Sclerostin Levels Associated with Inhibition of the Wnt/β-Catenin Signaling and Reduced Bone Turnover in Type 2 Diabetes Mellitus

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Context: Patients with type 2 diabetes (T2DM) have low bone turnover, poor bone quality, and circulating levels of sclerostin significantly higher than non-T2DM controls. There are no data on the possible association of sclerostin with β-catenin, a key component of the Wnt/β-catenin canonical signaling.

Objectives: The aim of the study was to evaluate the circulating β-catenin levels in T2DM patients and to analyze their relationship with sclerostin and bone turnover markers.

Design: This was a cross-sectional study.

Setting and Patients: The study was conducted at a clinical research center. Forty T2DM postmenopausal women were studied and compared with 40 healthy controls. Bone status was assessed by dual-energy x-ray absorptiometry measurements (bone mineral density) and by measuring bone alkaline phosphatase and carboxy-terminal telopeptide of type 1 collagen. Sclerostin and β-catenin were evaluated by an immunoenzymetric assay.

Results: Consistent with previous reports in T2DM subjects, we found sclerostin levels higher and bone turnover markers lower than controls. In our cohort of T2DM patients, β-catenin levels are significantly lower than in controls (median 1.22 pg/ml, 25th to 75th percentiles 0.50–2.80; and median 4.25 pg/ml, 25th to 75th percentiles 2.20–7.62, respectively; $P = 0.0002$). β-Catenin correlated negatively with sclerostin ($P < 0.0001$) and positively with bone alkaline phosphatase ($P = 0.0030$) only in T2DM patients and negatively with age in both groups. Eight of the 40 T2DM patients had vertebral fractures.

Conclusions: These results show for the first time that T2DM patients have serum concentrations of β-catenin lower than controls. The negative association of β-catenin with sclerostin suggests a biological effect of increased sclerostin on the Wnt signaling, which appears impaired in T2DM. **(*J Clin Endocrinol Metab* 97: 3744–3750, 2012)**

The relationship between diabetes mellitus and osteoporosis is currently under intense investigation due to the increasing prevalence of both diseases and to the evidence that type 2 diabetes mellitus (T2DM) is associated with an increased risk of fractures (1–3) that is higher than general population at a given bone mineral density (BMD) (4, 5). Recent reports propose that the Wnt signaling pathway may be implicated in this association (6). The Wnt/β-catenin (canonical) Wnt signaling is one of the three known pathways of Wnt signaling, and is also the most

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Abbreviations: B-ALP, Bone-specific alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; CTX, carboxy-terminal cross-linked telopeptide of type I collagen; Dkk-1, Dickkopf-1; FN, femoral neck; GSK-3, glycogen synthase-3; HbA1c, glycated hemoglobin; LRP, lipoprotein receptor-related protein; LS, lumbar spine; 25OHD, 25-hydroxyvitamin D; SF-36, 36-Item Short Form Health Survey; T2DM, type 2 diabetes mellitus; TH, total hip.

well characterized (7). The Wnt/β-catenin pathway is essential for normal osteogenesis (8–10). Activation of this pathway requires binding of Wnt ligands to the frizzled receptor and its coreceptors, the low-density lipoprotein receptor-related proteins (LRP)-5 and -6, leading to the down-regulation of glycogen synthase-3 (GSK-3) activity (11) and inhibition of β-catenin phosphorylation and proteosomal degradation, resulting in its accumulation in the cytoplasm and its translocation into the nucleus, in which it promotes the transcriptional response of Wnt target genes (12).

