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ORIGINAL ARTICLE

Inverse association of plasma leptin with cortical thickness at distal radius determined with a quantitative ultrasound device in patients with type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: Osteoporosis is known to be intimately related to sympathetic nerve activity. We examined the relationship of plasma leptin with cortical and trabecular bone components in patients with type 2 diabetes mellitus.

Materials and Methods: The present cross-sectional study included 182 type 2 diabetes mellitus patients (93 men, 89 women). Cortical thickness (CoTh) and trabecular bone mineral density (BMD) were determined at the 5.5% distal radius using an LD-100 ultrasonic bone densitometry device. Plasma leptin along with physical and laboratory measurements was simultaneously determined.

Results: Plasma leptin, but not body mass index (BMI), was inversely correlated with CoTh ($\rho = -0.487$, $P < 0.001$), while BMI, but not plasma leptin, was positively correlated with trabecular BMD ($\rho = 0.369$, $P < 0.001$). In multivariable regression analysis, after adjustments for age, sex, duration of diabetes, glycated hemoglobin A1c, albumin, estimated glomerular filtration rate, parathyroid hormone and handgrip strength, plasma leptin was inversely associated with CoTh ($\beta = -0.258$, $P < 0.001$), but not trabecular BMD. Furthermore, plasma leptin level retained a significant association with CoTh after further adjustment for BMI ($\beta = -0.237$, $P < 0.001$) and BMI plus waist-to-hip ratio ($\beta = -0.243$, $P < 0.001$). In contrast, the "sex × leptin" interaction was not significant ($P = 0.596$).

Conclusions: Leptin level in plasma, independent of BMI and BMI plus waist-to-hip ratio, was shown to be inversely associated with CoTh, but not trabecular BMD, suggesting that hyperleptinemia resulting from obesity might contribute to cortical porosis in patients with type 2 diabetes mellitus.

INTRODUCTION

Patients with type 2 diabetes mellitus have an elevated risk of bone fracture, even when adequate bone mineral density (BMD) is present[1–3], suggesting the involvement of impaired bone quality, but not reduced BMD, in the development of bone fragility in those patients. Using an LD-100 quantitative ultrasound (QUS) device, we recently showed that cortical thickness (CoTh), but not trabecular BMD (TrBMD), at the 5.5% distal radius was significantly reduced in type 2 diabetes mellitus patients as compared with individuals without diabetes[4], whereas reduced CoTh was found to be significantly associated with vertebral fracture in patients with type 2 diabetes mellitus[5]. In other similar studies[6,7], but not all[8], obese individuals have been shown to possess alterations in the structure and material properties of cortical bone, including higher cortical porosity along with reduced cortical area, bone mineral content, BMD and bone strength. Furthermore, the present authors and others have shown that obesity is a risk factor for lower CoTh in type 2 diabetes mellitus patients[4], as well as the general population[9], although the underlying

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mechanisms of the relationship of that with obesity have yet to be elucidated.

Leptin, a 16-kDa peptide hormone mainly derived from adipose tissue, was originally identified as a substance with activities to increase energy expenditure and suppress appetite[10,11], type 2 diabetes mellitus patients generally show higher levels of leptin in plasma than individuals without diabetes, due to increased adiposity and development of leptin resistance[12], although the level of leptin in plasma in those patients is a controversial issue[13,14]. In addition to its appetite-suppressing effect, leptin is known to activate the sympathetic nervous system through hypothalamic neurons expressing the leptin receptor[15]. Obesity is known to be closely associated with autonomic dysfunction[16], and we previously reported that the association of hyperleptinemia with autonomic dysfunction was more significant in type 2 diabetes mellitus as compared with non-diabetes patients, even though the plasma leptin level was lower in the former group[17], suggesting a strong involvement of plasma leptin in autonomic dysfunction occurring in association with type 2 diabetes mellitus.

Importantly, other reports have shown that activation of the sympathetic nervous system under the influence of leptin inhibits bone formation[18–20] and stimulates bone resorption[21], indicating involvement of leptin in the development of osteoporosis through sympathetic nerve activity. Those findings led us to examine here whether plasma leptin contributes to the pathophysiology of reduced CoTh in type 2 diabetes mellitus patients. Thus far, no known studies have investigated the associations of plasma leptin level and CoTh in type 2 diabetes mellitus diabetes. In the present study, we examined type 2 diabetes mellitus patients to analyze the relationship of the level of leptin in plasma with CoTh and TrBMD.

