 yr, time since diagnosis 1–26 yr, mean disease duration 9.7 ± 7.8 yr) referred to the Diabetes Unit of our department were included in the study. All patients had normal serum creatinine levels and no major comorbidities impairing normal daily activity. Glomerular filtration rate (GFR) was also calculated by Cockcroft-Gault equation and resulted above the threshold for chronic renal failure in all subjects, according to the Kidney Disease Outcomes Quality Initiative (KDOQI) Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease (20).

Age- and sex-matched controls (n = 83) were recruited from healthy volunteers [younger control cohort 1 (CT1), age range 25–48 yr] and subjects randomly selected from a population-based study [older cohort, control cohort 2 (CT2), age range 51–78 yr]. The latter group was obtained from an age-stratified random sampling of older men and postmenopausal women (between the ages of 50 and 80 yr) in primary care registers of Siena residents taking part in an epidemiological cohort study (21, 22). Conversely, CT1 subjects were randomly recruited from the personnel of our department. All included controls had normal glucose homeostasis as assessed by fasting glucose levels and glycosylated hemoglobin (HbA1c). Subjects with Paget's disease of bone, primary hyperparathyroidism, congestive heart failure, recent myocardial infarction, multiple myeloma, or other neoplasia were excluded from the study. Moreover, subjects were also excluded if they received treatment with antiresorptive or anabolic compounds for osteoporosis, previous (>2 months) and current corticosteroid therapy, or any other treatment known to affect bone metabolism. All patients with DM1 were on treatment with insulin, whereas DM2 patients were treated with oral antidiabetic agents alone (n = 31) or in combination with insulin (n = 9). The study was approved by the local Institutional Review Board, and written informed consent was obtained from all participants. General and clinical characteristics of patients and controls are reported in Table 1.

Clinical analysis

At recruitment, height (measured by stadiometer) and weight were recorded from all subjects, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Blood samples were collected in the morning after an overnight fast and stored at −70 C.

Serum concentrations of calcium (corrected for albumin concentration), phosphate, total alkaline phosphatase, and creatinine were measured using standard automated laboratory techniques. Levels of serum C-telopeptide of type I collagen (CTX) [serum CrossLaps; Immunodiagnostic Systems Ltd. (Boldon, Tyne, and Wear, UK); interassay coefficient of variation (CV) <3%; normal ranges 0.142–0.522 ng/ml, 0.166–0.476 ng/ml, and 0.251–0.761 ng/ml in males, premenopausal women, and postmenopausal women, respectively], intact osteocalcin [DiaSorin Diagnostics (Saluggia, Italy); interassay CV 7.1%, normal range 1.8–6.6 ng/ml], and bone-specific alkaline phosphatase (BALP) (Beckman Coulter, Fullerton, CA; with an interassay CV of <7.9%, normal range 9–21 μg/liter) were measured in serum samples, as markers of bone turnover. Moreover, circulating PTH (DiaSorin, Stillwater, MN; interassay CV <7.3%; normal range 10–60 pg/ml) and 25OHD (DiaSorin Diagnostics; sensitivity, 1.5 ng/ml; interassay CV <11%; normal range for vitamin D sufficiency, >30 ng/ml) were evaluated by RIA. Serum sclerostin levels were assessed using a quantitative sandwich ELISA from Biomedica (Biomedica Gruppe, Vienna, Austria), obtained from Pantec (Pantec Srl., Turin, Italy), with intraassay and interassay CV of 4 and 5.5%, respectively. This assay uses a polyclonal goat antihuman sclerostin antibody as a capture antibody and a biotin-labeled mouse monoclonal antisclerostin antibody for detection. Control and patient samples were run together. All assays were run in duplicate after one thaw, according to the manufacturer's instructions. At the time of blood sampling, areal BMD of the lumbar spine and the proximal femur was determined by a dual-energy x-ray absorptiometry device (Lunar Prodigy; GE Healthcare, Waukesha, WI).

