SERUM SCLEROSTIN LEVELS IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES MELLITUS

Sae Ishikawa[1)], Ikuko Takahashi[2)] and Tsutomu Takahashi[2)]*

[1)]*Division of Pediatrics, Akita Municipal General Hospital, Akita-shi, Akita, 010-0933, Japan*
[2)]*Department of Pediatrics, Akita University Graduate School of Medicine, Akita-shi, 010-8543, Japan*

(received 28 April 2023, accepted 1 June 2023)

Abstract
Type 1 diabetes mellitus (T1DM) is associated with an increased risk of incident fractures in children, which suggests a relationship between bone health and glucose metabolism. The Wnt signaling pathway plays a role in controlling osteoblastogenesis and bone formation, and is modulated by various endogenous inhibitors, including sclerostin, a Wnt signaling pathway inhibitor. A recent study demonstrated that glucose levels directly regulate osteocyte function through sclerostin expression, suggesting a potential mechanism for the negative effects of diabetes on bone quality. Thus, this study was conducted to assess the relationship between sclerostin levels and physical, metabolic, and endocrinological factors of bone metabolism in Japanese children and adolescents with T1DM. Twenty-two patients with T1DM (14.2±4.2 ys), and 16 age-, sex- and sex hormone-matched controls without diabetes mellitus (12.3±4.1 ys) were enrolled in this study. Serum sclerostin levels were significantly higher in patients with T1DM than in controls (p =0.013), without significant differences between sexes. Spearman's rho correlation revealed a significant positive association between serum sclerostin levels and height-standard deviation score (SDS) (p=0.011), Body mass index-SDS (p=0.014), and bone- specific alkaline phosphatase (p=0.003) in all participants. These results suggest that sclerostin plays a role in T1DM-related bone fragility in Japanese children and adolescents.

Keywords : adolescents, children, fracture risk, type 1 diabetes mellitus, sclerostin

Introduction

Type 1 diabetes mellitus (T1DM) increases the risk of bone fractures in adults[1,2)]. In a large UK cohort, T1DM was also demonstrated to be associated with an increased risk of incident fractures that begin in childhood and continue throughout one's life span[3)]. The mechanisms related to diabetic bone fragility have not been fully elucidated ; however, studies have shown that markers of both bone formation and resorption become abnormal in patients with diabetes, suggesting that diabetes mellitus is a condition of low bone turnover, which may consecutively lead to a more fragile bone[4)].

In adult T1DM, low bone formation is confirmed using biomarkers of bone turnover, such as serum osteocalcin[4)]. In T1DM children and adolescents, markers of bone formation, including osteocalcin and procollagen type-1 amino-terminal pro-peptide, and resorption, including C-terminal cross-linked telopeptide of type-1 collagen, were decreased, suggesting disturbed bone turnover in T1DM children and adolescents[5,6)]. In addition, bone growth is especially pronounced during puberty, with 25-50% of the peak bone mass accumulated[7)]. Bone maturation and the attainment of peak bone

Corresponding Author : Tsutomu Takahashi
Department of Pediatrics, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan.
Tel : +81-18-884-6159
Fax : +81-18-836-2620
E-mail : tomy@doc.med.akita-u.ac.jp

mass during puberty are important for fracture resistance in adulthood[7]. Decreased bone formation and insufficient mineralization during puberty due to T1DM may account for the lower peak bone mass acquisition observed in patients with T1DM[8,9]. T1DM has been reported to change or delay pubertal development, especially in the T1DM patients with poor glycemic control, which may lead to an increased fracture risk owing to lower bone mineral density, lower trabecular volume, and higher bone turnover than in healthy children[10,11].

The Wnt signaling pathway plays a role in the control of osteoblastogenesis and bone formation. Wnt ligands bind to low-density lipoprotein receptor proteins 5 and 6 (LRP5/6), which stimulate osteoblast differentiation[12]. Wnt signaling is modulated by various endogenous inhibitors, including sclerostin and dickkopf-1[12]. Sclerostin is a secreted glycoprotein primarily produced by osteocytes and has anti-anabolic effects on bone formation. Sclerostin binds to LRP5/6 receptors and inhibits the Wnt signaling pathway[13]. The inhibition of the Wnt pathway leads to decreased bone formation. In contrast, mutations in the *SOST* gene, which encodes the sclerostin protein, are associated with high bone mass, sclerosteosis, and van Buchem disease, an autosomal recessive skeletal disease characterized by uninhibited bone growth, especially in the mandible, skull, and ribs[13]. Recently, anti-sclerostin antibodies has been shown to be a therapeutic agent for bone mass recovery and fragility fracture prevention in low bone mass phenotypes such as osteoporosis[13].

High levels of sclerostin have been found in adult patients with type 2 diabetes mellitus, whereas adult patients with T1DM had either higher or comparable values of sclerostin levels with respect to controls[5]. A recent study using a mouse model and cultured osteocyte-like cells demonstrated that glucose levels directly regulate osteocyte function through sclerostin expression, suggesting a potential mechanism underlying the negative impact of diabetes on bone quality[14]. However, the mechanical involvement of sclerostin in low bone turnover in T1DM children and adolescents is still controversial since there is limited clinical data on these factors.

