Serum Sclerostin and Bone Turnover in Latent Autoimmune Diabetes in Adults

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Purpose: Bone formation is impaired in both type 1 diabetes and type 2 diabetes (T2D), whereas sclerostin, an antagonist of bone formation, is increased in T2D only. No data are available on latent autoimmune diabetes in adults (LADA), an autoimmune type of diabetes that may clinically resemble T2D at diagnosis. We evaluated serum sclerostin and bone turnover markers in LADA compared with those in T2D and whether metabolic syndrome (MetS) affects sclerostin in T2D or LADA.

Methods: This cross-sectional study included 98 patients with T2D and 89 with LADA from the Action LADA and Non Insulin Requiring Autoimmune Diabetes cohorts. Patients were further divided according to MetS status. Nondiabetic participants (n = 53) were used as controls. Serum sclerostin, bone formation (pro-collagen type 1 N-terminal propeptide [P1NP]), and bone resorption (C-terminal telopeptide of type I collagen [CTX]) were analyzed.

Results: Patients with T2D had higher sclerostin than did those with LADA [$P = 0.0008$, adjusted for sex and body mass index (BMI)], even when analysis was restricted to patients with MetS (adjusted $P = 0.03$). Analysis of T2D and LADA groups separately showed that sclerostin was similar between those with and those without MetS. However, a positive trend between sclerostin and number of MetS features was seen with T2D (P for trend = 0.001) but not with LADA. Patients with T2D or LADA had lower CTX than did controls ($P = 0.0003$) and did not have significantly reduced P1NP. Sclerostin was unrelated to age or hemoglobin A1c but was correlated with BMI ($\rho = 0.29$; $P = 0.0001$), high-density lipoprotein ($\rho = -0.23$; $P = 0.003$), triglycerides ($\rho = 0.19$; $P = 0.002$), and time since diagnosis ($\rho = 0.32$; $P < 0.0001$).

Conclusions: Patients with LADA presented lower bone resorption than did controls, similar to patients with T2D. Sclerostin is increased in T2D but not in LADA, suggesting possible roles on bone metabolism in T2D only. (***J Clin Endocrinol Metab*** **103: 1921–1928, 2018)**

Both type 1 diabetes (T1D) and type 2 diabetes (T2D) are associated with impaired bone metabolism and increased risk for fractures (1). A large variety of mechanisms have been proposed, including low bone turnover (2, 3).

The mechanism of reduced bone formation in diabetes is unclear, but emerging evidence implicates elevated levels of sclerostin. Sclerostin is a glycoprotein produced by osteocytes and is an antagonist of the osteoblastic

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Abbreviations: BMI, body mass index; CTX, C-terminal telopeptide of type I collagen; ELISA, enzyme-linked immunosorbent assay; GADA, glutamic acid decarboxylase autoantibodies; HDL, high-density lipoprotein; LADA, latent autoimmune diabetes in adults; MetS, metabolic syndrome; NIRAD, Non Insulin Requiring Autoimmune Diabetes; P1NP, pro-collagen type 1 N-terminal propeptide; T1D, type 1 diabetes; T2D, type 2 diabetes.

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bone formation through the inhibition of the Wnt/β-catenin pathway (4, 5). Clinical studies have consistently shown raised sclerostin levels in T2D (6–9). Although low bone formation is a feature of both T1D and T2D, clinical and experimental evidence has shown that sclerostin levels and/or expression are increased in T2D (10) but not in T1D (11). Furthermore, sclerostin is associated with clinical or biochemical surrogates of insulin resistance (a key feature of T2D), such as increased body weight or body mass index (BMI) (7) and the homeostasis model assessment–estimated insulin resistance index (12).

Latent autoimmune diabetes in adults (LADA) is an autoimmune type of adult-onset diabetes characterized by insulin independence at the time of diagnosis and positivity to circulating islet autoantibodies, most commonly glutamic acid decarboxylase autoantibodies (GADA) (13). Patients with LADA may resemble those with T2D at the onset because of insulin independence and the increased prevalence of metabolic syndrome (MetS) compared with T1D, representing about 10% of adults initially misdiagnosed as having T2D. Many immunologic and genetic features in LADA are similar to those found in T1D; thus, this form of diabetes is classified as a subtype of T1D. Recent efforts by the Action LADA and the Non Insulin Requiring Autoimmune Diabetes (NIRAD) study projects have improved the understanding of the biochemical, immunologic, metabolic, and clinical characteristics associated with LADA (14–17). To our knowledge, however, bone metabolic features of patients with LADA have been not investigated.

The aim of this study was to evaluate serum sclerostin and bone turnover markers in LADA in comparison with T2D. LADA is clearly different from T2D, in that LADA is associated with *HLA* genes, islet autoantibodies, reduced insulin secretion, and lower prevalence of MetS. On the basis of this and the evidence that Wnt is differently regulated in T1D compared with T2D, we hypothesized that patients with LADA would have a compromised skeletal phenotype, different from that in patients with T2D, and closer to that seen for T1D. Given the relatively high prevalence of MetS in both LADA and T2D and the potential impact of MetS features on sclerostin and bone turnover, we also studied whether sclerostin or bone turnover are affected by MetS associated with these two types of diabetes; our hypothesis was that they would be. We studied patients with LADA and T2D who had similar age, disease duration, and prevalence of MetS. Compared with T1D, LADA is the ideal model for studying bone turnover in the context of autoimmune diabetes because it is not affected by the juvenile age of onset, impaired peak bone mass, and insulin therapy typical of T1D.