The interest of Wnt signaling in diabetes had risen after the observation by Kanazawa *et al.* (13), who showed that a single polymorphism locus in the *WNT5B* gene caused susceptibility to type 2 diabetes in a Japanese population. More recent studies have shown that Wnt signaling impacts pancreatic β-cell function by regulating insulin secretion and viability (7). The Wnt/β-catenin canonical pathway is modulated by a number of factors that include, among others, soluble extracellular proteins such as the inhibitory factor 1 and secreted frizzled related proteins, which bind directly to Wnt proteins, preventing their interaction with receptors, and other secreted proteins, such as Dickkopf (Dkk-1) and sclerostin, which compete with the Wnt/β-catenin for binding to LRP5/6, disrupting (Dkk-1) or antagonizing (sclerostin) LRP5/6 mediated Wnt signaling (14). In humans, García-Martín *et al.* (6), van Lierop *et al.* (15), and Gennari *et al.* (16) have recently reported that, in patients with T2DM, circulating levels of sclerostin are increased, positively associated with duration of T2DM and glycated hemoglobin, and inversely related to bone turnover markers, suggesting that the Wnt signaling pathway may be impaired in these patients. This gives support to the hypothesis that osteocytes (which produce almost all the available sclerostin) may play a role also in glucose metabolism. Recent evidence has also implicated osteocytes as a major source of the osteoclastogenic cytokine receptor activator of nuclear factor-κB ligand, a cytokine that stimulates osteoclast maturation and activity, whose expression is also stimulated by sclerostin (17, 18). In this context, given the complexity of the interrelationships among all these factors, we reasoned that more information on changes in the Wnt system in diabetic patients could come from the measure of more than a single component of the Wnt-signaling pathway.

The objectives of this cross-sectional study were to evaluate the behavior of serum sclerostin, β-catenin, and Dkk-1 in a cohort of T2DM patients. In addition, we analyzed the relations of these products with bone turnover markers, BMD, and morphometric vertebral fractures.

Subjects and Methods

Study population

Our cross-sectional study included 40 postmenopausal women with T2DM and 40 healthy subjects as a control group. Diabetes was defined according to American Diabetes Association criteria (19). T2DM patients presenting to our community clinic for treatment of diabetes were recruited from June to September 2011. Controls were age- and sex-matched subjects recruited from the general population during the same period. All participants were Caucasian, free-living, aged 50–85 yr. Exclusion criteria were the following: 1) chronic diseases apart from T2DM, 2) known diseases affecting bone (Paget's disease, rheumatoid arthritis, hyperparathyroidism, hypercortisolism, malignant tumors, renal bone disease, chronic liver disease, and postransplantation bone disease), 3) use in the last 12 months of drugs affecting bone metabolism, including bisphosphonates, 4) chronic glucocorticoid use for more than 3 months, or 5) use in the last 12 months of thiazolidinediones. Ethical approval was obtained by the hospital ethical committee. All subjects gave informed consent before entering the study, which was performed in accordance with the Declaration of Helsinki.

Clinical evaluation

In all subjects we measured height and weight and calculated body mass index (BMI) using the Quetelet formula (weight in kilograms divided by the square of height in meters). Moreover, all patients and controls were asked to fill in the Italian version (20) of the 36-Item Short Form Health Survey (SF-36), which yields physical and mental health component scores and has been previously validated in patients with type 2 diabetes (21). Based on the results of the physical functioning domain, the study subjects were divided into two groups: sedentary (≤80) and nonsedentary (>80) according to a previous study (22).

Laboratory data

Biochemistries were measured in serum samples obtained in the morning after an overnight fast and stored at −30 C until the examination. Serum concentrations of total calcium (corrected for albumin concentration), phosphorus, creatinine, fasting plasma glucose (FPG), and glycated hemoglobin (HbA1c) were measured using standard laboratory techniques. 25-Hydroxyvitamin D (25OHD) was measured by chemiluminescence immunoassay (Liaison 25 OH Vitamin D Total; DiaSorin Inc., Stillwater, MN; intra- and interassay variability were less than 5 and 10%, respectively). Bone-specific alkaline phosphatase (B-ALP) was measured by immunoenzymetric assay (Ostase BAP; Immunodiagnostic Systems Ltd., Boldon, UK; intra- and interassay variability were less than 10%). Serum carboxy-terminal cross-linked telopeptide of type I collagen (CTX) was measured by an ELISA (Serum Crosslaps; Immunodiagnostic Systems; intra- and interassay variation was 2.5 and 1.8%, respectively). Sclerostin and Dkk-1 were measured by enzyme immunoassays using reagents provided by Biomedica Medizinprodukte (Wien, Austria). Intra- and interassay coefficients of variation for sclerostin were 5 and 4%, respectively. The detection limit was 2.6 pmol/liter. Mean concentration in normal subjects aged older than 50 yr for our laboratory is 43 pmol/liter. The detection limit for Dkk-1 is 0.38 pmol/liter. Intra- and interassay coefficients of variation are 7 and 9%, respectively. β-Catenin was measured using an immunoenzymatic assay developed by Cusabio Biotech Co. Ltd. (Newark, DE). The microtiter plate provided in this kit was precoated with an antibody specific to β-catenin. The β-catenin assay uses a biotin-conjugate antibody/horseradish peroxidase-avidin for detection of the analyte. This assay recognizes human β-catenin. No significant cross-reactivity or interference was ob-