This study was designed to evaluate the serum levels of sclerostin and their correlation with some physical, metabolic, and endocrinological factors of bone metabolism as well as the influence of glycemic control on bone health in Japanese children and adolescents with T1DM on intensive insulin therapy.

Methods

Study population

Twenty-two children and adolescents with T1DM on intensive insulin therapy (9 boys and 13 girls) and sex-matched 16 non-diabetes mellitus (non-DM) control patients (8 boys and 8 girls) who visited Akita University Hospital were included in this study. The study participants were enrolled between October 2021 and March 2022. The non-DM controls consisted of 14 patients with compensated hypothyroidism and 2 patients with non-DM obesity. Ethical approval was obtained from the Ethics Committee of Akita University's Graduate School of Medicine, Akita, Japan. Written informed consent was obtained from the parents or guardians of the patients, and the study was conducted in accordance with the Declaration of Helsinki.

All patients with T1DM were treated with intensive insulin therapy. Insulin analogs were administered to 22 patients, including 5 patients who were treated with an insulin pump. Rapid-onset and long-acting analog insulin were administered to 17 patients. The mean daily insulin dose of the entire group of patients reached 1.1±0.27 IU/kg body weight. The mean duration of diabetes was 6.7±4.2 years. The mean level of HbA1c in the group of patients with T1DM was 7.9±0.8%. Controls were selected from among non-DM patients whose disease was compensated with appropriate treatment in our outpatient clinic. All 14 patients with hypothyroidism were treated with levothyroxine, and euthyroidism was confirmed by laboratory tests. The mean daily levothyroxine dose of the entire group of patients reached 1.6±1.1 μg/kg body weight. The mean duration of treatment was 8.8±4.4 years. The mean levels of free triiodothyronine (fT3), free thyroxine (fT4), and thyroid stimulating hormone (TSH) were 3.94±0.47 pg/mL (reference range ; 3.10-4.50), 1.54±0.15 ng/dL (reference range ; 1.22-1.72), and 3.44±1.74 μIU/mL (reference range ; 0.75-5.95), respectively.

The height and weight of each patient were measured using a rigid stadiometer and calibrated balance scale, respectively. The height standard deviation score (height-SDS) was calculated from national normative data[15]. The body mass index (BMI) was calculated as weight in kilograms (kg) divided by the square of the height in meters (m^2). The BMI standard deviation score (BMI-SDS) was calculated from reference data for Japanese children[16].

Two groups of patients with T1DM and non-DM controls were enrolled in the study, with laboratory analysis of the patients' blood. Blood was collected in the state of fasting as outpatients and serum was stored frozen at <−20°C. Medical records were assessed for age, sex, height, weight, starting day of insulin therapy, starting day of other treatments, and laboratory data, including luteinizing hormone (LH), follicular stimulating hormone (FSH), estradiol (E2), testosterone, HbA1c, glucoalbumin (GA), C-peptide, bone-specific alkaline phosphatase (bone-ALP), 25-OH vitamin D, osteocalcin, adiponectin, and insulin-like growth factor-1 (IGF-I) levels. IGF-I standard deviation score (IGF-I-SDS) was calculated from the reference data for the normal Japanese population[17].

Serum sclerostin levels were determined using a Human Sclerostin ELISA Kit (Invitrogen). Briefly, 50 μL diluent solution was additionally poured to a conjugated 96-well plate containing 50 μL standards and samples in duplicate and incubated at 37°C for 3 hours. 100 μL of Mouse SOST conjugate was additionally poured to each well and incubated at 37°C for 1 h. 100 μL of substrate solution was added to each well and incubated in the dark at 37°C for 30 min. The reaction was stopped with the addition of 100 μL of stop solution and absorbance measured at 450 nm, with readings at 540 nm obtained for wavelength correction. Standards were used to interpolate the sclerostin concentration.

Table 1. Demographic and laboratory parameters of type 1 diabetes mellitus patients and non-type 1 diabetes mellitus controls