Methods

Patients

This was a cross-sectional study involving 89 patients with LADA and 98 with T2D. Serum samples from patients with LADA and those with T2D were obtained from the European Union Action LADA project (14) and the NIRAD study in Italy (18). Samples were selected where sufficient serum and data were available from four Action LADA centers in Spain, France, Belfast, and London and from the NIRAD group in Italy. The Action LADA multicenter study was performed to identify immune and clinical risk factors for adult-onset autoimmune diabetes, including its epidemiology, genetic susceptibility, metabolic characteristics, and clinical progression (13). Diabetes was designated according to standard criteria, and patients with LADA were defined as patients aged 30 to 70 years with GADAs who did not require insulin treatment for at least 6 months after diagnosis (13, 19). Patients came from Europe, and almost all of them were white (96% white, 3% Asian, 1% African, and 1% mixed race).

The NIRAD study is a nationwide survey sponsored by the Società Italiana di Diabetologia; its aim is to assess the prevalence and characteristics of autoimmune diabetes in adult patients attending diabetes clinics in Italy with a clinical diagnosis of non–insulin-requiring diabetes. Patients with adult-onset autoimmune diabetes were selected by using the following inclusion criteria: (1) an initial diagnosis of T2D according to the American Diabetes Association; (2) documented antibody positivity for GADA and/or tyrosine phosphatase–related islet antigen 2 autoantibodies (20); (3) no insulin requirement and no evidence of ketosis from diagnosis to screening time; and (4) time since diagnosis of 6 months to 5 years. All patients from the NIRAD study were unrelated and of exclusively Italian origin (with parents and grandparents of Italian origin). Exclusion criteria included prior insulin therapy, pregnancy, renal disease with a raised creatinine level or proteinuria, and the presence of any other severe disease. MetS was assessed according to the National Cholesterol Education Program criteria, as described below.

The T2D and LADA samples were selected where sufficient sample volume was available, aiming at ensuring a numeric balance among the following four groups: LADA with MetS, LADA without MetS, T2D with MetS, and T2D without MetS. There was no other selection at all. We selected from the Action LADA cohort patients with LADA who had MetS (n = 31) and those with LADA who did not have MetS (n = 29); from the NIRAD group, we selected patients with LADA who had MetS (n = 11) and patients with LADA who did not have MetS (n = 17). Overall, selected patients with T2D were limited by availability of sufficient sera (n = 60 from the Action LADA and n = 38 from the NIRAD group) and were of similar age, sex, and disease duration as the patients with LADA.

Waist circumference and blood pressure, which was measured at least twice in the sitting position, were assessed in each patient. Lipids and lipoproteins (total and high-density lipoprotein [HDL] cholesterol, triglycerides) were determined by standardized assays at each center. Patients with T2D or LADA were divided into four groups according to the presence or absence of MetS: (1) T2D with MetS (n = 57); (2) T2D without MetS (n = 41); (3) LADA with MetS (n = 42); and (4) LADA without MetS (n = 47). Sera from 53 individuals without diabetes (fasting blood glucose < 126 mg/dL) were used as

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control. Patients treated with thiazolidinediones or sodium glucose transporter 2 inhibitors were not included in this study. Control participants were recruited through the Endocrinology outpatient clinics of Universitá Campus Bio-Medico di Roma. Patients with diseases (*e.g.*, osteoporosis, hyperparathyroidism, hyper- or hypothyroidism, chronic kidney disease) or drugs (*e.g.*, glucocorticoids, bisphosphonates) known to affect bone metabolism were excluded.

Diagnostic criteria for MetS

MetS was assessed according to the National Cholesterol Education Program criteria (21), with modifications by the American Heart Association/National Heart, Lung, and Blood Institute (22), as follows: waist circumference > 102 cm in men and > 88 cm in women, triglycerides ≥ 150 mg/dL; HDL cholesterol < 40 mg/dL in men and < 50 mg/dL in women, blood pressure ≥ 130/85 mm Hg or use of antihypertensive medication, and fasting glucose ≥ 100 mg/dL. All diabetic patients in this study were identified as fulfilling the criteria for hyperglycemia. MetS was defined by the presence of three of five criteria, including blood glucose.

Sclerostin and bone turnover markers

Sclerostin serum levels were assessed by quantitative sandwich enzyme-linked immunosorbent assay (ELISA; Biomedica, Vienna, Austria). Bone turnover was evaluated by analyzing serum levels of a bone formation marker, the total procollagen type 1 *N*-terminal propeptide (P1NP), by ELISA (Biomedica) and a bone resorption marker, the C-terminal telopeptide of type I collagen (CTX), by ELISA (IDS, Boldon, United Kingdom). P1NP is a specific marker of bone formation, and CTX is an accurate marker of bone resorption. CTX and P1NP have been suggested by the International Osteoporosis Foundation as the appropriate bone markers for exploring bone resorption and formation in clinical and research settings (23).

Sample size and power calculation

Sample size was calculated for the primary aim on the hypothesis that circulating sclerostin would differ between T2D and LADA. The calculation was based on previously published data on increased circulating sclerostin in patients with T2D compared with nondiabetic controls (8). With a significance level of 0.05 and 80% power, the minimum sample size required was of 46 patients per group (T2D, LADA, and controls). To investigate the relationship between sclerostin and MetS status, the sample size was further increased on the basis of available serum and clinical/biochemical information on MetS status.