TABLE 1. General and clinical characteristics of study populations

	DM1	CT1	DM2	CT2
Subjects (n)	43	21	40	62
Males/females (n)	23/20	10/11	20/20	30/32
Age (yr)	43.7 ± 13.2[b]	34.6 ± 8.9[d]	62.7 ± 8.2	63.2 ± 6.9
BMI (kg/m^2)	22.9 ± 3.2[b]	24.1 ± 4.2	26.8 ± 4.0	25.7 ± 4.1
Creatinine (mg/dl)	1.02 ± 0.21	1.00 ± 0.11	0.99 ± 0.18	0.97 ± 0.15
HbA1c (%)	7.7 ± 0.9	4.9 ± 0.4[e]	7.2 ± 0.6	5.3 ± 0.5[h]
Fasting glucose (mg/dl)	132 ± 27	92 ± 8[e]	140 ± 29	93 ± 7[h]
GFR (ml/min)	88.9 ± 25.8	101.3 ± 20.1	79.4 ± 18.6	80.3 ± 16.2
LS BMD (g/cm^2)	1.098 ± 0.16	1.192 ± 0.15[c]	1.092 ± 0.19	1.052 ± 0.12
T score	−0.86 ± 1.1	−0.12 ± 1.2[c]	−0.87 ± 1.3	−1.20 ± 1.2
Z score	−0.54 ± 1.2	−0.18 ± 1.2	−0.17 ± 1.2	−0.14 ± 0.9
FN BMD (g/cm^2)	0.902 ± 0.13	1.055 ± 0.17[e]	0.922 ± 0.16	0.847 ± 0.12[f]
T score	−1.03 ± 1.0	+0.10 ± 1.2[e]	−0.78 ± 1.0	−1.35 ± 0.9[f]
Z score	−0.61 ± 0.9[a]	+0.12 ± 1.0[d]	+0.16 ± 0.9	−0.17 ± 0.9
BALP (μg/liter)	11.3 ± 4.1	12.7 ± 6.9	10.9 ± 4.2	14.0 ± 3.8[g]
OC (ng/ml)	3.4 ± 2.3	4.0 ± 1.8	3.6 ± 1.5	5.7 ± 1.1
CTX (ng/ml)	0.31 ± 0.15	0.586 ± 0.31[e]	0.272 ± 0.09	0.626 ± 0.21[h]
25OHD (ng/ml)	16.5 ± 8.9	34.7 ± 13.2[e]	15.1 ± 11.5	23.1 ± 9.8[f]
PTH (pg/ml)	33.0 ± 14.6	23.3 ± 2.1[d]	30.2 ± 14.8	25.4 ± 9.2

Data are expressed as means ± SD. GFR was calculated with the Cockcroft-Gault equation. FN, Femoral neck; LS, lumbar spine; OC, osteocalcin.

[a] $P < 0.01$ DM1 *vs.* DM2.

[b] $P < 0.001$ DM1 *vs.* DM2.

[c] $P < 0.05$ DM1 *vs.* CT1.

[d] $P < 0.01$ DM1 *vs.* CT1.

[e] $P < 0.001$ DM1 *vs.* CT1.

[f] $P < 0.05$ DM2 *vs.* CT2.

[g] $P < 0.01$ DM2 *vs.* CT2.

[h] $P < 0.001$ DM2 *vs.* CT2.

Statistical analysis

Data were summarized as means ± SD, and $P < 0.05$ was accepted as the value of significance. Quantitative variables were compared between the case and the control groups using ANOVA and analysis of covariance, with Fisher's protected least significant difference *post hoc* test. Qualitative variables were compared using standard χ^2 test. Logistic regression analysis was used to assess the independent association between sclerostin and bone turnover markers or calciotropic hormones. The relationship between sclerostin and other variables was evaluated further in DM1, DM2, and control groups using multivariate analysis.

