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Bone mass and bone resorption in postmenopausal women with type 2 diabetes mellitus

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Abstract

The aim of the present study was to examine the relationships between bone mass or bone resorption evaluated by urinary cross-linked N-telopeptides of type I collagen (NTx) concentration and known and potential contributors to bone mass or bone resorption such as sex hormones, age, duration of diabetes, glycemic control (hemoglobin A_{1c} [HbA_{1c}]), body mass index (BMI), severity of diabetic complications, smoking status, and current treatment of diabetes in postmenopausal women with type 2 diabetes mellitus (n = 196). In addition, the relationship of bone mass to pulse wave velocity, which is an earlier indicator of cardiovascular disease, was investigated in a subgroup of patients (n = 120). Bone mass was evaluated by the quantitative ultrasound method. A higher stiffness index indicates higher bone mass. Inverse correlations were found between the stiffness index and age ($r = -0.374$, $P < .0001$) and between the stiffness index and log (urinary albumin excretion) ($r = -0.170$, $P = .0398$), and a positive correlation was found between the stiffness index and serum dehydroepiandrosterone sulfate (DHEA-S) concentration ($r = 0.201$, $P = .0136$). No significant correlations were found between the stiffness index and duration of diabetes, HbA_{1c}, BMI, or serum estradiol concentration. No significant correlations were found between urinary NTx concentration and age, duration of diabetes, HbA_{1c}, BMI, serum estradiol concentration, or serum DHEA-S concentration. The stiffness index correlated inversely with urinary NTx concentration ($r = -0.262$, $P = .0002$). No significant correlation was found between the stiffness index and pulse wave velocity ($r = -0.165$, $P = .0714$). Multiple regression analysis demonstrated that serum DHEA-S concentration was an independent determinant of the stiffness index ($\beta = .207$, $P = .0428$). In conclusion, serum DHEA-S concentration correlated positively with bone mass, whereas glycemic control, BMI, or duration of diabetes did not correlate with bone mass or urinary NTx concentration in postmenopausal women with type 2 diabetes mellitus.

1. Introduction

Osteoporosis is an important health problem in postmenopausal women with a resulting increase in bone fragility and fractures. Bone loss is a chronic complication of diabetes-associated alterations in mineral and bone metabolism [1-4]. Available data concerning the association of bone mineral density (BMD) with type 2 diabetes mellitus are conflicting; type 2 diabetes mellitus has been reported to be associated with increased [5], unchanged [6], or decreased [1,7] BMD. Few studies have evaluated bone mass and bone resorption in postmenopausal women with type 2 diabetes mellitus [8]. Moreover, to our knowledge, no previous studies have investigated the relationships between factors, including age, duration of diabetes, hemoglobin A_{1c} (HbA_{1c}), body mass index (BMI), or severity of diabetic microangiopathy, and bone mass or urinary cross-linked N-telopeptides of type I collagen (NTx) concentration, a bone resorption marker, in postmenopausal women with type 2 diabetes mellitus.

Menopause results in a reduction in BMD, mainly because of increased bone resorption by estrogen deficiency [9]. To our knowledge, the relationships between serum sex hormones and bone mass or bone resorption have not been examined in postmenopausal women with type 2 diabetes mellitus. We therefore investigated the relationships between serum estradiol or dehydroepiandrosterone sulfate

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(DHEA-S) concentrations, which is a major source of estrogen after menopause, and bone mass or urinary NTx concentrations in postmenopausal women with type 2 diabetes mellitus.

Recently, the quantitative ultrasound (QUS) method, based on measurements of the ultrasound velocity in bone, has been proposed as a new screening tool for the evaluation of bone mass [10]. Moris et al [11] reported significant correlations between QUS of the calcaneus and dual-energy x-ray absorptiometry (DXA) of the lumbar spine. Hans et al [12] reported that low calcaneal ultrasonographic variables were able to predict an increased risk of hip fracture, with similar accuracy to low femoral BMD obtained by dual-photon x-ray absorptiometry. Moreover, QUS has substantial advantages compared with DXA in terms of safety, cost, and potential portability.