Statistical analysis

Continuous variables are presented as mean ± standard deviation. Normality was tested with the Shapiro-Wilk test. When data were not normally distributed, logarithmic transformation was performed. Categorical variables are expressed as absolute frequencies. For continuous variables, mean differences across groups were compared by generalized linear models/analysis of variance for normally distributed variables. Homoscedasticity was tested with the Levene and Brown-Forsythe test. For *post hoc* analyses, Tukey and Games-Howell tests were applied. Pearson (normal distribution) and Spearman (non-normal distribution) correlations were used to assess the correlations between serum sclerostin levels and other continuous variables. Multiple backward model linear regression analyses were performed to identify independent predictors of serum sclerostin levels (dependent variable, square root transformed) in the overall population and in the LADA and T2D groups. The models included time since diagnosis, BMI, HDL cholesterol levels, and triglycerides levels; age and sex were not included in the multiple linear regression model because they were not correlated with serum sclerostin levels. Furthermore, after adjustment for age and sex, the results did not change. A two-tailed P value < 0.05 was considered to indicate a statistically significant difference. A multiplicity adjusted P value was reported when multiple comparisons were performed. Data were analyzed with SAS software, version 9.4 (SAS Institute Inc., Cary, NC).

Results

Features of the studied population

Clinical and biochemical features of patients with T2D and LADA and nondiabetic controls are shown in Table 1. Overall, patients with LADA were younger and leaner than those with T2D [median BMI, 24.7 (range, 17.6 to 41.8) vs 28.3 (range, 18.0 to 44.5) kg/m^2, respectively; $P < 0.0001$], whereas time since diagnosis was similar in patients with LADA and T2D [median disease duration, 2.9 (range, 0 to 7) vs 2.0 (range, 0 to 11) years; $P = 0.25$). However, when divided according to presence or absence of MetS, the four groups were similar in terms of age and time since diagnosis (Table 1). The reference nondiabetic group consisted of 53 patients (36 women) without diabetes [age, 48.2 ± 21.1 years; median BMI, 26.2 (range, 18.5 to 41.0) kg/m^2]. Nondiabetic patients were of similar age to those with LADA but younger than those with T2D ($P < 0.001$); the nondiabetic control group consisted of more women than did the LADA and T2D groups ($P < 0.01$). The BMI of controls was not significantly different from that of patients with LADA or T2D. Five of 53 controls (9.4%) had impaired fasting glucose, 9 of 53 (17%) had a diagnosis of primary hypertension, 5 of 53 (9.4%) had dyslipidemia, and 9 of 53 (17%) were obese (BMI > 30 kg/m^2).

Circulating sclerostin in LADA and T2D

Patients with T2D had higher serum sclerostin than did patients with LADA or controls [29.8 ± 11.9 vs 23.0 ± 11.8 vs 24.3 ± 5.7 pmol/L; $P = 0.0001$ ($P \leq 0.002$ adjusted for sex and BMI)] (Fig. 1). In the combined group of diabetic patients, sclerostin tended to be higher in the group with MetS, but this finding was not significant (25.0 ± 12.7 vs 28.3 ± 12.8 pmol/L; $P = 0.08$). Among patients with MetS, serum sclerostin was higher in those with T2D than those with LADA [$P = 0.01$ ($P = 0.03$ adjusted for sex and BMI)]. When the T2D and LADA groups were analyzed separately, both groups had similar serum sclerostin between patients with and those without MetS ($P \geq 0.15$). However, when all diabetic

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Table 1. Clinical Characteristics of Patients With LADA and T2D, Categorized according to MetS Status, and of Nondiabetic Controls

	1	2	3	4	5		
	T2D		LADA				
Characteristic	With MetS	Without MetS	With MetS	Without MetS	Nondiabetic Controls	*P* Value Between T2D and LADA	*P* Value vs Group 5
Patients (n)	57	41	42	47	53		
Men/women (n/n)[a]	35/22	24/17	22/20	27/20	17/36	NS	<0.05
Age (y)[b]	52.7 ± 9.6	52.4 ± 9.2	51.5 ± 11.1	47.5 ± 11.2	48.2 ± 21.1	NS	NS
Time since diagnosis (y)[b]	2.9 ± 1.7	2.5 ± 1.8	2.7 ± 1.9	2.4 ± 2.3	NA	NS	NA
Waist circumference (cm)[b]	104.9 ± 9.1	90.2 ± 12.8	98.7 ± 12.4	82.3 ± 11.4	90.0 (24.0)	<0.0001	≤0.01
BMI (kg/m^2)[c]	30.4 (6.3)	25.6 (5.9)	27.6 (7.7)	22.6 (4.6)	26.2 (6.7)	<0.0001	<0.0001
HbA1c (%)[b]	6.7 ± 1.4	6.5 ± 1.2	7.9 ± 1.5	7.2 ± 1.5	NA	NS	NA
Creatinine (mg/dL)[b]	0.79 ± 0.13	0.80 ± 0.08	0.95 ± 0.26	0.85 ± 0.20	0.81 ± 0.17	NS	NS
Triglycerides (mg/dL)[c]	157.0 (139.0)	101.9 (35.4)	165.0 (124.0)	70.0 (31.3)	97.0 (53.0)	<0.0001	<0.0001
HDL cholesterol (mg/dL)[c]	37.1 (13.0)	52.6 (19.4)	46.4 (20.9)	65.0 (22.6)	54.5 (22.5)	<0.0001	<0.0001
Systolic blood pressure (mm Hg)[b]	134.9 ± 12.0	123.1 ± 13.4	134.3.1 ± 16.9	120.0 ± 11.1	123.7 ± 10.9	<0.0001	<0.0001
Diastolic blood pressure (mm Hg)[c]	80.0 (10.0)	80.0 (11.0)	77.0 (20.0)	75.5 (10.0)	75.0 (10.0)	0.005	NS

