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

Masafumi Kurajoh1*, Masaaki Inaba1, Koka Motoyama2, Nagato Kuriyama3, Etsuko Ozaki3, Teruhide Koyama3, Shinsuke Yamada1, Tomoaki Morioka1, Yasuo Imanishi1, Masanori Emoto1

1Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, 2Department of Diabetes, Osaka City General Hospital, Osaka, and 3Department of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan

Keywords
Cortical thickness, Leptin, Obesity

*Correspondence
Masafumi Kurajoh
Tel: +81-6-6645-3806
Fax: +81-6-6645-3808
E-mail address: m1155129@med.osaka-cu.ac.jp

J Diabetes Investig 2020; 11: 174–183
doi: 10.1111/jdi.13071

INTRODUCTION
Patients with type 2 diabetes mellitus have an elevated risk of bone fracture, even when adequate bone mineral density (BMD) is present4–3, 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 diabetes4, whereas reduced CoTh was found to be significantly associated with vertebral fracture in patients with type 2 diabetes mellitus5. In other similar studies6,7, 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 patients4, as well as the general population9, although the underlying

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 ($r = -0.487$, $P < 0.001$), while BMI, but not plasma leptin, was positively correlated with trabecular BMD ($r = 0.369$, $P < 0.001$). 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.
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\(^1^,\)\(^2^,\)\(^3^,\)\(^4^\), 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\(^5^,\)\(^6^,\)\(^7^,\)\(^8^\), although the level of leptin in plasma in those patients is a controversial issue\(^9^,\)\(^10^,\)\(^11^\). In addition to its appetite-suppressing effect, leptin is known to activate the sympathetic nervous system through hypothalamic neurons expressing the leptin receptor\(^12^,\)\(^13^,\)\(^14^\). Obesity is known to be closely associated with autonomic dysfunction\(^15^,\)\(^16^,\) 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\(^18^,\)\(^19^,\)\(^20^,\)\(^21^\) and stimulates bone resorption\(^22^,\)\(^23^\), 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\(^24^\). For glycemic control, the patients were being treated with dietary therapy alone (\(n = 20\)), metformin (\(n = 51\)), sulfonylurea (\(n = 54\)), glinides (\(n = 4\)), dipeptidyl peptidase-4 inhibitors (\(n = 68\)), \(\alpha\)-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–Barre 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 (\(\mu\)U/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%) |
| \(\alpha\)-Glucosidase inhibitor | 24 (13.2%) |
| Pioglitazone | 10 (5.5%) |
| GLP-1 receptor analog | 9 (4.9%) |
| Insulin | 71 (39.0%) |
| \(\alpha\)/\(\beta\)-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) | 127 (102–170) |
| TRACP-5b (U/L) | 341.0 (245.3–470.3) |
| 1,25(OH)\(_2\)D (pg/mL) | 11.1 (9.8–17.0) |
| e-ALP (U/L) | 440 (280–593) |
| Leptin (ng/mL) | 4.2 (1.8–9.3) |
| LD-100 (5.5% distal radius) | 368.0 (267.4–444.7) |
| CoTh (mm) | 167.6 (132.9–209.6) |

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\(^2\). 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

| Variables                                      | CoTh  | TrBMD |
|------------------------------------------------|-------|-------|
| 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\(^f\)                                      | -0.040 | 0.677  | 0.237  | 0.013  |
| HOMA-IR\(^f\)                                  | -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  |
| Sulfonlurea (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-Sb                                       | -0.182 | 0.014  | -0.331 | <0.001 |
| 1,25(OH)\(_2\)D                                | -0.040 | 0.596  | 0.063  | 0.401  |
| Leptin                                         | -0.487 | <0.001 | 0.038  | 0.612  |

\(^{1}\)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-Sb, tartrate-resistant acid phosphatase-Sb; TrBMD, trabecular bone mineral density.
TrBMD, expressed in mg/cm³, was estimated by quantifying the attenuation of ultrasound waves transmitted through bone and transmission velocity of fast waves propagated through trabecular bone structures. 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/m², and in another study with participants aged 50–86 years.