METHODS

Study design and participants

The present cross-sectional study was carried out at the Diabetes Center of Osaka City University Hospital (Osaka, Japan) between October 2011 and February 2017. We enrolled 182 consecutive patients with type 2 diabetes mellitus (93 men, 89 women) who had been admitted for evaluation of diabetic complications, education regarding caring for their condition and/or glycemic control. Type 2 diabetes mellitus was diagnosed based on criteria presented by the Japan Diabetes Society[22]. For glycemic control, the patients were being treated with dietary therapy alone (n = 20), metformin (n = 51), sulfonylurea (n = 54), glinides drugs (n = 4), dipeptidyl peptidase-4 inhibitors (n = 68), α-glucosidase inhibitors (n = 24), pioglitazone (n = 10), glucagon-like peptide-1 receptor agonists (n = 9) or insulin (n = 71; Table 1). Smoking status was determined based on self-reported history of cigarette smoking. We defined cerebrovascular disease as history of stroke (ischemic or hemorrhagic) diagnosed based on computed tomography or magnetic resonance imaging findings. Those who had malignancy, infection or acute illness; representative diseases known to have effects on sympathetic nerve activity, including Parkinson's disease, Guillain–Barré syndrome and multiple sclerosis; or taking such medications as anti-osteoporotic drugs or steroids that might have effects on bone metabolism were excluded from analysis. None had metabolic bone disease or a major condition that might influence bone metabolism or affect nutritional status.

Written informed consent was obtained from all participants before participation in this study. The study protocol was approved by the ethics review committee of Osaka City University Graduate School of Medicine (approval #308), and it was carried out in accordance with the principals of the Declaration of Helsinki.

Table 1 | Patient clinical characteristics

Age (years)	64 (53–71)
Male	93 (51.1%)
BMI (kg/m^2)	25.4 (22.4–28.6)
Waist-to-hip ratio	0.97 (0.92–1.00)
Handgrip strength (kg)	23.0 (17.5–28.6)
Past cerebrovascular disease	18 (9.9%)
Smoking habit	88 (48.4%)
Fasting plasma glucose (mg/dL)	113.5 (98.0–137.3)
HbA1c (%)	8.3 (7.3–9.8)
Immunoreactive insulin (μU/mL)†	7.9 (5.3–11.1)
HOMA-IR†	2.2 (1.5–3.4)
Duration of diabetes (years)	10 (3–20)
Diabetic neuropathy	102 (56.0%)
Medical treatment	
Metformin	51 (28.0%)
Sulfonylurea	54 (29.7%)
Glinides	4 (2.2%)
DPP4 inhibitor	68 (37.4%)
α-Glucosidase inhibitor	24 (13.2%)
Pioglitazone	10 (5.5%)
GLP-1 receptor analog	9 (4.9%)
Insulin	71 (39.0%)
α/β-Blocker	15 (8.2%)
Albumin (g/dL)	4.0 (3.7–4.3)
eGFR (mL/min/1.73 m^2)	66.9 (49.7–82.9)
Whole PTH (pg/mL)	20.4 (15.7–27.8)
BAP (U/L)	12.7 (10.2–17.0)
TRACP-5b (U/L)	341.0 (245.3–470.3)
1,25(OH)$_2$D (pg/mL)	44.0 (28.0–59.3)
Leptin (ng/mL)	4.2 (1.8–9.3)
LD-100 (5.5% distal radius)	
CoTh (mm)	3.68 (2.67–4.44)
TrBMD (mg/cm^3)	167.6 (132.9–209.6)

Total n = 182. Values are shown as the median (interquartile range) or number (%). †A total of 111 patients who did not receive insulin therapy. BAP, bone alkaline phosphatase; BMI, body mass index; CoTh, cortical thickness; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; HbA1c, glycated hemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; PTH, parathyroid hormone; TRACP-5b, tartrate-resistant acid phosphatase-5b; TrBMD, trabecular bone mineral density.

Physical measurements

We measured height and bodyweight. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured to the nearest centimeter at the level of the umbilicus in a standing position at the end of gentle expiration. Hip circumference was measured at the level of greatest posterior protuberance of the buttocks. Waist-to-hip ratio was calculated as waist circumference in centimeters divided by hip circumference in centimeters. Diabetic neuropathy was defined as the presence of two or more neuropathic symptoms, decreased distal sensation and unequivocally decreased or absent ankle reflexes[23]. Using a hand dynamometer, handgrip strength on the non-dominant side was measured by experienced research staff blinded to all biochemical and clinical data, during which the patient was instructed to apply as much handgrip pressure as possible, and repeated three times, with the highest score recorded in kilograms, using a method that we have previously reported[24,25].