For logistic regression analysis *vs.* age, the control groups CT1 and CT2 were merged and considered as a single group. As shown in Table 1, mean age significantly differed between DM1 or DM2 patients and control groups. This was mainly due to the different age of onset of DM1 and DM2, generally occurring in young and middle-aged individuals, respectively, thus making it unlikely to obtain three groups of age-matched DM1 or DM2 patients and controls. For this reason, differences in sclerostin levels between controls (CT1 plus CT2) and DM1 or DM2 groups were adjusted for age and BMI. Then age-matched analyses were also performed. Thus, DM2 patients were compared with the age-matched CT2 group. Conversely, the DM1 group comprised 12 subjects aged above 50 yr and, as evident in Table 1, showed a statistically significant difference in age with respect to either CT1 or CT2 group. Thus, we used the younger control group (CT1) for those under 50 yr (DM1a, n = 31, age range 24–49 yr, mean age 37.1 ± 7.2 yr) and the older control group (CT2) for those over 50 yr (DM1b, n = 12, age range 50–77 yr, mean age 62.2 ± 8.8 yr). Results from this analysis are summarized (see Fig. 2). All analyses were performed using Statistica version 5.1 (Statsoft, Tulsa, OK) and SPSS (release 6.1; SPSS, Chicago, IL).

Results

General and clinical characteristics of patients and controls are shown in Table 1. As is evident, all markers of bone turnover and calciotropic hormones were within the normal range in controls, as well as lumbar and femoral BMD (as shown by the Z score levels next to 0). Consistent with previous observations, BMD at the femoral neck was lower in DM1 but higher in DM2 with respect to control groups CT1 and CT2, respectively. Moreover, a significant reduction of BALP and CTX was observed in DM2 patients with respect to CT2. A similar reduction of CTX was observed in DM1 patients *vs.* CT1, whereas the reduction in BALP did not reach a statistically significant level. Of interest, in keeping with previous evidence (23), 25OHD levels were significantly lower in DM1 and DM2 patients than in CT1 and CT2 subjects, respectively. Consistent with this observation, a slight increase in PTH was

observed in both DM1 and DM2 patients compared with controls, with a statistically significant difference between DM1 and CT1. Similar results were observed when male and female cohorts were considered separately or when the age-matched DM1 groups (DM1a and DM1b) were considered (not shown).

In the overall cohort of subjects, circulating sclerostin levels were higher in males than in females and significantly increased with age and BMI in both genders (age: r = 0.31, $P < 0.005$ and r = 0.39, $P < 0.001$ in females and males, respectively; BMI: r = 0.34, $P < 0.005$ and r = 0.26, $P < 0.05$ in females and males, respectively). The positive correlation between sclerostin and age was maintained in controls and in DM1 patients but not in DM2 patients (Fig. 1), possibly due to the narrower age range of the latter group compared with the broader age range of DM1 subjects or controls. Conversely, the association between sclerostin and BMI was not significant in controls. Of interest, in DM2 subjects, sclerostin levels were positively correlated with years since diagnosis (r = 0.68; $P < 0.001$), whereas this association was not significant in DM1 patients. Finally, a trend for a positive correlation between sclerostin and HbA1c levels was observed in DM2 patients (r = 0.29; $P = 0.08$). Fasting glucose levels were not significantly associated with sclerostin in the overall group of diabetic patients as well as in DM1 and DM2 cohorts or in controls (not shown). Conversely, a significant and negative correlation between GFR and sclerostin was observed in controls (r = −0.34; $P < 0.005$) but not in DM1 or DM2 patients (r = −0.25 and r = −0.13, respectively).

In all study groups, bone turnover markers were not significantly correlated with serum sclerostin, except that BALP that was negatively associated with sclerostin in control males (r = −0.60; $P < 0.05$). Moreover, sclerostin levels were higher in DM2 than in controls or DM1 patients, and this difference persisted when adjustments were made for age and BMI (Fig. 2A) or when CT1 and CT2 subgroups were considered separately (Fig. 2, B and C). Moreover, a similar trend approaching statistical significance ($P = 0.06$) was observed between DM1a and CT1 groups (Fig. 2D).

Consistent with previous clinical and experimental observations, sclerostin levels were negatively correlated with serum PTH in nondiabetic patients (r = −0.30; $P < 0.01$) independently of age and gender (Fig. 3A). Conversely, an opposite but nonsignificant trend between PTH and sclerostin was observed in both DM1 (r = 0.26; $P = 0.09$) and DM2 (r = 0.32; $P = 0.07$) groups (Fig. 3, B and C). No correlation between sclerostin and BMD or bone mineral content was observed in controls as well as in DM1 or DM2 cohorts (not shown).