The aim of the present study was to examine the relationships between bone mass or bone resorption evaluated by urinary NTx concentration and known and potential contributors to bone mass or bone resorption such as sex hormones, age, duration of diabetes, glycemic control (HbA_{1c}), BMI, severity of diabetic complications, smoking status, and current treatment of diabetes in postmenopausal women with type 2 diabetes mellitus.

2. Subjects and methods

2.1. Subjects

This study consisted of 196 consecutive postmenopausal women with type 2 diabetes mellitus recruited from the outpatient clinic of Kyoto Prefectural University of Medicine. Type 2 diabetes mellitus was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [13]. Retinopathy was graded as follows: no diabetic retinopathy, NDR; simple diabetic retinopathy, SDR; and proliferative diabetic retinopathy, PDR. Nephropathy was graded as follows: normoalbuminuria, urinary albumin excretion less than 30 mg/g Cr; microalbuminuria, 30 to 300 mg/g Cr; or macroalbuminuria, more than 300 mg/g Cr. Smoking status was recorded as nonsmoker, past smoker, or current smoker according to a self-administered questionnaire. Cardiovascular disease (CVD) was defined as the presence of previous myocardial infarction or cerebral infarction based on the clinical history or physical examination. Patients with secondary causes of osteoporosis and those with disorders known to affect mineral metabolism (thyroid dysfunction, liver or kidney disease) were excluded. We also excluded patients medicated with drugs known to interfere with calcium metabolism, such as corticosteroids, thyroid hormone, or vitamin D. No patients took hormone replacement therapy.

2.2. Experimental design

The relationships between bone mass or urinary NTx concentration and age, duration of diabetes, glycemic control (HbA_{1c}), BMI, severity of diabetic retinopathy, severity of diabetic nephropathy defined by urinary albumin excretion, smoking status, or current treatment of diabetes were evaluated. In addition, the relationships between bone mass or urinary NTx concentration and serum estradiol or DHEA-S concentration were investigated. Moreover, the relationship of bone mass to pulse wave velocity (PWV), which is an earlier indicator of CVD, was investigated in a subgroup of patients (n = 120). Approval for the study was obtained from the local research ethics committee, and informed consent was obtained from all participants.

2.3. Biochemical analyses

Serum DHEA-S concentrations (reference range, 50-1160 ng/mL) were measured by Coat-A-Count DHEA-S kits (Diagnostic Products, Los Angeles, CA). Intra- and interassay coefficients of variance (CV) for serum DHEA-S concentrations were as stated previously [14]. Serum estradiol concentrations (reference range, <21 pg/mL) were measured by an electrochemiluminescence immunoassay using a Modular Analytics <EE> system (Roche Diagnostics, Tokyo, Japan). Intra-assay CVs were 3.7% and 2.7%, and the interassay CVs were 3.2% and 2.8% for estradiol concentrations of 32.5 and 474 pg/mL, respectively. Urinary NTx concentrations were measured using an enzyme-linked immunosorbent assay (Osteomark; Ostex International, Seattle, WA) and expressed as nanomoles of bone collagen equivalent per millimole of creatinine (nmol BCE/mmol Cr) (reference range, 14.3-89.0 nmol BCE/mmol Cr). The detection limit for NTx was 7.5 nmol BCE/L. Intra- and interassay CV for NTx concentrations of 320 nmol BCE/L and 1680 nmol BCE/L were 6.2% and 7.2% for the low concentration, and 4.5% and 2.5% for the high concentration, respectively. Blood samples were obtained in the morning. Urinary albumin and creatinine concentration were determined in an early morning spot urine test. Urinary albumin excretion was measured with an immunoturbidimetric assay. A mean value for urinary albumin excretion was determined from 3 urine collections. Serum total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods [15-17]. Hemoglobin A_{1c} was assayed using high-performance liquid chromatography [18]. Mean values for serum lipid concentrations, blood pressure, or HbA_{1c} obtained during the previous year were used for statistical analysis.