Measurement of bone densitometry with an ultrasonic device

Ultrasonic measurements of the non-dominant side of the 5.5% distal radius were carried out using an LD-100 bone densitometer device (Oyo Electric, Kyoto, Japan), which allows determination of CoTh and TrBMD[26,27]. The LD-100 system consists of two ultrasonic transducers located coaxially in the forward direction and is equipped with a computer system. The transducers move simultaneously for scanning, during which one transmits ultrasound signals through the objective region and the other receives those signals. As noted in our previous studies[26,27], the propagation time of echo waves and slow waves was used. CoTh, expressed in millimeters, was estimated by analyzing reflected and transmitted ultrasonic signals, whereas

Table 2 | Simple regression analysis of factors associated with bone densitometry determined by LD-100

Variables	CoTh		TrBMD	
	ρ	*P*	ρ	*P*
Age	−0.368	<0.001	−0.569	<0.001
Male = 1, Female = 0	0.652	<0.001	0.134	0.070
BMI	−0.008	0.915	0.369	<0.001
Waist-to-hip ratio	0.174	0.019	0.177	0.017
Handgrip strength	0.650	<0.001	0.395	<0.001
Past cerebrovascular disease (yes = 1, no = 0)	0.078	0.292	0.023	0.762
Smoking habit (yes = 1, no = 0)	0.321	<0.001	0.161	0.030
Fasting plasma glucose	0.117	0.115	0.152	0.040
HbA1c	0.182	0.014	0.114	0.126
IRI[†]	−0.040	0.677	0.237	0.013
HOMA-IR[†]	−0.033	0.732	0.270	0.004
Duration of diabetes	−0.131	0.079	−0.245	0.001
Diabetic neuropathy (yes = 1, no = 0)	−0.037	0.622	−0.148	0.046
Metformin (yes = 1, no = 0)	−0.124	0.095	0.110	0.138
Sulfonylurea (yes = 1, no = 0)	0.067	0.367	0.008	0.918
Glinides (yes = 1, no = 0)	0.122	0.100	0.035	0.643
DPP4 inhibitor (yes = 1, no = 0)	−0.019	0.794	−0.066	0.377
α-Glucosidase inhibitor (yes = 1, no = 0)	0.117	0.117	−0.026	0.723
Pioglitazone (yes = 1, no = 0)	0.077	0.302	0.052	0.483
GLP-1 receptor analog (yes = 1, no = 0)	0.014	0.849	0.126	0.089
Insulin (yes = 1, no = 0)	−0.065	0.386	−0.153	0.039
α/β-Blocker (yes = 1, no = 0)	−0.052	0.485	0.004	0.953
Albumin	0.067	0.371	0.103	0.168
eGFR	−0.067	0.369	0.109	0.143
Whole PTH	−0.013	0.857	−0.058	0.439
BAP	−0.068	0.359	−0.055	0.458
TRACP-5b	−0.182	0.014	−0.331	<0.001
$1,25(OH)_2D$	−0.040	0.596	0.063	0.401
Leptin	−0.487	<0.001	0.038	0.612

[†]A total of 111 patients who did not receive insulin therapy. BAP, bone alkaline phosphatase; BMI, body mass index; CoTh, cortical thickness; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; HbA1c, glycated hemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; PTH, parathyroid hormone; TRACP-5b, tartrate-resistant acid phosphatase-5b; TrBMD, trabecular bone mineral density.

TrBMD, expressed in mg/cm^3, was estimated by quantifying the attenuation of ultrasound waves transmitted through bone and transmission velocity of fast waves propagated through trabecular bone structures[28–30]. The LD-100 is a new QUS system that recently received approval for use as medical equipment in Japan. The results obtained with the device have been validated based on a significant correlation with values obtained by peripheral quantitative computed tomography in Japanese individuals with a mean BMI of 21.5 kg/m^2[26], and in another study with participants aged 50–86 years[27].

Laboratory measurements

Blood samples were obtained in the morning after an overnight fast. Fasting plasma glucose, glycated hemoglobin A1c, and serum levels of albumin and creatinine were analyzed using a standard laboratory method at the Central Laboratory of Osaka City University Hospital[31]. Estimated glomerular filtration rate was calculated using an equation for Japanese individuals, as previously described[32]. The level of whole parathyroid hormone in serum was measured using a whole parathyroid hormone assay (Scantibodies Laboratory Inc., Santee, CA, USA), as previously described[33]. The level of immunoreactive insulin in serum was determined using an electrochemiluminescence immunoassay (Roche Diagnostics K.K., Tokyo, Japan). Homeostatic model assessment of insulin resistance (HOMA-IR) index values were calculated with the following formula[34,35]; (fasting immunoreactive insulin [IU/mL] × fasting plasma glucose [mg/dL] / 405). Serum bone alkaline phosphatase levels were determined with an enzyme immunoassay (ALKPHASE-B; Metra Biosystems, Mountain View, CA, USA)[36]. Measurement of serum osteocalcin was carried out using a two-site immunoradiometric assay kit (Mitsubishi Kagaku Bioclinical Laboratories, Tokyo, Japan)[37], while that of tartrate-resistant acid phosphatase-5b (TRACP-5b) activity was carried out with a fragment absorbed immunocapture enzymatic assay method using two monoclonal antibodies[38]. The serum level of 1,25 $(OH)_2D$ was measured using radioimmunoassay findings[33]. Plasma leptin levels were determined using an enzyme-linked immunosorbent assay kit (R&D Systems Inc., Minneapolis, MN, USA), as previously reported[17,39].

