

Relation between the insulin lowering rate and changes in bone mineral density: Analysis among subtypes of type 1 diabetes mellitus

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Keywords

Bone mineral density, Subtypes, Type 1 diabetes mellitus

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ABSTRACT

Aims/Introduction: The bone mineral density in patients with type 1 diabetes mellitus is reduced due to impaired insulin secretion. However, it is unclear whether the rate of bone mineral density reduction is affected by the type 1 diabetes mellitus subtype. This study aimed to clarify the difference in bone mineral density across type 1 diabetes mellitus subtypes: slowly progressive (SP), acute-onset (AO), and fulminant (F).

Methods: This was a retrospective, single-center, cross-sectional study conducted on 98 adult type 1 diabetes mellitus patients. The main outcome included the bone mineral density Z-score (BMD-Z) measured at the lumbar spine and femoral neck.

Results: The lumbar spine BMD-Z was lower in the acute-onset than in the slowly progressive subtype ($P = 0.03$). No differences were observed when compared with the fulminant subtype. The femoral neck BMD-Z tended to be higher in the slowly progressive than in the acute-onset and fulminant subtypes. Multiple regression analyses showed that the lumbar spine BMD-Z was associated with subtypes (AO vs SP) ($P = 0.01$), but not subtypes (F vs SP), adjusted for sex, duration, retinopathy, and C-peptide immunoreactivity (CPR). When the patients were divided into disease duration tertiles, in the first and second tertiles, the CPR levels were lower in the acute-onset or fulminant than in the slowly progressive subtype. In contrast, the lumbar spine and femoral neck BMD-Z differed between the acute-onset and slowly progressive only in the second tertiles (both $P < 0.01$), with a similar tendency between the fulminant and slowly progressive subtypes.

Conclusions: Among the type 1 diabetes mellitus subtypes, bone mineral density undergoes time-dependent changes, which reveals that the bone mineral density decline follows the impaired insulin secretion. These results provide novel insights into the association between the low insulin exposure duration and bone mineral density.

INTRODUCTION

Patients with type 1 diabetes mellitus have decreased bone mineral density in both the lumbar spine and the femoral neck. Therefore, a higher fracture risk has been implicated in type 1 diabetes mellitus^{1,2}, particularly in older adult populations

(4- to 6-fold higher risk)^{3–6} and postmenopausal women (12-fold increase in a hip fracture) compared with the general population⁷. In a recent meta-analysis of adult type 1 diabetes mellitus, a lower bone mineral density was demonstrated in both the lumbar spine (-0.035 g/cm^2) and the femoral neck (-0.055 g/cm^2)⁸, while normal or higher bone mineral density was shown in those with type 2 diabetes mellitus.

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An endocrinology literature review indeed confirmed that the bone turnover markers, including serum osteocalcin, bone-specific alkaline phosphatase, procollagen type 1 propeptide amino-terminal, and crosslinking telopeptides of type 1 collagen C-terminal, are lower in patients with insulin-dependent diabetes mellitus (DM)^{9,10}, which supports the theory that insulin is thought to play an important role in bone formation¹¹. Furthermore, C-peptide immunoreactivity (CPR), a marker of endogenous insulin secretion, was positively correlated with bone mineral density in both the lumbar spine and femoral neck in type 1 diabetes mellitus¹². Serum CPR levels were positively associated with bone mineral density in a cohort of postmenopausal women without diabetes mellitus¹³, indicating the important role of endogenous insulin levels in bone metabolism.

Type 1 diabetes mellitus is a disease primarily caused by the destruction of pancreatic beta cells, which leads to insulin deficiency, and requires lifelong exogenous insulin replacement therapy. According to the diagnostic criteria of the Japan Diabetes Society^{14–16}, three pathogenic definitions of type 1 diabetes mellitus have been demonstrated: slowly progressive (SP), acute-onset (AO), and fulminant (F). In patients with acute-onset and fulminant type 1 diabetes mellitus, lifelong insulin treatment initiation is required soon after the diagnosis because of the marked acute progression of insulin deficiency^{15,16}. Conversely, patients with slowly progressive type 1 diabetes mellitus develop an insulin-dependent state from 6 months to several years after onset because of a gradual decrease in insulin secretory capacity¹⁴.

As the decline in endogenous insulin secretion occurs differently in each subtype, the differences in bone mineral density between the subtypes could be a suitable model to represent the effect of the duration of low insulin exposure on bone mineral density. However, few studies have examined the bone mineral density reduction according to the type 1 diabetes mellitus classification. Here, we conducted a cross-sectional single-institute study to clarify the difference in bone mineral density across type 1 diabetes mellitus subtypes.

MATERIALS AND METHODS

Patients, study design, and data collection

This was a retrospective, single-center, cross-sectional study conducted at Kobe University Hospital. A total of 170 Japanese adult patients with type 1 diabetes mellitus admitted to our unit for evaluation of diabetic complications between January 2008 and April 2019 and who underwent bone mineral density measurements were enrolled in this study. A total of 18 patients were excluded based on the following criteria: estimated glomerular filtration rate (eGFR) of <30 mL/min/1.73 m²; disease duration of <1 year; and a medical history of osteoporosis, including treatment with bisphosphonates, selective estrogen receptor modulators, denosumab, and recombinant human parathyroid hormone (Figure 1). Cases that could not be categorized into a definite subtype based on the

medical records were also excluded. The remaining 98 patients were then classified into three groups according to their subtypes based on the diagnostic criteria of the Japan Diabetes Society: acute-onset ($n = 51$), slowly progressive ($n = 35$), and fulminant ($n = 12$)^{14–16}. Blood samples were collected early in the morning. We reviewed the medical records and documented the baseline characteristics, including laboratory findings. This study was approved by the Research Ethics Committee of Kobe University Hospital (permit no.: B210070). This study was conducted retrospectively, and all procedures were part of routine medical care. The patients had the option of an opt-out process, where patients were provided with information explaining the data to be collected and the purpose of the study and were given the opportunity to withdraw.

