1. Title page

(1) the title

Serum dipeptidyl peptidase-4 is associated with multiple vertebral fractures in type 2 diabetes mellitus

(2) a short title

DPP-4 and vertebral fracture

(3)(4) the names of the authors and the departments

Author's names and institutions

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2. Abstract

Objective

Patients with type 2 diabetes mellitus (T2DM) have a high risk of fracture although they have slightly higher bone mineral density (BMD). There is no evidence that dipeptidyl peptidase-4 (DPP-4) is involved in the bone fragility of the patients. The aim of this study is to investigate the association between serum DPP-4 levels and vertebral fractures (VFs) in men with T2DM.

Design

We conducted a cross-sectional study and investigated the relationships between serum DPP-4 levels versus BMD at lumbar spine, femoral neck and radius, bone turnover markers, and presence of VFs in 204 Japanese male patients.

Results

Multiple regression analyses adjusted for confounders such as age, duration of diabetes, body mass index, serum creatinine, HbA1c, serum albumin, log(alanine transaminase), and log(C-reactive protein) showed that serum DPP-4 was positively associated with bone formation markers (bone-specific alkaline phosphatase and osteocalcin) as well as a bone resorption marker [tartrate-resistant acid phosphatase 5b (TRACP5b)] (β=0.25, $p<0.01$; β=0.17, $p<0.05$; and β=0.30, $p<0.01$, respectively), but not BMD at each site. Multivariate logistic regression analyses adjusted for the confounders described above revealed that serum DPP-4 levels were associated with the presence of multiple VFs (odds ratio 1.61, 95% confidential interval 1.05-2.49 per SD increase, $p<0.05$). This association was still significant after additional adjustment for any sites of BMD or bone turnover markers except for TRACP-5b.

Conclusions

We firstly showed that high level of serum DPP-4 is associated with prevalent multiple VFs independently of BMD and bone formation in men with T2DM.

3. Introduction

Accumulative evidence has shown that type 2 diabetes mellitus (T2DM) increases a risk of osteoporotic fracture in spite of high or normal bone mineral density (BMD) (1). These findings suggest that the deterioration of bone quality, which can't be defined by BMD, may contribute to the increased fracture risk in T2DM (2, 3). Both vertebral and hip fractures are most important osteoporotic fractures because they frequently occur and enhance the mortality of the elderly people as high as 6- to 9-fold (4, 5). The increased mortality is more prominent in men than in women (4), and absolute risk of subsequent fracture after an initial one in men is higher than or similar to that in women (6). Therefore, it is no less important to predict the risk of vertebral and hip fractures in diabetic subjects than in non-diabetic counterparts, especially in men. However, the detailed mechanism of bone quality deterioration still remains unclear. Further, there is no evidence what parameters are useful to estimate the risk of fracture in men with T2DM.

Since a meta-analysis showed that the treatment with Dipeptidyl peptidase-4 (DPP-4) inhibitors could be associated with reduction of fracture risk in T2DM (7), DPP-4 and its substrates attract widespread attention in the field of bone biology. DPP-4 is a 110 kDa serine protease and a transmembrane protein of 766 amino acids present on the cell surface. It is also present in soluble form in the circulation blood and body fluid. DPP-4 claves and removes the penultimate L-proline or L-alanine dipeptides from various substrates such as gastric inhibitory polypeptide (GIP), glucagon-like peptide (GLP)-1, GLP-2, insulin-like growth factor (IGF)-1, peptide YY, neuropeptide Y, and Interleukin-2 (8-10). Recent studies have shown that DPP-4 is expressed in adipocytes and potentially represents an important source of DPP-4 in obesity (11). Furthermore, serum DPP-4 levels are higher in patients with T2DM than in control group, and are associated with glucose control status (12). Therefore, it is considered that DPP-4 plays important roles in glucose metabolism and diabetic complications. Based on the conception, we hypothesized that serum DPP-4 levels might be involved in the risk of osteoporotic fracture in T2DM. However, to our knowledge, there are no studies on the effects of DPP-4 on bone.

In the present study, we aimed to investigate whether serum DPP-4 levels are associated with BMD and bone turnover markers, and whether high DPP-4 levels are associated with increased risk of vertebral fractures (VFs) in men with T2DM.

