Plasma DPP4 Activities Are Associated With Osteoporosis in Postmenopausal Women With Normal Glucose Tolerance

Tianpeng Zheng, Liuxue Yang, Yihong Liu, Hongbo Liu, Jian Yu, Xiaoxi Zhang, and Shenghua Qin

Department of Endocrinology and Metabolism (T.Z., L.Y., J.Y.), Affiliated Hospital of Guilin Medical University, Guangxi 541001, People's Republic of China; Diabetic Centre of Control and Prevention (Y.L.), The People's Liberation Army 520 Hospital, Sichuan 621000, China; Department of Laboratory Medicine (H.L.), Affiliated Hospital of Guilin Medical University, Guangxi 541001, People's Republic of China; Center of Diabetic Systems Medicine (T.Z., X.Z.), Guilin Medical University, Guangxi 541001, People's Republic of China; and Medical Examination Center (S.Q.), Affiliated Hospital of Guilin Medical University, Guangxi 541001, People's Republic of China

Context: Inflammation, insulin resistance, dyslipidemia, and glucagon-like peptide-1 (GLP-1) are risk factors for osteoporosis. Dipeptidyl peptidase-4 (DPP4) is a newly identified adipokine related to these risk factors.

Objective: To investigate the association between plasma DPP4 activities and osteoporosis.

Design, Setting, and Patients: This was a cross-sectional study conducted in Guilin, China. A total of 744 postmenopausal women with normal glucose tolerance were studied.

Main Outcome Measures: Plasma DPP4 activity, inflammatory markers, blood lipids, homeostatic model assessment of insulin resistance (HOMA-IR), active GLP-1, bone turnover markers, and bone mineral density (BMD) were measured in all participants.

Results: Participants in the highest quartile of DPP4 activity had higher triglyceride, total cholesterol, HOMA-IR, IL-6, high-sensitivity C-reactive protein (hs-CRP), C-terminal telopeptide of type I collagen, and osteocalcin and lower BMD (lumbar spine and femoral neck) and active GLP-1 compared with participants in the lowest quartile ($P < .05$). DPP4 activities were associated positively with triglyceride, total cholesterol, HOMA-IR, IL-6, hs-CRP, C-terminal telopeptide of type I collagen, and osteocalcin and negatively with active GLP-1 and BMD ($P < .05$). In the highest DPP4 quartile, osteoporosis risk was significantly higher (odds ratio, 3.01; 95% confidence interval, 1.66–5.43) than in the lowest quartile after adjustment for potential confounders. The risk for osteoporosis increased more with higher levels of DPP4 activity, HOMA-IR, IL-6, and hs-CRP ($P < .05$), but not with higher levels of triglyceride and total cholesterol or lower levels of active GLP-1.

Conclusions: This study shows that increased DPP4 activities are independently associated with osteoporosis. The mechanisms may be partly explained by the effect of DPP4 on inflammation and insulin resistance. **(*J Clin Endocrinol Metab* 100: 3862–3870, 2015)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received May 8, 2015. Accepted August 4, 2015.
First Published Online August 10, 2015

Abbreviations: BMD, bone mineral density; BMI, body mass index; CI, confidence interval; CTX, C-terminal telopeptide of type I collagen; DPP4, dipeptidyl peptidase-4; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide-1; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; NGT, normal glucose tolerance; OCN, osteocalcin; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio; TC, total cholesterol; TG, triglyceride.

Osteoporosis is defined as a disease characterized by low bone mass and microarchitectural deterioration of trabecular bone leading to enhanced bone fragility. It is a common disease that affects humans around the world, especially postmenopausal women. Assessing osteoporosis risk and identifying a potential target for osteoporosis intervention can have profound socioeconomic significance for public health.

It is now clear that multiple traditional risk factors such as genetics (1, 2), advanced age, low intake of calcium and vitamin D, smoking, and suboptimal physical activity contribute to the development of osteoporosis. In addition, accumulated clinical or basic evidence revealed that inflammation, altered lipid profiles, and insulin resistance also play critical roles in the changes in bone turnover and bone mass that occur in osteoporosis (3–6). Moreover, emerging evidence supports the contribution of glucagon-like peptide-1 (GLP-1), an incretin hormone secreted from L cells located in the distal ileum and colon, to bone metabolism (7).

Dipeptidyl peptidase-4 (DPP4) is a widely expressed multifunctional serine peptidase that exists as a membrane-anchored cell surface protein or in a soluble form in the plasma (8). In addition to its role in the degradation of numerous substrates such as GLP-1 (9), it has also been identified as a novel adipokine playing crucial roles in the development of dyslipidemia, inflammation, and insulin resistance (10–13), all of which have been suggested to be involved in the pathogenesis of osteoporosis. Furthermore, a meta-analysis by Monami et al (14) suggested that DPP4 inhibitors could have a protective effect on bone. Therefore, it is reasonable to speculate that DPP4 activity may be positively correlated with osteoporosis in postmenopausal women; however, no study has evaluated whether DPP4 may serve as a risk marker for osteoporosis in postmenopausal women and to what extent it is associated with osteoporosis.

Consequently, in this study, we aimed to evaluate the association between plasma DPP4 activities and osteoporosis in a cross-sectional population study of 744 Chinese postmenopausal women. The relationship between DPP4 activities and novel risk factors for osteoporosis mentioned above was also evaluated because we attempted to further explain the mechanism for such an association from a clinical perspective. Because hyperglycemia may have a mutual effect with DPP4 and impair bone metabolism (15, 16), introducing an additional confounding factor into this study, the current study was performed in a group of postmenopausal women with normal glucose tolerance (NGT).