2.4. Quantitative ultrasound evaluation

The QUS measurements were carried out with the Achilles EXPRESS ultrasonometer (GE Healthcare Lunar, Madison, WI). The Achilles measures SOS (speed of sound in meters per second) and BUA (broadband ultrasound attenuation in decibels per megahertz), a measure of frequency-dependent attenuation of the ultrasound wave passing through the heel. The stiffness index (reference range, >60 in postmenopausal women), a variable derived

Table 1
Clinical characteristics of postmenopausal women with type 2 diabetes mellitus

	Mean ± SD
n	196
Age (y)	69.7 ± 7.1
Age at onset (y)	55.0 ± 11.8
Duration of diabetes (y)	14.6 ± 10.7
BMI (kg/m^2)	22.4 ± 3.4
HbA_{1c} (%)	7.4 ± 1.1
Systolic blood pressure (mm Hg)	133 ± 14
Diastolic blood pressure (mm Hg)	72 ± 9
Total cholesterol (mg/dL)	207 ± 31
Triglyceride (mg/dL)	118 ± 68
HDL cholesterol (mg/dL)	60 ± 9
Smoking (none/past/current)	169/11/16
Nephropathy (normo-/micro-/macroalbuminuria)	107/64/25
Retinopathy (NDR/SDR/PDR)	123/36/37
CVD (−/+)	167/29
Current treatment (diet/OHA/insulin)	16/115/65
DHEA-S (ng/mL)	623 ± 360
Estradiol (pg/mL)	13.1 ± 5.5

Data are expressed as mean ± SD or as number of patients. HDL indicates high-density lipoprotein; OHA, oral hypoglycemic agent.

from a combination of SOS and BUA, was calculated by the analysis software according to the equation: 0.67 BUA + 0.28 SOS − 420 [19]. Higher stiffness index indicates higher bone mass. We evaluated the stiffness index of the right heel using the scanning protocol provided by the manufacturer.

2.5. Measurement of pulse wave velocity

Brachial-ankle (ba)PWV was measured using a Colin Waveform analyzer (form PWV/ABI; Colin Medical Technology, Komaki, Japan). Subjects were examined in the supine position after 5 minutes of bed rest. The cuffs were adapted to both of the brachium and ankle, and pressure waveforms of both the brachial and ankle arteries were recorded by an oscillometric method, after placing a plethysmographic sensor at both of the brachium and ankle. The baPWV was calculated by time-phase as distance/time (cm/s). The time delay between the arrival of the pulse wave at the brachium and ankle at each side was measured automatically by gating the pulse wave to the peak of the R wave of the electrocardiogram. The distance between the brachium and ankle at each side was estimated based on body height and adjusted for average Japanese body composition. Details of the method have been described elsewhere [20]. After bilateral determination of baPWV, the higher value was taken as representative for each subject.

Table 2
Correlation between the stiffness index or urinary NTx concentration and other variables

	Stiffness		NTx	
	r	*P*	*r*	*P*
Age	−0.362	<.0001	0.061	.3787
Duration of diabetes	−0.124	.1081	−0.042	.5910
BMI	−0.098	.2258	−0.156	.0528
HbA_{1c}	0.012	.8755	−0.139	.0770
Log (urinary albumin excretion)	−0.170	.0398	0.095	.2547
Estradiol	−0.137	.1330	0.015	.8715
DHEA-S	0.201	.0136	−0.062	.4562