served. The minimum detectable amount of human β-catenin is 0.39 pg/ml. The detection range is 0.62–40 pg/ml. The commercial kit does not provide samples with known concentration, so we had to assess our own intra- and interassay variability, which were both less than 10%. In this β-catenin assay, 100% of samples of healthy subjects showed detectable values. In our healthy controls, β-catenin serum range was 4.2–8.6 pg/ml.

Bone density and vertebral fracture evaluation

Areal BMD at lumbar spine (LS) L_2-L_4 and the proximal femur [femoral neck (FN) and total hip (TH)] was measured in all subjects by dual-energy x-ray absorptiometry using a Lunar Prodigy DPX densitometer (GE Healthcare, Madison, WI). The coefficient of variation is less than 1.5% for all sites. BMD measurements were performed by the same operator. Lateral standardized spinal x-ray films of the thoracic and lumbar spine were taken in the same week as the serum collection for morphometric analysis and interpreted according to the semiquantitative method by Genant *et al.* (23).

Statistical analysis

Descriptive statistics and significance levels were analyzed using the GraphPad InStat version 4 for Windows (GraphPad, San Diego, CA). Power analysis was performed by GraphPad StatMate2 (GraphPad). The analysis indicates that a sample size of 40 in each group has a 80% power to detect a difference between means of 4.49 with a significance level (α) of 0.05 (two tailed). Data for continuous variables are expressed as means ± SD. The normal distribution of values for different parameters was verified with the Kolmogorov-Smirnov test. Pearson linear regression analysis (normal distribution) or Spearman test (non-normal distribution) was used for association studies. Comparisons of continuous variables between groups were carried out using a Student's *t* test or Wilcoxon test, as appropriate. Comparisons of categorical variables between groups were performed using the χ^2 test. A multiple regression analysis was used to determine the influence of one independent variable after correcting for others. All models were adjusted for age. $P < 0.05$ was considered as statistically significant.

Results

Table 1 shows the general characteristics, clinical, biochemical, and densitometric data of patients and controls. T2DM patients and controls were comparable for age, calcium, phosphate, and creatinine. The average of duration of T2DM was 10.05 ± 5.36 yr. T2DM patients were treated with oral antidiabetic agents alone (n = 31) or with insulin (n = 9). As expected, T2DM patients had significantly higher levels of FPG and HbA1c ($P < 0.001$ *vs.* controls). Diabetic patients also had a BMI (kilograms per square meter) higher than controls (31.16 ± 5.06 *vs.* 26.55 ± 4.77; $P < 0.001$). According to the physical functioning domain of the SF-36 questionnaire, 33 patients and 31 control subjects were considered sedentary (less than 80 on the scale). All markers of bone turnover, as well as lumbar and femoral BMD, were within the normal range in controls. 25OHD serum levels in T2DM patients were lower than in controls (17.14 ± 7.11 *vs.* 24.34 ± 7.56 ng/ml; $P <$

TABLE 1. General characteristics and clinical, biochemical, and densitometric data of patients and controls