4. Methods

Patientss

The subjects in this cross-sectional study were 204 Japanese men with T2DM. We consecutively enrolled the subjects who visited Shimane University Hospital for evaluation or treatment of T2DM from 2009 to 2013. None of the participants had renal dysfunction (estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2), and had taken DPP-4 inhibitor and GLP-1 receptor agonist, and drugs kwon to influence bone and calcium metabolism like vitamin D, bisphosphonate, and glucocorticoid. This study was approved by the ethical review board of Shimane University Faculty of Medicine and complied with the Helsinki Declaration. All subjects agreed to participate in the study and gave written informed consent.

Biochemical measurements

After an overnight fasting, blood and urine samples were collected. Hemoglobin A_{1C} (HbA_{1C}) (NGSP) was determined by high-performance liquid chromatography. The serum concentration of albumin (Alb), creatinine (Cr), alanine transaminase (ALT), C-reactive protein (CRP) were measured by automated techniques at the central laboratory of our hospital. Estimated glomerular filtration rate (eGFR) was calculated using the equation proposed by the Modification of Diet in Renal Disease Study with modified coefficients for Japanese. Bone-specific alkaline phosphatase (BAP) and Tartrate-resistant acid phosphatase-5b (TRACP-5b) were measured by enzyme immuno assay (EIA) (normal range of males, 13.0-33.9 U/L, 170-590 mU/dL, respectively). Total osteocalcin (OC) was measured by radioimmunoassay assay (RIA) (normal range 2.5–13 ng/mL). Undercarboxylated osteocalcin (ucOC) was measured by the electrochemiluminescence immunoassay (normal range, <4.5 ng/mL). Serum DPP-4 concentration was determined by using commercially available diagnostic kits (R&D Systems, Minneapolis, MN). Serum samples was diluted a 100-fold dilution. Samples were added to the wells of microtiter plates, which were coated with a monoclonal antibody against DPP-4. After incubation of the sample for 2 h at room temperature, a horseradish-peroxidase–conjugated polyclonal antibody against DPP-4 was added and the plates were incubated for another 2 h. The assay was developed with peroxidase substrate for 30 min at room temperature in the dark. Absorbance was measured at 450 nm, and a reference wavelength of 540 nm was used. Standard curves for DPP-4 were prepared by using serial dilutions of exogenous recombinant human DPP-4. The coefficient of variation (CV) of DPP-4 measurements was <10%.

BMD measurements

The BMD values of the lumbar spine (LS), femoral neck (FN), and one third of the radius (1/3R) were measured by dual-energy X-ray absorptiometry using the QDR-4500 system (Hologic, Inc., Waltham, MA). The BMD was measured at the non-dominant hip and wrist specifically. The BMD values were automatically calculated from the bone area (in square centimeters) and

bone mineral content (in grams) and expressed as an absolute value in grams per square centimeter. The CVs of measurement of BMD at the lumbar spine (LS), femoral neck (FN), and one third of the radius (1/3R) were 0.9, 1.7, and 1.9%, respectively. The Z-scores were expressed relative to the standard deviation (SD) of the age- and gender-matched normal Japanese BMD values, and were calculated on the basis of the reference values for the Japanese population provided by the producer of the device.

Radiography

Lateral X-ray films of the thoracic and lumbar spine (LS) were taken at the same week of the serum collection, in all subjects. The anterior, central, and posterior heights of each of the 13 vertebral bodies from Th4-L4 were measured. A vertebral fracture (VF) was diagnosed if at least one of three height measurements along the length of the same vertebrae had decreased by >20% compared to the height of the nearest uncompressed vertebral body (13). None of the subjects had a history of serious trauma.

Statistical Analysis

All data were expressed as mean ± SD for each index. Because ALT, CRP and bone turnover markers showed a markedly skewed distribution, logarithmic (log) transformation of these values were carried out before performing correlation and regression analysis. The statistical significance between the two groups was determined using the unpaired *t*-test. Multiple logistic regression analyses were performed after adjustments for the variables shown in Tables. The statistical analyses were performed using StatView (Abacus Concepts, Inc., Berkeley, CA). A p value less than 0.05 was considered to be significant.