Subjects and Methods

Subjects

A total of 744 postmenopausal women aged 47–76 years (mean ± SD, 59.5 ± 7.8), who had undergone routine health examinations at the Medical Examination Center of the Affiliated Hospital of Guilin Medical University between 2013 and 2014, were enrolled for analysis in this study. All subjects visited the Medical Examination Center spontaneously for routine health examinations consisting of extensive screening tests for the early detection of diabetes, hypertension, metabolic syndrome, osteoporosis, malignancy, and other age-related diseases. Menopause was defined as the absence of menstruation for at least 1 year and was confirmed by serum FSH measurement. Inclusion criteria were: 1) postmenopausal woman with NGT; 2) long-term residence (≥5 y) in China's Guangxi province; and 3) ability to give informed consent. Exclusion criteria were: 1) subjects who had taken drugs that could affect bone metabolism for more than 3 months or at any time within 12 months before the enrollment, such as vitamin D, calcium, systemic glucocorticoids, bisphosphonate, or hormone therapy; 2) subjects who had any disease that could affect bone metabolism or DPP4 activity, such as thyroid diseases, hyperparathyroidism, rheumatoid arthritis, chronic obstructive pulmonary disease/asthma, diabetes, nonalcoholic fatty liver disease, acute inflammatory diseases, stroke, myocardial infarction, and heart, liver, and respiratory dysfunction; and 3) subjects with incomplete data (study population data that were not completely determined mainly refer to the study population who failed to complete the questionnaire or the absence of any physical or metabolic indicators).

The study was approved by the Drugs/Medical Apparatus and Instruments Ethics Committee at the Affiliated Hospital of Guilin Medical University, and all subjects gave their informed consent. This study was registered on the Chinese Clinical Trial Registry (ChiCTR-EPC-14005273).

Clinical and laboratory measurements

A standard questionnaire was administered by trained staff to the participants to record demographic characteristics, lifestyle risk factors, a self-reported medical history, and medications known to affect bone metabolism. Measurements of body weight and height, waist and hip circumference, body mass index (BMI), waist/hip ratio, and blood pressure have been described previously (17). Subjects were instructed to maintain their usual physical activity and diet for at least 3 days before undergoing an oral glucose tolerance test. After an overnight fast of ≥ 10 hours, venous blood samples were collected to measure serum calcium, serum phosphorus, serum 25-hydroxyvitamin D [25(OH)D], fasting plasma glucose (FPG), fasting insulin, blood lipids (including total cholesterol [TC], triglycerides [TGs], low-density lipoprotein cholesterol [LDL-C], and high-density lipoprotein cholesterol [HDL-C]), IL-6, high-sensitivity C-reactive protein (hs-CRP), fasting active GLP-1, C-terminal telopeptide of type I collagen (CTX), osteocalcin (OCN), and DPP4 activity. Blood samples were also drawn 30 and 120 minutes after a 75-g glucose load to measure glucose and insulin concentrations.

Calcium intake was assessed using a 24-hour dietary recall questionnaire administered by a trained dietician. The results were calculated using a food composition table from China (Institute of Nutrition and Food Safety, 2002) (18).

Serum calcium and phosphorus were examined using an autoanalyzer (Roche Cobas 8000). Plasma glucose levels, insulin, TC, TG, HDL-C, LDL-C, IL-6, hs-CRP, DPP4 activity and active GLP-1 were measured as previously described (12, 13, 15). The serum concentrations of OCN (Roche Diagnostics) and CTX (Roche Diagnostics) were measured using an electrochemical luminescence immunoassay according to the manufacturer's instructions. Serum 25(OH)D levels were measured with an RIA (DiaSorin Inc).

The areal bone mineral density (BMD) (g/cm^2) of all women was measured at the lumbar spine (L1–L4) and femoral neck by dual-energy x-ray absorptiometry (Lunar Prodigy). According to the World Health Organization, osteoporosis is diagnosed by a T-score ≤ -2.5 SD at any of the sites on the lumbar spine or femoral neck.

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as previously reported (15).

Statistical analysis

All of the statistical analyses were performed using the SPSS 16.0 software (SPSS Inc). Normally distributed data were expressed as means ± SD, whereas variables with a skewed distribution were reported as median (interquartile range) and log-transformed to approximate normality before analysis. Categorical variables were represented by frequency and percentage. We divided the study population into quartiles of plasma DPP4 activity with cutoff points of 12.80, 17.66, and 22.83. Clinical and biochemical characteristics were compared by an analysis of covariance, χ^2, or t test. Associations between continuous variables were tested by partial correlation analyses. Multivariate logistic regression models were used to estimate the odds ratios (ORs) for osteoporosis, elevated TG, TC, HOMA-IR, IL-6, hs-CRP, CTX, and OCN and decreased active GLP-1. According to Chinese guidelines on the prevention and treatment of dyslipidemia in adults, elevated TG and TC were defined as TG $\geq$ 1.70 mmol/L and TC $\geq$ 5.18 mmol/L, respectively (19). Owing to a lack of current global guidelines regarding the normal range of HOMA-IR, IL-6, hs-CRP, CTX, OCN, and active GLP-1, the upper quartiles of HOMA-IR, IL-6, hs-CRP, CTX, and OCN were defined as elevated, whereas the lower quartiles of GLP-1 were defined as decreased. Potential confounding variables including age, BMI, smoking, alcohol consumption, leisure-time physical activity, family history of diabetes, serum calcium, phosphorus, calcium intake, serum 25(OH)D, TG, active GLP-1, HOMA-IR, and IL-6 were controlled in the regression models. The variables included in the multivariate regression models were those that were statistically significant in univariate analyses or were biologically relevant. In all statistical tests, two-sided P values $< .05$ were considered significant.

Results

Clinical and laboratory characteristics

Among the 744 participants included in this study, 140 (18.8%) had osteoporosis. The prevalence of osteoporosis according to DPP4 quartiles was 11.4, 9.6, 23.0, and 31.5%, respectively. The subjects with higher DPP4 activity were more likely to be older ($P = .003$). With respect to metabolic parameters, the subjects in the higher DPP4 quartiles exhibited higher levels of BMI, TG, TC, fasting insulin, HOMA-IR, IL-6, hs-CRP, CTX, and OCN and lower active GLP-1 and BMD (lumbar spine and femoral neck) compared with subjects in the lowest quartile (all $P < .05$). Smoking, leisure-time physical activity, alcohol consumption, family history of diabetes, calcium intake, serum 25(OH)D, serum calcium, phosphorus, LDL-C, HDL-C, and FPG did not differ significantly across DPP4 activity categories (Table 1).