	T2DM patients	Controls	*P*
n	40	40	
Age (yr)	63.68 ± 8.39	62.12 ± 7.99	ns
BMI	31.16 ± 5.06	26.55 ± 4.77	<0.001
Sedentary (n/%)	33/82.5	31/77.5	ns
Smoking habit (n/%)	1/2.5	2/5	ns
Menopausal age (yr)	49.26 ± 5.05	48.45 ± 4.98	ns
Parity (n)	4.06 ± 2.13	3.98 ± 2.01	ns
Diabetes duration (yr)	10.05 ± 5.36		
HbA1c (%)	7.27 ± 0.51	4.94 ± 0.48	<0.001
Vertebral fractures (n/%)	8/20	3/7.5	ns
BMD LS (g/cm^2)	1.110 ± 0.185	1.099 ± 0.156	ns
T-score LS	−0.52 ± 1.54	−0.61 ± 1.43	ns
BMD FN (g/cm^2)	0.881 ± 0.140	0.819 ± 0.111	<0.05
T-score FN	−0.72 ± 1.14	−1.32 ± 1.17	<0.05
BMD TH (g/cm^2)	0.977 ± 0.130	0.943 ± 0.120	ns
T-score TH	−0.14 ± 1.07	−0.50 ± 1.23	ns
Calcium corrected for albumin (mg/dl)	9.47 ± 0.36	9.68 ± 0.44	ns
Phosphorus (mg/dl)	3.89 ± 0.44	3.74 ± 0.38	ns
Creatinine (mg/dl)	0.92 ± 0.18	0.90 ± 0.13	ns
FPG (mg/dl)	149.11 ± 38.35	93.32 ± 10.25	<0.001
25OHD (ng/ml)	17.14 ± 7.11	24.34 ± 7.56	<0.001
B-ALP (μg/liter)	15.48 ± 5.69	19.72 ± 9.66	0.0261
CTX (ng/ml)	0.40 ± 0.25	0.64 ± 0.43	0.0032
Sclerostin (pmol/liter)	53.18 ± 10.94	47.50 ± 12.62	<0.05
Dkk-1 (pmol/liter)	12.90 ± 10.27	9.07 ± 5.68	<0.05
β-Catenin (pg/ml)	2.43 ± 2.82	5.94 ± 5.53	0.0002

Data for continuous variables are presented as mean ± SD. FPG, Fasting plasma glucose; ns, not significant.

TABLE 2. Correlations of sclerostin in T2DM patients and controls

	T2DM patients		Controls	
	r	P	r	P
β-Catenin	−0.7469	<0.0001	0.1010	ns
Dkk-1	−0.1384	ns	0.3527	0.02
BMD LS	−0.3965	ns	−0.3246	ns
BMD FN	−0.1283	ns	−0.1494	ns
B-ALP	0.0086	ns	−0.1511	ns
CTX	0.3773	0.0164	0.0423	ns
Age	0.8513	<0.0001	0.8258	<0.001
BMI	−0.2376	ns	−0.4256	ns
Diabetes duration	0.4648	<0.001		

ns, Not significant.

0.001). T2DM patients had femoral neck BMD significantly higher than controls ($P < 0.01$). Sclerostin serum levels in T2DM patients were 53.18 ± 10.94 pmol/liter (median 52.50; 25th to 75th percentiles 45–60).