	T1DM (n=22)	non-T1DM (n=16)	p
Age (ys)	14.2±4.2 (6.5-20.2)	12.2±4.1 (6.7-18.3)	0.145
Male/Female	9/13	8/8	0.651
Height SDS	0.52±1.2 (-1.5-3.5)	−0.37±1.28 (−3.1-1.4)	0.018*
BMI SDS	0.69±0.86 (-0.7-2.5)	0.49±1.19 (−1.4-2.6)	0.281
LH (mIU/mL)	3.6±4.2 (<0.1-17.2)	2.2±2.6 (<0.1-8.8)	0.312
(male/female)	1.8±1.2 (0.1-4.26)/4.9±5.1 (0.1-9.6)	2.2±1.8 (0.1-4.89)/2.2±3.3 (0.1-8.79)	(0.297/ 0.238)
FSH (mIU/mL)	3.8±2.1 (1.0-7.9)	5.0±6.7 (1.3-28.8)	0.715
(male/female)	2.9±1.3 (1.48-5.81)/4.4±2.3 (0.96-7.85)	6.7±9.3 (1.88-28.77)/3.3±1.9 (1.33-6.3)	(0.606/ 0.122)
E2 (pg/mL)	44.9±51.2 (<10-186)	24.1±22.4 (<10-96)	0.129
(male/female)	19.8±8.7 (<10-39)/62.3±61.1 (<10-186)	21.0±13.1 (<10-40.0)/27.3±29.7 (<10-96)	(0.815/ 0.140)
Testosterone (ng/mL)	1.7±1.9 (<0.04-5.73)	1.8±2.6 (0.04-7.59)	0.51
(male/female)	3.5±1.8 (0.04-5.73)/0.4±0.2 (0.17-0.7)	3.5±3.2 (0.05-7.59)/0.2±0.3 (0.05-0.83)	(0.479/ 0.161)
Duration of insulin therapy (y)	6.7±4.2 (1.8-19.8)	N.D.	
HbA1 (%)	7.9±0.8 (6.5-10.4)	N.D.	
GA (%)	24.2±4.1 (17.1-33.8)	12.4±1.0 (10.9-14.0)	<0.001*
C-peptide (ng/mL)	0.15±0.21 (0.1-0.2)	2.30±1.10 (0.70-4.00)	<0.001*
Sclerostin (pmol/L)	19.5±8.0 (8.1-36.1)	14.9±4.0 (7.9-20.9)	0.013*
Bone-ALP (μg/L)	54.5±43.1 (9.8-136)	62.5±39.1 (6.8-123)	0.51
25-OH vitamin D (ng/mL)	17.8±5.5 (7.8-28.6)	15.1±4.1 (8.9-24.7)	0.088
Osteocalcin (ng/mL)	38.5±29.6 (3.8-114)	50.4±40.0 (7.8-142)	0.404
Adiponectin (μg/mL)	11.6±5.6 (3.0-20.4)	10.9±5.2 (3.2-21.4)	0.336
IGF-I (ng/mL)	217.6±105.7 (41-503)	251.3±110.6 (99-481)	0.352
IGF-I SDS	−1.2±1.6 (−5.3-1.3)	−0.3±1.0 (−2.2-1.8)	0.017*

T1DM : patients with type 1 diabetes mellitus, non-T1DM : patients without type 1 diabetes mellitus, *<0.05

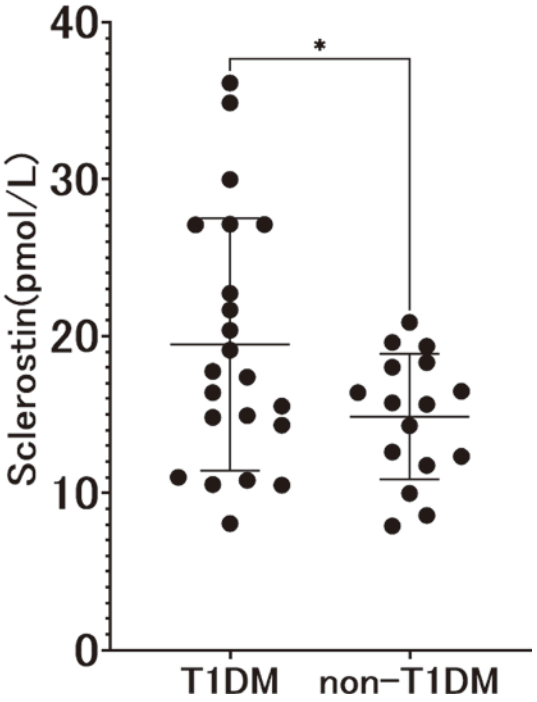

Figure 1. Serum sclerostin levels of children and adolescents with T1DM and non-DM controls
Serum sclerostin levels were significantly higher in patients with T1DM (T1DM) than in those without DM controls (non-T1DM) (*p=0.013).

Statistical Analysis

Data were analyzed using the IBM SPSS statistics 28.0 software package, and the results are presented as the mean±standard deviation. Skewness estimation of the data was performed using the Shapiro-Wilk test. An independent t-test was performed for normally distributed parameters. Mann-Whitney U tests were used for parameters without a normal distribution. Depending on the skewness of the data, Spearman's rho correlation or Pearson's correlation test was used to examine the correlation between parameters. Statistical significance was set at $p<0.05$.