Correlation analysis between DPP4 activity and other variables

Partial correlation analysis demonstrated that DPP4 activity was positively associated with, TC, HOMA-IR, IL-6, hs-CRP, CTX, and OCN and was negatively associated with active GLP-1 and BMD (lumbar spine and femoral neck) in osteoporosis participants, non-osteoporosis participants, and all participants after adjustment for age, BMI, current smoking, alcohol consumption, leisure-time physical activity, family history of diabetes, serum calcium, serum phosphorus, calcium intake, and serum 25(OH)D (all $P < .05$) (Table 2).

Associations between DPP4 activity and osteoporosis

As presented in Table 3, the ORs for increased blood lipids, insulin resistance, inflammation, bone turnover markers, and decreased active GLP-1 were higher with increasing DPP4 quartiles ($P < .05$ for trend). In the highest DPP4 quartiles, the ORs were 1.92 (95% confidence interval [CI], 1.21, 3.04) for elevated TG, 2.31 (1.39, 3.86) for elevated HOMA-IR, 2.40 (1.44, 4.00) for elevated IL-6, 2.92 (1.73, 4.92) for elevated hs-CRP, 4.55 (2.76, 7.50) for elevated CTX, 1.89 (1.12, 3.19) for elevated OCN, 2.59 (1.61, 4.18) for decreased active GLP-1, and 3.57 (2.01, 6.33) for osteoporosis after adjusting for age, BMI, current smoking, alcohol consumption, leisure-time physical activity, family history of diabetes, serum calcium, serum phosphorus, calcium intake, and serum 25(OH)D (all $P < .05$) (Tables 3 and 4). Interestingly, further adjustment for HOMA-IR and IL-6 reduced the magnitude of the ORs for osteoporosis [3.01 (1.66, 5.43); $P < .05$], but this association was not attenuated by additional adjustment for TG or active GLP-1 (Table 4).

The risk of osteoporosis was more pronounced among participants with rising DPP4 activity and higher levels of HOMA-IR (Figure 1C), higher levels of IL-6 (Figure 1D), and higher levels of hs-CRP (Figure 1E); however, this increasing trend of osteoporosis risk was not observed in higher levels of TG (Figure 1A) and TC (Figure 1B) and in lower levels of active GLP-1 (Figure 1F). Even in the lowest quartile of HOMA-IR, IL-6, and hs-CRP, the risks for

Table 1. Characteristics of Study Participants According to DPP4 Quartiles

Characteristics	Q1	Q2	Q3	Q4	*P* Value
n	185	188	187	184	
DPP4 cutoff points	<12.79	12.80–17.65	17.66–22.82	≥22.83	
DPP4 activity, nmol/min/mL	8.93 ± 2.61	15.41 ± 1.39	20.25 ± 1.54	26.61 ± 2.85	<.001
Age, y	58.0 ± 7.7	59.8 ± 7.5	59.2 ± 7.8	61.0 ± 8.1	.003
Weight, kg	56.5 ± 9.1	57.2 ± 10.8	57.2 ± 10.0	59.5 ± 11.0	.030
Height, cm	156.7 ± 7.4	158.1 ± 8.6	157.2 ± 7.9	156.8 ± 8.7	.295
BMI, kg/m^2	23.07 ± 3.51	22.86 ± 3.91	23.14 ± 3.65	24.16 ± 3.82	.004
Cigarette smoking, %					.960
Never	84.9	82.4	81.3	81.5	
Past	6.5	8.0	8.0	9.2	
Current	8.6	9.6	10.7	9.2	
Alcohol consumption, %	12.4	10.1	13.9	9.2	.473
Leisure-time physical activity, %	63.8	56.9	63.1	58.2	.424
Family history of diabetes, %	13.5	16.0	15.5	19.6	.458
Calcium intake, mg/d[a]	442.1 ± 147.0	474.9 ± 192.1	468.5 ± 162.3	464.7 ± 181.5	.223
Serum 25(OH)D, ng/mL[a]	19.4 ± 5.8	18.5 ± 7.0	18.8 ± 7.9	17.6 ± 6.3	.055
Serum calcium, mmol/L[a]	2.31 ± 0.10	2.29 ± 0.09	2.30 ± 0.09	2.31 ± 0.11	.210
Serum phosphorus, mmol/L[a]	1.24 ± 0.18	1.24 ± 0.15	1.23 ± 0.16	1.25 ± 0.15	.723
TG, mmol/L[a]	1.29 (0.92,1.70)	1.29 (0.96,1.90)	1.40 (1.04,1.91)	1.57 (1.18,2.00)	.003
TC, mmol/L[a]	5.20 ± 0.92	5.28 ± 1.07	5.35 ± 0.99	5.55 ± 1.05	.027
LDL-C, mmol/L[a]	3.21 ± 0.80	3.18 ± 0.89	3.31 ± 0.87	3.34 ± 0.87	.456
HDL-C, mmol/L[a]	1.57 ± 0.33	1.53 ± 0.36	1.55 ± 0.34	1.50 ± 0.32	.357
FPG, mmol/L[a]	4.77 ± 0.52	4.70 ± 0.47	4.79 ± 0.44	4.85 ± 0.49	.090
Fasting insulin, μU/mL[a]	6.32 (4.96,8.10)	6.46 (4.64,8.45)	6.85 (5.31,8.59)	7.84 (6.11,9.73)	<.001
HOMA-IR[a]	1.33 (1.02,1.84)	1.34 (0.94,1.77)	1.49 (1.07,1.85)	1.67 (1.27,2.13)	<.001
IL-6, pg/mL[a]	1.21 ± 0.25	1.28 ± 0.25	1.32 ± 0.20	1.36 ± 0.31	<.001
hs-CRP, mg/L[a]	1.11 ± 0.26	1.17 ± 0.22	1.19 ± 0.20	1.24 ± 0.16	<.001
Active GLP-1, pmol/L[a]	3.39 ± 1.13	3.12 ± 0.95	3.10 ± 0.92	2.97 ± 1.04	.001
Bone turnover markers					
CTX, ng/mL[a]	0.24 (0.20,0.31)	0.27 (0.23,0.33)	0.33 (0.28,0.49)	0.48 (0.34,0.64)	<.001
OCN, ng/mL[a]	13.5 (11.4,18.6)	20.4 (16.0,25.1)	21.8 (18.1,27.6)	22.2 (17.8,26.0)	<.001
BMD values					
Lumbar spine, g/cm^2 [a]	1.02 ± 0.14	1.01 ± 0.12	0.97 ± 0.11	0.97 ± 0.14	<.001
Femoral neck, g/cm^2 [a]	0.84 ± 0.11	0.84 ± 0.10	0.81 ± 0.08	0.79 ± 0.12	<.001