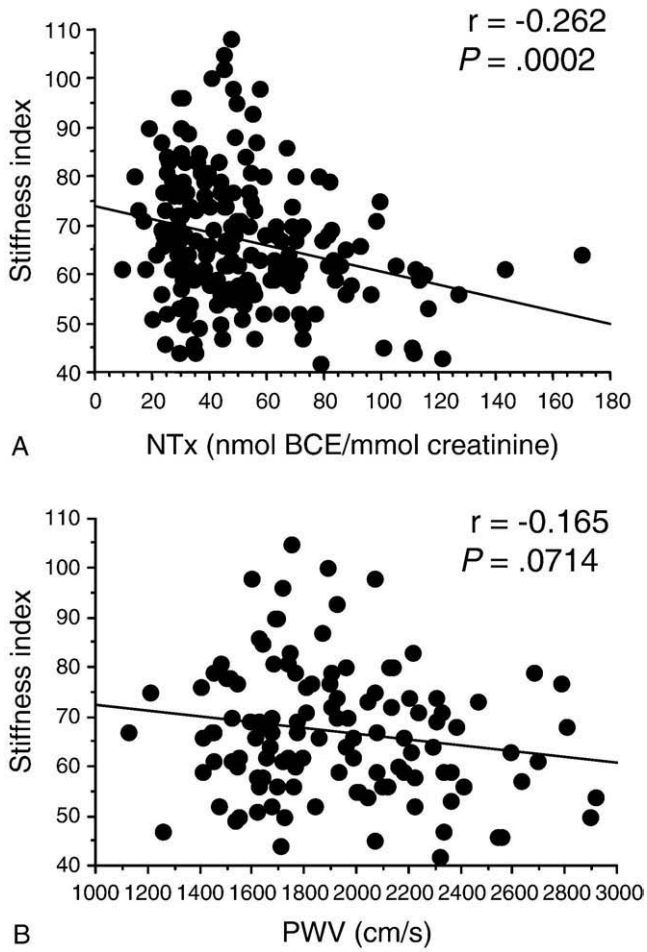

Fig. 1. Correlation between the stiffness index and urinary cross-linked NTx concentration (A) and between the stiffness index and PWV (B) in postmenopausal women with type 2 diabetes mellitus.

2.6. Statistical analysis

Means and frequencies of potential confounding variables were calculated, and unpaired Student *t* tests or analyses of variance were conducted, to assess statistical significance of differences between patients with and without CVD, between patients treated with and without insulin, according to diabetic microangiopathy, or according to smoking status, using Stat View software (version 5.0; SAS Institute, Cary, NC). Because urinary albumin excretion showed a skewed distribution, logarithmic (log) transformation was carried out before performing correlation analysis. The relationships between the stiffness index or urinary NTx concentration and age, duration of diabetes, glycemic control, serum DHEA-S concentration, and other variables were examined by

Pearson correlation analyses. All continuous variables are presented as the mean ± SD. To examine the effects of factors on the stiffness index, the following factors, which were known and potential contributors to bone mass, were considered as independent variables for multiple regression analysis: serum DHEA-S concentration, duration of diabetes, BMI, HbA_{1c}, and smoking status. A P value of less than .05 was considered statistically significant.

3. Results

The clinical characteristics of the 196 postmenopausal women with type 2 diabetes enrolled in this study are shown in Table 1. Mean urinary NTx concentration (reference range, 14.3-89.0 nmol BCE/mmol Cr), stiffness index (reference range, >60), and PWV (reference range, <1400 cm/s) were 51.8 ± 26.1 nmol BCE/mmol Cr, 67.0 ± 13.3, and 1914 ± 376 cm/s, respectively. Correlation between the stiffness index or urinary NTx concentration and other variables are shown in Table 2.

The stiffness index correlated inversely with urinary NTx concentration ($r = -0.262$, $P = .0002$; Fig. 1A). No significant correlation was found between the stiffness index and PWV ($r = -0.165$, $P = .0714$; Fig. 1B). Multiple regression analysis, covariates of which were serum DHEA-S concentration, duration of diabetes, BMI, HbA_{1c}, and smoking status, demonstrated that serum DHEA-S concentration was an independent determinant of the stiffness index ($\beta = .207$, $P = .0428$).

