High Sclerostin and Dickkopf-1 (DKK-1) Serum Levels in Children and Adolescents With Type 1 Diabetes Mellitus

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Context: Childhood type 1 diabetes mellitus (T1DM) is associated with decreased bone mass. Sclerostin and dickkopf-1 (DKK-1) are Wnt inhibitors that regulate bone formation.

Objective: To evaluate sclerostin and DKK-1 levels in T1DM children and to analyze the influence of glycemic control on bone health.

Design and setting: Cross-sectional study conducted at a clinical research center.

Participants: One hundred and six T1DM subjects (12.2 ± 4 years), 66 on multiple daily injections (MDIs) and 40 on continuous subcutaneous infusion of insulin (CSII), and 80 controls.

Results: The average bone transmission time (BTT) and amplitude-dependent speed of sound (AD-SoS) *z* scores were lower in patients with diabetes than in controls. Significantly increased DKK-1 (3593 ± 1172 *vs* 2652 ± 689 pg/mL; $P < 0.006$) and sclerostin (29.45 ± 12.32 *vs* 22.53 ± 8.29; $P < 0.001$) levels were found in patients with diabetes with respect to controls, particularly in patients on MDI compared with ones on CSII. Glycemic control was improved in CSII patients compared with MDI ones ($P < 0.001$) and was also associated with significantly higher BMI-SDS ($P < 0.002$) and BTT *z* scores ($P < 0.02$). With adjustment for age, multiple linear regression analysis of DKK-1 and sclerostin as dependent variables showed that levels of glycated hemoglobin, glucose, 25(OH) vitamin D, osteocalcin, and parathyroid hormone; years of diabetes; and BMI-SDS and AD-SoS *z* score were the most important predictors ($P < 0.0001$).

Conclusions: Our study highlighted (1) the high serum levels of DKK-1 and sclerostin in T1DM children and their relationship with altered glycemic control and (2) the effect of CSII on improvement of glycemic control and bone health in T1DM children. **(*J Clin Endocrinol Metab* 102: 1174–1181, 2017)**

Type 1 diabetes mellitus (T1DM) is associated with decreased bone mass and microarchitectural bone alterations (1). Bone loss can begin in childhood at the onset of diabetes (2), leading to a lower peak of bone mass and growth impairment with a consequent increase in risks for osteoporosis and fractures later in life (3, 4). The mechanisms underlying bone fragility in T1DM are not completely understood. Reduced bone formation due to decreased differentiation and function of osteoblasts (OBs) has been demonstrated (5, 6), whereas osteoclasts

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Abbreviations: AD-SoS, amplitude-dependent speed of sound; ALP, alkaline phosphatase; B-ALP, bone alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; BMI-SDS, body mass index-standard deviation score; BTT, bone transmission time; Ca, calcium; CSII, continuous subcutaneous infusion of insulin; CTX, C-terminal telopeptide of type I collagen; DKK-1, dickkopf-1; HbA1c, glycated hemoglobin; IGF-1, insulin growth factor-1; MDI, multiple daily injection; OB, osteoblast; PTH, parathyroid hormone; QUS, 1uantitative ultrasonography; SDS, standard deviation score; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

appear unaltered or decreased (7). Potential factors leading to suppression of bone formation in T1DM include the lack of osteoanabolic pancreatic hormones, in particular insulin (8), low levels of IGF-1 (9), chronic hyperglycemia (10), and the accumulation of advanced glycation end products (11). There are conflicting data about the impact of long-term glycemic control on bone in T1DM (12). However, it has been demonstrated that poor glycemic control has consequences on bone properties (13).

Recently, the canonical Wnt-signaling pathway has been shown to play a major role in the control of osteoblastogenesis and bone formation (14). Wnt ligands bind to the frizzled receptor complex and the coreceptor's low-density lipoprotein receptor-related proteins 5 and 6, which stimulates the differentiation of OBs. Wnt signaling is modulated by various endogenous inhibitors, including sclerostin and dickkopf-1 (DKK-1). High levels of sclerostin have been found in adult patients with type 2 diabetes mellitus (T2DM) (15, 16), whereas adult T1DM patients had either higher (17) or comparable values of sclerostin with respect to controls (18). The few data on pediatric populations showed that T1DM children had similar levels of sclerostin with respect to controls (19). High circulating levels of DKK-1 have been found in adult T2DM patients with cardiovascular disease (20), whereas there are no available data on DKK-1 serum levels in T1DM subjects.