Data were expressed as means ± SD, median (interquartile range), or percentage for normally distributed continuous various, abnormally distributed continuous variables, and categorical variables, respectively. Cigarette smoking was defined as having smoked at least 100 cigarettes in one's lifetime. Alcohol consumption was defined as consumption of ≥ 30 g of alcohol per week for 1 year or more. Regular leisure-time physical activity was defined as participation in ≥ 30 minutes of moderate or vigorous activity per day at least 3 days per week.

[a] Adjusted for age and BMI.

osteoporosis were 2.57- to 5.37-fold higher in the highest DPP4 quartile than in the lowest quartile.

Discussion

In this study, we found a strong association between DPP4 activity and the risk of osteoporosis and its pathogenic factors such as insulin resistance, inflammation, and decreased levels of active GLP-1 in postmenopausal women with NGT. Moreover, the underlying mechanisms may be partly explained by the effect of DPP4 on inflammation and insulin resistance, not on alter lipids profile or fasting active GLP-1.

Although previous studies suggest that DPP4 inhibitors may exert a protective effect on bone (14), the precise relationship between DPP4 activity and bone metabolism remains poorly understood. In the present study, our data support a significant association between rising DPP4 activity and osteoporosis risk, as estimated by BMD of the lumbar spine and femoral neck. Therefore, we hypothesize that plasma DPP4 activity may be a predictive biomarker of osteoporosis-related phenotypes. This hypothesis was supported by our cross-sectional study, which showed that DPP4 activity correlated positively with CTX and OCN, two classic bone turnover markers representing bone resorption and formation, respectively, suggesting that high DPP4 activity associates with a high bone turnover rate.

What interested us most was the independent positive association between DPP4 activity and osteoporosis risk. Although we cannot evaluate the causal relationship of these two in this cross-sectional study, we still tried to

Table 2. Partial Correlation Coefficients Among DPP4 Activities, Metabolic Features, and BMD

	Non-osteoporosis Participants		Osteoporosis Participants		All Participants	
	r	*P*	r	*P*	r	*P*
TG	0.176	<.001	0.164	.063	0.170	<.001
TC	0.113	.006	0.283	.001	0.148	<.001
LDL-C	0.062	.130	0.054	.544	0.062	.092
HDL-C	−0.031	.447	−0.056	.527	−0.059	.113
HOMA-IR	0.221	<.001	0.271	.002	0.254	<.001
IL-6	0.186	<.001	0.178	.043	0.223	<.001
hs-CRP	0.180	<.001	0.222	.011	0.212	<.001
Active GLP-1	−0.150	<.001	−0.226	.010	−0.159	<.001
CTX	0.351	<.001	0.383	<.001	0.379	<.001
OCN	0.377	<.001	0.304	<.001	0.332	<.001
BMD (femoral neck)	−0.123	.003	−0.228	.009	−0.255	<.001
BMD (lumbar spine)	−0.129	.002	−0.273	.002	−0.277	<.001

All correlation coefficients were calculated after adjustment for age, BMI, current smoking, alcohol consumption, leisure-time physical activity, family history of diabetes, serum calcium, serum phosphorus, calcium intake, and serum 25(OH)D.

explain this association by asking whether the elevation in DPP4 activity leads to an increased risk of osteoporosis or vice versa.

A positive relationship between DPP4 activity and dyslipidemia has been demonstrated in previous animal and human studies (11, 20, 21). Similarly, our data further indicated that the ORs for increased plasma TG and TC were higher with increasing DPP4 activity quartiles in postmenopausal women with NGT, but when potential confounders such as age, BMI, and life risk factors were adjusted, the association between DPP4 activity and TC was lost. Previous studies regarding the relationship between an altered lipid profile and osteoporosis were not concordant in postmenopausal women. Some findings (4, 5) supported an inverse relationship between dyslipidemia and BMD, whereas others did not (22, 23). In this cross-sectional study, regardless of the positive relationship found between TC, TG, and DPP4, the ORs for osteopo-

Table 3. Adjusted ORs and 95% CIs for Increased Levels of Blood Lipids, Insulin Resistance, Inflammation, Bone Turnover Markers, and Decreased Active GLP-1 According to DPP4 Quartiles