Statistical analysis

Values are expressed as the number (%) or median (interquartile range). Spearman's correlation coefficient test was carried out to determine correlations between continuous variables. Multivariable regression analyses were carried out to evaluate the associations of bone densitometry parameters with various clinical parameters, including plasma leptin, BMI and waist-to-hip ratio. Plasma leptin levels and HOMA-IR were logarithmically transformed, while the duration of diabetes including values of zero were logarithmically transformed (log $[x + 1]$) before carrying out multivariable regression analysis, due to its skewed distribution. We incorporated a two-factor interaction term (sex × log leptin) to assess the effect of sex difference on the relationship between leptin level and bone densitometry parameters. The variance inflation factor was determined to estimate multicollinearity for each predictor. All statistical analyses were carried out using the Statistical Package for the Social Sciences (PASW Statistics version 22.0; SPSS Inc. Chicago, IL, USA). All reported *P*-values are two-tailed and were considered statistically significant at a level <0.05.

RESULTS

Clinical characteristics of type 2 diabetes mellitus patients

The characteristics of the enrolled type 2 diabetes mellitus patients (n = 182) are shown in Table 1. The median values

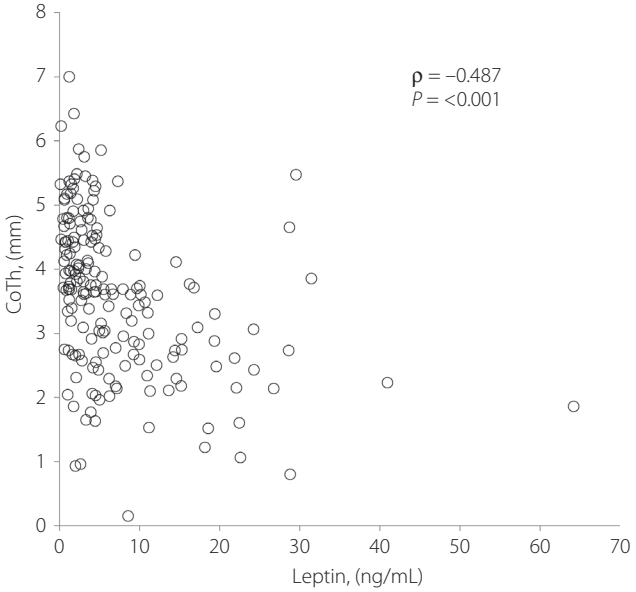

Figure 1 | Relationship of plasma leptin level with cortical thickness (CoTh).

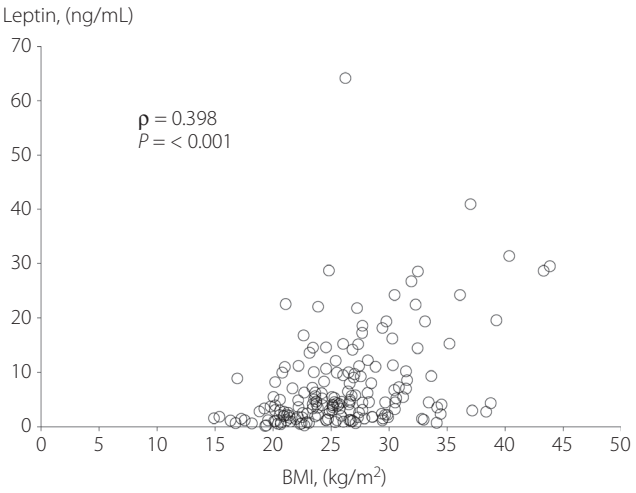

Figure 2 | Relationship of body mass index (BMI) with plasma leptin level.

for fasting plasma glucose, glycated hemoglobin A1c, BMI and waist-to-hip ratio were 113.5 mg/dL, 8.3%, 25.4 kg/m^2 and 0.97, respectively, whereas those for plasma leptin, CoTh and TrBMD were 4.2 ng/mL, 3.68 mm and 167.6 mg/cm^3, respectively.

Simple correlations of plasma leptin, clinical parameters, and bone parameters with CoTh and TrBMD in type 2 diabetes mellitus patients

Table 2 shows a summary of simple correlations of various clinical factors found with CoTh and TrBMD in the 182 type 2 diabetes mellitus patients, as determined by LD-100 findings. Plasma leptin, but not BMI, was significantly and inversely correlated with CoTh ($\rho = -0.487$, $P < 0.001$; also shown in Figure 1), despite the significant and positive correlation of BMI with leptin in plasma ($\rho = 0.398$, $P < 0.001$; Figure 2). In contrast, BMI, but not plasma leptin, was significantly and positively correlated with TrBMD ($\rho = 0.369$, $P < 0.001$). Clinical variables inversely correlated with both CoTh and TrBMD were age, duration of type 2 diabetes mellitus, and TRACP-5b, whereas factors found to be positively correlated were male sex, waist-to-hip ratio, handgrip strength and smoking status.