Waist circumference: 1 vs 2, 1 vs 4, 2 vs 3, 2 vs 4, 3 vs 4, $P \leq 0.03$; BMI: 1 vs 2, 1 vs 3, 1 vs 4, 2 vs 4 and 3 vs 4, $P \leq 0.019$; triglycerides: 1 vs 2, 1 vs 3, 2 vs 3, 2 vs 4 and 3 vs 4, $P \leq 0.01$; HDL cholesterol: 1 vs 2, 1 vs 4, 2 vs 4 and 3 vs 4, $P \leq 0.007$; systolic blood pressure: 1 vs 2, 1 vs 4, 2 vs 3 and 3 vs 4, $P \leq 0.007$; diastolic blood pressure: 1 vs 2 and 1 vs 4, $P \leq 0.048$.

Abbreviations: HbA1c, hemoglobin A1c; NA, not available/not applicable; NS, not significant.

[a]Data are expressed as absolute frequencies.

[b]Data are expressed as mean ± standard deviation.

[c]Data are log trasformed and are expressed as median (interquartile range).

individuals were included in the analysis and divided into four groups according to diabetes type and MetS (namely, LADA without MetS, LADA with MetS, T2D without MetS, T2D with MetS), we found a trend for increased sclerostin from LADA without MetS toward T2D with MetS ($P < 0.0001$ for trend) (Fig. 2). In the whole group of diabetic patients, sclerostin progressively increased with the number of MetS features ($P = 0.002$ for trend); when analysis was performed according to diabetes type, sclerostin increased with the number of MetS features in patients with T2D ($P = 0.001$ for trend) but not in those with LADA.

Bone turnover markers in LADA and T2D

Patients with T2D had 11% and 13% lower P1NP compared with patients with LADA or nondiabetic controls, respectively, although the differences were not significant (57.3 ± 16.6 vs 64.2 ± 19.5 vs 66.5 ± 25.2 pg/mL, respectively). The bone resorption marker CTX was 43% lower in patients with diabetes, either T2D or LADA, than in nondiabetic controls (0.16 ± 0.06 vs 0.16 ± 0.10 vs 0.28 ± 0.16 ng/mL, respectively; $P = 0.0003$) (Fig. 3). When LADA and T2D were divided according to the presence of MetS, we found no significant differences in P1NP or CTX levels across the four groups. Levels of bone turnover markers were unrelated to age, time since diagnosis, BMI, or other clinical and biochemical parameters in all study groups. Bone turnover markers were not correlated with serum sclerostin ($0.006 < \rho < 0.04$; $P \geq 0.67$) in all study groups.

Relationship of sclerostin with clinical and biochemical features

In the control group, serum sclerostin were unrelated with age ($\rho = 0.08$; $P = 0.63$), BMI ($\rho = -0.06$; $P = 0.73$), or blood glucose ($\rho = 0.21$; $P = 0.22$). In the overall cohort of patients with diabetes, serum sclerostin was similar between men and women ($P = 0.92$) and was unrelated to age ($\rho = 0.05$; $P = 0.49$), hemoglobin A1c ($\rho = 0.27$; $P = 0.053$), or creatinine ($\rho = -0.18$; $P = 0.16$) but increased significantly with BMI ($\rho = 0.29$; $P = 0.0001$), time since diagnosis ($\rho = 0.32$; $P < 0.0001$), and triglycerides ($\rho = 0.19$; $P = 0.02$) and was inversely correlated with HDL cholesterol ($\rho = -0.23$; $P = 0.003$). Multiple regression analysis of the overall population with diabetes showed that time since diagnosis ($\beta = 0.19$; $P = 0.002$) and triglycerides ($\beta = 0.003$; $P = 0.03$), but not BMI and HDL cholesterol, were independent predictors of sclerostin levels (Supplemental Table 1). Altogether, time since diagnosis and triglycerides explained 11% of sclerostin variance.