This study was designed to evaluate (1) serum levels of sclerostin and DKK-1 and their correlation with markers of bone metabolism and quantitative ultrasound measurements of bone density and (2) the influence of glycemic control on bone health in children and adolescents with T1DM.

Methods

Patients

This cross-sectional study included 106 children and adolescents (53 males) diagnosed with T1DM according to the American Diabetes Association (21) who were referred to the Endocrinology Unit of the Pediatric Clinic of the University of Bari (Italy) between April 2014 and May 2015. At recruitment, the patients had an average age of 12.2 ± 4 years and a T1DM duration of 4.4 ± 3.1 years. Sixty-six patients received insulin with multiple daily injections (MDIs) and 40 with continuous subcutaneous infusion of insulin (CSII). The CSII and MDI groups were not different with regard to age, sex, or diabetes duration. The MDI protocol consisted of intensive insulin therapy (3 to 4 injections per day) of long- and short-acting analogs (glargine and aspart) and home blood glucose measurements (3 to 4 times per day). CSII was started according to the criteria of the Ministry of Health Pump Committee, and the patients were on CSII for at least 6 months at the time of the study (to minimize the effect of the honeymoon period). All pumps used ultra-short–acting insulin aspart. Glycemic control was monitored by glycated hemoglobin (HbA1c) levels, and T1DM subjects were classified as having good or poor glycemic control (HbA1c >8.0%; 64 mmol/mol) according to the American Diabetes Association (21). A control group of 80 healthy subjects with the same age and sex distribution as the group of T1DM subjects were enrolled on a voluntary basis in the outpatient clinic or were among those referred to our hospital for minor surgery or electrocardiographic screening. Study entry criteria for children and adolescents with T1DM were (i) age >5 years and (ii) disease duration of >2 years. Exclusion criteria from the study for both patients and controls were use of vitamin and mineral supplements or dietary restrictions, the coexistence of chronic diseases with a possible impact on bone metabolism (*e.g.,* pituitary insufficiency, hypothyroidism or hyperthyroidism, Cushing syndrome, and celiac disease), and use of medications affecting bone turnover (*e.g.,* corticosteroids, antipsychotic, or neuroleptic drugs). Subjects who had sustained a fracture in the 3 months preceding the study were also excluded. Written informed consent was obtained from children's parents or their legal guardians. All procedures were in accordance with the guidelines of the Declaration of Helsinki on human experimentation.

Anthropometric measurements

Height and weight for every patient were measured using standard techniques. Body mass index (BMI) was calculated as the ratio of weight/height^2. Height, weight, and BMI were converted to age- and sex-specific *z* scores on the basis of reference data published by the Centers for Disease Control and Prevention (22). Puberty was classified according to Tanner's staging system (23).

Biochemical measurements

A venous blood sample was drawn from all participants at 08:00 AM after a 12-hour fast. The serum samples from patients and controls were stored in aliquots at −20°C for subsequent assay, with measurements made immediately after thawing. All serum bone markers were measured in the same assay run in order to avoid interassay variance. Calcium, phosphorus, and alkaline phosphatase (ALP) concentrations were measured with the nephelometric method. Serum active intact parathyroid hormone (PTH) and 25(OH) vitamin D were measured by immunological tests based on the principle of chemiluminescence using commercial kits (Liaison assay; DiaSorin, Stillwater, MN). Osteocalcin serum concentration was measured by enzyme immunoassay using a commercial kit (IBL International GmbH, Hamburg, Germany). C-terminal telopeptide of type I collagen, DKK-1, and sclerostin were also assessed (Serum CrossLaps; Immunodiagnostics Systems, Fountain Hills, AZ; R&D Systems, Minneapolis, MN; and Biomedica Medizinprodukte, GmbH & Co KG, Vienna, Austria, respectively) with intra-assay coefficients of variation of ≤1.9, ≤4%, and ≤6.5, respectively.