Biochemical measurements

Anthropometric parameters such as body mass index (BMI) were collected for each patient along with biochemical data, including platelets, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, cholinesterase, thyroid stimulating hormone (TSH), free thyroxine (FT4), Ca (correction value), phosphate (P), insulin-like growth factor I (IGF-I), and hemoglobin A1c (HbA1c). Postprandial C-peptide immunoreactivity (ppCPR), and C-peptide immunoreactivity during a glucagon stimulating test (GST-CPR) were measured to evaluate the capacity of insulin secretion. The serum ppCPR levels were measured 2 h after breakfast. Serum ppCPR was not available in three cases, and 95 cases were measured (35 (100%), 49 (96%), 11 (91%) cases in the slowly progressive, acute-onset, and fulminant groups, respectively). The GST-CPR levels were measured 6 min after the intravenous injection of 1 mg glucagon, and 62 cases were measured (28 (80%), 30 (58%), and 4 (33%) cases in the slowly progressive, acute-onset, fulminant groups, respectively). The serum IGF-I levels were measured using an immunoradiometric assay (IRMA, Daiichi Radioisotope Laboratories, Tokyo, Japan). The IGF-I standard deviation (SD) score was calculated based on age- and sex-matched healthy Japanese individuals¹⁷. The eGFR was calculated from the serum creatinine levels.

Other covariates

The onset of type 1 diabetes mellitus, disease duration, and a previous history of cardiovascular diseases were determined by medical review. Evaluation of diabetic neuropathy was based on symptoms, quantitative sensory testing (vibration and monofilament test), and quantitative motor testing (patellar and ankle reflexes)¹⁸. Diabetic retinopathy was categorized as non-proliferative, pre-proliferative, or proliferative¹⁹. Diabetic nephropathy was assessed by measuring microalbumin levels in 24 h urine (normal value: <30 mg/day). Microalbuminuria and macroalbuminuria were diagnosed if the albumin excretion rate was 30–300 mg/day or > 300 mg/day, respectively²⁰.

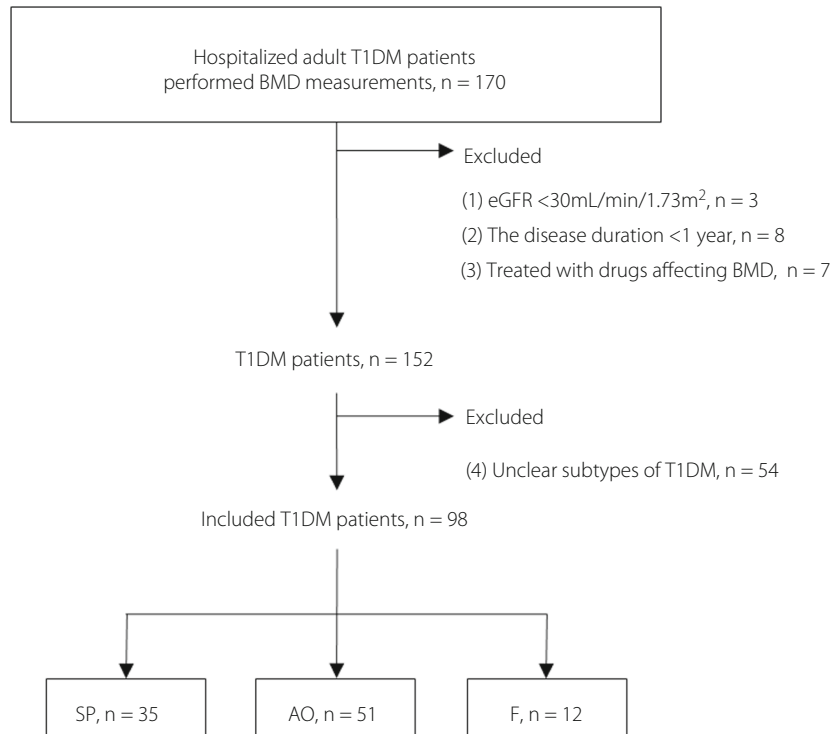


Figure 1 | Flow diagram of included patients. AO, acute-onset; BMD, bone mineral density; F, fulminant; SP, slowly progressive; T1DM, type 1 diabetes mellitus.

Bone mineral density

The bone mineral density of the lumbar spine (L2–L4) and the femoral neck were measured using dual-energy x-ray absorptiometry (DXA; Horizon A DXA System) simultaneously with blood sampling. The coefficients of variation were 1.44% and 2.10% for the lumbar spine and femoral neck, respectively. The bone mineral density Z-score (BMD-Z), obtained from the DXA measurement, represents the number of SDs by which the bone mineral density in an individual differs from the mean value for sex and age. A total of 15 cases were excluded because calcification in the vertebral body made it difficult to assess the bone mineral density accurately. The remaining 83 and 98 cases in the lumbar spine and femoral neck BMD-Z groups, respectively, were analyzed.

Analysis based on disease duration tertiles

Since it takes ≥ 6 months for changes in bone metabolism to become apparent²¹, patients with disease durations of < 1 year were excluded from this study. In the previous reports, the association between the duration of diabetes and the complications was compared in tertiles, as the duration of diabetes can show a skewed distribution^{22,23}. To clarify the long-term effects of endogenous insulin reduction on bone mineral density changes, the patients were divided according to the tertile of disease duration; first tertile (< 6 years [excluding the first year])

($n = 33$), second tertile (6–14 years) ($n = 34$), and third tertile (≥ 15 years) ($n = 31$).