The stiffness index did not differ between patients with and without CVD (64.4 ± 14.4 vs 67.5 ± 13.0, $P = .2496$), and between patients treated with insulin and patients treated without insulin (66.7 ± 15.1 vs 67.1 ± 12.6, $P = .8467$). In addition, the stiffness index did not differ according to severity of diabetic retinopathy (67.2 ± 12.8 vs 69.2 ± 10.5 vs 66.7 ± 14.1 for patients with NDR, SDR, and PDR, respectively) or according to the smoking status (66.8 ± 12.3 vs 65.1 ± 12.1 vs 64.4 ± 13.0 for patients with nonsmoker, past smoker, and current smoker, respectively). Stiffness indexes were 68.6 ± 12.6, 63.6 ± 12.8, and 64.6 ± 13.5 for patients with normoalbuminuria, microalbuminuria, and macroalbuminuria, respectively. The stiffness index was higher in patients with normoalbuminuria than that in patients with microalbuminuria ($P = .0305$). Urinary NTx concentration did not differ between patients with and without CVD (52.2 ± 28.6 vs 51.7 ± 25.8 nmol BCE/mmol Cr, $P = .9287$) and between patients treated with insulin and patients treated without insulin (52.3 ± 30.3 vs 51.6 ± 24.5 nmol BCE/mmol Cr, $P = .8621$). In addition, urinary NTx concentration did not differ according to the severity of diabetic nephropathy (53.3 ± 24.2 vs 47.8 ± 28.5 vs 62.2 ± 36.0 nmol BCE/mmol Cr for patients with normoalbuminuria, microalbuminuria, and macroalbuminuria, respectively), according to the severity of diabetic retinopathy (53.1 ± 25.5 vs 46.5 ± 33.6 vs 51.6 ± 27.0 nmol BCE/mmol Cr for patients with NDR, SDR, and PDR, respectively), or according to smoking status (52.1 ± 25.9 vs 50.2 ± 22.8 vs 48.3 ± 18.0 nmol BCE/mmol Cr for patients with nonsmoker, past smoker, and current smoker, respectively).

4. Discussion

Available data concerning the association of BMD with type 2 diabetes mellitus are equivocal; in various studies, type 2 diabetes mellitus has been reported to be associated with increased [5], unchanged [6], or decreased [1,7] BMD. Such varying results may be related to differences in the population of patients with type 2 diabetes mellitus, including sex, the proportion of postmenopausal women, age, degree of insulin resistance, or ethnic group. We evaluated the relationships between bone mass or bone resorption and serum sex hormones or PWV as well as between bone mass or bone resorption and other variables including age, duration of diabetes, glycemic control (HbA_{1c}), or BMI in postmenopausal women with type 2 diabetes mellitus in the present study.

Both osteoporosis and CVD are major public health problems that adversely impact morbidity and mortality. Accumulating evidence indicates a link between osteoporosis and CVD [21-23], suggesting some shared pathophysiologic mechanisms underlying the 2 diseases. In addition to cardiovascular risk factors such as age, dyslipidemia, oxidative stress, hypertension, and diabetes, sex hormone deficiency also regulate bone remodeling [24]. Menopause is one of the most important risk factors for osteoporosis in women. Although many reports have elucidated the pathophysiologic characteristics of abnormal bone mass or bone resorption in patients with type 2 diabetes mellitus, the relationships between serum sex hormones and bone mass or bone resorption have not been examined in postmenopausal women with type 2 diabetes mellitus. We therefore evaluated the relationships between serum estradiol or DHEA-S concentrations and bone mass or urinary NTx concentration in postmenopausal women with type 2 diabetes mellitus. We found a weak but significant positive correlation between serum DHEA-S concentration and the stiffness index. Multiple regression analysis also identified serum DHEA-S concentration as an independent determinant of the stiffness index. Because decreased concentrations of DHEA-S are responsible for aging, adjusting for age to assess the relationship between serum DHEA-S concentration and the stiffness index can be considered an overadjustment. In fact, if we included age as a covariate in the multiple regression model, age was an independent determinant of stiffness index ($\beta = -.463$, $P < .0001$), whereas serum DHEA-S concentration was not ($\beta = 0.066$, $P = .4966$). We thus performed multiple regression analysis to assess the combined effect of variables on the stiffness index using the following factors: serum DHEA-S concentration, duration of diabetes, BMI, HbA_{1c}, and smoking status. Low