5. Results

Baseline characteristics of the subjects

Baseline characteristics of the subjects are shown in Table 1. The averages of age and diabetes duration were 59.7 and 11.6 years, respectively. The numbers of patient who had been taking sulfonylurea, metformin, alpha-glucosidase inhibitors, pioglitazone, and insulin administration were 53, 33, 16, 12, and 59, respectively. Out of 204 subjects, 71 had vertebral fractures (VFs) (34.8%) and 37 had more than two vertebral fractures (VFs) (18.1%). This rate of vertebral fracture (VF) on this cohort is higher than that of general population for Japanese men of mean age about 60 years.

Association between serum DPP-4 levels versus BMD and bone turnover markers

First, we analyzed simple correlations between serum DPP-4 levels and various parameters including BMD and bone turnover markers (Table 2). Serum DPP-4 levels were significantly and negatively correlated with age, positively with Alb and log(ALT). Moreover, DPP-4 levels were significantly and positively correlated with log(BAP), log(ucOC) and log(TRACP-5b), while they were not correlated with BMD at any site.

Next, multiple regression analyses adjusted for age, duration of diabetes, body mass index (BMI), Alb, log(ALT), creatinine, HbA1c, and log(CRP) were performed to examine independent association between DPP-4 levels and BMD as well as bone turnover markers (Table 3). Serum DPP-4 levels were significantly and positively correlated with log(BAP), log(OC), log(ucOC) and log(TRACP-5b), whereas they were not associated with any sites of BMD.

Comparison of serum DPP-4 levels between subjects with and without vertebral fractures (VFs)

We compared demographic and biochemical parameters including serum DPP-4 levels between subjects with and without vertebral fractures (VFs) (Table 4). Patients with vertebral fractures (VFs) and with multiple vertebral fractures (VFs) were significantly older and had lower FN-BMD, FN-Z score and 1/3R-BMD than patients without vertebral fractures (VFs). Serum DPP-4 levels of patients with multiple vertebral fractures (VFs) were marginally, but not significantly, higher than those of patients without vertebral fractures (VFs).

Next, we did multivariate logistic regression analyses adjusted for confounding factors such as age, duration of diabetes, BMI, albumin, log(ALT), creatinine, log(CRP), and HbA1c (Table 5). It was found that serum DPP-4 levels were significantly and positively associated with the presence of multiple vertebral fractures (VFs). This association was still significant after additional adjustment for LS-, FN-, 1/3R-BMD, log(BAP), log(OC), or log(ucOC), whereas the association between serum DPP-4 levels and the presence of multiple vertebral fractures (VFs) became nonsignificant when log(TRACP-5b) was added as an independent variable in the multivariate logistic

151 regression analysis. Because anti-diabetic drugs might influence the risk of vertebral fracture (VF), we performed logistic regression
152 analyses with additional adjustment for use of pioglitazone, sulfonylurea, alfa-glucosidase inhibitor, metformin, or insulin. We found that
153 the association between serum DPP-4 levels and the presence of multiple vertebral fractures (VFs) was still significant (data not shown).

6. Discussion

Previous studies have shown that the fracture risk is increased in T2DM independently of BMD, suggesting that diabetes-related bone fragility is caused by deterioration of bone quality. In this study, we found that serum DPP-4 levels were positively associated with bone turnover markers, but not BMD. Moreover, elevated serum DPP-4 levels were associated with increased risk of prevalent multiple vertebral fractures (VFs) even after additional adjustment for BMD. These findings suggest that serum DPP-4 is involved in the deterioration of bone quality in T2DM.

Several *in vivo* studies on the roles of DPP-4 in bone have been reported. Kyle *et al.* reported that mice lacking *DPP-4* gene showed no remarkable phenotype of bone volume and structure (14), suggesting that DPP-4 may not be relevant to bone homeostasis in normal condition. In contrast, Glorie *et al.* reported that inhibition of DPP-4 by sitagliptin did not affect bone parameters in normal male rats; however, bone volume, cancellous bone structure and mechanical resistance estimated by three-point bending test were significantly improved in streptozotocin-induced diabetic male rats treated with sitagliptin compared with controls (15). In the present study, serum DPP-4 levels were associated with an increased risk of multiple vertebral fractures (VFs) in diabetic patients. The findings from previous and our studies suggest that inactivation of DPP-4 may have a positive impact on bone strength especially under diabetic condition. In fact, a previous meta-analysis showed a significant risk reduction of the incidence of fracture in patients treated with DPP-4 inhibitors compared with other oral hypoglycemic agent (Odds ratio 0.60) (7). Therefore, further longitudinal studies are necessary to examine whether serum DPP-4 levels predict the incidence of future fracture in T2DM, and whether DPP-4 inhibitors prevent it.