Statistical analysis

All statistical analyses were performed using SPSS Statistics ver. 26.0 software (IBM Corporation, Armonk, NY, USA). The results are presented as the mean \pm SD. All continuous variables were analyzed using statistical graphs such as histograms, and the Shapiro–Wilk normality test was performed to test the normality of the data distribution. Pearson's correlation coefficient and Spearman's rank correlation coefficient were used for correlation analysis between two variables of normally and non-normally distributed data, respectively. One-way analysis of variance with *post-hoc* Bonferroni correction was used to compare the differences in normally distributed data. The Mann–Whitney *U* test and Kruskal–Wallis test were used to compare the differences in non-normally distributed data. For categorical variables, differences were analyzed using the chi-squared test or Fisher's exact test. In the simple regression analyses, the relation with BMD-Z, and subtypes (AO vs SP, and F vs SP) was performed. Previous studies indicated bone mineral density was related with disease duration²⁴, retinopathy^{24,25}, and CPR¹². The multiple regression analyses were performed to adjust for sex, disease duration, retinopathy, and ppCPR, as the confounding factors. Values of $P < 0.05$ indicated statistical significance.

RESULTS

Participant characteristics

Among the 98 adult patients with type 1 diabetes mellitus, the BMD-Z distributions demonstrated that the bone mineral density in the lumbar spine (53%) and femoral neck (62%) were lower than the average bone density of age-matched controls (Table 1, Figure 2, and Table S1). Both the age at the time of this study and the disease onset were higher in the slowly progressive (57.9 ± 13.2 years and 46.8 ± 14.8 years) and the fulminant (51.4 ± 15.1 years and 43.5 ± 15.9 years) groups than in the acute-onset group (39.1 ± 14.6 years and

27.5 ± 14.8 years) (both $P < 0.01$). The disease duration did not differ across the three groups ($P = 0.31$). No differences were observed among the three groups in terms of sex, BMI, and HbA1c levels. The AST and ALT levels were significantly higher in the slowly progressive group than in the acute-onset group ($P = 0.03$ and $P < 0.01$, respectively). As expected, individuals in the slowly progressive group had significantly higher ppCPR and GST-CPR values than those in the acute-onset (both $P < 0.01$) and fulminant (both $P < 0.01$) groups. Diabetic retinopathy was more complicated in the slowly progressive group, followed by the acute-onset group; this was not observed

Table 1 | Clinical characteristics of patients with type 1 diabetes mellitus according to disease subtype

Variable	SP (n = 35)	AO (n = 51)	F (n = 12)	p1	p2	p3	p4
Age (years)	57.9 ± 13.2	39.1 ± 14.6	51.4 ± 15.1	<0.01	<0.01	0.73	0.05
Female, n (%)	21 (60%)	38 (74%)	8 (66%)	0.38			
Postmenopause, (%)	14 (40%)	11 (21%)	4 (33%)	0.17			
Age at onset (years)	46.8 ± 14.8	27.5 ± 14.8	43.5 ± 15.9	<0.01	<0.01	0.50	<0.01
Duration of diabetes (years)	11.0 ± 10.1	11.5 ± 7.0	7.9 ± 5.0	0.31	–	–	–
Time to insulin treatment (years)	2.6 ± 4.6	0.1 ± 0.5	0.0 ± 0.0	<0.01	<0.01	<0.01	1.00
BMI (kg/m ²)	21.9 ± 3.8	22.0 ± 2.9	22.6 ± 3.6	0.51	–	–	–
Grave's disease, n (%)	3 (8%)	10 (19%)	0 (0%)	0.15	–	–	–
PLT (10 ⁴ /mL)	22.1 ± 5.8	28.9 ± 31.0	21.7 ± 6.5	0.07	–	–	–
Alb (g/dL)	4.1 ± 0.4	4.2 ± 0.5	4.1 ± 0.2	0.80	–	–	–
eGFR (mL/min)	84.7 ± 22.2	89.6 ± 27.4	81.5 ± 17.2	0.17	–	–	–
AST (U/L)	26.7 ± 24.0	20.0 ± 9.6	24.7 ± 8.4	<0.01	0.03	1.00	0.05
ALT (U/L)	25.6 ± 22.8	17.5 ± 13.2	22.0 ± 16.2	<0.01	<0.01	1.00	0.42
ALP (U/L)	252.8 ± 89.1	219.9 ± 78.4	198.8 ± 80.5	0.08	–	–	–
ChE (U/L)	302.6 ± 93.0	314.0 ± 76.9	290.5 ± 87.8	0.64	–	–	–
TSH (mU/mL)	1.59 ± 1.38	2.61 ± 6.22	1.75 ± 0.77	0.50	–	–	–
FT4 (ng/dL)	1.04 ± 0.16	1.19 ± 1.42	0.94 ± 0.17	0.66	–	–	–
Ca (mg/dL)	9.0 ± 1.5	9.2 ± 0.3	9.2 ± 0.5	0.88	–	–	–
P (mg/dL)	3.4 ± 0.5	3.5 ± 0.5	3.5 ± 0.6	0.95	–	–	–
IGF-I SDS	−1.08 ± 1.94	−0.96 ± 1.02	−1.46 ± 1.28	0.33	–	–	–
HbA1c (%)	8.7 ± 1.6	8.3 ± 2.0	8.0 ± 1.3	0.41	–	–	–
ppCPR (ng/mL)	1.60 ± 1.99	0.17 ± 0.38	0.01 ± 0.03	<0.01	<0.01	<0.01	0.23
GST-CPR (ng/mL)	1.41 ± 1.39	0.23 ± 0.33	0.01 ± 0.03	<0.01	<0.01	<0.01	0.35
TDD (U/day)	27.9 ± 14.4	35.6 ± 16.7	42.3 ± 27.4	0.04	0.19	0.06	0.75
TDD/kg (U/kg)	0.40 ± 0.26	0.61 ± 0.28	0.72 ± 0.47	<0.01	<0.01	<0.01	0.67
Retinopathy, n (%)	11 (31%)	8 (15%)	0 (0%)	0.03	–	–	–
Nephropathy (non, micro and macro), n (%)	34 (97%), 0 (0%), 1 (3%)	44 (86%), 4 (8%), 3 (6%)	11 (92%), 1 (8%), 0 (%)	0.35	–	–	–
Neuropathy, n (%)	18 (51%)	15 (29%)	4 (33%)	0.12	–	–	–
CVD, n (%)	3 (8%)	1 (1%)	1 (8%)	0.30	–	–	–
LS BMD (g/cm ²)	0.99 ± 0.18	0.95 ± 0.12	0.88 ± 0.17	0.41	–	–	–
LS BMD Z-score	0.38 ± 1.08	−0.25 ± 0.96	−0.35 ± 1.01	0.02	0.03	0.15	1.00
FN BMD (g/cm ²)	0.69 ± 0.11	0.70 ± 0.12	0.65 ± 0.08	0.47	–	–	–
FN BMD Z-score	0.03 ± 1.01	−0.44 ± 1.00	−0.56 ± 0.70	0.05	–	–	–