Results

Characteristics of the patients with T1DM and non-DM controls are summarized in Table 1. Patients with T1DM had significantly higher levels of GA and lower levels of C-peptide than non-DM controls ($P<0.001$, respectively). There were no differences in age, sex, or BMI-SDS between patients with T1DM and non-DM controls. There was also no difference in the levels of LH, FSH, E2, and testosterone between patients with T1DM and non-DM controls. The non-DM controls were comparable to the patients with T1DM in terms of age, sex, BMI-SDS, and sex hormones. Significant differences in height SDS scores were observed between the two groups. There was also a significant difference in the IGF-I-SDS between the two groups. There were no differences in bone-ALP, 25-OH vitamin D, osteocalcin, adiponectin, or IGF-I levels between the two groups. However, there was a significant difference in the IGF-I-SDS between the two groups.

Serum sclerostin levels were significantly higher in patients with T1DM than in non-DM controls ($p=0.013$) (Table 1). Distribution of serum sclerostin levels were presented in Figure 1. There were no differences in serum sclerostin levels between the sexes in any of the participants, patients with T1DM, or non-DM controls (data not shown).

Spearman's rho correlation revealed a significant positive association between serum sclerostin level and height-SDS ($\rho=0.407$, $P=0.011$), BMI-SDS ($\rho=0.394$, $P=0.014$), and bone-ALP ($\rho=0.463$, $P=0.003$) in all participants (Table 2, Figure 2, Figure 3, and Figure 4). The Pearson correlation test revealed a significant positive association between serum sclerostin level and height-SDS ($r=0.470$, $P=0.027$) and bone-ALP level ($r=0.575$, $p=0.005$) in patients with T1DM (data not shown). The Pearson correlation test revealed a significant positive association between height SDS and IGF-I SDS ($r=0.743$, $p<0.001$) in patients with T1DM (Figure 5). The Pearson correlation test revealed no significant positive association between serum sclerostin levels and any factors in non-DM controls (data not shown).

Discussion

In our cross-sectional study comparing T1DM with non-DM patients, significant differences in SDS-height were observed between the two groups. In addition,

Table 2. Spearman's rho correlation between serum sclerostin and clinical data in all of the subjects

	T1DM+nonT1DM	Sclerostin	
	n=38	ρ	p
Age (years)	13.38±4.22	−0.203	0.223
Sex (male/female)	17/21	−0.186	0.264
Height-SDS	0.14±1.30	0.407	0.011*
BMI-SDS	0.61±1.00	0.394	0.014*
LH (mIU/mL)	3.03±3.64	−0.059	0.723
FSH (mIU/mL)	4.28±4.58	−0.072	0.669
E2 (pg/mL)	36.16±42.42	−0.211	0.203
Testosterone (ng/mL)	1.74±2.22	0.145	0.386
GA (%)	19.23±6.67	0.139	0.404
C-peptide (ng/mL)	1.06±1.29	−0.187	0.260
Bone-ALP (μg/L)	57.86±41.15	0.463	0.003*
25OH-D (ng/mL)	16.67±5.09	0.242	0.143
Osteocalcin (ng/mL)	43.51±5.41	0.246	0.137
Adiponectin (μg/mL)	11.29±5.41	−0.171	0.305
IGF-I (ng/mL)	232±107.62	0.26	0.115
IGF-I-SDS	−0.82±1.42	0.261	0.114

*<0.05

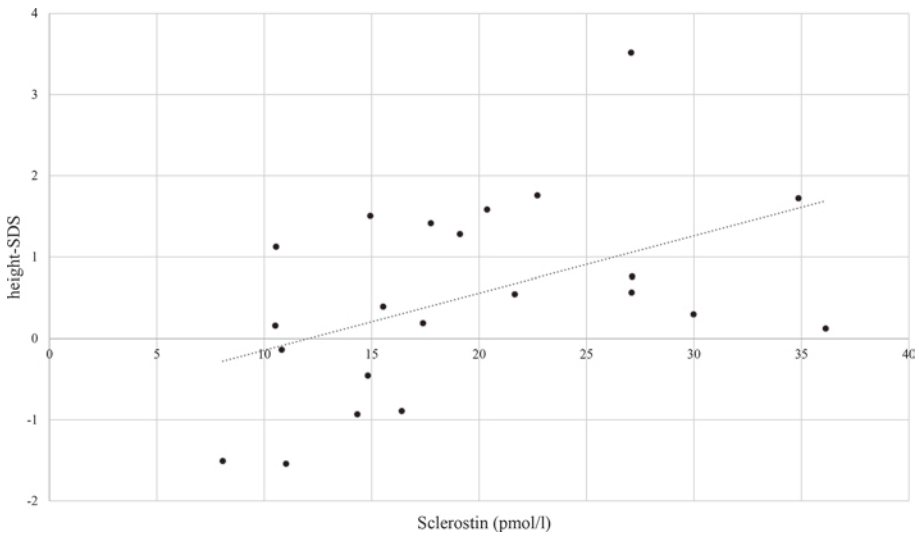

Figure 2. Association between sclerostin and height-SDS in all study participants
Spearman's rho correlation revealed a significant positive association between serum sclerostin and height-SDS (ρ=0.407, P=0.011) in all of the participants.