Plasma leptin, but not BMI or waist-to-hip ratio, associated with CoTh

To examine whether the level of leptin in plasma was significantly associated with CoTh independent of the main confounding factors, multivariable regression analyses were carried out (Table 3). In basic model 1, which included age, sex, log (duration of type 2 diabetes mellitus + 1), glycated hemoglobin A1c, albumin, estimated glomerular filtration rate, handgrip strength, whole parathyroid hormone level and log (plasma leptin level) as covariates, plasma leptin level was significantly and inversely associated with CoTh. When log (plasma leptin level) was replaced with BMI (model 2), BMI also emerged as a significant factor inversely associated with CoTh. When log (plasma leptin level) and BMI were simultaneously included (model 3), plasma leptin level, but not BMI, retained a significant association with CoTh. Finally, when waist-to-hip ratio was added to model 3 (model 4), log (plasma leptin level), but not BMI or waist-to-hip ratio, retained a significant association with CoTh. In each model, age and estimated glomerular filtration rate were significantly and inversely associated, whereas male sex and handgrip strength were significantly and positively associated with CoTh. The variance inflation factor values were <5 for each of the predictors, indicating no multicollinearity between the variables (Table 3). Although sex was significantly associated with CoTh, the "sex × leptin level" interaction was not significant ($P = 0.596$), whereas plasma leptin level was significantly and inversely associated with CoTh in both men and women ($\beta = -0.254$, $P = 0.030$ and $\beta = -0.240$, $P = 0.017$, respectively), suggesting that sex does not have an effect on the relationship between the level of leptin in plasma and CoTh. Furthermore, that leptin level ($\beta = -0.271$, $P = 0.015$) was significantly associated with CoTh, even in post-menopausal women with type 2 diabetes mellitus ($n = 72$).

Plasma leptin associated with CoTh independent of other confounding factors

To further examine whether the association of plasma leptin level with CoTh was independent of other confounding factors, multivariable regression analyses were again carried out (Table 4). When diabetic neuropathy (model 1), past cerebrovascular disease (model 2), smoking status (model 3), 1,25

Table 3 | Multiple regression analysis of factors associated with cortical thickness determined by LD-100

Variables	Model 1			Model 2			Model 3			Model 4		
	β	*P*	VIF	β	*P*	VIF	β	*P*	VIF	β	*P*	VIF
Age	−0.321	<0.001	1.604	−0.370	<0.001	1.850	−0.336	<0.001	1.888	−0.340	<0.001	1.896
Sex (male/female, 1/0)	0.314	<0.001	2.085	0.412	<0.001	1.831	0.319	<0.001	2.116	0.296	<0.001	2.451
Log (type 2 diabetes mellitus duration + 1)	−0.009	0.867	1.409	−0.041	0.487	1.384	−0.011	0.845	1.412	−0.010	0.865	1.414
HbA1c	0.069	0.203	1.274	0.061	0.276	1.284	0.066	0.228	1.285	0.061	0.263	1.297
Albumin	0.090	0.103	1.322	0.059	0.299	1.298	0.088	0.112	1.327	0.091	0.103	1.330
eGFR	−0.260	<0.001	1.949	−0.275	<0.001	1.960	−0.264	<0.001	1.964	−0.260	<0.001	1.972
Handgrip strength	0.236	0.001	2.024	0.257	<0.001	2.039	0.241	0.001	2.048	0.251	<0.001	2.112
Whole PTH	−0.074	0.190	1.399	−0.050	0.393	1.388	−0.072	0.205	1.405	−0.066	0.246	1.423
Log leptin	−0.258	<0.001	1.355				−0.237	<0.001	1.870	−0.243	<0.001	1.892
BMI				−0.158	0.005	1.252	−0.039	0.541	1.728	−0.069	0.340	2.293
Waist-to-hip ratio										0.054	0.393	1.741
Adjusted R^2/*P*	0.587/<0.001			0.557/<0.001			0.586/<0.001			0.585/<0.001		

Values shown represent standardized partial regression coefficient (β values), level of significance and variance inflation factor (VIF). Each model included age, sex, log (duration of type 2 diabetes mellitus + 1), glycated hemoglobin A1c (HbA1c), albumin, estimated glomerular filtration rate (eGFR), handgrip strength and whole parathyroid hormone (PTH) level as covariates. For the models, log (leptin) (model 1), body mass index (BMI) (model 2), log (leptin) and BMI (model 3), and log (leptin), BMI and waist-to-hip ratio (model 4) were added. R^2, coefficient of determination.