Data are presented as mean ± standard deviation. Statistical significance was set at $P < 0.05$. p1: all groups, p2: SP vs AO, p3: SP vs F, p4: AO vs F. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AO, acute-onset; AST, aspartate aminotransferase; BMD, bone mineral density; BMI, body mass index; ChE, cholinesterase; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; F, fulminant; FN, femoral neck; FT4, free thyroxine; GST-CPR, C-peptide immunoreactivity during the glucagon stimulation test; HbA1c, hemoglobin A1c; IGF-I, insulin-like growth factor I; LS, lumbar spine; PLT, platelets; ppCPR, postprandial C-peptide immunoreactivity; SP, slowly progressive; TDD, total daily insulin dose; TSH, thyroid stimulating hormone.

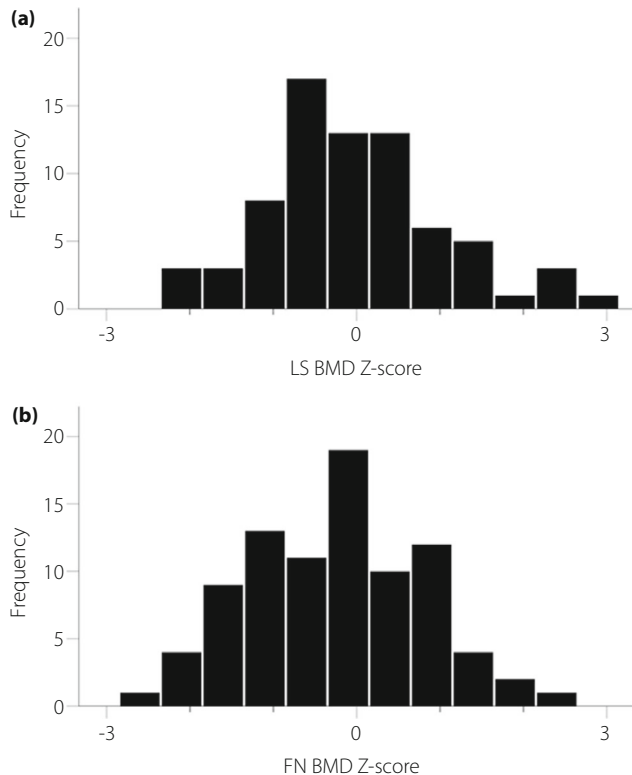


Figure 2 | Distribution of the (a) lumbar spine and (b) femoral neck BMD Z-score among patients with type 1 diabetes mellitus. BMD, bone mineral density; FN, femoral neck; LS, lumbar spine; T1DM, type 1 diabetes mellitus.

in the fulminant group ($P = 0.03$). No differences were observed for other diabetic microangiopathies, such as neuropathy ($P = 0.12$) and nephropathy ($P = 0.35$).

Bone mineral density among the three type 1 diabetes mellitus subtypes

The lumbar spine BMD-Z was lower in the acute-onset (-0.25 ± 0.96) group than in the slowly progressive group (0.38 ± 1.08 , $P = 0.03$), while neither the slowly progressive nor the acute-onset group differed from the fulminant group (-0.35 ± 1.01) ($P = 0.15$ and $P = 1.00$, respectively). The femoral neck BMD-Z tended to be lower in the acute-onset (-0.44 ± 1.00) and fulminant (-0.56 ± 0.70) groups than in the slowly progressive group (0.03 ± 1.01) (Table 1).

Analysis of disease duration, bone mineral density, and endogenous insulin levels among the three type 1 diabetes mellitus subtypes

We further investigated the detailed association between long-term low endogenous insulin secretion and bone mineral density, analyzing the patients in all three subtypes. A correlation analysis was performed among disease duration, CPR, and BMD Z-score in all three groups (Figure 3). The disease

duration was negatively correlated with ppCPR in both the slowly progressive ($r = -0.44$, $P < 0.01$) and acute-onset ($r = -0.35$, $P = 0.01$) groups, and with GST-CPR in the slowly progressive group ($r = -0.51$, $P < 0.01$), but not in the fulminant group (Figure 3a, b). In contrast, the disease duration was not correlated with the lumbar spine or femoral neck BMD-Z in either the slowly progressive ($P = 0.38$, $P = 0.87$), acute-onset ($P = 0.81$, $P = 0.08$), or fulminant groups ($P = 0.58$, $P = 0.12$) (Figure 3c, d). No correlations were shown between the lumbar spine or the femoral neck BMD-Z and ppCPR or GST-CPR in all three groups (Figure 3e-h).