**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. Estimated glomerular filtration rate was calculated using an equation for Japanese individuals, as previously described. 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. 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: (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). Measurement of serum osteocalcin was carried out using a two-site immunoradiometric assay kit (Mitsubishi Kagaku Bioclinical Laboratories, Tokyo, Japan), 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. The serum level of 1,25(OH)₂D was measured using radioimmunoassay findings. Plasma leptin levels were determined using an enzyme-linked immunosorbent assay kit (R&D Systems Inc., Minneapolis, MN, USA), as previously reported.

**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...
for fasting plasma glucose, glycated hemoglobin A1c, BMI and waist-to-hip ratio were 113.5 mg/dL, 8.3%, 25.4 kg/m² and 0.97, respectively, whereas those for plasma leptin, CoTh and TrBMD were 4.2 ng/mL, 3.68 mm and 167.6 mg/cm³, 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

| Variables                      | Model 1     | Model 2     | Model 3     | Model 4     |
|-------------------------------|-------------|-------------|-------------|-------------|
| Age                           | $-0.321$    | $-0.370$    | $-0.336$    | $-0.340$    |
| Sex (male/female, 1/0)        | $0.314$     | $0.412$     | $0.319$     | 0.296       |
| Log (duration of type 2 diabetes mellitus + 1) | $-0.009$    | $-0.041$    | $-0.011$    | $-0.010$    |
| HbA1c                         | $0.069$     | $0.061$     | $0.066$     | $0.061$     |
| Albumin                       | $0.090$     | $0.059$     | $0.088$     | $0.091$     |
| eGFR                          | $-0.260$    | $-0.275$    | $-0.264$    | $-0.260$    |
| Handgrip strength             | $0.236$     | $0.257$     | $0.241$     | $0.251$     |
| Whole PTH                     | $-0.074$    | $-0.050$    | $-0.072$    | $-0.066$    |
| Log leptin                    | $-0.258$    | $-0.039$    | $-0.237$    | $-0.069$    |
| BMI                           | $-0.158$    | $0.054$     | $0.158$     | $0.587$     |
| Waist-to-hip ratio            | $0.587$     | $0.557$     | $0.586$     | $0.558$     |

Values shown represent standardized partial regression coefficient ($\beta$ 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)\textsubscript{2}D 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 (\( \beta = 0.198, \ P = 0.069 \) and \( \beta = -0.034, \ P = 0.750 \), 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)\textsubscript{2}D 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 (\( \rho = -0.220, \ P = 0.003 \)) and TRACP-5b (\( \rho = -0.219, \ P = 0.003 \)), but not with bone alkaline phosphatase level (\( \rho = 0.035, \ P = 0.636 \)).

**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\textsuperscript{10,40}. Consistent with those reports, plasma leptin level was positively correlated with BMI and waist-to-hip ratio (\( P = 0.146, \ P = 0.049 \)) in the present type 2 diabetes mellitus patients, as well as a previously reported general population\textsuperscript{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\textsuperscript{18–20}.

Other studies have provided evidence showing that an increase in sympathetic activity might be intimately involved in the suppression of bone formation\textsuperscript{42} simultaneously with stimulation of bone resorption\textsuperscript{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                        | \( \beta \) | \( P \)   |
|----------------------------------|-------------|----------|
| Model 1                          |             |          |
| Log leptin (yes = 1, no = 0)     | -0.243      | <0.001   |
| Diabetic neuropathy              | -0.006      | 0.916    |
| Model 2                          |             |          |
| Log leptin (yes = 1, no = 0)     | -0.244      | <0.001   |
| Past cerebrovascular disease     | 0.020       | 0.700    |
| Model 3                          |             |          |
| Log leptin (yes = 1, no = 0)     | -0.243      | <0.001   |
| Smoking habit (yes = 1, no = 0)  | -0.011      | 0.839    |
| Model 4                          |             |          |
| Log leptin (yes = 1, no = 0)     | -0.248      | <0.001   |
| 1,25(OH)\textsubscript{2}D       | -0.044      | 0.458    |
| Model 5                          |             |          |
| Log leptin (yes = 1, no = 0)     | -0.240      | <0.001   |
| Use of pioglitazone              | 0.060       | 0.227    |
| Model 6                          |             |          |
| Log leptin (yes = 1, no = 0)     | -0.328      | <0.001   |
| Use of insulin (yes = 1, no = 0) | 0.051       | 0.384    |
| Model 7\textsuperscript{†}       |             |          |
| Log leptin (yes = 1, no = 0)     | -0.228      | 0.018    |
| Log HOMA-IR                      | 0.018       | 0.831    |
| Model 8                          |             |          |
| Log leptin (yes = 1, no = 0)     | -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 (\( \beta \)) 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)\textsubscript{2}D 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). \textsuperscript{†}A total of 111 patients who did not receive insulin therapy.
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. In our previous study, we showed that the level of leptin in plasma was not significantly different between diabetes patients with and without neuropathy, while the present results showed that plasma leptin level was not significantly different between diabetes patients with and without neuropathy. Previous studies including ours have shown that obesity is inversely associated with CoTh at the distal radius in type 2 diabetes mellitus patients, as well as in women in the general population, although other studies have noted no relationship between obesity and CoTh in children, young adults, or the general population. 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 porosity.