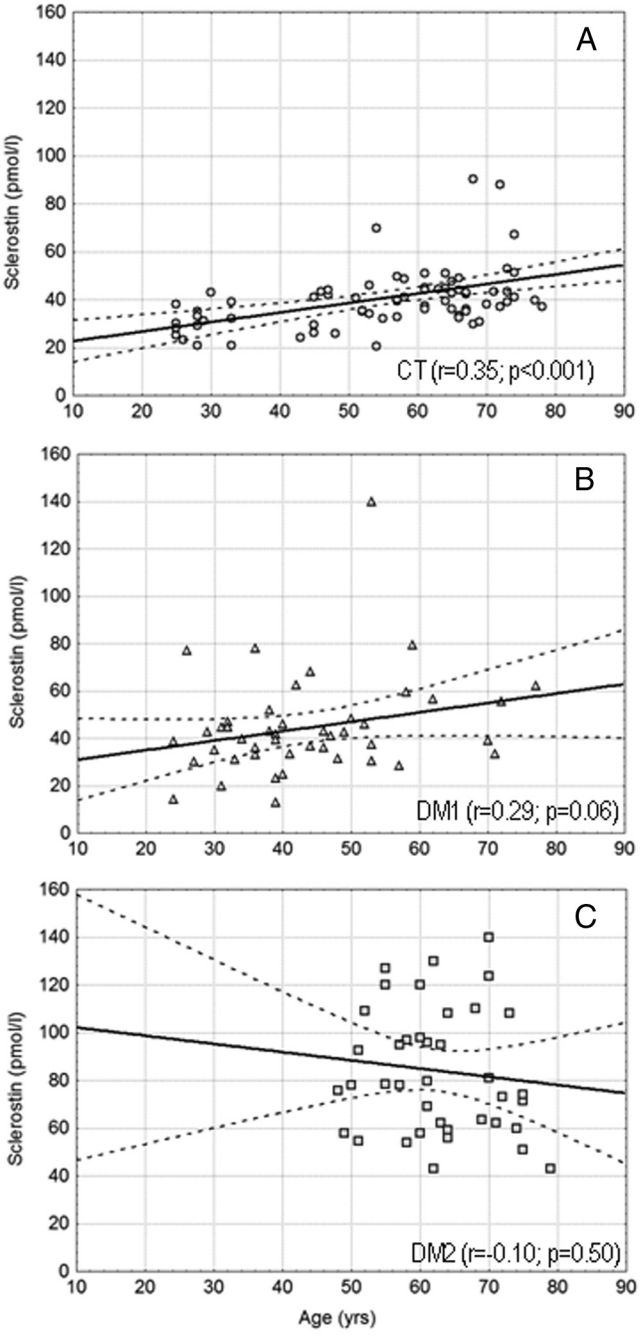

FIG. 1. Correlation of serum sclerostin with age in controls (CT) (A); and patients with DM1 (B) or DM2 (C). *Dotted lines* indicate 95% confidence intervals.

Using multivariate analysis, we found that age ($\beta = 0.40$; $P < 0.001$) and PTH ($\beta = -0.30$; $P < 0.01$) were independent predictors of sclerostin in controls after adjusting for BMI and 25OHD. In multivariate analysis, GFR was not significantly associated with sclerostin, suggesting that the significant correlation observed with linear regression analysis was dependent on the age-related decrease in GFR. In diabetic patients, we included HbA1c, fasting glucose levels, and years since diagnosis as additional factors in multivariate analysis. In DM1, age ($\beta =$