The simple regression analyses showed that the lumbar spine BMD-Z tended to be lower in the acute-onset group than in the slowly progressive group ($P = 0.05$). The multiple regression analysis adjusted for sex, disease duration, retinopathy, and ppCPR showed that the lumbar spine BMD-Z was significantly lower in the acute-onset group than in the slowly progressive group ($P = 0.01$) (Table 2a). In contrast, no associations were shown between these variables and the femoral neck BMD-Z. Since menstrual status has strong effect on bone mineral density, we further extracted women from these subjects and divided them into pre- and post-menopausal groups and analyzed the data. In premenopausal women, after being adjusted for disease duration, retinopathy, and ppCPR, both the lumbar spine and the femoral neck BMD-Z were lower in the fulminant group than in the slowly progressive group ($P = 0.02$, $P = 0.03$) (Table 2b). Furthermore, the lumbar spine BMD-Z tended to be lower in the acute-onset group than in the slowly progressive group ($P = 0.05$). However, no association was shown between the lumbar spine or femoral neck BMD-Z and the type 1 diabetes mellitus subtypes in postmenopausal women (Table 2c).

Analysis based on disease duration tertiles

To visualize the effect of the duration of low insulin exposure on bone mineral density, we divided the participants into three subgroups according to the disease duration tertiles (first tertile (<6 years [excluding the first year]), second tertile (6–14 years), and third tertile (≥ 15 years)). The ppCPR, GST-CPR, lumbar spine BMD-Z, and femoral neck BMD-Z among the three subtypes were compared (Table 3). In the first tertile, both the ppCPR and GST-CPR levels were lower in the acute-onset group than in the slowly progressive group (both $P < 0.01$), as expected. In the meantime, the ppCPR was lower in the fulminant group than in the slowly progressive group ($P < 0.01$). The lumbar spine and femoral neck BMD-Z were similar among the three groups. In the second tertile, ppCPR and GST-CPR levels were also lower in the acute-onset ($P = 0.02$, $P = 0.01$) and fulminant groups ($P = 0.02$, $P = 0.02$). Intriguingly, a decline in the lumbar spine and femoral neck BMD-Z in the acute-onset group was shown (both $P < 0.01$). In the third tertile, no differences of ppCPR, GST-CPR, lumbar spine BMD-Z and femoral neck BMD-Z were indicated among all subtypes, suggesting that a bone

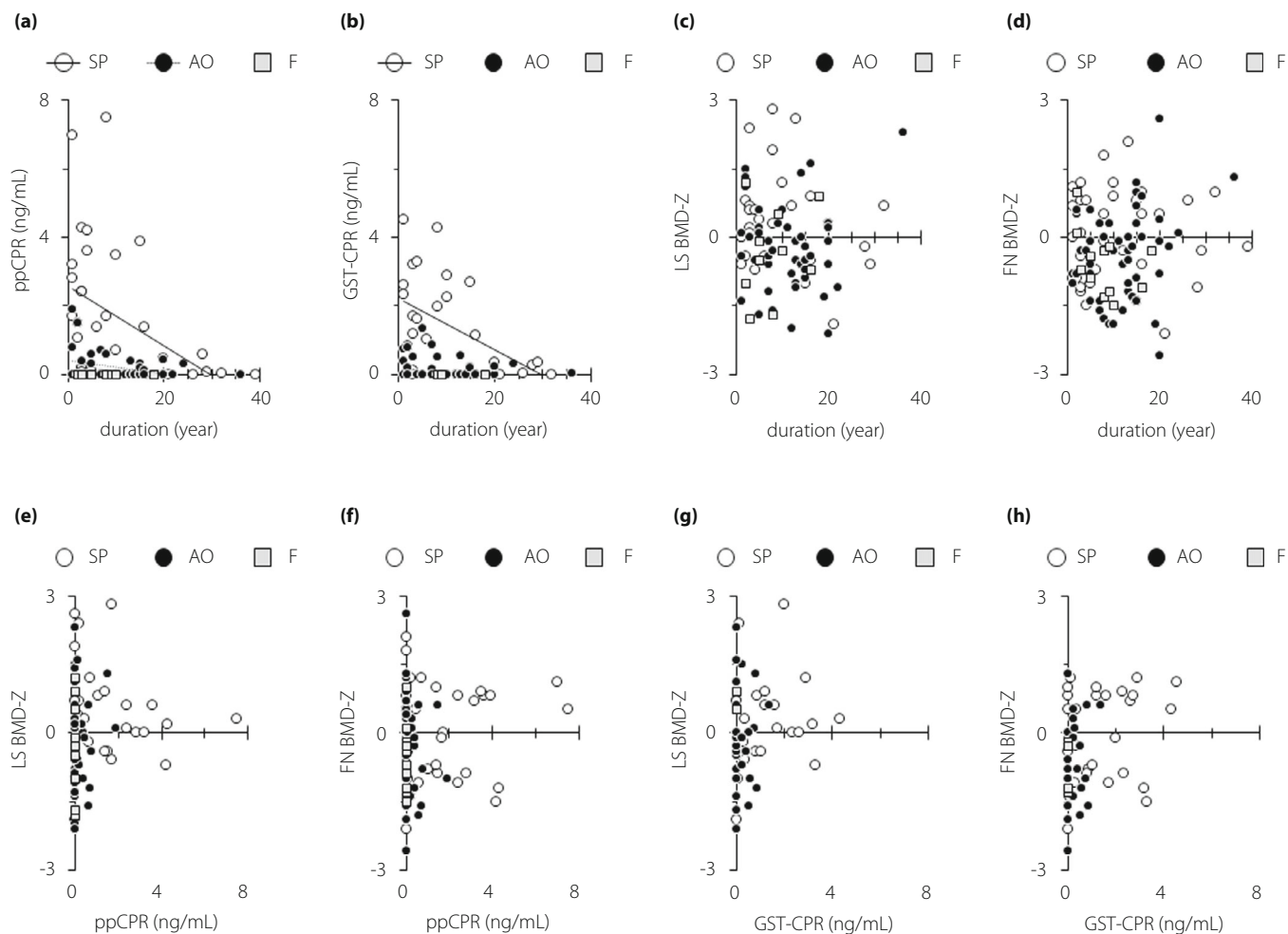


Figure 3 | Scatter plot representing individual data point correlations among disease duration, CPR, and BMD Z-score. AO, acute-onset; BMD-Z, bone mineral density Z-score; FN, femoral neck; GST-CPR, C-peptide immunoreactivity during glucagon stimulating test; LS, lumbar spine; ppCPR, postprandial C-peptide immunoreactivity; SP, slowly progressive.

mineral density decline occurs years after the disruption of endogenous insulin secretion.