Discussion

This study reports on sclerostin and bone turnover in patients affected with LADA. We found that sclerostin is

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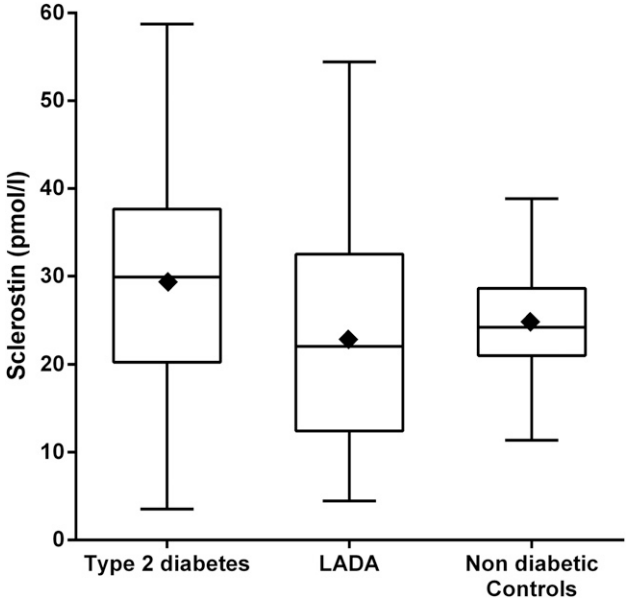


Figure 1. Sclerostin in patients with T2D, patients with LADA, and nondiabetic controls. Patients with T2D had higher sclerostin than did patients with LADA ($P = 0.0007$) or controls ($P = 0.002$). Box plots show the 25th and 75th percentiles, and the horizontal line shows the median (50th percentile). Bars outside the box indicate the minimum and maximum value. Diamond symbol represents the mean.

increased in T2D but not in LADA, whereas the bone resorption marker CTX was equally reduced, compared with controls, in both types of diabetes. These data indicate that low bone resorption is a feature of both LADA and T2D. MetS did not affect bone turnover markers in either LADA or T2D. In contrast, sclerostin was positively associated with the number of MetS features in patients with T2D, suggesting a relationship between MetS severity and T2D.

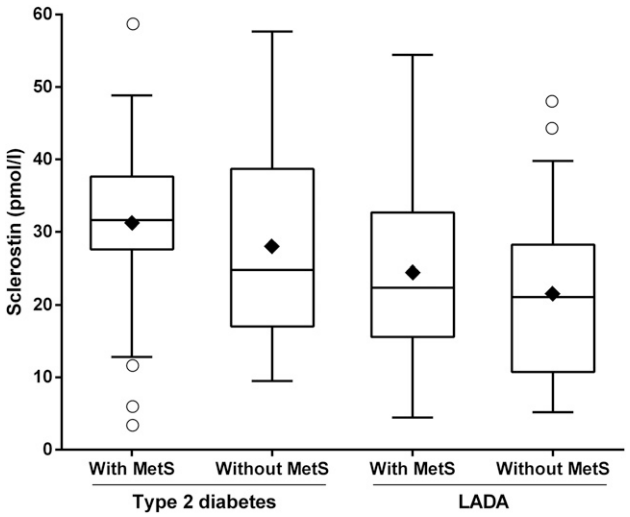


Figure 2. Sclerostin in T2D and LADA according to MetS status. When T2D and LADA were analyzed separately, in both groups the presence of MetS did not significantly influence serum sclerostin ($P \geq 0.15$); however, in patients with MetS, serum sclerostin was higher among those with T2D than those with LADA ($P = 0.01$). Patients with T2D who had MetS had higher sclerostin than did patients with LADA who did not have MetS ($P = 0.001$). A trend for increased sclerostin across the four groups is shown, from LADA without MetS to T2D with MetS ($P < 0.0001$ for trend). Box plots show the 25th and 75th percentiles, and the horizontal line shows the median (50th percentile). Bars outside the box indicate the minimum and maximum value. Circle and diamond symbols represent outliers and mean, respectively.

This study found that bone resorption is reduced in patients with LADA compared with controls. Previous studies have shown consistently lower levels of CTX and the bone formation marker osteocalcin in patients with T1D and T2D compared with controls, regardless of diabetes type, suggesting that both bone resorption and formation are reduced in these types of diabetes (24, 25). Our data follow a similar trend, showing that patients with LADA, as well as patients with T2D, have lower CTX than do controls. As highlighted by a recent meta-analysis, most studies have reported reduced P1NP levels in patients with T2D compared with nondiabetic controls (24). In our study, we found a nonsignificant reduction of P1NP in patients with T2D compared with those with LADA and nondiabetic controls. However, the magnitude of P1NP reduction (>10%) was similar to that reported by the meta-analysis of Hygum *et al.* (24) in a comparison of patients with T2D to nondiabetic controls. This may suggest that the P1NP difference found in our study did not reach statistical significance, probably because of increased variance among the groups or the small sample size.

Despite the similar reduction in bone turnover, we found that serum sclerostin was increased in T2D but not in LADA. The role of sclerostin in diabetic bone turnover is controversial, and there are no data in LADA so far. According to the literature, our data clearly mirror those reported by other groups in T1D. Patients with T1D have sclerostin levels similar to those reported previously (7, 26, 27) or only slightly higher than in controls (28). Conversely, increased sclerostin has been consistently found in individuals with T2D (8, 29, 30). In a recent meta-analysis, the magnitude of sclerostin increase in comparison with controls was four times higher than that reported for T1D (24). In the study by Gennari *et al.* (7), sclerostin was increased in patients with T2D but not in those with T1D despite the similar reduction of bone turnover in both groups. This is consistent with the experimental evidence that sclerostin expression is downregulated in a mouse model of T1D (11), whereas the *SOST*/sclerostin gene is upregulated in T2D rats (10). Taken together, these findings may support a different role for sclerostin in the impairment of bone metabolism associated with T2D or autoimmune diabetes (including LADA and T1D). Drake *et al.* (31) have shown that peripheral serum sclerostin correlates with bone marrow plasma levels. Although sclerostin is a locally active molecule, we may speculate that circulating levels could reflect activity in the bone microenvironment. In T2D, the