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, it has not been unanimously supported. 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, 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 association of plasma leptin level with BMD after adjustment for BMI, as well as for BMI and waist-to-hip ratio.

| Variables                                      | Model 1      | Model 2      | Model 3      | Model 4      |
|------------------------------------------------|--------------|--------------|--------------|--------------|
| Age (male/female, 1/0)                         | β 0.480      | β 0.427      | β 0.436      | β 0.437      |
| Sex                                             | <0.001       | <0.001       | <0.001       | <0.001       |
| Location                                       | 1.604        | 1.384        | 1.729        | 2.085        |
| HbA1c                                          | 0.040        | 0.050        | 0.048        | 0.047        |
| Albumin                                        | 0.022        | 0.035        | 0.028        | 0.028        |
| eGFR                                           | 0.180        | 0.168        | 0.170        | 0.169        |
| Handgrip strength                              | 0.265        | 0.248        | 0.252        | 0.254        |
| Whole PTH                                      | 0.030        | 0.018        | 0.023        | 0.025        |
| Log leptin                                     | 0.120        | 0.140        | 0.061        | 0.102        |
| BMI                                             | 0.090        | 0.040        | 0.464        | 0.269        |
| Waist-to-hip ratio                             | 1.355        | 1.252        | 1.870        | 2.293        |
| Adjusted ICC/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), waist-to-hip ratio 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.
associations 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. 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.

ACKNOWLEDGMENTS
We thank the patients for their participation in this study. We are also grateful for the staff of Osaka City University Hospital for their help with patient recruitment, as well as collection and recording of clinical information. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 17K16169 to Masafumi Kurajoh; No. 23791041 to Koka Motoyama), and a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No. 15K08925 to Koka Motoyama).

DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. Inaba M, Okuno S, Kumedya Y, et al. Increased incidence of vertebral fracture in older female hemodialyzed patients with type 2 diabetes mellitus. *Calcif Tissue Int* 2005; 76: 256–260.
2. Yamamoto M, Yamaguchi T, Yamauchi M, et al. Diabetic patients have an increased risk of vertebral fractures independent of BMD or diabetic complications. *J Bone Miner Res* 2009; 24: 702–709.
3. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. *Osteoporos Int* 2007; 18: 427–444.
4. Nakamura M, Inaba M, Yamada S, et al. Association of decreased handgrip strength with reduced cortical thickness.
in Japanese female patients with type 2 diabetes mellitus. Sci Rep 2018; 8: 10767.

5. Mishima T, Motoyama K, Imajishi Y, et al. Decreased cortical thickness, as estimated by a newly developed ultrasound device, as a risk for vertebral fracture in type 2 diabetes mellitus patients with eGFR of less than 60 mL/min/1.73 m². Osteoporos Int 2015; 26: 229–236.

6. Liu CT, Broe KE, Zhou Y, et al. Visceral adipose tissue is associated with bone microarchitecture in the Framingham osteoporosis study. J Bone Miner Res 2017; 32: 143–150.