DISCUSSION

This study was the first to report the difference in bone mineral density associated with the three pathological type 1 diabetes mellitus subtypes (SP, AO, and F). Since these subtypes have different time courses for insulin dependence, the analysis of bone mineral density across the groups appears to be an excellent model for demonstrating the pathophysiological association between impaired endogenous insulin secretion and reduced bone mineral density in this beta-cell dysfunction disorder.

The lower bone mineral density levels in the present type 1 diabetes mellitus participants were consistent with those reported in a previous meta-analysis among other races⁷, indicating that race-based differences in bone metabolism are not significant. This finding also suggests that these participants

were suitable for further investigations as a model of bone research in type 1 diabetes mellitus. A low bone mineral density in type 1 diabetes mellitus is associated with endogenous insulin deficiency, as well as low BMI and diabetic complications^{26,27}. In this study, the disease duration, BMI, and microvascular disease did not differ across the three subtypes, except for diabetic retinopathy. Regarding macrovascular disease, the previous history of cardiovascular disease did not differ across the subtypes, suggesting that these subtypes were suitable for analyzing the relationship between endogenous insulin secretion and bone mineral density in type 1 diabetes mellitus. The lower lumbar spine bone mineral density in the acute-onset group than in the slowly progressive group, despite the younger age, indicates that the decrease in endogenous insulin secretion contributes more to bone mineral density than age. Multiple regression analysis revealed that the subtype of type 1 diabetes mellitus was an independent factor associated

Table 2 | Multiple regression analyses of determinants of the lumbar spine and femoral neck bone mineral density in patients with slowly progressive, acute-onset and fulminant type 1 diabetes mellitus

Dependent variable	Independent variable	Simple regression analysis			Multiple regression analysis		
		β value	B [95% CI]	P-value	β value	B [95% CI]	P-value
(a) All patients							
Lumbar spine BMD Z-score	AO vs SP	-0.21	-0.44 [-0.89, 0.00]	0.05	-0.35	-0.75 [-1.35, -0.15]	0.01
	F vs SP	-0.10	-0.34 [-1.04, 0.36]	0.33	-0.24	-0.82 [-1.74, 0.08]	0.07
	Sex	0.10	0.24 [-0.25, 0.73]	0.32	0.13	0.31 [-0.20, 0.83]	0.23
	Duration	-0.04	0.00 [-0.03, 0.02]	0.69	-0.02	-0.03 [-0.03, 0.03]	0.85
	Retinopathy	0.06	0.17 [-0.39, 0.74]	0.55	0.01	0.03 [-0.62, 0.69]	0.92
Femoral neck BMD Z-score	ppCPR	0.11	0.09 [-0.90, 0.28]	0.30	-0.06	-0.05 [-0.28, 0.18]	0.66
	AO vs SP	-0.16	-0.32 [-0.72, 0.07]	0.10	-0.14	-0.28 [-0.80, 0.24]	0.28
	F vs SP	-0.10	-0.31 [-0.92, 0.29]	0.30	-0.07	-0.23 [-1.01, 0.54]	0.55
	Sex	-0.03	-0.06 [-0.49, 0.36]	0.76	0	0.02 [-0.42, 0.46]	0.93
	Duration	0.11	0.01 [-0.01, 0.03]	0.27	0.08	0.01 [-0.02, 0.03]	0.51
Retinopathy		0.22	0.56 [0.07, 1.06]	0.02	0.17	0.43 [-0.15, 1.03]	0.14
	ppCPR	0.14	0.10 [-0.04, 0.24]	0.16	0.13	0.09 [-0.08, 0.27]	0.30
(b) Premenopausal women							
Lumbar spine BMD Z-score	AO vs SP	-0.19	-0.46 [-1.30, -0.37]	0.27	-0.51	-1.23 [-2.50, 0.04]	0.05
	F vs SP	-0.22	-0.84 [-2.19, 0.49]	0.20	-0.51	-1.98 [-3.74, -0.22]	0.02
	Duration	-0.13	-0.02 [-0.09, 0.04]	0.46	0.02	0.00 [-0.08, 0.09]	0.91
	Retinopathy	-0.20	-0.67 [-1.86, 0.51]	0.25	-0.17	0.59 [-1.92, 0.74]	0.37
	ppCPR	0.13	0.15 [-0.26, 0.57]	0.46	-0.20	-0.24 [-0.77, 0.29]	0.36
Femoral neck BMD Z-score	AO vs SP	0.03	0.06 [-0.46, 0.58]	0.81	-0.40	-0.63 [-1.45, 0.19]	0.12
	F vs SP	-0.18	-0.43 [-1.19, 0.33]	0.25	-0.49	-1.12 [-2.19, -0.06]	0.03
	Duration	0.04	0.00 [-0.03, 0.04]	0.79	0.05	0.00 [-0.04, 0.05]	0.77
	Retinopathy	-0.15	-0.34 [-1.11, 0.41]	0.36	-0.19	-0.43 [-1.25, 0.38]	0.29
	ppCPR	-0.17	-0.13 [-0.39, 0.13]	0.31	-0.43	-0.34 [-0.68, 0.00]	0.05
(c) Postmenopausal women							
Lumbar spine BMD Z-score	AO vs SP	-0.19	-0.37 [-1.26, 0.50]	0.38	-0.18	-0.37 [-1.66, 0.91]	0.54
	F vs SP	-0.04	-0.10 [-1.26, 1.06]	0.85	0.04	0.12 [-1.47, 1.72]	0.87
	Duration	0.22	0.02 [-0.02, 0.07]	0.30	0.28	0.03 [-0.03, 0.09]	0.29
	Retinopathy	0.36	0.80 [-0.13, 1.73]	0.09	0.30	0.67 [-0.53, 1.87]	0.25
	ppCPR	0.08	0.06 [-0.29, 0.43]	0.69	0.18	0.14 [-0.35, 0.64]	0.53
Femoral spine BMD Z-score	AO vs SP	-0.19	-0.46 [-1.39, 0.47]	0.32	0	0.00 [-1.21, 1.19]	0.98
	F vs SP	-0.04	-0.15 [-1.49, 1.18]	0.81	0.10	0.34 [-1.28, 1.97]	0.66
	Duration	0.21	0.02 [-0.02, 0.07]	0.25	0.18	0.02 [-0.03, 0.08]	0.43
	Retinopathy	0.39	1.04 [0.09, 1.98]	0.03	0.36	0.94 [-0.31, 2.20]	0.13
	ppCPR	0.09	0.06 [-0.21, 0.35]	0.62	0.25	0.18 [-0.18, 0.54]	0.31