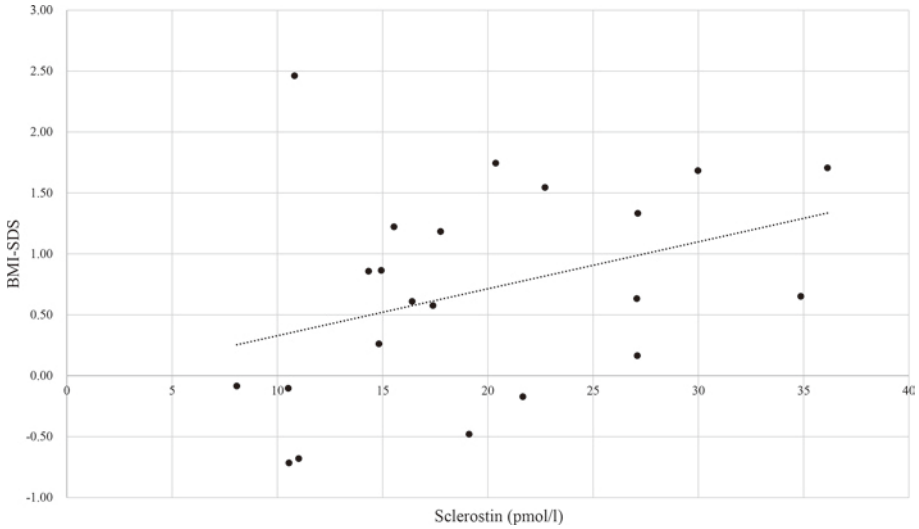

Figure 3. Association between sclerostin and bone-ALP levels in all study participants
Spearman's rho correlation revealed a significant positive association between serum sclerostin and bone-ALP levels (ρ=0.463, P=0.003) in all of the participants.

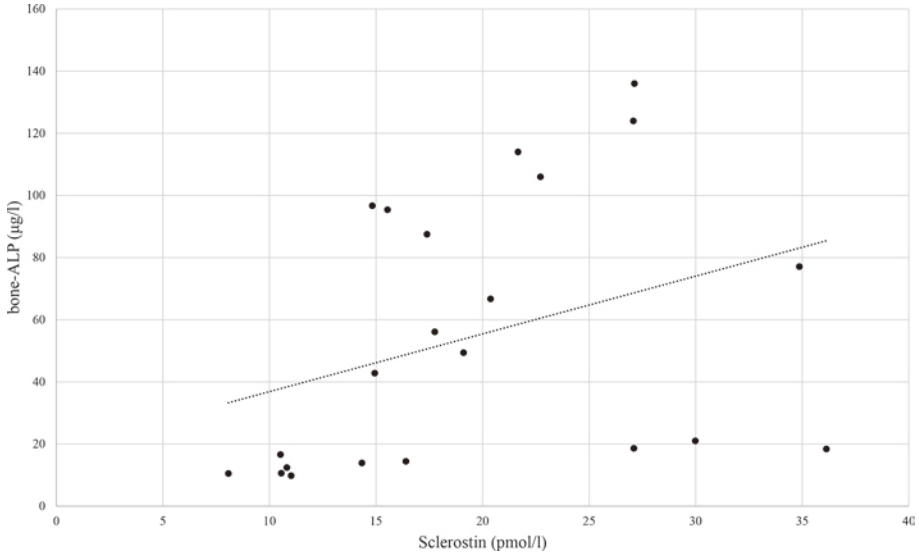

Figure 4. Association between sclerostin and BMI-SDS in all of the study participants
Spearman's rho correlation revealed a significant positive association between serum sclerostin levels and BMI-SDS (ρ=0.394, P=0.014) in all study participants.

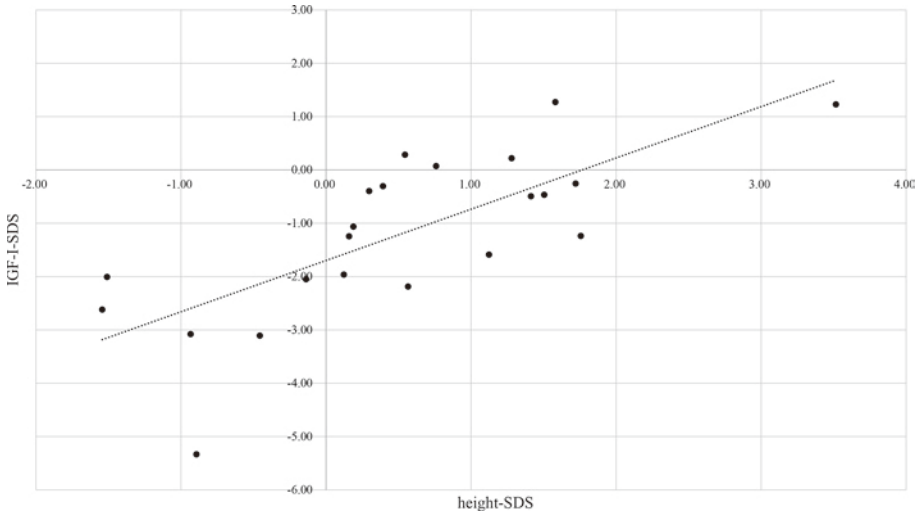

Figure 5. Association between height-SDS and IGF-I-SDS in patients with T1DM
The Pearson correlation test revealed a significant positive association between the height SDS and IGF-I-SDS scores ($r=0.743$, $p<0.001$) in the patients with T1DM