7. Pollock NK, Laing EM, Baile CA, et al. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. Am J Clin Nutr 2007; 86: 1530–1538.

8. Vandewalle S, Taes Y, Van Helvoirt M, et al. Bone size and bone strength are increased in obese male adolescents. J Clin Endocrinol Metab 2013; 98: 3019–3028.

9. Ng AC, Melton LJ 3rd, Atkinson EJ, et al. Relationship of adiposity to bone volumetric density and microstructure in men and women across the adult lifespan. Bone 2013; 55: 119–125.

10. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998; 395: 763–770.

11. Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425–432.

12. Bandaru P, Shankar A. Association between plasma leptin levels and diabetes mellitus. Metab Syndr Relat Disord. 2011; 9: 19–23.

13. Vinagre I, Sanchez-Quesada JL, Sanchez-Hernandez J, et al. Inflammatory biomarkers in type 2 diabetic patients: effect of glycemic control and impact of LDL subfraction phenotype. Cardiovasc Diabetol 2014; 13: 34.

14. Tatti P, Masselli L, Buonanno A, et al. Leptin levels in diabetic and nondiabetic subjects. Endocrine 2001; 15: 305–308.

15. Simonds SE, Pyor JT, Ravussin E, et al. Leptin mediates the increase in blood pressure associated with obesity. Cell 2014; 159: 1404–1416.

16. Peterson HR, Rothschild M, Weinberg CR, et al. Body fat and the activity of the autonomic nervous system. N Engl J Med 1998; 318: 1077–1083.

17. Kurajoh M, Koyama H, Kadoya M, et al. Plasma leptin level is associated with cardiac autonomic dysfunction in patients with type 2 diabetes: HSCAA study. Cardiovasc Diabetol 2015; 14: 117.

18. Elefteriou F, Takeda S, Ebihara K, et al. Serum leptin level is a regulator of bone mass. Proc Natl Acad Sci USA 2004; 101: 3258–3263.

19. Takeda S, Elefteriou F, Levasseur R, et al. Leptin regulates bone formation via the sympathetic nervous system. Cell 2002; 111: 305–317.

20. Ducy P, Amling M, Takeda S, et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell 2000; 100: 197–207.

21. Kondo H, Nifuji A, Takeda S, et al. Unloading induces osteoblastic cell suppression and osteoclastic cell activation to lead to bone loss via sympathetic nervous system. J Biol Chem 2005; 280: 30192–30200.

22. Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus, Seino Y, Nanjo K, et al. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. J Diabetes Investig 2010; 1: 212–228.

23. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010; 33: 2285–2293.

24. Nakagawa C, Inaba M, Ishimura E, et al. Association of increased serum ferritin with impaired muscle strength/quality in hemodialysis patients. J Ren Nutr 2016; 26: 253–257.

25. Inaba M, Kurajoh M, Okuno S, et al. Poor muscle quality rather than reduced lean body mass is responsible for the lower serum creatinine level in hemodialysis patients with diabetes mellitus. Clin Nephrol 2010; 74: 266–272.

26. Sai H, Iguchi G, Tobimatsu T, et al. Novel ultrasonic bone densitometry based on two longitudinal waves: significant correlation with pQCT measurement values and age-related changes in trabecular bone density, cortical thickness, and elastic modulus of trabecular bone in a normal Japanese population. Osteoporos Int 2010; 21: 1781–1790.

27. Mano I, Horii K, Hagino H, et al. Estimation of in vivo cortical bone thickness using ultrasonic waves. J Med Ultrason 2015; 42: 315–322.

28. Kuriyama N, Inaba M, Ozaki E, et al. Association between loss of bone mass due to short sleep and leptin-sympathetic nervous system activity. Arch Gerontol Geriatr 2017; 70: 201–208.

29. Yamamoto T, Otani T, Hagino H, et al. Measurement of human trabecular bone by novel ultrasonic bone densitometry based on fast and slow waves. Osteoporos Int 2009; 20: 1215–1224.

30. Otani T. Quantitative estimation of bone density and bone quality using acoustic parameters of cancellous bone for fast and slow waves. Jpn J Appl Phys 2005; 44: 4578–4582.