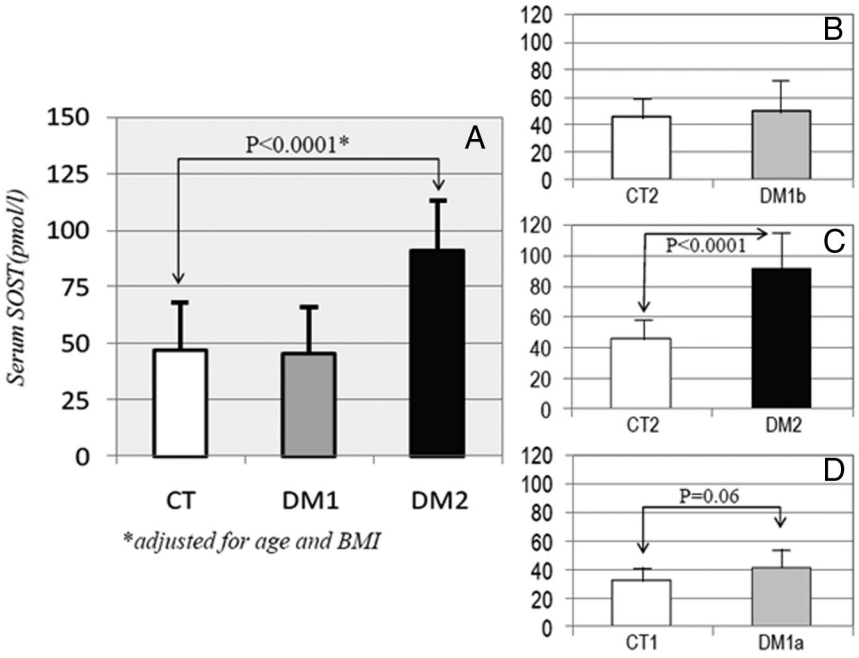

FIG. 2. Serum sclerostin levels in cases and controls. A, Differences according to diagnosis were tested considering a single control group (CT), adjusting for age and BMI;. B–D, given the wider age range, patients with DM1 were divided into two subgroups (DM1a, n = 31, age range 24–49 yr; and DM1b, n = 12, age range 50–77 yr). Then age-matched analyses were performed between DM1b *vs.* CT2 (B); DM2 *vs.* CT2 (C); and DM1a *vs.* CT1 (D).

0.35; $P < 0.05$) was an independent predictor of sclerostin, whereas in DM2, the only independent predictor of sclerostin was represented by the years since diagnosis ($\beta = 0.46$; $P < 0.005$).

Discussion

Despite several clinical and experimental observations suggesting an increased skeletal fragility in both DM1 and DM2 patients, the pathophysiology of reduced bone strength in diabetes remains to be clarified in detail and might differ at least in part between DM1 and DM2. The results of the present study confirm previous clinical evidence showing a condition of low bone turnover in DM1 and DM2. This was associated with decreased BMD in DM1 but not in DM2 patients, which showed normal or even higher BMD levels than controls. Moreover, we show for the first time a marked increase in circulating sclerostin levels in DM2 patients, with mean sclerostin concentrations more than 2-fold higher in most DM2 patients than in age- and sex-matched controls. Such increase in sclerostin levels was comparable or even higher to that observed in immobilized patients (19) and could explain, at least in part, the parallel decrease in bone formation markers observed in our cohort of DM2 patients. In fact, sclerostin is a recently discovered Wnt antagonist that is almost entirely produced by osteocytes and plays a major role in the suppression of bone formation. Together with other factors such as Dickkopf1, sclerostin can bind to LRP5 and LRP6 leading to the inhibition of the Wnt/β-catenin signaling pathway in the osteoblast (24). This in turn leads to reduced osteoblast proliferation, differentiation, and lifespan (9, 25, 26). Consistent with these experimental data, several clinical observations clearly indicated a relative increase in bone formation and enhanced bone mass during conditions of impaired sclerostin secretion and/or enhanced Wnt/β-catenin signaling, whereas low bone formation was described with reduced Wnt/β-catenin signaling (9, 27–29). Thus, our data point toward an increase in sclerostin levels as a potential cause of the reduction of bone formation in DM2. Although a similar reduction in bone formation markers was observed also in DM1 patients, sclerostin did not significantly differ between DM1 and controls. This suggests that partly different mechanisms are implicated in the pathogenesis of skeletal fragility in DM1 and DM2. Indeed, a similar trend approaching statistical significance was evident in the subgroup of DM1 patients aged below 50 yr than in the group of age-matched controls. Additional studies in larger samples will be required to clarify this issue.