Of interest, we found that serum DPP-4 levels were positively associated with bone resorption marker, TRACP5b, and that the association between serum DPP-4 and vertebral fractures (VFs) became nonsignificant after additional adjustment for TRACP5b. These findings suggest that DPP-4 dominantly affects bone resorption, resulting in the increased risk of vertebral fractures (VFs). An *in vivo* study showed that the treatment with sitagliptin significantly decreased a bone resorption marker, serum C-terminal peptide of collagen I, but not a bone formation marker, OC, in diabetic rats (15). Furthermore, a clinical interventional study demonstrated that the treatment with sitagliptin significantly decreased another bone resorption marker, urinary deoxypyridinoline, but not OC, leading to a gain of L-BMD in postmenopausal women with T2DM (16). These findings suggest that DPP-4 stimulates bone resorption and then weakens bone strength. However, the mechanism how DPP-4 affects bone resorption is still unclear.

DPP-4 is a complex enzyme that exists as a membrane-anchored cell surface peptidase that transmits intracellular signals and as a second smaller soluble form present in the circulation (17). Cell surface DPP-4 on monocyte/macrophage is reported to play roles in inflammatory response and cell migration (18, 19). Thus, there is a possibility that DPP-4 itself has direct effects on bone forming cells such as osteoblasts, osteocytes and osteoclasts. However, because *DPP-4* null mice showed no specific phenotype of bone, we hypothesize that soluble form of DPP-4 and its substrates may be important to regulate bone metabolism under the condition of diabetes.

DPP-4 cleaves several hormones such as GIP, GLP-1, GLP-2, IGF-1 and peptide YY, which may be related to bone metabolism. Because serum DPP-4 levels are increased in patients with T2DM (12), the action of these hormones on bone may be decreased. GIP and GLP-1 are identified as gut hormones and stimulate insulin secretion from pancreatic β cells. In fact, because the functions of GIP and GLP-1 are decreased in diabetes (20), hypoglycemic agents stimulating GIP and GLP-1 signals are frequently used to lower blood glucose levels in clinical settings. Previous studies using genetically modified mice have shown that GIP enhances bone formation and inhibits bone resorption, resulting in a gain of bone mass (21-23). Further, knockout mice of GLP-1 receptor showed a significant increase in osteoclast activity and a reduction in cortical bone mass (24). On the other hand, IGF-I is known to play important roles in bone remodeling (25, 26). Although the association between serum DPP-4 and IGF-I is unknown in human, IGF-I levels in serum and cortical bone were significantly reduced in spontaneously diabetic rats, which displayed a significant decrease in BMD at long bone metaphyses and vertebrae (27). We previously reported that serum IGF-I levels were inversely associated with the presence of vertebral fractures (VFs) in T2DM (28, 29). Therefore, further studies are needed to clarify the effects of DPP-4 on bone through the substrates of DPP-4 in future.

There are several limitations in this study. First, we analyzed only subjects who visited Shimane University Hospital, a tertiary center, for treatment of diabetes mellitus. Therefore, the participants enrolled in this study might have relatively severe states of the disorder and might not be representative of Japanese patients with the disorder. Second, more than 50% of subjects were treated. Therefore, we cannot exclude the possibility that the treatment of diabetes affected serum DPP-4 level and the occurrence of vertebral fracture (VF). Third, we focused on male patients in this study. Because an *in vivo* study reported that effects of DPP-4 inhibition on bone in female mice were greater than in male mice (14), we have to examine the association between DPP-4 and fractures in women. Fourth, the conclusions of this study are weakened by its cross-sectional design. It is necessary to pay attention to that, in a cross-sectional study, causal relationships cannot generally be referred. Finally, we unfortunately didn't measure the hormones cleaved by DPP-4 in this study.

In conclusion, we found for the first time that high levels of serum DPP-4 were associated with the increased risk of multiple vertebral fractures (VFs) independently of BMD in men with T2DM. Moreover, the present findings suggest that bone resorption might be involved in the DPP-4-related bone fragility.