Quantitative ultrasonography (QUS)

Bone quality was assessed by QUS measurements performed with a DBM Sonic 1200 bone profiler (Igea S.r.l., Carpi, MO, Italy) employing a sound frequency of 1.25 MHz. QUS is a radiation-free technique that evaluates bone mineral status in the peripheral skeleton by measuring the amplitude-dependent speed of sound (AD-SoS), which reflects the ultrasound velocity inside the bone, and the bone transmission time (BTT), reflecting the bone characteristics without the interference of the

soft tissue. It has also been demonstrated that sex, age, BMI, and pubertal stage are independent predictors of AD-SoS and BTT in both sexes; thus, although a possible effect of pubertal stage on bone age may be considered, phalangeal QUS is a useful tool to assess bone mineral status with a very small confounding effect (24). QUS was performed on the second to the fifth proximal phalanges of the nondominant hand, and the mean value per person was calculated. QUS was performed by the same operator on the second to the fifth proximal phalanges of the nondominant hand and were expressed as *z* scores calculated on the basis of the normal values for age and sex obtained in a large Italian population sample (24).

Statistical analyses

For statistical analyses, the Statistical Package for the Social Sciences for Windows, version 22.0 (SPSS Inc., Chicago, IL) was used. Results are presented as means ± standard deviation. The Kolmogorov-Smirnov test was applied to determine the normality of the distribution of the studied parameters. In parameters with normal distribution, mean values were compared using the unpaired Student *t* test, whereas linear correlations were calculated with the Pearson correlation coefficient. In parameters with skewed distribution, significance was assessed with the Mann-Whitney test and Spearman correlation coefficient. Comparisons among groups were performed by analysis of variance. Finally, multiple regression analyses were performed to identify the relative strength of each biochemical and clinical variable in predicting DKK-1 and sclerostin levels. The limit of statistical significance was set at 0.05.

Results

Clinical characteristics, glycemic control, bone metabolism markers, and parameters of bone quality of the study group are shown in Table 1. Diabetic patients showed a statistically significant reduction of serum 25(OH) vitamin D levels with respect to controls ($P < 0.01$). In T1DM patients, the average AD-SoS *z* score and BTT *z* score were within the normal range but were reduced with respect to controls. Glucose serum levels significantly correlated with pubertal stage ($r = -0.110$; $P < 0.001$), 25(OH) vitamin D level ($r = -0.076$; $P < 0.025$), and BTT *z* score values ($r = -0.213$; $P < 0.0001$). Additionally, the years of diabetes significantly correlated with HbA1c% ($r = 0.239$; $P < 0.040$) and after adjustment for age also with 25(OH) vitamin D level ($r = -0.128$; $P < 0.0001$). Moreover, the high HbA1c% correlated with pubertal stage ($r = 0.186$; $P < 0.0001$), osteocalcin ($r = -0.165$; $P < 0.0001$), 25(OH) vitamin D ($r = -0.110$; $P < 0.001$), AD-SoS *z* score ($r = -0.138$; $P < 0.005$), and BTT *z* score ($r = -0.159$; $P < 0.001$).

DKK-1 and sclerostin serum levels

To better understand the pathogenic mechanisms that are involved in bone loss in T1DM subjects, we evaluated sclerostin and DKK-1 serum levels. Significantly increased serum levels of sclerostin [29.45 ± 12.32 *vs* 22.53 ± 8.29; $P < 0.001$; Fig. 1(A)] and DKK-1 [3593 ± 1172 pg/mL *vs* 2652 ± 689 pg/mL; $P < 0.006$; Fig. 1(B)] were found in T1DM patients with respect to controls. Serum sclerostin levels were higher in male patients than in female ones (30.75 ± 7.92 *vs* 28.09 ± 15.87; $P < 0.01$) and were positively correlated with age ($r = 0.115$; $P < 0.036$).