31. Morioka T, Emoto M, Yarnazaki Y, et al. Plasma soluble leptin receptor levels are associated with pancreatic beta-cell dysfunction in patients with type 2 diabetes. J Diabetes Investig 2018; 9: 55–62.

32. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009; 53: 982–992.

33. Kurajoh M, Inaba M, Yamada S, et al. Association of increased active PTH(1-84) fraction with decreased GFR and serum Ca in predialysis CRF patients: modulation by serum 25-OH-D. Osteoporos Int 2008; 19: 709–716.

34. Emoto M, Nishizawa Y, Maekawa K, et al. Homeostasis model assessment as a clinical index of insulin resistance in
type 2 diabetic patients treated with sulfonylureas. *Diabetes Care* 1999; 22: 818–822.

35. Yokoyama H, Emoto M, Fujiwara S, et al. Quantitative insulin sensitivity check index and the reciprocal index of homeostasis model assessment in normal range weight and moderately obese type 2 diabetic patients. *Diabetes Care* 2003; 26: 2426–2432.

36. Gomez B Jr, Ardakan I, Ju J, et al. Monoclonal antibody assay for measuring bone-specific alkaline phosphatase activity in serum. *Clin Chem* 1995; 41: 1560–1566.

37. Yamada S, Inaba M, Kurajoh M, et al. Utility of serum tartrate-resistant acid phosphatase (TRACP5b) as a bone resorption marker in patients with chronic kidney disease: independence from renal dysfunction. *Clin Endocrinol* 2008; 69: 189–196.

38. Ohashi T, Igarashi Y, Mochizuki Y, et al. Development of a novel fragments absorbed immunocapture enzyme assay system for tartrate-resistant acid phosphatase 5b. *Clin Chim Acta* 2007; 376: 205–212.

39. Morioka T, Emoto M, Yamazaki Y, et al. Leptin is associated with vascular endothelial function in overweight patients with type 2 diabetes. *Cardiovasc Diabetol* 2014; 13: 10.

40. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996; 334: 292–295.

41. Gomez JM, Maravall FJ, Gomez N, et al. Interactions between serum leptin, the insulin-like growth factor-I system, and sex, age, anthropometric and body composition variables in a healthy population randomly selected. *Clin Endocrinol* 2003; 58: 213–219.

42. Elefteriou F. Neuronal signaling and the regulation of bone remodeling. *Cell Mol Life Sci* 2005; 62: 2339–2349.

43. Thomas T, Burguera B, Melton LJ 3rd, et al. Role of serum leptin, insulin, and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women. *Bone* 2001; 29: 114–120.

44. Kovacs CP, Molnar MZ, Czira ME, et al. Associations between serum leptin level and bone turnover in kidney transplant recipients. *Clin J Am Soc Nephrol* 2010; 5: 2297–2304.

45. Lorentzon M, Landin K, Mellstrom D, et al. Leptin is a negative independent predictor of areal BMD and cortical bone size in young adult Swedish men. *J Bone Miner Res* 2006; 21: 1871–1878.

46. Burghardt AJ, Issever AS, Schwartz AV, et al. High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2010; 95: 5045–5055.

47. Dimitri P, Jacques RM, Paggiosi M, et al. Leptin may play a role in bone microstructural alterations in obese children. *J Clin Endocrinol Metab* 2015; 100: 594–602.

48. Blum M, Harris SS, Must A, et al. Leptin, body composition and bone mineral density in premenopausal women. *Calcif Tissue Int* 2003; 73: 27–32.

49. Di Monaco M, Vallero F, Di Monaco R, et al. Fat body mass, leptin and femur bone mineral density in hip-fractured women. *J Endocrinol Invest* 2003; 26: 1180–1185.

50. Zoico E, Zamboni M, Adami S, et al. Relationship between leptin levels and bone mineral density in the elderly. *Clin Endocrinol* 2003; 59: 97–103.

51. Dennison EM, Syddall HE, Fall CH, et al. Plasma leptin concentration and change in bone density among elderly men and women: the Hertfordshire Cohort Study. *Calcif Tissue Int* 2004; 74: 401–406.