7. Declaration of interest

Masakazu Notsu, Ippei Kanazawa, Sayuri Tanaka, Toru Yamaguchi, and Toshitsugu Sugimoto declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

8. Funding

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9. Author contributions

None

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vertebral fractures in postmenopausal women with type 2 diabetes mellitus. Osteoporos Int, 22, 1191-1198.

12. Figure legends

None

13. Tables

Other file

14. Figures

None

Table 1 Baseline characteristics of the subjects

Age (years)	59.7 ± 15.0
Duration of diabetes (years)	11.6 ± 11.9
Height（cm）	165.3 ± 8.0
Weight（kg）	68.1 ± 14.8
Body mass index（kg/m^2）	24.8 ± 4.5
Albumin (g/dL)	4.1 ± 0.7
ALT (U/L)	31 ± 24
Creatinine (μmol/L)	74.3 ± 25.6
eGFR (ml/min/1.73m^2)	81.9 ± 27.6
Fasting plasma glucose (mmol/L)	8.9 ± 2.7
HbA1c (%)	9.3 ± 2.3
CRP (mg/dL)	0.32 ± 0.78
DPP-4 (ng/mL)	801.7 ± 211.8
BAP (U/L)	31.3 ± 12.5
OC (ng/mL)	5.4 ± 2.9
ucOC (ng/mL)	3.17 ± 3.48
TRACP5b (mU/dL)	307 ± 154
L2-4 BMD (g/cm^2)	1.033 ± 0.212
FN BMD (g/cm^2)	0.760 ± 0.143
1/3R BMD (g/cm^2)	0.719 ± 0.093

ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c, CRP, C-reactive protein; DPP-4, dipeptidyl peptidase-4; BAP, bone-specific alkaline phosphatase; OC, osteocalcin; ucOC, undercarboxylated osteocalcin, TRACP-5b, Tartrate-resistant acid phosphatase-5b, L, lumber, FN, femoral neck; 1/3R, one-third of the radius

Table 2 Correlations between serum DPP-4 levels and various variables

Independent variables	*r*	*p*
Age	-0.16	0.026
Duration of diabetes	-0.02	0.765
Height	0.02	0.822
Weight	0.11	0.109
Body mass index	0.13	0.056
Albumin	0.20	0.004
Log(ALT)	0.30	<0.001
Creatinine	-0.10	0.107
eGFR	0.09	0.221
HbA1c	0.11	0.110
Log(CRP)	-0.08	0.306
Log(BAP)	0.27	<0.001
Log(OC)	0.11	0.145
Log(ucOC)	0.15	0.041
Log(TRACP5b)	0.19	0.047
L2-4 BMD	0.03	0.682
L2-4 Z score	-0.05	0.482
FN BMD	0.08	0.243
FN Z score	0.09	0.191
1/3R BMD	0.13	0.064
1/3R Z score	0.06	0.369

The correlations between serum DPP-4 levels versus bone mineral density, bone metabolic marker, or other variables. DPP-4, dipeptidyl peptidase-4; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c, CRP, C-reactive protein; BAP, bone-specific alkaline phosphatase; OC, osteocalcin; ucOC, undercarboxylated osteocalcin, TRACP-5b, Tartrate-resistant acid phosphatase-5b, L, lumber, FN, femoral neck; 1/3R, one-third of the radius

Table 3 Multiple regression analyses between serum DPP-4 levels versus BMD and bone turnover markers

Independent variables	β	R^2	p
Log(BAP)	0.25	0.140	<0.001
Log(OC)	0.17	0.145	0.030
Log(ucOC)	0.21	0.161	0.007
Log(TRACP5b)	0.30	0.357	0.002
L2-4 BMD	0.02	0.119	0.825
FN BMD	-0.06	0.337	0.395
1/3R BMD	0.04	0.371	0.527

Multiple regression analysis was performed between serum DPP-4 levels versus BMD at each skeletal site and bone turnover markers adjusted for age, duration of diabetes, body mass index, albumin, log(ALT), creatinine, HbA1c, and log(CRP).
DPP-4, dipeptidyl peptidase-4; ALT, alanine transaminase; HbA1c, hemoglobin A1c, CRP, C-reactive protein; BAP, bone-specific alkaline phosphatase; OC, osteocalcin; ucOC, undercarboxylated osteocalcin, TRACP-5b, Tartrate-resistant acid phosphatase-5b, L, lumber, FN, femoral neck; 1/3R, one-third of the radius