Simple and multiple regression analyses are shown in (a) all patients, (b) premenopausal women, and (c) postmenopausal women. The β value, the B value [95% CI], the P-value, and VIF are shown in the Table. 95% CI, 95% confidence interval; AO, acute-onset; B, unstandardized partial regression coefficient; BMD, bone mineral density; F, fulminant; FN, femoral neck; LS, lumbar spine; ppCPR, postprandial C-peptide immunoreactivity; SP, slowly progressive; VIF, variance inflation factor; β , standardized partial regression coefficient.

with a reduced lumbar spine bone mineral density in adjusting for sex, disease duration, diabetic retinopathy, and current CPR, suggesting that the degree of endogenous insulin decline is important for bone mineral density. In the analysis of premenopausal women, the subtypes were associated with a reduced bone mineral density, but not in postmenopausal women. Estrogen deficiency itself can significantly impact the bone mineral density decline in postmenopausal patients. In addition, the glycemic control of diabetes mellitus was poorer in postmenopausal than in premenopausal women, leading to

more diabetic microvascular complications including neuropathy in the present study. Furthermore, the distribution of subtypes significantly differed between premenopausal and postmenopausal patients. Therefore, in addition to insulin deficiency, various other factors may affect the differences in bone mineral density between subtypes in premenopausal and postmenopausal women in the present study.

The multiple regression analysis indicated that subtypes of type 1 diabetes mellitus were associated with the lumbar spine bone mineral density, but not with that of the femoral neck.

Table 3 | CPR and BMD-Z in the three groups categorized by disease duration groups

Disease duration tertiles	Measurement	SP		AO		F		p1	p2	p3	p4
		n	mean ± SD	n	mean ± SD	n	mean ± SD				
First	ppCPR	15	2.32 ± 1.95	13	0.42 ± 0.63	5	0.00 ± 0.00	<0.01	<0.01	<0.01	0.20
	GST-CPR	12	1.85 ± 1.38	10	0.39 ± 0.45	0	–	<0.01	<0.01		
	LS BMD Z-score	13	0.31 ± 0.79	12	0.07 ± 0.98	5	–0.44 ± 1.11	0.29			
	FN BMD Z-score	15	–0.20 ± 0.93	13	–0.41 ± 0.65	5	–0.18 ± 0.75	0.78			
Second	ppCPR	8	1.84 ± 2.57	19	0.09 ± 0.21	5	0.00 ± 0.00	0.01	0.02	0.02	1.00
	GST-CPR	6	2.07 ± 1.49	13	0.15 ± 0.28	3	0.00 ± 0.00	<0.01	0.01	0.02	1.00
	LS BMD Z-score	7	1.30 ± 1.19	19	–0.46 ± 0.79	3	–0.50 ± 1.11	<0.01	<0.01	0.19	1.00
	FN BMD Z-score	8	0.67 ± 1.00	21	–0.85 ± 0.75	5	–0.90 ± 0.60	<0.01	<0.01	0.06	1.00
Third	ppCPR	12	0.54 ± 1.13	17	0.08 ± 0.13	1	0.10	0.32			
	GST-CPR	10	0.48 ± 0.85	7	0.13 ± 0.13	1	0.06	0.76			
	LS BMD Z-score	8	–0.28 ± 0.92	14	–0.25 ± 1.12	2	0.10 ± 1.13	0.88			
	FN BMD Z-score	12	–0.09 ± 1.03	17	0.03 ± 1.28	2	–0.70 ± 0.56	0.50			

Data are presented as mean ± standard deviation. Statistical significance was set at $P < 0.05$. p1: all groups, p2: SP vs AO, p3: SP vs F, p4: AO vs F. AO, acute-onset; BMD, bone mineral density; BMD-Z, bone mineral density Z-score; CPR, C-peptide immunoreactivity; F, fulminant; FN, femoral neck; GST-CPR, C-peptide immunoreactivity during glucagon stimulating test; LS, lumbar spine; ppCPR, postprandial C-peptide immunoreactivity; SD, standard deviation; SP, slowly progressive.

The lumbar spine and femoral neck have different proportions of the trabecular bone and cortical bone. Insulin is known to have a positive effect on both trabecular and cortical bone in a mouse model of type 1 diabetes mellitus²⁸. In contrast, decreased IGF-I levels in type 1 diabetes mellitus may have a different impact on the lumbar spine and the femoral neck because serum IGF-I levels are positively associated more strongly with cortical bone than with trabecular bone²⁹. Moreover, bone mineral density measurements of cortical bone is limited by dual-energy x-ray absorptiometry³⁰. Different modalities such as high-resolution quantitative computed tomography may provide additional insight into the discrepancy between the lumbar spine and the femoral neck bone mineral density among type 1 diabetes mellitus subtypes.