	Q1	Q2	Q3	Q4
Elevated TG				
Model 1	1.0	1.31 (0.83, 2.06) 0.243	1.42 (0.91, 2.23) 0.126	2.02 (1.30, 3.15) 0.002
Model 2	1.0	1.32 (0.83, 2.10) 0.238	1.41 (0.89, 2.23) 0.141	1.92 (1.21, 3.04) 0.005
Elevated TC				
Model 1	1.0	0.85 (0.57, 1.28) 0.437	1.14 (0.76, 1.71) 0.538	1.58 (1.05, 2.40) 0.030
Model 2	1.0	0.86 (0.56, 1.31) 0.474	1.11 (0.73, 1.69) 0.638	1.55 (1.00, 2.40) 0.050
Elevated HOMA-IR				
Model 1	1.0	1.05 (0.63, 1.75) 0.855	1.24 (0.75, 2.04) 0.405	2.48 (1.55, 3.98) <0.001
Model 2	1.0	1.12 (0.66, 1.93) 0.671	1.37 (0.81, 2.32) 0.243	2.31 (1.39, 3.86) 0.001
Elevated IL-6				
Model 1	1.0	1.82 (1.10, 3.03) 0.021	1.78 (1.07, 2.97) 0.026	2.50 (1.52, 4.11) <0.001
Model 2	1.0	1.79 (1.07, 2.99) 0.027	1.75 (1.04, 2.92) 0.034	2.40 (1.44, 4.00) 0.001
Elevated hs-CRP				
Model 1	1.0	1.64 (0.98, 2.77) 0.061	1.81 (1.08, 3.03) 0.025	3.01 (1.83, 4.95) <0.001
Model 2	1.0	1.69 (0.99, 2.90) 0.054	1.86 (1.09, 3.16) 0.022	2.92 (1.73, 4.92) <0.001
Decreased active GLP-1				
Model 1	1.0	1.32 (0.82, 2.13) 0.258	2.51 (1.59, 3.97) <0.001	2.35 (1.48, 3.73) 0.001
Model 2	1.0	1.35 (0.83, 2.19) 0.234	2.61 (1.64, 4.16) <0.001	2.59 (1.61, 4.18) 0.001
Elevated CTX				
Model 1	1.0	0.66 (0.37, 1.19) 0.164	1.62 (0.97, 2.70) 0.063	4.46 (2.75, 7.22) <0.001
Model 2	1.0	0.66 (0.37, 1.20) 0.175	1.63 (0.97, 2.73) 0.063	4.55 (2.76, 7.50) <0.001
Elevated OCN				
Model 1	1.0	1.51 (0.91, 2.50) 0.115	2.20 (1.35, 3.60) 0.002	1.78 (1.08, 2.94) 0.023
Model 2	1.0	1.61 (0.95, 2.71) 0.075	2.28 (1.38, 3.78) 0.001	1.89 (1.12, 3.19) 0.017

Data are expressed as OR (95% CI) + *P* value, unless stated otherwise. Model 1 is the crude model. Model 2 represents model 1 + age + BMI + current smoking + alcohol consumption + leisure-time physical activity + family history of diabetes + serum calcium + serum phosphorus + calcium intake + serum 25(OH)D.

Table 4. Adjusted ORs and 95% CIs for Osteoporosis According to DPP4 Quartiles

	Q1	Q2	Q3	Q4
DPP4 activity, nmol/mL/min	<12.79	12.80–17.65	17.66–22.82	≥22.83
Osteoporosis, n (%)	21 (11.4)	18 (9.6)	43 (23.0)	58 (31.5)
Model 1	1.0	0.83 (0.43, 1.61) 0.575	2.33 (1.32, 4.11) 0.003	3.60 (2.07, 6.23) <0.001
Model 2	1.0	0.88 (0.45, 1.74) 0.729	2.43 (1.37, 4.34) 0.003	3.57 (2.01, 6.33) <0.001
Model 3	1.0	0.89 (0.45, 1.75) 0.741	2.45 (1.37, 4.38) 0.002	3.60 (2.02, 6.41) <0.001
Model 4	1.0	0.91 (0.46, 1.80) 0.794	2.52 (1.41, 4.51) 0.002	3.75 (2.10, 6.70) <0.001
Model 5	1.0	0.88 (0.45, 1.74) 0.719	2.40 (1.34, 4.27) 0.003	3.35 (1.87, 5.99) <0.001
Model 6	1.0	0.83 (0.42, 1.63) 0.586	2.18 (1.21, 3.91) 0.009	3.01 (1.66, 5.43) <0.001

Data are expressed as OR (95% CI) + *P* value, unless stated otherwise. Model 1 is the crude model. Model 2 represents model 1 + age + BMI + current smoking + alcohol consumption + leisure-time physical activity + family history of diabetes + serum calcium + serum phosphorus + calcium intake + serum 25(OH)D. Model 3 is model 2 + TG. Model 4 is model 2 + active GLP-1. Model 5 is model 2 + HOMA-IR. Model 6 is model 5+ IL-6.

rosis risk increased with rising levels of DPP4 activity but not with TC or TG. We believed that this divergence may result from the race, sample size, different duration, and severity of dyslipidemia to some extent. More importantly, it might be more appropriate to consider dyslipidemia as an associated condition rather than a pathogenetic factor for osteoporosis, and in-depth analysis and/or a prospective trial assessing the risk of osteoporosis with altered lipids profile is still needed.

A recent study by Shin et al (6) showed that HOMA-IR was inversely associated with BMD, indicating that insulin resistance was a negative predictor for bone health. Consistent with their result, a positive relationship between insulin resistance and risk of osteoporosis was also observed in our study. However, this positive relationship was not compatible with previous findings that insulin resistance with compensating hyperinsulinemia has been shown to result in increased bone mass (24, 25). One possible reason for this difference is that potential confounding factors may or may not be sufficiently controlled because some studies found that positive relationships between hyperinsulinemia or insulin resistance and bone mass were attenuated and became nonsignificant after adjustment for body weight or BMI (6).

Bone has now been recognized as an insulin target organ, and insulin receptor signaling in osteoblasts has been found to be important for proliferation, differentiation, and survival of osteoblasts; interruption of osteoblastic insulin signaling in insulin-resistant subjects could directly result in a reduction of bone mass (26, 27). A previous study has demonstrated that DPP4 may impair insulin sensitivity and leads to insulin resistance in an autocrine and paracrine fashion (10). In this study, we also found a positive correlation between DPP4 activity and insulin resistance, estimated by HOMA-IR. More importantly, the risk of osteoporosis was more pronounced among participants with rising DPP4 activity and higher levels of HOMA-IR. Because DPP4 may be a pathogenic factor for insulin resistance, it could promote osteoporosis development by impairing insulin sensitivity of osteoblastic cell; however, because of the nature of our study, this speculation remains to be clarified by further research. In addition, we found that the ORs for osteoporosis according to DPP4 quartiles were not substantially attenuated by additional adjustment for HOMA-IR, even within the lowest HOMA-IR quartile. The risks for osteoporosis were 4.00-fold higher in the highest DPP4 quartile than in the lowest quartile, suggesting that an elevated DPP4 activity could promote osteoporosis risk through a pathway not fully overlapping with insulin resistance.