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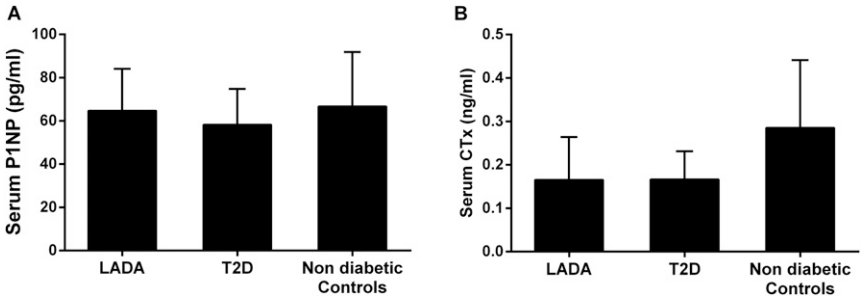


Figure 3. Bone turnover markers in patients with T2D, patients with LADA, and nondiabetic controls. (A) Patients with T2D had 11% and 13% lower P1NP compared with patients with LADA or nondiabetic controls, respectively, although the differences were not significant. (B) The bone reabsorption marker CTX was significantly reduced in patients with either type of diabetes compared with controls ($P \leq 0.006$). Data are presented as mean ± standard deviation.

increased sclerostin release by osteocytes may attenuate osteoclastogenesis via inhibition of the canonical Wnt pathway. On the other hand, this pathway may not be significantly affected by the autoimmune types of diabetes (LADA and T1D), where other T1D/LADA-specific elements, such as insulin deficiency (32), autoimmunity (33, 34), or an intrinsic osteoblast defect, may be the primary cause. This is plausible considering that T1D, T2D, and LADA present many differences in genetic, metabolic, and immunologic profile. It follows that bone metabolism/turnover is an additional element that can differentiate these forms of diabetes.

MetS is more common in T2D than in autoimmune diabetes, and its features are clearly linked to insulin resistance (35). In this study, sclerostin and bone turnover markers were similar between patients with MetS and those without MetS, regardless of diabetes type. However, when all diabetic individuals were included in the analysis and divided into four groups according to diabetes type and MetS (namely, LADA without MetS, LADA with MetS, T2D without MetS, T2D with MetS), we found a trend for increased sclerostin from LADA without MetS toward T2D with MetS. Such increase may reflect an association with the progressive increase in insulin resistance across the four groups analyzed, where LADA without MetS is the group with the lowest degree of insulin resistance, whereas T2D with MetS is the group characterized by the highest degree of insulin resistance, respectively. Of note, in patients with T2D we found a correlation between sclerostin and the number of MetS features. These observations may partially resemble those provided by Daniele *et al.* (12), who showed a correlation between sclerostin and insulin resistance in skeletal muscle, liver, and adipose tissue. Sclerostin has been studied in association with other features of MetS, such as body weight, providing mixed results. A positive correlation with body weight or BMI has been shown in one report (7), whereas others have found no association (6, 9), such as in the current study, or even increased levels in response to weight loss, as also recently shown by our group (36).

According to multivariate analysis, time since diagnosis (which is an estimate of disease duration) was the strongest predictor of sclerostin levels. This may imply that when diabetes progresses, inhibition of Wnt/β-catenin pathway by sclerostin becomes more substantial, leading to impaired bone turnover. Although bone turnover markers did not correlate with disease duration in our study, other researchers have shown a negative correlation between diabetes duration and markers of bone formation (37) and resorption (38).

Our study has several strengths and limitations. This analysis of bone turnover and sclerostin was performed in a large and well-characterized population of patients with LADA. The study population consisted of patients with relatively short time since diagnosis without chronic complications. The significance of our findings may be limited by the lack of bone mineral density measurements and by the cross-sectional nature of the study. An additional limitation is the lack of data on postmenopausal status, vitamin D status, or treatment with bisphosphonates or other bone-active drugs in patients with T2D and LADA, all factors that may alter bone turnover and/or sclerostin levels. Additional studies with larger cohorts (cross-sectional and longitudinal and at-risk individuals) are required to assess the role of sclerostin and the Wnt pathway on bone turnover and fragility in diabetes.

In conclusion, our findings indicate that bone resorption is reduced in patients with T2D and LADA compared with nondiabetic controls, whereas circulating sclerostin is increased in patients with T2D only. These data suggest that pathways involved in bone metabolism are different between the two types of diabetes. Furthermore, MetS does not seem to affect bone turnover in either types of diabetes, whereas its features may additively influence sclerostin in T2D. Larger longitudinal studies are needed to confirm these findings and to explore the potential role of sclerostin and Wnt pathway on bone fragility associated with diabetes.