$(OH)_2D$ level (model 4), pioglitazone prescription (model 5), insulin (model 6) and HOMA-IR (model 7) were added to the above-mentioned model 4, plasma leptin retained a significant association with CoTh. Furthermore, when α/β-blocker prescription was added (model 8), that level continued to show a significant association with CoTh, although it tended to be higher (9.9 [1.9–15.2] vs 4.0 [1.8–8.6], $P = 0.067$) in patients being administered an α/β-blocker ($n = 15$), as compared with those who were not ($n = 167$).

No independent association of plasma leptin, BMI or waist-to-hip ratio with TrBMD

When the same multivariate analysis model was used to elucidate clinical variables associated with TrBMD (Table 5), log (plasma leptin level) emerged as borderline positive (model 1). However, when BMI or BMI and waist-to-hip ratio were simultaneously included with log (plasma leptin level), neither plasma leptin level, BMI nor waist-to-hip ratio retained a significant association with TrBMD (models 3 and 4). Furthermore, the "sex × leptin level" interaction was not significant ($P = 0.830$), and the level of leptin in plasma was not significantly associated with TrBMD (β = 0.198, $P = 0.069$ and β = –0.034, $P = 0.750$, respectively) in either men or women, suggesting that sex does not have an effect on the relationship between leptin level and TrBMD. We also noted that plasma leptin did not have a significant association with TrBMD after further adjustment with diabetic neuropathy (model 1), past cerebrovascular disease (model 2), smoking status (model 3), $1,25(OH)_2D$ level (model 4), pioglitazone prescription (model 5), insulin (model 6), HOMA-IR (model 7) or α/β-blocker prescription (model 8; Table 6).

Correlations of plasma leptin with bone metabolic markers

Correlations between plasma leptin level and bone metabolic markers were also evaluated. Leptin level was found to be significantly and inversely correlated with osteocalcin (ρ = –0.220, $P = 0.003$) and TRACP-5b (ρ = –0.219, $P = 0.003$), but not with bone alkaline phosphatase level (ρ = 0.035, $P = 0.636$).

DISCUSSION

The present results showed that the level of leptin in plasma, but not BMI or waist-to-hip ratio, is independently and inversely associated with LD-100-determined CoTh, but not TrBMD, at the 5.5% distal radius in type 2 diabetes mellitus patients (Figure 1; Tables 3–6). In addition, they suggest that leptin has an inhibitory effect preferentially on cortical bone components, in contrast to trabecular bone components, in these patients. Furthermore, it is possible that the mechanism by which leptin has effects on cortical bone might be explained by its stimulatory effect on sympathetic nerve activity, as the inverse association was independent of BMI and waist-to-hip ratio (Tables 3,4).

The level of leptin in plasma is known to be positively associated with adiposity caused by development of leptin resistance[10,40]. Consistent with those reports, plasma leptin level was positively correlated with BMI and waist-to-hip ratio (ρ = 0.146, $P = 0.049$) in the present type 2 diabetes mellitus patients, as well as a previously reported general population[41]. Additionally, basic studies have shown that leptin binds to its specific receptors in the hypothalamus to activate sympathetic nerve function, resulting in inhibition of bone formation by β2-adrenergic receptors on osteoblasts in mice[18–20].

Other studies have provided evidence showing that an increase in sympathetic activity might be intimately involved in the suppression of bone formation[42] simultaneously with stimulation of bone resorption[21]. Thus, it is speculated that the higher plasma leptin activity seen in leptin-resistant obese type 2 diabetes mellitus patients, which represents increased sympathetic nerve activity, is associated with reduced BMD. In

Table 4 | Inverse association of plasma leptin with cortical thickness determined by LD-100 independent of other confounding factors

Variables	β	*P*
Model 1		
Log leptin	–0.243	<0.001
Diabetic neuropathy (yes = 1, no = 0)	–0.006	0.916
Model 2		
Log leptin	–0.244	<0.001
Past cerebrovascular disease (yes = 1, no = 0)	0.020	0.700
Model 3		
Log leptin	–0.243	<0.001
Smoking habit (yes = 1, no = 0)	–0.011	0.839
Model 4		
Log leptin	–0.248	<0.001
$1,25(OH)_2D$	–0.044	0.458
Model 5		
Log leptin	–0.240	<0.001
Use of pioglitazone (yes = 1, no = 0)	0.060	0.227
Model 6		
Log leptin	–0.328	<0.001
Use of insulin (yes = 1, no = 0)	0.051	0.384
Model 7†		
Log leptin	–0.228	0.018
Log HOMA-IR	0.018	0.831
Model 8		
Log leptin	–0.244	<0.001
Use of α- or β-blocker (yes = 1, no = 0)	0.003	0.956

Values shown represent standardized partial regression coefficient (β values) and level of significance. Model 1 included age, sex, log (duration of type 2diabetes mellitus + 1), glycated hemoglobin A1c, albumin, estimated glomerular filtration rate, handgrip strength, whole parathyroid hormone level, body mass index, waist-to-hip ratio, log (plasma leptin level) and diabetic neuropathy as covariates. Diabetic neuropathy was replaced with past cerebrovascular disease (model 2), smoking (model 3), $1,25(OH)_2D$ level (model 4), use of pioglitazone (model 5), use of insulin (model 6), log (homeostatic model assessment of insulin resistance [HOMA-IR]) (model 7), and use of α- or β-blocker (model 8). †A total of 111 patients who did not receive insulin therapy.