Sclerostin serum levels in controls were 47.50 ± 12.62 pmol/liter (median 47.5; 25th to 75th percentiles 40–58). The difference was significant, with $P < 0.05$. β-Catenin serum concentrations in diabetic patients were 2.43 ± 2.82 pg/ml (median 1.22; 25th to 75th percentiles 0.50–2.80). β-Catenin serum levels in controls were 5.94 ± 5.53 pg/ml (median 4.25; 25th to 75th percentiles 2.20–7.62). The difference was significant ($P = 0.0002$). T2DM had serum concentrations of Dkk-1 of 12.90 ± 10.27 pmol/liter (median 8.45; 25th to 75th percentiles 7.0–12.23), significantly higher than controls (9.07 ± 5.68 pmol/liter; median 7.5; 25th to 75th percentiles 6.57–9.45, $P < 0.05$). Both B-ALP and CTX levels were reduced in diabetic patients if compared with controls. Mean B-ALP was 15.48 ± 5.69 *vs.* 19.72 ± 9.66 μg/l ($P = 0.0261$), and mean CTX was 0.40 ± 0.25 *vs.* 0.64 ± 0.43 ng/ml ($P = 0.0032$). Tables 2 and 3 show correlations of sclerostin and β-catenin in T2DM patients and controls. Sclerostin serum levels were positively correlated with age in T2DM patients ($P < 0.0001$) and controls ($P < 0.001$) and positively correlated with years since diagnosis in T2DM ($P < 0.001$). A significant negative correlation was observed between sclerostin and β-catenin ($r = −0.7469$; $P < 0.0001$) (Fig. 1) and between sclerostin and CTX ($P = 0.0164$). Sclerostin serum levels positively correlated with Dkk-1 in controls ($P = 0.02$). A significant positive correlation was observed between β-catenin and B-ALP ($r = 0.4658$; $P = 0.0030$) (Fig. 1). No relationship was found between serum sclerostin, BMI, and physical activity in the overall cohort of subjects. β-Catenin was also negatively associated with age ($P < 0.001$). Multiple regression analysis was then performed in T2DM patients with β-catenin as the outcome. The potential determinant variables considered were sclerostin, age, HbA1c levels, and years since diagnosis. We found that only age ($\beta = −0.04906$; $P = 0.0075$; SE = 0.1309) and sclerostin ($\beta = −0.1835$; $P = 0.0075$; SE = 0.0909) were independent predictors of β-catenin in T2DM patients. The calculated variance inflation factor confirms that the x variables are independent of each other, excluding multicollinearity problems in the model. We were not able to find any significant correlation between BMD at any site and the biochemical parameters measured in the patients and in controls. No subject had a nonvertebral fracture history; vertebral fractures were found in eight of the 40 diabetic patients (20%) and in three controls (7.5%).

TABLE 3. Correlations of β-catenin in T2DM patients and controls

	T2DM patients		Controls	
	r	P	r	P
Sclerostin	−0.7469	<0.0001	0.1010	ns
Dkk-1	−0.2486	ns	0.2874	ns
BMD LS	0.3176	ns	0.2455	ns
BMD FN	0.0062	ns	0.0167	ns
B-ALP	0.4658	0.0030	0.0260	ns
CTX	−0.1281	ns	0.1086	ns
Age	−0.6864	<0.001	−0.5768	0.002
BMI	−0.0246	ns	−0.0376	ns
Diabetes duration	−0.0870	ns		

ns, Not significant.

Discussion

Our results confirm and extend recent studies of circulating sclerostin in patients with T2DM and show for the first time that in T2DM the increase of serum sclerostin is associated with a significant decrease in β-catenin serum concentration, suggesting that increased sclerostin has a causative effect in impairing the functionality of the Wnt canonical signaling in these patients. We also found Dkk-1 circulating levels significantly higher than in controls. Both B-ALP and CTX were significantly reduced in our T2DM cohort, confirming that a generalized reduction in bone turnover takes place in this population, eventually leading to poor bone quality and to increased skeletal fragility, despite normal or even greater bone mass than expected according to sex and age (16, 24). Eight of 40 T2DM had in fact a morphometric vertebral fracture, a figure that appears in line with the observations by García-Martín (6) and by other authors (2, 3, 16, 25). The pathogenesis of increased bone fragility in T2DM remains to be clarified. Other factors, such as changes in calcium homeostasis and an increase in advanced glycation end-product or nonenzymatic cross-links within collagen fibers have been proposed as possible contributors to the