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References

1. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL; IOF Bone and Diabetes Working Group. Mechanisms of diabetes mellitus-induced bone fragility. *Nat Rev Endocrinol*. 2017;**13**(4):208–219.
2. Napoli N, Strollo R, Paladini A, Briganti SI, Pozzilli P, Epstein S. The alliance of mesenchymal stem cells, bone, and diabetes. *Int J Endocrinol*. 2014;**2014**:690783.
3. Epstein S, Defeudis G, Manfrini S, Napoli N, Pozzilli P; Scientific Committee of the First International Symposium on Diabetes and Bone. Diabetes and disordered bone metabolism (diabetic osteodystrophy): time for recognition. *Osteoporos Int*. 2016;**27**(6): 1931–1951.
4. Semënov M, Tamai K, He X. SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. *J Biol Chem*. 2005;**280**(29):26770–26775.
5. Sutherland MK, Geoghegan JC, Yu C, Turcott E, Skonier JE, Winkler DG, Latham JA. Sclerostin promotes the apoptosis of human osteoblastic cells: a novel regulation of bone formation. *Bone*. 2004;**35**(4):828–835.
6. Yamamoto M, Yamauchi M, Sugimoto T. Elevated sclerostin levels are associated with vertebral fractures in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2013;**98**(10):4030–4037.
7. Gennari L, Merlotti D, Valenti R, Ceccarelli E, Ruvio M, Pietrini MG, Capodarca C, Franci MB, Campagna MS, Calabrò A, Cataldo D, Stolakis K, Dotta F, Nuti R. Circulating sclerostin levels and bone turnover in type 1 and type 2 diabetes. *J Clin Endocrinol Metab*. 2012;**97**(5):1737–1744.
8. Gaudio A, Privitera F, Battaglia K, Torrisi V, Sidoti MH, Pulvirenti I, Canzonieri E, Tringali G, Fiore CE. Sclerostin levels associated with inhibition of the Wnt/β-catenin signaling and reduced bone turnover in type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2012; **97**(10):3744–3750.
9. García-Martín A, Rozas-Moreno P, Reyes-García R, Morales-Santana S, García-Fontana B, García-Salcedo JA, Muñoz-Torres M. Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2012;**97**(1):234–241.
10. Nuche-Berenguer B, Moreno P, Portal-Nuñez S, Dapía S, Esbrit P, Villanueva-Peñacarrillo ML. Exendin-4 exerts osteogenic actions in insulin-resistant and type 2 diabetic states. *Regul Pept*. 2010; **159**(1-3):61–66.
11. Portal-Núñez S, Lozano D, de Castro LF, de Gortázar AR, Nogués X, Esbrit P. Alterations of the Wnt/beta-catenin pathway and its target genes for the N- and C-terminal domains of parathyroid hormone-related protein in bone from diabetic mice. *FEBS Lett*. 2010;**584**(14):3095–3100.
12. Daniele G, Winnier D, Mari A, Bruder J, Fourcaudot M, Pengou Z, Tripathy D, Jenkinson C, Folli F. Sclerostin and insulin resistance in prediabetes: evidence of a cross talk between bone and glucose metabolism. *Diabetes Care*. 2015;**38**(8):1509–1517.
13. Leslie RD, Kolb H, Schloot NC, Buzzetti R, Mauricio D, De Leiva A, Yderstraede K, Sarti C, Thivolet C, Hadden D, Hunter S, Schernthaner G, Scherbaum W, Williams R, Pozzilli P. Diabetes classification: grey zones, sound and smoke: action LADA 1. *Diabetes Metab Res Rev*. 2008;**24**(7):511–519.
14. Hawa MI, Thivolet C, Mauricio D, Alemanno I, Cipponeri E, Collier D, Hunter S, Buzzetti R, de Leiva A, Pozzilli P, Leslie RD; Action LADA Group. Metabolic syndrome and autoimmune diabetes: action LADA 3. *Diabetes Care*. 2009;**32**(1):160–164.
15. Pham MN, Hawa MI, Pfleger C, Roden M, Schernthaner G, Pozzilli P, Buzzetti R, Scherbaum WA, Seissler J, Kolb H, Hunter S, Leslie RD, Schloot NC; Action LADA Study Group. Pro- and anti-inflammatory cytokines in latent autoimmune diabetes in adults, type 1 and type 2 diabetes patients: action LADA 4. [published correction appears in *Diabetologia*. 2012;55(2):534] *Diabetologia*. 2011;**54**(7):1630–1638.
16. Zampetti S, Capizzi M, Spoletini M, Campagna G, Leto G, Cipolloni L, Tiberti C, Bosi E, Falorni A, Buzzetti R; NIRAD Study Group. GADA titer-related risk for organ-specific autoimmunity in LADA subjects subdivided according to gender (NIRAD study 6). *J Clin Endocrinol Metab*. 2012;**97**(10):3759–3765.
17. Zampetti S, Campagna G, Tiberti C, Songini M, Arpi ML, De Simone G, Cossu E, Cocco L, Osborn J, Bosi E, Giorgino F, Spoletini M, Buzzetti R; NIRAD Study Group. High GADA titer increases the risk of insulin requirement in LADA patients: a 7-year follow-up (NIRAD study 7). *Eur J Endocrinol*. 2014;**171**(6):697–704.
18. Buzzetti R, Di Pietro S, Giaccari A, Petrone A, Locatelli M, Suraci C, Capizzi M, Arpi ML, Bazzigaluppi E, Dotta F, Bosi E; Non Insulin Requiring Autoimmune Diabetes Study Group. High titer of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. *Diabetes Care*. 2007;**30**(4):932–938.
19. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 1997;**20**(7): 1183–1197.
20. Petrone A, Suraci C, Capizzi M, Giaccari A, Bosi E, Tiberti C, Cossu E, Pozzilli P, Falorni A, Buzzetti R; NIRAD Study Group. The protein tyrosine phosphatase nonreceptor 22 (PTPN22) is associated with high GAD antibody titer in latent autoimmune diabetes in adults: Non Insulin Requiring Autoimmune Diabetes (NIRAD) Study 3. *Diabetes Care*. 2008;**31**(3):534–538.
21. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*. 2001;**285**(19):2486–2497.
22. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa FAmerican Heart AssociationNational Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National