Table 5 | Multiple regression analysis of factors associated with trabecular bone mineral density determined by LD-100

Variables	Model 1			Model 2			Model 3			Model 4		
	β	*P*	VIF	β	*P*	VIF	β	*P*	VIF	β	*P*	VIF
Age	−0.480	<0.001	1.604	−0.427	<0.001	1.850	−0.436	<0.001	1.888	−0.437	<0.001	1.896
Sex (male/female, 1/0)	−0.016	0.854	2.085	−0.054	0.506	1.831	−0.031	0.727	2.116	−0.036	0.701	2.451
Log (type 2 diabetes mellitus duration + 1)	−0.061	0.397	1.409	−0.049	0.492	1.384	−0.056	0.434	1.412	−0.056	0.438	1.414
HbA1c	0.040	0.563	1.274	0.050	0.469	1.284	0.048	0.479	1.285	0.047	0.492	1.297
Albumin	0.022	0.751	1.322	0.035	0.608	1.298	0.028	0.690	1.327	0.028	0.685	1.330
eGFR	−0.180	0.034	1.949	−0.168	0.049	1.960	−0.170	0.046	1.964	−0.169	0.048	1.972
Handgrip strength	0.265	0.002	2.024	0.248	0.005	2.039	0.252	0.004	2.048	0.254	0.004	2.112
Whole PTH	0.030	0.678	1.399	0.018	0.803	1.388	0.023	0.743	1.405	0.025	0.732	1.423
Log leptin	0.120	0.090	1.355				0.061	0.464	1.870	0.059	0.478	1.892
BMI				0.140	0.040	1.252	0.109	0.171	1.728	0.102	0.269	2.293
Waist-to-hip ratio										0.013	0.869	1.741
Adjusted R^2/*P*	0.337/<0.001			0.343/<0.001			0.341/<0.001			0.337/<0.001		

Values shown represent standardized partial regression coefficient (β values), level of significance and variance inflation factor. Each model included age, sex, log (duration of type 2 diabetes mellitus + 1), glycated hemoglobin A1c (HbA1c), albumin, estimated glomerular filtration rate (eGFR), handgrip strength and whole parathyroid hormone (PTH) level as covariates. For the models, log (leptin) (model 1), body mass index (BMI) (model 2), log (leptin) and BMI (model 3), and log (leptin), BMI and waist-to-hip ratio (model 4) were added.

support of this notion, findings have been presented showing that treatment with propranolol, a β-blocker, suppressed a reduction in bone mass induced by unloading, while isoproterenol, a β-agonist, reduced bone mass in loaded mice[21]. In our previous study, we showed that the level of leptin in plasma was not significantly different between diabetes patients with and without neuropathy[17], while the present results showed that plasma leptin level was not significantly different between diabetes patients with and without neuropathy (4.0 [1.8–10.0] vs 4.5 [1.8–8.7], $P = 0.995$). In addition, diabetic neuropathy was not significantly associated with either CoTh or TrBMD (Tables 4,6), suggesting that the condition does not have effects on the association of plasma leptin with those parameters. As for bone metabolic markers, plasma leptin levels were inversely correlated with osteocalcin, a marker of bone formation, as well as the bone resorption marker, TRACP-5b, findings consistent with previous reports that showed an inverse association of plasma leptin with bone metabolic markers[43,44]. Together, these results provide important evidence for the role of sympathetic nervous tone to mediate unloading-induced bone loss by suppression of bone formation, rather than enhancement of resorption.

In a previous study, we found a significant and inverse association of plasma leptin level with LD-100-determined CoTh at the distal radius in middle-aged healthy individuals[28]. Furthermore, an investigation of Swedish young adults found that plasma leptin level was inversely associated with CoTh at the distal radius and tibia, as determined by peripheral quantitative computed tomography[45]. Examination of the association between these parameters in type 2 diabetes mellitus patients is important, because of the higher plasma leptin level caused by leptin resistance[12], as well as increased cortical porosity[46] associated with the disease.

Previous studies including ours have shown that obesity is inversely associated with CoTh at the distal radius in type 2 diabetes mellitus patients[4], as well as in women in the general population[9], although other studies have noted no relationship between obesity and CoTh in children[47], young adults[45] or the general population[6]. Consistent with those reports, the present multivariate analysis showed BMI to be a significant factor inversely associated with CoTh in the absence of log (plasma leptin level) as an independent variable (Table 3, model 2). Of importance, the addition of log (plasma leptin level) eliminated the association of BMI with CoTh (Table 3, model 3), suggesting that hyperleptinemia, but not obesity, is a factor contributing to reduced CoTh in type 2 diabetes mellitus patients. These results might explain, at least in part, the higher risk of bone fracture observed in type 2 diabetes mellitus patients, resulting in cortical porosis.