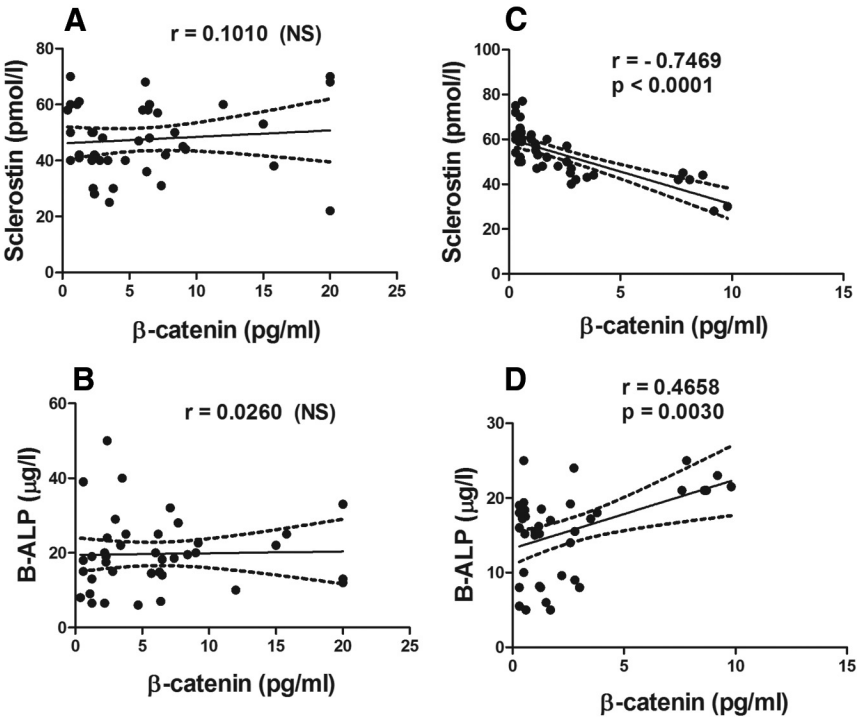

FIG. 1. Univariate correlation (Pearson analysis) between β-catenin and sclerostin and between β-catenin and B-ALP serum levels, in controls (A and B) and T2DM patients (C and D).

deterioration in the structural and mechanical properties of bone and to a decrease in bone strength (25–28). The recognition of the role of the osteocyte-produced sclerostin in bone metabolism has stimulated a number of studies on the effects of this protein on bone strength. These studies have been enabled by the development of validated commercial assays for measuring it in humans.

The recent report by Drake *et al.* (29), which showed a significant correlation between bone marrow plasma and serum concentration of sclerostin, confirmed that although sclerostin is a locally active molecule, circulating levels are of clinical relevance and can be used to explore the association between serum sclerostin and indexes of bone metabolism in patients with various bone disorders. Studies in patients with disorders of parathyroid gland function (30) and in immobilized subjects (31) as well as in diabetic patients (6, 15, 16) have detected high circulating levels of sclerostin, associated with bone turnover markers in some studies (30–32) but not in others (15). These previous studies, however, do not provide information on the bioactivity of circulating sclerostin, nor do they prove the pathophysiological significance of high levels of sclerostin in a clinical setting.

Our finding that the increase of sclerostin in T2DM patients is significantly associated with a reduction of circulating β-catenin may provide further *in vivo* evidence of how sclerostin works in the proposed model of bone mass regulation by LRP receptors (33). Although there are many unanswered questions regarding the role of LRP5 in bone, it is clear that sclerostin is a direct antagonist of Wnt signaling. Wnt ligand binds to the LRP5/LRP6, promoting the activation of the intracellular protein Disheveled. Activated Disheveled inhibits GSK-3β with consequent disassociation of the multiprotein degradation complex and inhibition of β-catenin degradation. Sclerostin is a ligand for the LRP5/LRP6 complex and competes with Wnt, preventing its binding. Downstream of the LRP is the key signaling molecule β-catenin, which has been shown to be important in osteoblast differentiation, proliferation, and apoptosis (34). The unimpeded GSK-3β combines with the multiprotein complex, phosphorylating β-catenin and leads to its degradation (35, 36). If this proposed pattern holds *in vivo* in humans, our observation that serum levels of B-ALP (which reflect osteoblast activity and bone formation) are reduced in T2DM patients may account for a disruption of the Wnt/β-catenin signaling pathway, with a consequent impairment of the regulation of the transcriptional activity of several genes in the osteoblast, including B-ALP (37). This hypothesis is in line with the observation by Devarajan-Ketha *et al.* (38), who observed that sclerostin inhibits the bioactivity of alkaline phosphatase in a rat bone model. Our T2DM patients also had a serum concentration of Dkk-1 higher than controls. Dkk-1 is a well-recognized Wnt signaling inhibitor with direct binding affinity to LRP5. Although the LRP-mediated regulation of such a major osteometabolic pathway is expected to have a number of modulators, the role of Dkk-1 in diabetes remains to be clarified. The lack of a significant correlation between the Dkk-1 and β-catenin levels in fact argues against a major contribution of this protein to the impairment of the Wnt-signaling pathway in this setting.