there was a significant difference in the IGF-I-SDS between the two groups. The GH/IGF-I axis is recognized as one of the most important axes that play roles in bone growth. Several studies have reported abnormalities in the GH/IGF-I axis in patients with T1DM[18]. Some studies have shown that IGF-I levels in patients with T1DM are significantly lower at every developmental stage compared with matched controls[18]. IGF-I levels in patients with T1DM are also negatively correlated with glycemic control, particularly during puberty[18]. Thus, the difference in height-SDS and IGF-I-SDS may have originated from the endocrinological characteristics of patients with T1DM in our study.

In the present study, the results showed that serum sclerostin levels were significantly higher in patients with T1DM than in age-, sex-, and sex hormone-matched non-DM controls. These results suggest that sclerostin is associated with glucose metabolism. In a previous study by Tseniditis et al., there were no differences in serum sclerostin levels between T1DM children and adolescents and their healthy peers[19]. However, serum sclerostin levels were reported to be higher in adult patients with T1DM, including men and premenopausal women, than in the control group, irrespective of sex[20]. Sclerostin levels have also been reported to be higher in prediabetic individuals than in healthy adults[21]. Since then, four studies have investigated serum sclerostin levels in T1DM children and adolescents compared with controls, and two studies showed significantly increased levels of serum sclerostin in patients with T1DM than in non-DM controls ; however, in the other two studies, the serum sclerostin levels were not significantly higher in patients with T1DM than in controls[22-25].

Serum sclerostin was first studied by Kirman in healthy children, who showed that serum sclerostin levels were higher in boys than in girls and declined in both sexes following the onset of puberty[25]. Following this report, serum sclerostin levels were studied in patients with T1DM by adjusting for sex and pubertal stage using Tanner stages 1-5. Three studies reported negative results regarding the significance of sclerostin in patients with T1DM[19,23,25]. Instead of the pubertal stages, our study evaluated patients by directly determining sex hormone levels of LH, FSH, E2, and testosterone and showed a significant elevation of serum sclerostin levels in children and adolescents with T1DM. In addition, our report is the first study on serum sclerostin levels in the Japanese population. Bone mineral density and bone metabolism are known to be influenced by ethnicity or genetic background, suggesting that there could be some

influence of ethnic or genetic background on the study results[27]. Therefore, our study suggests that ethnicity may not influence the relationship between serum sclerostin levels and T1DM on bone health in children.

In our study, no significant correlation was observed between serum sclerostin levels and HbA1c in children and adolescents with T1DM. The five studies described above showed controversial results regarding the correlation between serum sclerostin levels and glycemic control in children with T1DM and adolescents[19,22-25]. One of the studies showed a negative association between serum sclerostin levels and HbA1c[24]. Another highlighted high serum levels of sclerostin in children with T1DM and its relationship with altered glycemic control as well as the effect of continuous subcutaneous insulin infusion (CSII) on the improvement of glycemic control and bone health in children with T1DM[22]. However, the other three studies and our study did not show a significant correlation between serum sclerostin levels and glycemic control.

Spearman's rho correlation revealed a significant positive association between serum sclerostin level and height-SDS, BMI-SDS ($\rho=0.394$, $P=0.014$), and bone-ALP level in all of the participants. Bone ALP levels have been investigated in studies on bone health in T1DM children, adolescents, and adults. In a study of biochemical markers of bone turnover in older adults with T1DM including 245 patients and 104 controls, there was no difference in bone-ALP between the two groups ; however, a lower estimated glomerular filtration rate (eGFR) was reported to be significantly associated with higher bone-ALP levels[28]. In two studies on child bone health in patients with T1DM, bone ALP was measured, and there were no significant differences in bone ALP between children with T1DM and controls[22,25]. In our study, as shown in Table 1, there was no difference in bone-ALP levels between the two groups. Bone-ALP levels is one of the markers of bone formation, whereas osteocalcin is another marker of bone formation. A study of sclerostin and bone health in children showed a strong positive association between serum sclerostin levels and osteocalcin levels in a group of children with T1DM before and after adjusting for age, BMI-SDS, and Tanner stage, suggesting that osteocalcin seems to modulate the participation of sclerostin in metabolic regulation in T1DM[24]. Our study suggests that bone-ALP might be another possible modulator of the metabolic regulation of sclerostin in T1DM. Further studies are needed to clarify the significance of sclerostin as a regulator of bone metabolism in T1DM.