Furthermore, with adjustment for age, sclerostin levels significantly correlated with height-SDS ($r = 0.379$;

Table 1. Clinical and Biochemical Data of Study Group

Variable	Controls	T1DM	*P*
N (M/F)	80 (43/37)	106 (53/53)	NS
Age, y	11.8 ± 3.4	12.2 ± 3.4	NS
Tanner stage (I, II, III, IV, V)	30, 6, 14, 9, 21	40, 9, 17, 11, 29	NS
T1DM duration, y	—	4.4 ± 3.1	—
Height-SDS	0.35 ± 1.06	0.11 ± 1.02	NS
BMI-SDS	0.24 ± 0.80	−0.08 ± 1.01	NS
Glucose, mg/dL	86.44 ± 7.2	187 ± 84	0.001
HbA1c%	—	8.13 ± 0.94	—
HbA1c, mmol/mol	—	64.3 ± 13.5	—
IGF-1, ng/mL	—	279.8 ± 119.9	—
PTH, pg/mL	19.33 ± 6.5	18.74 ± 6.8	NS
Phosphorus, mg/dL	4.7 ± 0.6	4.37 ± 0.6	NS
Ca, mg/dL	9.47 ± 0.4	9.50 ± 0.4	NS
B-ALP, μg/L	78.26 ± 13.84	81.20 ± 37.98	NS
25(OH) vitamin D, ng/mL	38.64 ± 14.70	22.10 ± 9.3	0.01
CTX, ng/mL	1.60 ± 0.45	1.56 ± 0.64	NS
Osteocalcin, ng/mL	36.67 ± 24.5	46.88 ± 21.6	NS
BTT *z* score	0.13 ± 0.80	−0.14 ± 1.04	0.04
Ad-SoS *z* score	0.40 ± 0.85	−0.06 ± 1.12	0.04
Sclerostin, pmol/L	22.53 ± 8.29	29.45 ± 12.32	0.001
DKK-1, pg/mL	2652 ± 689	3593 ± 1172	0.006

AD-SoS *z* score or BTT *z* score <−2.0: low bone mineral status (24).

Abbreviations: B-ALP, bone alkaline phosphatase; Ca, calcium; CTX, C-terminal telopeptide of type I collagen; NS, not significant; SDS: standard deviation score.

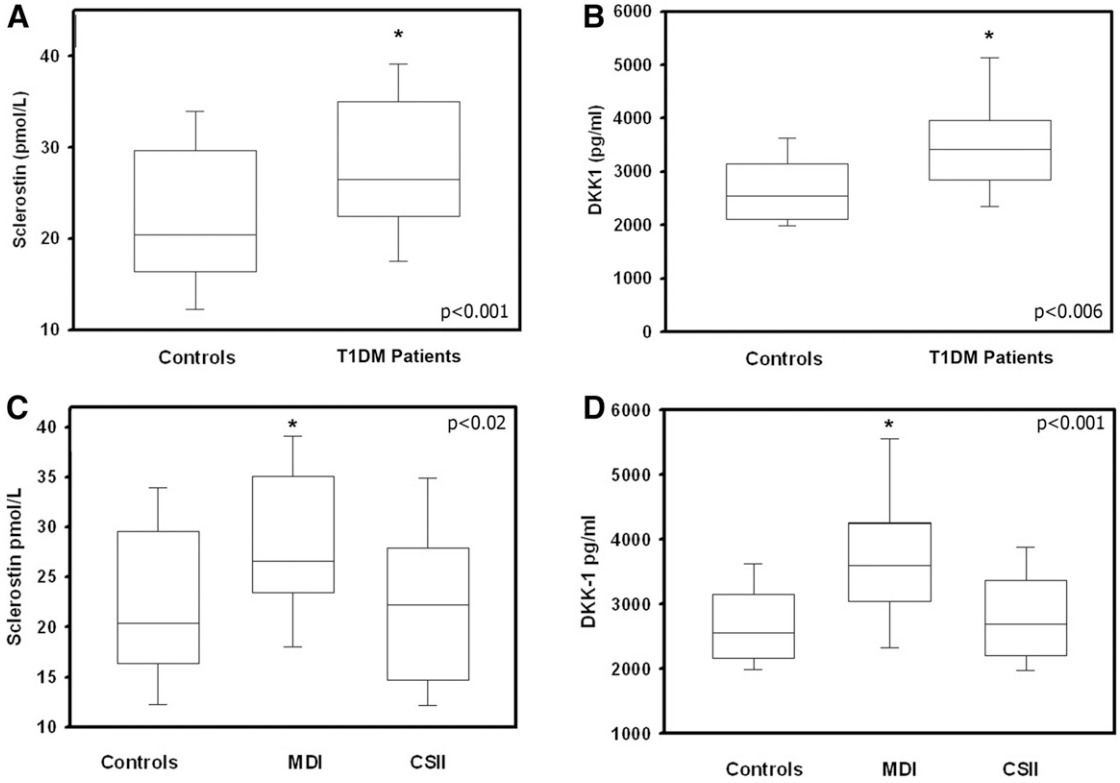

Figure 1. High levels of sclerostin and DKK-1 in T1DM patients. In controls and all patients with T1DM, the serum levels of (A) sclerostin and (B) DKK-1 were measured by enzyme-linked immunosorbent assay. In patients on MDIs and CSII, the serum levels of (C) sclerostin and (D) DKK-1 were also detected.