It is generally acknowledged that inflammation has been suggested to play an important role in the pathogenesis of postmenopausal osteoporosis (3). Proinflammatory cytokines such as TNF-α, hs-CRP, and IL-6 are associated with the development of osteoporosis through stimulation of osteoclastogenesis and subsequent bone resorption (28–30). In line with these results, our data indicated that the risk of osteoporosis increased significantly with higher levels of hs-CRP and IL-6, and this trend gets more pronounced among subjects with both higher levels of inflammatory markers and DPP4 activity. Because a causal relationship between DPP4 and inflammation has been demonstrated by previous studies (31, 32), we speculate that DPP4 might promote the development of osteoporosis partly through its proinflammatory function.

Interestingly, further adjustment for HOMA-IR and IL-6 yielded only a reduction of the osteoporosis risk across the DPP4 activity quartiles. Thus, increased DPP4 activities in the individuals with high osteoporosis risk may not be merely a consequence of enhanced insulin resistance and inflammation. Considering the multiple pleiotropic effects of DPP4 activity, the mutual effects between DPP4 activities and other unknown factors might also exert some influence on the development of osteoporosis in postmenopausal women with NGT.

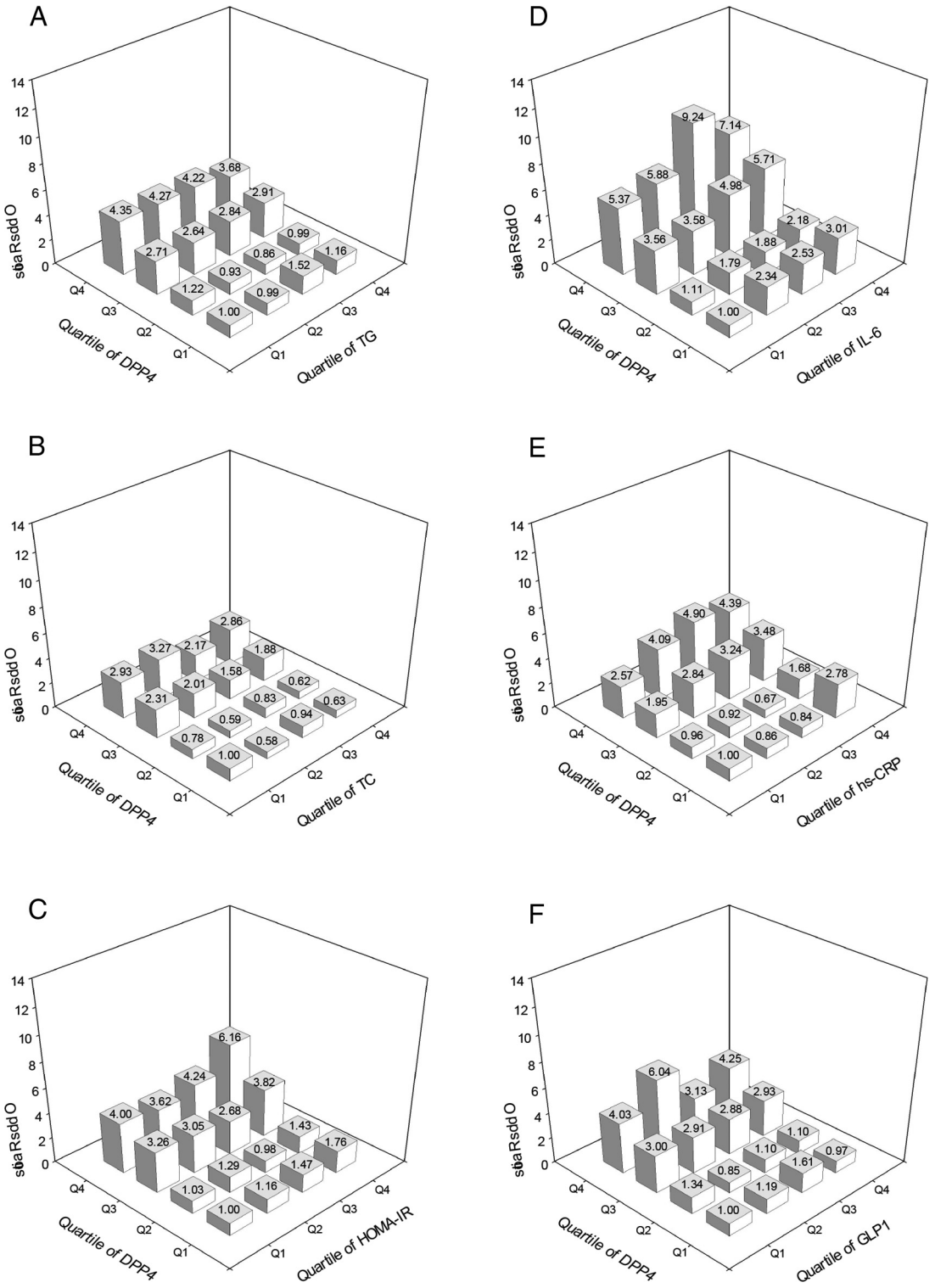

Figure 1. Adjusted ORs for osteoporosis according to the quartiles of DPP4 and TG (A), DPP4 and TC (B), DPP4 and HOMA-IR (C), DPP4 and IL-6 (D), DPP4 and hs-CRP (E), and DPP4 and active GLP-1 (F). Data are adjusted for age, BMI, current smoking, alcohol consumption, leisure-time physical activity, family history of diabetes, serum calcium, serum phosphorus, calcium intake, and serum 25(OH)D.