In our T2DM patients, we observed a decrease in the bone resorption marker CTX. In patients with sclerosteosis (a bone sclerosing dysplasia caused by loss of function mutation in the *SOST* gene encoding for sclerostin), the lack of sclerostin leads to unrestrained bone formation, characterized by high levels of both procollagen type 1 amino-terminal propeptide (a marker of osteoblast activity) and β-CTX (a marker of bone resorption) (39). Conversely, it is reasonable to assume that enhanced sclerostin production may lead to a generalized reduction in bone

turnover. The mechanism by which T2DM is associated with increased levels of sclerostin remains obscure. First, it is not clear whether this increase occurs as a result of increased synthesis rather than decreased degradation or clearance of glycosylated or glycated molecules. Second, because sclerostin levels are tightly regulated by mechanical strain, its concentration increases during skeletal unloading in the mice (40) and after prolonged immobilization in man (31). In our study we evaluated the physical functioning by the SF-36 and observed similar values in T2DM patients and controls, but the hypothesis that the increase of sclerostin serum levels could be contributed to by decreased mechanical loading of the skeleton in T2DM patients still needs to be explored. Human and animal studies have shown that PTH is a negative regulator of sclerostin (29, 41). Serum sclerostin levels are higher in hypoparathyroidism than in primary hyperparathyroidism and normal controls (30). In addition, either intermittent or continuous infusion of PTH 1–34 decreases serum sclerostin levels in postmenopausal women and in healthy subjects (29, 42). In T2DM patients, PTH serum levels are reported either lower (24) or slightly higher than in controls (16), probably due to lower 25OHD levels; however, sclerostin levels remain paradoxically high. It would seem reasonable to hypothesize that the catabolic actions of PTH (not measured in the present study) predominate in T2DM, with little or no influence on the sclerostin-mediated Wnt-signaling pathway associated with its anabolic action (32). Sclerostin serum levels are also influenced by age (6, 16). However, as in our study, sclerostin was positively associated with age in both groups, and age does not help explain the difference observed between patients and age-matched control subjects.

A potential limitation in the interpretation of the results is the lack of standardization of sclerostin assay. McNulty *et al.* (43) examined the performance of two commercially available immunoassays kits for sclerostin and found different concentrations of this protein in both serum and plasma samples. However, because the same sclerostin assay was used in our study for comparing T2DM patients and controls, this limitation could be minimized. New evidence shows that Wnt signaling has a role in endocrine pancreas development and in regulating the function of mature β-cells, including insulin secretion (7). A discussion on this particular point is beyond the scope of the present study, although the fact that several components of the canonical Wnt-signaling pathway are also members of other signaling pathways in β-cells, suggests that a physiological regulation of glucose metabolism requires intact Wnt-signaling pathways. Osteocytes, which are now recognized as active regulators of almost every phase of mineral handling by bone, may thus participate also in glucose homeostasis by the osteocyte-secreted product, sclerostin.

Our observational study has some limitations. First, these analyses are cross-sectional, and can show only association. Second, we studied a small group of subjects, possibly resulting in reduced statistical power. Third, the T2DM population has a mean age of 63.68 $\pm$ 8.39 yr; the pathophysiological model proposed should therefore be confirmed in a younger population. More research is needed to attempt to connect these preliminary observations with other established pathways of calcium and glucose metabolism in a consistent unique physiological paradigm.

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