In patients with slowly progressive type 1 diabetes mellitus, it takes >6 months to become insulin-dependent, while immediate insulin initiation at the time of diagnosis is required for those with acute-onset and fulminant type 1 diabetes mellitus^{14–16}. No correlations were shown between bone mineral density and CPR, and the multiple regression analyses showed that type 1 diabetes mellitus subtypes were associated with bone mineral density, suggesting that the accumulation of lower insulin, not current insulin secretion, appears to affect the lowering of the bone mineral density. When the participants were divided based on disease duration tertiles, the insulin secretion was differentially impaired among all subtypes in the first and second tertiles, but not the third tertile. In contrast, differences in bone mineral density between acute-onset and slowly progressive subtypes were shown only in the second subtype, with a similar tendency between fulminant and slowly progressive. These results suggest that the change in bone mineral density, which was associated with a transient change in insulin levels,

followed sometime later. A longitudinal analysis of bone mineral density is required to clarify this speculation. A previous longitudinal study showed that the rate of decline in bone mineral density did not differ between type 1 diabetes mellitus patients and healthy controls for 2 years follow-up³¹, suggesting that 2 years may not be sufficient to evaluate the effect of impaired insulin secretion on bone mineral density.

Insulin has been demonstrated to exert anabolic effects on bone physiology. In fetal rats, insulin-induced bone collagen synthesis occurred in osteoblasts, where insulin receptors were expressed³². In an *in vivo* model, insulin receptor substrate (one of the main substrates of insulin) knockout mice exhibited a marked reduction in bone formation, indicating the importance of insulin signaling in bone metabolism^{33,34}. Decreased endogenous insulin levels are associated with lower IGF-I levels due to both portal insulinopenia and hepatic growth hormone resistance³⁵. Serum IGF-I levels were found to be lower in type 1 diabetes mellitus patients than in healthy controls³⁶. In the present study, low IGF-I levels were also observed in most patients, as described previously. Since IGF-I also has an anabolic effect on bone, low IGF-I levels were thought to contribute to the decrease in bone mineral density more in acute-onset than in slowly progressive type 1 diabetes mellitus. However, no difference was observed between these subtypes, suggesting that IGF-I had less of an effect on the results of this study.

Acute-onset and slowly progressive type 1 diabetes mellitus are pathogenically associated with autoimmune diseases³⁷, but fulminant type 1 diabetes mellitus may not be. In autoimmune-related diabetes, elevated inflammatory cytokines, such as interleukin-1 and tumor necrosis factor- α , have been shown to increase bone resorption and to reduce bone formation, resulting in decreased bone mineral density³⁸. Patients with type 1

diabetes mellitus often have other autoimmune diseases, such as Graves' disease, which also promotes bone resorption. In our study, the complication rate of Graves' disease was similar across all subtypes without differences in either the serum TSH or FT4 levels, indicating that it had little involvement in the present study. Slowly progressive type 1 diabetes mellitus is a disease that shares many common conditions with latent autoimmune diabetes mellitus in adults (LADA)³⁹. Compared with classical adult-onset type 1 diabetes mellitus such as acute-onset type 1 diabetes mellitus, patients with LADA have higher rates of microvascular complications and metabolic syndrome, which is thought to cause a lower bone mineral density^{40–42}. LADA has been reported to be associated with a higher risk of osteoporosis than type 2 diabetes mellitus, with lower bone mineral density⁴³. In this study, there were no differences between the slowly progressive and acute-onset groups in either BMI or the complication rate of microangiopathy aside from retinopathy, which was not extracted as an independent variable for bone mineral density reduction in the multivariate regression analyses. This study was a cross-sectional study, and glycemic control was not evaluated over time. Since chronic poor glycemic control contributes to the progression of diabetic microvascular complications, further analysis is needed to clarify the association between long-term glycemic control and bone mineral density. Elevated liver enzymes, which were negatively correlated with bone mineral density, were higher in the slowly progressive group than in the acute-onset group. Since the slowly progressive group had a higher bone mineral density than the acute-onset group, the effect of liver damage on bone mineral density in the acute-onset group did not need to be considered.

This study had several limitations. First, this study had a cross-sectional, retrospective design. Long-term longitudinal studies are needed to investigate the association between endogenous insulin decline and the bone mineral density reduction. However, in long-term longitudinal studies, it is often difficult to match factors other than endogenous insulin secretion, such as BMI and the complication rate of microangiopathy, among patients with different subtypes of type 1 diabetes mellitus; thus, it is difficult to clarify the association between the decline in endogenous insulin secretion and the reduction in bone mineral density. Second, this study included both adult and childhood-onset cases, which have been shown to contribute to bone metabolism in later life⁴⁴. The age of onset was lower in the acute-onset group than in the other groups in this study, which may have affected the results. However, the bone mineral density did not differ between the acute-onset and slowly progressive groups in the later years of disease duration. Finally, the sample size of this study, especially fulminant type 1 diabetes mellitus or patients with longer duration, was small. Fulminant type 1 diabetes mellitus was firstly proposed in 2000⁴⁵. Therefore, the long-term follow-up cases were limited. The advantage of the present study is that it was

conducted at a single center using the same measurement systems, including those for bone mineral density.

In conclusion, this is the first study to observe the variation in the lowering of bone mineral density between the type 1 diabetes mellitus subtypes, acute-onset, slowly progressive, and fulminant and demonstrates that bone mineral density in the acute-onset group was lower than that in the slowly progressive group. The bone mineral density lowering rates were strongly associated with type 1 diabetes mellitus subtypes, indicating that the rate of endogenous insulin decline may be an important determinant of bone mineral density reduction in this disease. This is also the largest study involving adult Japanese patients with type 1 diabetes mellitus, showing that bone mineral density in those with type 1 diabetes mellitus is lower than that in a similarly aged healthy population. Further longitudinal prospective studies are needed to clarify these important clinical pathologies.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Clinical characteristics of premenopausal and postmenopausal women with type 1 diabetes mellitus