Some basic research has confirmed the presence of GLP-1 receptor in human osteoblastic cell lines, and GLP-1 may play a role in either direct or indirect modulation of postprandial bone metabolism (33). However, some clinical trials did not show that GLP-1 receptor agonists had a significant impact on total BMD in patients with T2DM (34). In this study, although a significant and inverse relationship was found between DPP4 activity and fasting active GLP-1 level in postmenopausal women, we did not find any significant association between increased

risks of osteoporosis and decreased fasting active GLP-1 levels as well. The OR for osteoporosis according to DPP4 quartiles was not reduced after further adjustment of fasting active GLP-1. The reasons for this discrepancy could as summarized as follows. First, it is generally accepted that postprandial GLP-1 levels are much higher than fasting levels. A previous study by Bjarnason et al (35) has shown that the bone turnover response to oral glucose was substantially greater than that after iv glucose, suggesting a potential role for postprandial GLP-1 after nutrient ingestion. In addition, octreotide, a long-acting analog of somatostatin inhibiting the basal and postprandial secretion of GLP-1, has been demonstrated to abolish nutrient-induced suppression in bone turnover (36). Consequently, we hypothesize that it might be more appropriate to evaluate the association between osteoporosis and incretin hormones using postprandial GLP-1 rather than fasting GLP-1. However, this hypothesis remains to be validated because there is no direct clinical evidence indicating that GLP-1 contribution to bone homeostasis is mainly postprandial. Second, the protective effect of GLP-1 on bone metabolism was mostly observed in cell or animal studies, whereas this study was conducted in a population of postmenopausal women. The effect of GLP-1 on bone metabolism might differ in various species. Further clinical studies are still needed to investigate the influence of the incretin system on bone homeostasis in humans.

Because our study is cross-sectional, we cannot draw a causal conclusion that increased DPP4 activities promote the development of osteoporosis. The parallel increase in DPP4 activity and osteoporosis risk could also be interpreted in an opposite way, however. Work completed to date has demonstrated that DPP4 might interact indirectly with bone resorption and formation, but no direct evidence links bone back to the regulation of DPP4 activity.

Some limitations of our study should also be considered. First, this study is an epidemiological cross-sectional study, and somehow it fails to address the causal role of DPP4 in the pathogenesis of osteoporosis. Second, postprandial GLP-1 levels and regulators of calcium homeostasis such as PTH were not measured in this study. Third, information on fragility fractures is lacking. Finally, as with any epidemiological study, the main issue is related with the presence of many concomitant factors that may influence the relationship between DPP4 and osteoporosis and actually limit clinical significance of the results, although every effort was made to adjust for relevant DPP4 activity and bone health risk factors. This point and the cross-sectional nature of the study make our data preliminary to some extent.

In conclusion, we provide the first evidence that increased DPP4 activities are independently associated with osteoporosis in postmenopausal women with NGT. From a clinical perspective, we speculate that the underlying mechanisms may be partly explained by the effect of DPP4 on inflammation and insulin resistance, not by an altered lipid profile or active fasting GLP-1. Moreover, independent of the mechanism, an elevated DPP4 activity in postmenopausal women could be a marker or a warning signal predicting the development of osteoporosis. If this issue could be better addressed by future studies with different endpoints (eg, fractures, evaluation of bone quality), it may have clinical significance for the early identification of postmenopausal women at high risk of osteoporosis.

Acknowledgments

Address all correspondence and requests for reprints to: Tianpeng Zheng, MD, Department of Endocrinology and Metabolism, Affiliated Hospital of Guilin Medical University, 15 Le Qun Road, Guilin, Guangxi 541001, China. E-mail: w19831120@126.com.

This study was supported by grants from National Natural Science Foundation of China (Grant NO. 81560144/H0713), the scientific research and technology development projects of Science and Technology Department of Guangxi province (Grant NO. 1598012-13) and Guangxi Province Department of Education (Grant NO. KY2015LX247).

Trial Registration No.: ChiCTR-EPC-14005273.

Disclosure Summary: None of the authors reports a duality of interest.

References

1. **Lee SH, Kang MI, Ahn SH, et al.** Common and rare variants in the exons and regulatory regions of osteoporosis-related genes improve osteoporotic fracture risk prediction. *J Clin Endocrinol Metab*. 2014;99:E2400–E2411.
2. **Xiao SM, Kung AW, Sham PC, Tan KC.** Genetic analysis of recently identified osteoporosis susceptibility genes in southern Chinese. *J Clin Endocrinol Metab*. 2013;98:E1827–E1834.
3. **Lorenzo J, Horowitz M, Choi Y.** Osteoimmunology: interactions of the bone and immune system. *Endocr Rev*. 2008;29:403–440.
4. **Makovey J, Chen JS, Hayward C, Williams FM, Sambrook PN.** Association between serum cholesterol and bone mineral density. *Bone*. 2009;44:208–213.
5. **Orozco P.** Atherogenic lipid profile and elevated lipoprotein (a) are associated with lower bone mineral density in early postmenopausal overweight women. *Eur J Epidemiol* 2004;19:1105–1112.
6. **Shin D, Kim S, Kim KH, Lee K, Park SM.** Association between insulin resistance and bone mass in men. *J Clin Endocrinol Metab*. 2014;99:988–995.
7. **Gilbert MP, Pratley RE.** The impact of diabetes and diabetes medications on bone health. *Endocr Rev*. 2015;36:194–213.
8. **Matteucci E, Giampietro O.** Dipeptidyl peptidase-4 (CD26): knowing the function before inhibiting the enzyme. *Curr Med Chem*. 2009;16:2943–2951.
9. **Drucker DJ, Nauck MA.** The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*. 2006;368:1696–1705.
10. **Lamers D, Famulla S, Wronkowitz N, et al.** Dipeptidyl peptidase 4