$P < 0.0001$), BMI-SDS ($r = -0.310$; $P < 0.0001$), pubertal stage ($r = 0.094$; $P < 0.025$), HbA1c% ($r = 0.185$, $P < 0.0001$), glucose ($r = 0.161$; $P < 0.0001$), years of diabetes ($r = 0.188$; $P < 0.0001$), calcium ($r = 0.267$; $P < 0.0001$), phosphorus ($r = -0.219$; $P < 0.0001$), creatinine ($r = 0.116$; $P < 0.006$), PTH ($r = 0.118$; $P < 0.007$), 25(OH) vitamin D ($r = -0.226$; $P < 0.0001$), AD-SoS z score ($r = -0.154$; $P = 0.02$), ALP ($r = 0.141$; $P < 0.001$), osteocalcin ($r = 0.254$; $P < 0.0001$), and IGF-1 ($r = 0.157$; $P < 0.0001$).

Serum DKK-1 levels significantly correlated with HbA1c% ($r = 0.257$; $P < 0.0001$). With adjustment for age, we found that DKK-1 levels correlated with glucose ($r = -0.137$; $P < 0.0001$), years of diabetes ($r = 0.115$; $P = 0.001$), BMI-SDS ($r = 0.178$; $P < 0.0001$), calcium ($r = 0.124$; $P = 0.001$), PTH ($r = 0.119$; $P = 0.016$), AD-SoS z score ($r = -0.160$; $P = 0.001$), and osteocalcin ($r = -0.079$; $P = 0.02$).

MDI *vs* CSII therapy: bone health and glycemic control

Our cohort of T1DM patients can be divided in 2 subgroups according to the different therapy: MDI or CSII (Table 2). First, we found that glycemic control was better in CSII patients than in MDI ones. In particular, both glucose levels and HbA1c% were significantly reduced in CSII patients compared with MDI patients. This improvement was also associated with higher BMI-SDS and BMD in CSII patients than in MDI patients. Interestingly, in parallel with BMD "rescue," DKK-1 and sclerostin levels also reached the controls' level in CSII patients [Fig. 1(C–D)]. On the contrary, levels of DKK-1 and sclerostin were very high in the MDI group, suggesting the important role of the type of therapy on bone health and glycemic control in T1DM patients. Although bone health was improved in CSII patients, 25(OH) vitamin D levels remained low as in MDI patients compared with controls. Interestingly, in CSII patients, 25(OH) vitamin D serum levels significantly correlated with serum glucose levels ($r = -0.175$; $P < 0.01$).

Multiple regression analyses

Multiple linear regression analyses were performed to investigate the factors associated with high DKK-1 and sclerostin serum levels in patients (Table 3).

With adjustment for age, multiple linear regression analysis for DKK-1 as a dependent variable showed that levels of HbA1c%, glucose, 25(OH) vitamin D, osteocalcin, and PTH and years of diabetes, AD-SoS z score, and BMI-SDS were the most important predictors ($r = 0.552$; $P < 0.0001$).

With adjustment for age, multiple linear regression analysis for sclerostin as a dependent variable showed that levels of HbA1c%, glucose, 25(OH) vitamin D,