The association of circulating leptin level with BMD determined based on dual-energy X-ray absorptiometry is controversial. While some studies have reported that the level of leptin in circulation is inversely associated with dual-energy X-ray absorptiometry-determined BMD[48,49], it has not been unanimously supported[50,51]. In the present study, the leptin level in plasma was not independently associated with TrBMD after adjustment for BMI, as well as for BMI and waist-to-hip ratio. Furthermore, our previous results determined with an LD-100 showed a close positive association of handgrip strength with CoTh, but not trabecular BMD, at the 5.5% distal radius in both female type 2 diabetes mellitus patients and non-diabetes healthy female participants[4], leading us to conclude that mechanical stress decreased by unloading suppresses bone formation and stimulates bone resorption preferentially in cortical bone components. These observations might explain the inverse

Table 6 | Non-significant association of plasma leptin with trabecular bone mineral density determined by LD-100

Variables	β	*P*
Model 1		
Log leptin	0.058	0.487
Diabetic neuropathy (yes = 1, no = 0)	–0.024	0.735
Model 2		
Log leptin	0.052	0.529
Past cerebrovascular disease (yes = 1, no = 0)	0.098	0.125
Model 3		
Log leptin	0.061	0.463
Smoking habit (yes = 1, no = 0)	0.117	0.098
Model 4		
Log leptin	0.064	0.447
$1,25(OH)_2D$	0.045	0.553
Model 5		
Log leptin	0.061	0.469
Use of pioglitazone (yes = 1, no = 0)	0.037	0.558
Model 6		
Log leptin	0.061	0.471
Use of insulin (yes = 1, no = 0)	–0.012	0.866
Model 7†		
Log leptin	0.142	0.271
Log HOMA-IR	0.090	0.344
Model 8		
Log leptin	0.061	0.475
Use of α- or β-blocker (yes = 1, no = 0)	–0.007	0.912

Values shown represent standardized partial regression coefficient (β values) and level of significance. Model 1 included age, sex, log (duration of type 2 diabetes mellitus + 1), glycated hemoglobin A1c, albumin, estimated glomerular filtration rate, handgrip strength, whole parathyroid hormone level, body mass index, waist-to-hip ratio, log (plasma leptin level) and diabetic neuropathy as covariates. Diabetic neuropathy was replaced with past cerebrovascular disease (model 2), smoking (model 3), $1,25(OH)_2D$ level (model 4), use of pioglitazone (model 5), use of insulin (model 6), log (homeostatic model assessment of insulin resistance [HOMA-IR]) (model 7) and use of α- or β-blocker (model 8). †A total of 111 patients who did not receive insulin therapy.

association noted between plasma leptin level and CoTh, but not TrBMD in the present cohort.

The present study had some important limitations. First, the design was cross-sectional, thus, even though relationships were explored in predictive terms, the results cannot be interpreted in regard to causal relationships. Second, the LD-100 is a newly-developed device used for measurement of bone parameters related to cortical bone separately from trabecular bone, and has yet to be well established. In contrast, a series of recent studies, including ours, have shown that LD-100 findings are clinically relevant for estimation of cortical bone components[26,27]. Furthermore, the effect of fat mass volume at the measured site or obesity on values obtained with the LD-100 were not fully examined, and we had no access to data from a control group, such as individuals without type 2 diabetes mellitus with a similar level of obesity. Additional studies that include non-diabetic obese participants are required to clarify the role of leptin in regulation of CoTh and TrBMD. Third, although BMI and waist-to-hip ratio were measured, visceral adiposity was not quantitatively determined, thus we could not analyze the precise effects of visceral fat accumulation together with plasma leptin level on bone densitometry findings. Fourth, the type 2 diabetes mellitus patients in the present study had received various drugs for diabetes, hypertension or dyslipidemia, some of which might have had effects on bone metabolism, thus potentially affecting the results. Furthermore, drugs that have effects on sympathetic nerve activity, such as serotonin noradrenalin reuptake inhibitors, were not fully investigated. Additional investigations are required to clarify the role of leptin in the development of cortical porosis. Finally, the present cohort consisted of nearly exclusively Japanese patients with type 2 diabetes mellitus, thus it is unclear whether the findings can be generalized to other ethnic groups.

In conclusion, the present results showed that plasma leptin level is inversely associated with CoTh, but not TrBMD, in patients with type 2 diabetes mellitus, independent of obesity. Furthermore, they suggest that hyperleptinemia resulting from obesity might contribute to cortical porosis in individuals affected by type 2 diabetes mellitus.

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DISCLOSURE

The authors declare no conflict of interest.

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