Based on studies of sclerostin in bone health in T1DM, serum sclerostin levels could be a biomarker of low bone turnover as a complication of glucose metabolism disorders, such as T1DM. Some bone biomarkers are known to display diurnal variation in humans. However, the rhythmicity of serum sclerostin over a 24-hour interval demonstrates that sclerostin levels do not appear to be rhythmic in men[29]. Therefore, serum sclerostin levels could be a useful biomarker for screening for low bone turnover in children and adolescents with T1DM.

In summary, sclerostin and bone ALP levels may play important roles in the relationship between bone and glucose metabolism in children and adolescents. We suggest that serum sclerostin levels could be a possible biomarker of a state of low bone turnover in children and adolescents with T1DM.

The limitations of our study include its small sample size. Therefore, the conclusions of our study must be applied cautiously to other populations. Another limitation is the lack of data on bone mineral density among the participants of the study. Therefore, our suggestions about the potential role of sclerostin in bone formation are only speculations. We should plan more eligible design of the study including larger sample size, a study of bone mineral density, and even normal controls without underlying health conditions, different from our study using the controls with compensated hypothyroidism and non-DM obesity.

Conclusion

Our results suggest a relationship between sclerostin levels and bone health in children and adolescents with T1DM. Further investigation is required to determine whether an increase in sclerostin levels could be a potential cause of the regulation of bone formation in T1DM.

Conflicts of interest

None of the authors have conflicts of interest to declare.

References

1) Hough, F.S., Pierroz, D.D., Cooper, C. and Ferrari, S.L. (2016) IOF CSA Bone and Diabetes Working Group. MECHANISMS IN ENDOCRINO LOGY: mechanisms and evaluation of bone fragility in type 1 diabetes mellitus. *Eur. J. Endocrinol.*, **174**, R127-R138.
2) Shah, V.N., Shah, C.S. and Snell-Bergeon, J.K. (2015) Type 1 diabetes and risk of fracture : meta-analysis and review of the literature. *Diabet. Med.*, **32**, 1134-1142.
3) Weber, D.R., Haynes, K., Leonard, M.B., Willi, S.M. and Denburg, M.R. (2015) Type 1 diabetes is associated with an increased risk of fracture across the life span ; a population-based cohort study using The Health Improvement Network (THIN). *Diabetes Care.*, **38**, 1913-1920.
4) Hygum, K., Starup-Linde, J., Harsløf, T., Vestergaard, P. and Langdahl, B.L. (2017) MECHANISMS IN ENDOCRINOLOGY: Diabetes mellitus, a state of low bone turnover-a systematic review and meta-analysis. *Eur. J. Endocrinol.*, **176**, R137-R157.
5) Janner, M. and Saner, C. (2022) Impact of type 1 diabetes mellitus on bone health in children. *Horm. Res. Paediatr.*, **95**, 205-214.
6) Madsen, J.O.B., Herskin, C.W., Zerahn, B., Jørgensen, N.R., Olsen, B.S., Pociot, F. and Johannesen, J. (2020) Decreased markers of bone turnover in children and adolescents with type 1 diabetes. *Pediatr. Diabetes*, **21**, 505-514.
7) Fuusager, G., Milandt, N., Shanbhogue, V.V., Hermann, A.P., Schou, A.J. and Christesen, H.T. (2020) Lower estimated bone strength and impaired bone microarchitecture in children with type 1 diabetes. *B.M.J. Open Diabetes Res. Care*, **8**. e001384.
8) Massé, P.G., Pacifique, M.B., Tranchant, C.C., Arjmandi, B.H., Ericson, K.L., Donovan, S.M., Delvin, E. and Caissie, M. (2010) Bone metabolic abnormalities associated with well-controlled type 1 diabetes (IDDM) in young adult women : a disease complication often ignored or neglected. *J. Am. Coll. Nutr.*, **29**, 419-429.
9) Neumann, T., Hofbauer, L.C., Rauner, M., et al. (2014) Clinical and endocrine correlates of circulating sclerostin levels in patients with type 1 diabetes mellitus. *Clin. Endocrinol. (Oxf)*, **80**, 649-655.
10) Rohrer, T., Stierkorb, E., Heger, S., et al. (2007) Delayed pubertal onset and development in German children and adolescents with type 1 diabetes : cross-sectional analysis of recent data from the DPV diabetes documentation and quality management system. *Eur. J. Endocrinol.*, **157**, 647-653.
11) Franceschi, R., Longhi, S., Cauvin, V., et al. (2018) Bone geometry, quality, and bone markers in children with type 1 diabetes mellitus. *Calcif. Tissue Int.*, **102**, 657-665.
12) Brunetti, G., D'Amato, G., Chiarito, M., Tullo, A., Colaianni, G., Colucci, S., Grano, M. and Faienza, M.F. (2019) An update on the role of RANKL-RANK/osteoprotegerin and WNT-β-catenin signaling pathways in pediatric diseases. *World J. Pediatr.*, **15**, 4-11.
13) Dreyer, T.J., Keen, J.A., Wells, L.M. and Roberts, S.T. (2023) Novel insights on the effect sclerostin on bone and other organs. *J. Endocrinol.*, **257**, e220209.
14) Pacicca, D.M., Brown, T., Watkins, D., Kover, K., Yan, Y., Prideaux, M. and Bonewald, L. (2019) Elevated glucose acts directly on osteocytes to increase sclerostin expression in diabetes. *Sci. Rep.*, **9**, 17353.
15) Isojima, T., Kato, N., Ito, Y., Kanzaki, S. and Murata, M. (2016) Growth standard charts for Japanese children with mean and standard deviation (SD) values based on the year 2000 national survey. *Clin. Pediatr. Endocrinol.*, **25**, 71-76.
16) Kato, N., Takimoto, H. and Sudo, N. (2011) The cubic function for spline smoothed L, S, and M values for BMI reference data of Japanese children. *Clin. Pediatr. Endocrinol.*, **20**, 47-49.
17) Isojima, T., Shimatsu, A., Yokoya, S., et al. (2012) Standardized centile curves and reference intervals of serum insulin-like growth factor-I (IGF-I) levels in a normal Japanese population using the LMS method. *Endocr. J.*, **59**, 771-780.
18) Raisingani, M., Preneet, B., Kohn, B. and Yakar, S. (2017) Skeletal growth and bone mineral acquisition in type 1 diabetic children ; abnormalities of the GH/IGF-1 axis. *Growth Horm. I.G.F. Res.*, **34**,