Table 2. Clinical and Biochemical Data of Diabetic Patients: MDI *vs* CSII Group

Variable	MDI	CSII	*P*
N (M/F)	66 (32/34)	40 (21/19)	NS
Age, y	12.18 ± 3.21	12.26 ± 3.76	NS
T1DM duration, y	4.23 ± 3.10	4.92 ± 3.13	NS
CSII, y	—	2.73 ± 1.74	—
Height-SDS	−0.24 ± 1.04	0.09 ± 0.95	NS
BMI-SDS	−0.28 ± 0.92	0.47 ± 1.03	0.002
Glucose, mg/dL	195.35 ± 70.60	141.97 ± 44.14	0.001
HbA1c%	8.35 ± 0.98	7.66 ± 0.66	0.001
HbA1c, mmol/mol	65.18 ± 13.13	60.29 ± 7.18	0.04
IGF-1	276 ± 115	288 ± 135	NS
PTH, pg/mL	18.66 ± 6.16	18.98 ± 8.74	NS
Phosphorus, mg/dL	4.32 ± 0.57	4.49 ± 0.69	NS
Ca, mg/dL	9.54 ± 0.41	9.39 ± 0.42	NS
B-ALP, μg/L	80.78 ± 36.43	82.39 ± 43.12	NS
25(OH) vitamin D, ng/mL	22.78 ± 9.91	20.08 ± 5.35	NS
CTX, ng/mL	1.57 ± 0.62	1.53 ± 0.72	NS
Osteocalcin, ng/mL	46.04 ± 19.71	45.22 ± 23.80	NS
BTT *z* score	−0.34 ± 1.61	0.25 ± 0.85	0.04
AD-SoS *z* score	0.09 ± 1.01	0.38 ± 0.85	0.04
DKK-1, pg/mL	3744 ± 1266	2962 ± 986	0.001
Sclerostin, pmol/L	30.25 ± 13.10	22.53 ± 8.29	0.02

Abbreviation: NS, not significant.

osteocalcin, and PTH and years of diabetes, AD-SoS *z* score, and BMI-SDS were the most important predictors ($r = 0.498$; $P < 0.0001$).

Discussion

In the current study, we demonstrated 2 important findings: (1) DKK-1 and sclerostin are 2 key markers of altered bone turnover in T1DM children and adolescents, levels of which increased if metabolic control was altered, and (2) CSII-treated patients showed improved bone turnover together with better metabolic controls with respect to MDI-treated patients.

DKK-1 and sclerostin as markers of altered bone turnover in T1DM children and adolescents

DKK-1 and sclerostin are critical regulators of bone mass; in fact, higher levels of these Wnt-signaling inhibitors have been found in several bone diseases (14). T1DM patients show low bone turnover status,

Table 3. Multivariate Analysis Models

	Dependent Variable	Independent Variable	β	*P*	*r*
Model 1				0.0001	
					0.552
	DKK-1	HbA1c%	−0.191	0.008	
		Glucose levels	0.040	0.007	
		25(OH) vitamin D	−0.011	0.013	
		AD-SoS *z* score	−0.541	0.0001	
		Osteocalcin	−0.842	0.0001	
		Years of diabetes	0.312	0.0001	
		PTH	0.041	0.04	
		BMI, SDS	0.037	0.201	
Model 2				0.0001	
					0.498
	Sclerostin	HbA1c%	0.321	0.0001	
		Glucose levels	0.222	0.0001	
		25(OH) vitamin D	−0.302	0.0001	
		AD-SoS *z* score	−0.295	0.0001	
		Osteocalcin	0.288	0.007	
		Years of diabetes	0.047	0.04	
		PTH	0.083	0.02	
		BMI, SDS	−0.100	0.079	

β = regression coefficient.

particularly with impaired bone formation (5, 6). During the last few years, a critical link between bone metabolism and energy control has been established (25, 26). Our report demonstrated DKK-1 involvement in T1DM, whereas other studies measured sclerostin levels in diabetes with contrasting results. In particular, in contrast with our results, Tsentidis *et al.* (19) found that T1DM children/adolescents and controls had similar levels of sclerostin, which showed a Gaussian distribution. Although we used the same enzyme-linked immunosorbent assay kit, the different results could be due to the lower number of patients tested by Tsentidis *et al.* compared with our numbers. However, consistent with results by Tsentidis *et al.* (19), sclerostin levels were higher in male patients than in female patients and correlated with bone formation markers and pubertal stage. Neumann *et al.* (17) reported that both males and females with T1DM had higher serum levels of sclerostin compared with controls, but they did not find a correlation between serum sclerostin levels and markers of bone metabolism. Garcia-Martin *et al.* (27) found that in T2DM men, but not in women, serum sclerostin levels were higher than in controls. Gennari *et al.* (18) reported no differences in serum sclerostin levels in adult T1DM patients compared with controls. In our study, DKK-1 and sclerostin levels correlated with AD-SoS *z* score. Previous studies showed conflicting results. In particular, Tsentidis *et al.* showed that in T1DM children and adolescents, sclerostin levels correlated with BMD *z* scores (19). It has also been proposed that serum sclerostin is not associated with bone mass in young people, but is in elderly people, arguing for a different expression or effect in the phases of bone growth and modeling as opposed to bone remodeling. Older men with T1DM did not show correlations of sclerostin serum levels with bone mass as demonstrated in healthy subjects (17).