Despite the above observations, we did not detect any association between sclerostin and markers of bone formation or BMD in our cohorts of patients, whereas a negative association between serum sclerostin and BALP (a marker of bone formation) was observed in the male cohort of controls. Because circulating sclerostin levels have been negatively associated with estrogen but not androgen levels (16, 18), it is likely that gender-related differences in sex steroid concentrations may explain the observed differences in the degree of correlation between bone markers and sclerostin in female *vs.* male controls. Moreover, although a larger study reported a negative correlation between sclerostin and bone formation markers in postmenopausal women but not men (14), other studies showed no correlation (16, 30) or even a positive association (31). The reason for these contrasting results remains unclear. Conversely, the lack of association between sclerostin and bone formation markers in DM2 patients could be in part related to the marked increase in sclerostin in this specific cohort, with levels well above the normal range in most patients. This might suggest that

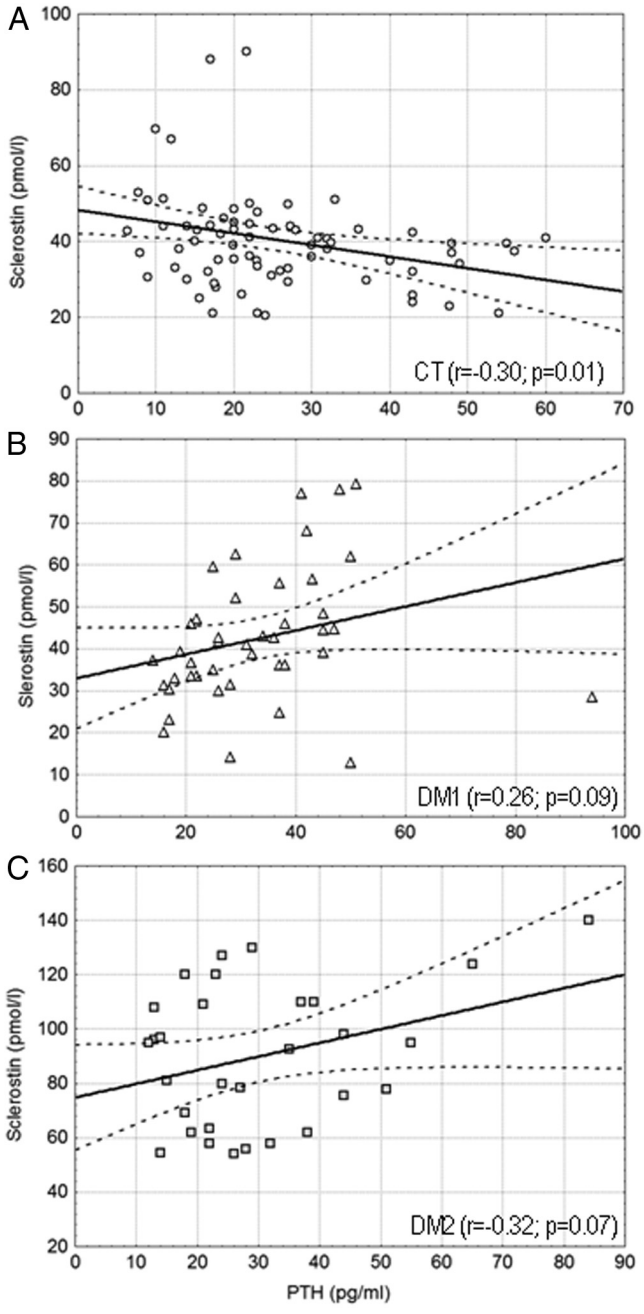

FIG. 3. Correlation of serum sclerostin with PTH in controls (CT) (A) and patients with DM1 (B) or DM2 (C). *Dotted lines* indicate 95% confidence intervals.

above a certain threshold, the suppressive effect of sclerostin on bone formation is not linear, likely due to a saturation point for sclerostin effects on bone cells. However, additional studies will be required to clarify more specifically the dose-relationship between circulating sclerostin levels and the suppression of bone formation.