13–21.

19) Tsentidis, C., Gourgiotis, D., Kossiva, L., Marmarinos, A., Doulgeraki, A. and Karavanaki, K. (2017) Increased levels of Dickkopf-1 are indicative of Wnt/β-catenin downregulation and lower osteoblast signalin in children and adolescents with type 1 diabetes mellitus, contributing to lower bone mineral density. *Osteoporos. Int.*, **28**, 945–953.

20) Catalano, A., Pintaudi, B., Morabito, N., Giunta, L., Loddo, S., Corrado, F., D'Anna, R., Lasco, A. and Di Benedetto A. (2017) Wnt antagonist sclerostin and Dickkopt-1 in gestational diabetes. *Diabetes Metab.*, **43**, 375–377.

21) Daniele, G., Winnier, D., Mari, A., Bruder, J., Fourcaudot, M., Pengou, Z., Tripathy, D., Jenkinson, C. and Folli, F. (2015) Sclerostin and insulin resistance in prediabetes : evidence of a cross talk between bone and glucose metabolism. *Diabetes Care*, **38**, 1509–1517.

22) Faienza, M.F., Ventura, A., Delvecchio, M., et al. (2017) High sclerostin and Dickkopf-1 (DKK-1) serum levels in children and adolescents with type 1 diabetes mellitus. *J. Clin. Endocrinol. Metab.*, **102**, 1174–1181.

23) Tsentidis, C., Gourgiotis, D., Kossiva, L., Marmarinos, A., Doulgeraki, A. and Karavanaki, K. (2017) Increased levels of Dickkopf-1 are indicative of Wnt/β-catenin downregulation and lower osteoblast signaling in children and adolescents with type 1 diabetes mellitus, contributing to lower bone mineral density. *Osteoporos. Int.*, **28**, 945–953.

24) Wędrychowicz, A., Sztefko, K. and Starzyk, J.B. (2019) Sclerostin and its association with insulin resistance in children and adolescents. *Bone*, **120**, 232–238.

25) Kurban, S., Eklioglu, B.S. and Selver, M.B. (2022) Investigation of the relationship between serum sclerostin and dickkopf-1 protein levels with bone turnover in children and adolescents with type-1 diabetes mellitus. *J. Pediatr. Endocrinol. Metab.*, **35**, 673–679.

26) Kirmani, S., Amin, S., McCready, L.K., Atkinson, E.J., Melton, L.J. 3rd., Müller, R. and Khosla, S. (2012) Sclerostin levels during growth in children. *Osteoporos. Int.*, **23**, 1123–1130.

27) Medina-Gómez, C., Chesi, A., Heppe, D.H., et al. (2015) BMD Loci Contribute to Ethnic and Developmental Differences in Skeletal Fragility across Populations : Assessment of Evolutionary Selection Pressures. *Mol. Biol. Evol.*, **32**, 2961–2972.

28) Rubin, M.R., de Boer, I.H., Backlund, J.C., et al. (2022) Biochemical markers of bone turnover in older adults with type 1 diabetes. *J. Clin. Endocrinol. Metab.*, **107**, e2405–e2416.

29) Swanson, C., Shea, S.A., Wolfe, P., Markwardt, S., Cain, S.W., Munch, M., Czeisler, C.A., Orwoll, E.S. and Buxton, O.M. (2017) 24-hour profile of serum sclerostin and its association with bone biomarkers in men. *Osteoporos. Int.*, **28**, 3205–3213.