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Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005;**112**(17):2735–2752.
23. Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A, McClung M, Morris HA, Silverman S, Trenti T, Wahl DA, Cooper C, Kanis JA; IOF-IFCC Bone Marker Standards Working Group. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int*. 2011;**22**(2):391–420.
24. Hygum K, Starup-Linde J, Harsløf T, Vestergaard P, Langdahl BL. Mechanisms in endocrinology: diabetes mellitus, a state of low bone turnover - a systematic review and meta-analysis. *Eur J Endocrinol*. 2017;**176**(3):R137–R157.
25. Starup-Linde J, Eriksen SA, Lykkeboe S, Handberg A, Vestergaard P. Biochemical markers of bone turnover in diabetes patients–a meta-analysis, and a methodological study on the effects of glucose on bone markers. *Osteoporos Int*. 2014;**25**(6):1697–1708.
26. Tsentidis C, Gourgiotis D, Kossiva L, Marmarinos A, Doulgeraki A, Karavanaki K. Sclerostin distribution in children and adolescents with type 1 diabetes mellitus and correlation with bone metabolism and bone mineral density. *Pediatr Diabetes*. 2016;**17**(4):289–299.
27. Catalano A, Pintaudi B, Morabito N, Di Vieste G, Giunta L, Bruno ML, Cucinotta D, Lasco A, Di Benedetto A. Gender differences in sclerostin and clinical characteristics in type 1 diabetes mellitus. *Eur J Endocrinol*. 2014;**171**(3):293–300.
28. Neumann T, Hofbauer LC, Rauner M, Lodes S, Kästner B, Franke S, Kiehntopf M, Lehmann T, Müller UA, Wolf G, Hamann C, Sämann A. Clinical and endocrine correlates of circulating sclerostin levels in patients with type 1 diabetes mellitus. *Clin Endocrinol (Oxf)*. 2014;**80**(5):649–655.
29. Ardawi MS, Akhbar DH, Alshaikh A, Ahmed MM, Qari MH, Rouzi AA, Ali AY, Abdulrafee AA, Saeda MY. Increased serum sclerostin and decreased serum IGF-1 are associated with vertebral fractures among postmenopausal women with type-2 diabetes. *Bone*. 2013;**56**(2):355–362.
30. van Lierop AH, Hamdy NA, van der Meer RW, Jonker JT, Lamb HJ, Rijzewijk LJ, Diamant M, Romijn JA, Smit JW, Papapoulos SE. Distinct effects of pioglitazone and metformin on circulating sclerostin and biochemical markers of bone turnover in men with type 2 diabetes mellitus. *Eur J Endocrinol*. 2012;**166**(4):711–716.
31. Drake MT, Srinivasan B, Mödder UI, Peterson JM, McCready LK, Riggs BL, Dwyer D, Stolina M, Kostenuik P, Khosla S. Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. *J Clin Endocrinol Metab*. 2010;**95**(11):5056–5062.
32. Campos Pastor MM, López-Ibarra PJ, Escobar-Jiménez F, Serrano Pardo MD, García-Cervigón AG. Intensive insulin therapy and bone mineral density in type 1 diabetes mellitus: a prospective study. *Osteoporos Int*. 2000;**11**(5):455–459.
33. Sugai E, Cherñavsky A, Pedreira S, Smecuol E, Vazquez H, Niveloni S, Mazure R, Mauriro E, Rabinovich GA, Bai JC. Bone-specific antibodies in sera from patients with celiac disease: characterization and implications in osteoporosis. *J Clin Immunol*. 2002;**22**(6):353–362.
34. Strollo R, Rizzo P, Spoletini M, Landy R, Hughes C, Ponchel F, Napoli N, Palermo A, Buzzetti R, Pozzilli P, Nissim A. HLA-dependent autoantibodies against post-translationally modified collagen type II in type 1 diabetes mellitus. *Diabetologia*. 2013;**56**(3):563–572.
35. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet*. 2010;**375**(9710):181–183.
36. Strollo R, Soare A, Manon Khazrai Y, Di Mauro A, Palermo A, Del Toro R, Fallucca S, Giovanna Belluomo M, Dugo L, Pianesi M, Pozzilli P, Napoli N. Increased sclerostin and bone turnover after diet-induced weight loss in type 2 diabetes: a post hoc analysis of the MADIAB trial. *Endocrine*. 2017;**56**(3):667–674.
37. Abd El Dayem SM, El-Shehaby AM, Abd El Gafar A, Fawzy A, Salama H. Bone density, body composition, and markers of bone remodeling in type 1 diabetic patients. *Scand J Clin Lab Invest*. 2011;**71**(5):387–393.
38. Reyes-García R, Rozas-Moreno P, López-Gallardo G, García-Martín A, Varsavsky M, Avilés-Perez MD, Muñoz-Torres M. Serum levels of bone resorption markers are decreased in patients with type 2 diabetes. *Acta Diabetol*. 2013;**50**(1):47–52.

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