Table 4 Comparison of demographic and biochemical parameters, bone turnover markers, and BMD between subjects with and without vertebral fractures

	Without VFs	With VFs	*p*	With multiple VFs	*p*
Number of the subjects	133	71		37	
Age (years)	57.3±15.1	64.3±13.7	0.001	62.9±14.1	0.046
Duration of diabetes (years)	10.8±11.3	13.1±13.0	0.198	13.4±9.9	0.208
Body mass index (kg/m^2)	25.2±4.7	24.1±4.1	0.121	24.4±4.0	0.397
Albumin (g/dL)	4.2±0.6	4.1±0.7	0.286	4.0±0.7	0.117
ALT (U/L)	31.3±23.6	29.9±24.3	0.702	29.9±24.5	0.757
Creatinine (μmol/L)	75.1±26.5	74.3±25.6	0.833	73.4±25.6	0.759
eGFR (ml/min/1.73m^2)	83.0±28.1	79.9±26.6	0.444	81.5±27.6	0.776
CRP (mg/dL)	0.32±0.82	0.33±0.70	0.991	0.35±0.73	0.850
HbA1c (%)	9.4±2.4	9.0±2.0	0.168	8.9±2.1	0.270
DPP-4 (ng/mL)	802.0±197.9	801.0±237.2	0.972	859.1±276.5	0.159
BAP (U/L)	30.6±11.3	32.6±14.4	0.281	34.1±17.3	0.147
OC (ng/mL)	5.4±2.8	5.2±3.0	0.665	5.2±3.0	0.752
ucOC (ng/mL)	3.4±4.1	2.8±1.9	0.352	3.1±1.9	0.701
TRACP5b (mU/dL)	292.6±117.4	326.1±192.0	0.270	307.0±140.5	0.639
L2-4 BMD (g/cm^2)	1.039±0.219	1.022±0.199	0.580	1.004±0.171	0.371
L2-4Z score (SD)	0.52±1.17	0.38±1.14	0.416	0.29±1.08	0.264
FN BMD (g/cm^2)	0.776±0.141	0.730±0.157	0.034	0.727±0.156	0.069
FN Z score (SD)	-0.69±1.12	-1.07±1.21	0.024	-1.10±1.18	0.054
1/3R BMD (g/cm^2)	0.733±0.087	0.692±0.099	0.003	0.690±0.096	0.010
1/3R Z score (SD)	0.57±1.42	0.26±1.81	0.186	0.05±1.77	0.064

P values v.s subjects without VFs

DPP-4, dipeptidyl peptidase-4; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c, CRP, C-reactive protein; BAP, bone-specific alkaline phosphatase; OC, osteocalcin; ucOC, undercarboxylated osteocalcin, TRACP-5b, Tartrate-resistant acid phosphatase-5b, L, lumber, FN, femoral neck; 1/3R, one-third of the radius

Table 5 Association between serum DPP-4 levels and the presence of multiple vertebral fractures

OR(95% CI)	*p*	adjusted variables
1.27 (0.91-1.79)	0.165	None
1.61 (1.05-2.49)	0.030	Model1
1.63 (1.06-2.53)	0.026	Model1 + L2-4 BMD
1.60 (1.03-2.49)	0.036	Model1 + FN BMD
1.63 (1.06-2.52)	0.028	Model1 + 1/3R BMD
1.59 (1.01-2.51)	0.045	Model1 + log(BAP)
1.64 (1.05-2.56)	0.031	Model1 + log(OC)
1.56 (1.00-2.42)	0.049	Model1 + log(ucOC)
0.96 (0.50-1.84)	0.909	Model1 + log(TRACP5b)

Model 1: adjusted for age, duration of diabetes, body mass index, albumin, log(ALT), creatinine, log(CRP), and HbA1c

DPP-4, dipeptidyl peptidase-4; ALT, alanine transaminase; HbA1c, hemoglobin A1c, CRP, C-reactive protein; BAP, bone-specific alkaline phosphatase; OC, osteocalcin; ucOC, undercarboxylated osteocalcin, TRACP-5b, Tartrate-resistant acid phosphatase-5b, L, lumber, FN, femoral neck; 1/3R, one-third of the radius