In our DM2 cohort, we also observed a decrease in the bone resorption marker CTX. Although the relationship between sclerostin and osteoclast activity remains to be clarified in detail, other studies identified a possible association between sclerostin levels and bone resorption (14, 15). In keeping with this hypothesis, an increase in bone resorption markers was described in patients with impaired sclerostin secretion due to van Buchem disease (32), suggesting that due to compensatory mechanisms, a relative increase in bone resorption can be associated with the enhanced bone formation typical of this disorder. Together with additional indications from experimental studies (33), these data suggest that due to similar compensatory mechanisms, enhanced sclerostin production (at least with levels well above the normal range) might lead to a generalized reduction in bone turnover over the long term. As counterpart, recent observations with monoclonal antibodies against sclerostin in animal models or postmenopausal women clearly demonstrated that an acute reduction in sclerostin levels leads to an increase in bone formation and a suppression in bone resorption (11–13, 34).

Several systemic and local factors have been implicated as possible regulators of sclerostin expression and release by the osteocyte. Among them, PTH has been shown to decrease sclerostin expression both *in vitro* and *in vivo* (35). In fact, PTH suppressed the transcription of the *SOST* gene *in vitro* (36), and a consistent reduction of sclerostin levels was observed in mice overexpressing a constitutively active PTH receptor 1 variant (35, 36). Moreover, continuous infusion of PTH to mice markedly decreased *SOST* expression and sclerostin levels in vertebral bone (37). A similar although transient finding was also reported with intermittent PTH injection (36). These experimental data have been confirmed more recently by different clinical studies. In some cohorts of osteoporotic and nonosteoporotic subjects, serum PTH levels were inversely correlated with circulating sclerostin (16, 30, 31), whereas low sclerostin concentrations were described in patients with primary hyperparathyroidism (38–40). In addition, either intermittent or continuous infusions of PTH 1–34 decreased circulating sclerostin levels in postmenopausal women and healthy men (17, 41). Importantly, although a negative association between sclerostin and PTH levels was observed in controls (consistent with the above observations), we did not detect a similar association in our cohorts of diabetic subjects. On the contrary, a trend for a positive association between sclerostin and PTH was observed in both DM1 and DM2 patients. Indeed, PTH levels were slightly higher in DM1 and DM2 than in controls (achieving statistical significance in DM1), likely due to the lower 25OHD levels. This increase in PTH in diabetic subjects, under normal circumstances, might have led to reduced rather than increased sclerostin levels. Even though further prospective and experimental observations will be required to clarify this issue, our findings suggest that the transcriptional suppression of

sclerostin production by PTH may be impaired in diabetes. Indeed, a previous histomorphometric analysis on diabetic and nondiabetic patients with renal osteodystrophy might indirectly support this hypothesis (42). In fact, a positive correlation between PTH and bone apposition rate or bone formation rate was observed only in the nondiabetic group, suggesting that the lower bone formation in diabetic patients may have arisen in part from a failure of PTH to promote osteoblast activity. However, the molecular mechanisms underlying the lack of correlation between PTH and sclerostin in diabetes remain to be demonstrated and might be at least in part mediated by variation in glucose or insulin levels. Of interest, single experimental studies demonstrated that high glucose levels impair the bone cell response to PTH (43), whereas insulin treatment potentiates the skeletal effects of PTH in streptozotocin-induced diabetic rats (44). This could also explain the positive correlation between sclerostin and HbA1c that we observed in DM2 patients. Possibly, the use of different treatments (*i.e.* insulin *vs.* oral antidiabetic agents) might differentially affect sclerostin levels. However, even though in our DM2 cohort we did not detect any difference in sclerostin levels in relation to the treatment, this hypothesis has to be verified in larger and prospective samples.

In summary, this study demonstrates an increase in circulating sclerostin in DM2 patients compared with age-matched controls. Moreover, the negative correlation between PTH and sclerostin (demonstrated in previous observations) was lost in DM1 and DM2 patients. Additional experimental and clinical studies in larger and prospective samples will be required to confirm our data and identify the underlying pathogenetic mechanism. This could be particularly important not only for a better understanding of the causes of skeletal fragility in diabetes but also for its potential therapeutic implications, providing the basis for the use of the monoclonal antibody against sclerostin.

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