Sclerostin and DKK-1 showed different correlations, probably because sclerostin is expressed only by osteocytes, whereas DKK-1 is expressed by preosteoblasts, OBs, and osteocytes. Thus, the increase in DKK-1 levels in T1DM patients probably reflected the altered activity of all the cells of osteoblastic lineage, whereas the sclerostin increase was linked only to osteocyte activity. The important role of both molecules is also sustained by a recent article on a mouse model showing that a bispecific antibody targeting sclerostin and DKK-1 promoted bone mass accrual and fracture repair with a superior effect compared with monotherapies (28).

Glycemic controls and markers of bone metabolism

It has been found that patients with diabetes are prone to vitamin D deficiency (29), and moreover, poor glycemic control has been shown to impair the response of OBs and osteoclasts to $1,25(OH)_2$ vitamin D_3 in T2DM (30). In our study, we found an inverse correlation between glycemic control and 25(OH) vitamin D levels as well as between sclerostin and 25(OH) vitamin D levels. Our results are also sustained by another study in premenopausal T1DM women that demonstrated that poor glycemic control was associated with decreased BMD but with normal bone resorption markers, suggesting uncoupled turnover (31). Other studies suggested that improvement in glycemic control can result in increased bone turnover markers of resorption and formation in both male and female patients with diabetes, possibly mediated by increased levels of IGF-1 (32). As an additional mechanism, poor glycemic control has been associated with early low BMD, increased osteoprotegerin mRNA expression, and low osteocalcin concentration in children and adolescents with TIDM (33).

Effects of glycemic control on bone health in our diabetic subjects

In our study, subjects treated with CSII had better glycemic control than subjects on an MDI regimen, and we found high levels of sclerostin and DKK-1 especially in those subjects treated with MDI. Conversely, T1DM subjects on CSII showed serum levels of sclerostin and DKK-1 comparable to those of controls. Over the last 15 years, the growing use of insulin pump therapy, particularly in children, has been determined by improvements in pump technology and the availability of insulin analogs, together with the results of the Diabetes Control and Complications Trial, which recognized the benefit of improved glycemic control (34, 35). In a meta-analysis comparing CSII and MDI, it has been reported that HbA1c decreased more in patients treated with CSII in all trials. In a long-term study on a large number of children with T1DM, sustained improvement in glycemic control was demonstrated in the CSII group compared with a matched cohort using injections (36). Furthermore, in a recent retrospective, international, multicenter study, Mameli *et al.* (37) associated the use of CSII with greater improvement in HbA1c after 1 year of treatment and during the 7-year follow-up. Furthermore, it has been reported that the amelioration of glycemic control is clinically significant, resulting in reductions of microvascular complications (38) as well as an increase in BMI-SDS (39). We have demonstrated the association between improved glycemic control and bone health in T1DM patients who are on CSII. In particular, BMD reached the level of control subjects, although 25(OH) vitamin D levels remained low in patients. It might be possible that a longer duration of the CSII treatment causes levels of vitamin D to increase to normal values. Giving supplements of vitamin D to these subjects to prevent bone alterations from happening might also be needed. On the

contrary, it was interesting to observe that DKK-1 and sclerostin were improved in subjects with better glycemic control. Thus, the measurement of serum levels could represent a valid instrument to simultaneously monitor glycemic and bone status in T1DM patients.

Consistently, our multiple regression analyses showed that both DKK-1 and sclerostin levels were best predicted by glycemic control and bone markers as well as by BMI-SDS.

In conclusion, our study highlighted (1) the involvement of DKK-1 and sclerostin in the altered glycemic control and bone metabolism of T1DM patients and (2) the association of CSII with good bone metabolism in T1DM patients.

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