is a novel adipokine potentially linking obesity to the metabolic syndrome. *Diabetes*. 2011;60:1917–1925.
11. **Conarello SL, Li Z, Ronan J, et al.** Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc Natl Acad Sci USA*. 2003;100:6825–6830.
12. **Zheng T, Baskota A, Gao Y, Chen T, Tian H, Yang F.** Increased plasma DPP4 activities predict new-onset hyperglycemia in Chinese over a four-year period: possible associations with inflammation. *Metabolism*. 2015;64:498–505.
13. **Zheng T, Chen T, Liu Y, Gao Y, Tian H.** Increased plasma DPP4 activity predicts new-onset hypertension in Chinese over a 4-year period: possible associations with inflammation and oxidative stress. *J Hum Hypertens*. 2015;29:424–429.
14. **Monami M, Dicembrini I, Antenore A, Mannucci E.** Dipeptidyl peptidase-4 inhibitors and bone fractures: a meta-analysis of randomized clinical trials. *Diabetes Care*. 2011;34:2474–2476.
15. **Zheng T, Gao Y, Baskota A, Chen T, Ran X, Tian H.** Increased plasma DPP4 activity is predictive of prediabetes and type 2 diabetes onset in Chinese over a four-year period: result from the China National Diabetes and Metabolic Disorders Study. *J Clin Endocrinol Metab*. 2014;99:E2330–E2334.
16. **Wittrant Y, Gorin Y, Woodruff K, et al.** High d(+)glucose concentration inhibits RANKL-induced osteoclastogenesis. *Bone*. 2008; 42:1122–1130.
17. **Yang W, Lu J, Weng J, et al.** Prevalence of diabetes among men and women in China. *N Engl J Med*. 2010;362:1090–1101.
18. **Yang Y, Wang G, Pan X.** Chinese food composition table. Beijing, China: Beijing Medical University Press; 2002.
19. **Joint Committee for Developing Chinese guidelines on Prevention and Treatment of Dyslipidemia in Adults.** Chinese guidelines on prevention and treatment of dyslipidemia in adults [in Chinese]. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2007;35:390–419.
20. **Flock G, Baggio LL, Longuet C, Drucker DJ.** Incretin receptors for glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide are essential for the sustained metabolic actions of vildagliptin in mice. *Diabetes*. 2007;56:3006–3013.
21. **Tremblay AJ, Lamarche B, Deacon CF, Weisnagel SJ, Couture P.** Effect of sitagliptin therapy on postprandial lipoprotein levels in patients with type 2 diabetes. *Diabetes Obes Metab*. 2011;13:366–373.
22. **Go JH, Song YM, Park JH, Park JY, Choi YH.** Association between serum cholesterol level and bone mineral density at lumbar spine and femur neck in postmenopausal Korean women. *Korean J Fam Med*. 2012;33:166–173.
23. **Li S, Guo H, Liu Y, et al.** Relationships of serum lipid profiles and bone mineral density in postmenopausal Chinese women. *Clin Endocrinol (Oxf)*. 2015;82:53–58.
24. **Abrahamsen B, Rohold A, Henriksen JE, Beck-Nielsen H.** Correlations between insulin sensitivity and bone mineral density in nondiabetic men. *Diabet Med*. 2000;17:124–129.
25. **Hanley DA, Brown JP, Tenenhouse A, et al.** Associations among disease conditions, bone mineral density, and prevalent vertebral deformities in men and women 50 years of age and older: cross-sectional results from the Canadian Multicentre Osteoporosis Study. *J Bone Miner Res*. 2003;18:784–790.
26. **de Paula FJ, Horowitz MC, Rosen CJ.** Novel insights into the relationship between diabetes and osteoporosis. *Diabetes Metab Res Rev*. 2010;26:622–630.
27. **Fulzele K, Riddle RC, DiGirolamo DJ, et al.** Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. *Cell*. 2010;142:309–319.
28. **Collin-Osdoby P, Rothe L, Anderson F, Nelson M, Maloney W, Osdoby P.** Receptor activator of NF-κ B and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. *J Biol Chem*. 2001;276:20659–20672.
29. **Laird E, Ward M, McSorley E, Strain JJ, Wallace J.** Vitamin D and bone health: potential mechanisms. *Nutrients*. 2010;2:693–724.
30. **Ishii S, Cauley JA, Greendale GA, et al.** C-reactive protein, bone strength, and nine-year fracture risk: data from the Study of Women's Health Across the Nation (SWAN). *J Bone Miner Res*. 2013; 28:1688–1698.
31. **Cordero OJ, Salgado FJ, Viñuela JE, Nogueira M.** Interleukin-12 enhances CD26 expression and dipeptidyl peptidase IV function on human activated lymphocytes. *Immunobiology*. 1997;197:522–533.
32. **Ikushima H, Munakata Y, Iwata S, et al.** Soluble CD26/dipeptidyl peptidase IV enhances transendothelial migration via its interaction with mannose 6-phosphate/insulin-like growth factor II receptor. *Cell Immunol*. 2002;215:106–110.
33. **Pacheco-Pantoja EL, Ranganath LR, Gallagher JA, Wilson PJ, Fraser WD.** Receptors and effects of gut hormones in three osteoblastic cell lines. *BMC Physiol*. 2011;11:12.
34. **Bunck MC, Eliasson B, Cornér A, et al.** Exenatide treatment did not affect bone mineral density despite body weight reduction in patients with type 2 diabetes. *Diabetes Obes Metab*. 2011;13:374–377.
35. **Bjarnason NH, Henriksen EE, Alexandersen P, Christgau S, Henriksen DB, Christiansen C.** Mechanism of circadian variation in bone resorption. *Bone*. 2002;30:307–313.
36. **Clowes JA, Allen HC, Prentis DM, Eastell R, Blumsohn A.** Octreotide abolishes the acute decrease in bone turnover in response to oral glucose. *J Clin Endocrinol Metab*. 2003;88:4867–4873.