serum DHEA-S concentration also appears to be associated with increased risk of CVD in women [25]. The positive correlation between serum DHEA-S concentration and the stiffness index may partly account for the association between osteoporosis and CVD in women with type 2 diabetes mellitus. Estrogen deficiency is an important factor that causes osteoclast activation [26]. Estrogen inhibits cytokines that activate osteoclasts, such as interleukin 1, interleukin 6, and tumor necrosis factor α [27]. All these cytokines have also been implicated in atherogenesis [28]. No significant correlation was found between serum estradiol concentration and the stiffness index or urinary NTx concentration in the present study. Serum estradiol concentrations do not necessarily reflect tissue-level activity, as peripherally formed estradiol is partially metabolized in situ; thus, not all of the estradiol enters the general circulation. In postmenopausal women, almost all active sex steroids are made locally in peripheral tissues. Dehydroepiandrosterone sulfate is a major source of estrogen in postmenopausal women. Therefore, serum DHEA-S concentration is the parameter directly reflecting the level of sex steroid. A decline in serum DHEA-S concentration with age may partly explain the age-related increases in risk of osteoporosis as well as CVD. Dehydroepiandrosterone replacement therapy has been reported to improve BMD [29,30]. Women with low concentrations of serum DHEA-S may be candidates for DHEA replacement therapy to prevent osteoporosis as well as atherosclerosis.

Some studies have found an association between BMD decrease and the degree of glycemic control [31]. Metabolic effects of poor glycemic control in patients with type 2 diabetes mellitus, such as hypercalciuria, may lead to an increase in net bone resorption [2]. However, no correlation was found between glycemic control and the stiffness index or urinary NTx concentration in the present study. The stiffness index or urinary NTx concentrations did not differ between patients with CVD and those without, despite an apparent association between osteoporosis and CVD. Moreover, no significant correlation was found between the stiffness index and PWV, an earlier indicator of CVD. An inverse correlation was found between the stiffness index and log (urinary albumin excretion) in women with type 2 diabetes mellitus in the present study. Clausen et al [32] also reported that BMD was reduced in male patients with increased urinary albumin excretion, considering diabetic osteopenia to be a progressive disorder related to the development of diabetic nephropathy, associated with decreased creatinine clearance and consequently increased parathyroid hormone.

Clinical management of osteoporosis is important for preservation of the quality of life in elderly female patients with type 2 diabetes mellitus, considering that metabolic derangements and increased likelihood of falling resulting from diabetes are related to high risk of fracture [33].

Limitations of the present study include the cross-sectional design and a relatively small number of patients. We could not compare the stiffness index between postmenopausal diabetic women and age-matched nondiabetic women. In addition, we did not evaluate physical activity or alcohol consumption, both of which could affect bone mass and resorption. Furthermore, we evaluated bone mass by QUS rather than DXA. Bone mineral density assessed by DXA is an established marker for osteoporosis. Quantitative ultrasound measurements of bone have been recently proposed as a new, radiation-free, noninvasive technique to screen and identify patients at risk for osteoporosis [10]. Moris et al [11] demonstrated that significant correlations were found between QUS of the calcaneus and DXA of the lumbar spine. Hans et al [12] reported that low calcaneal ultrasonographic variables were able to predict an increased risk of hip fracture, with similar accuracy to low femoral BMD obtained by dual-photon x-ray absorptiometry. Quantitative ultrasound measures bone mass, which can be modified not only by bone density, but also by bone architecture and quality [34]. Furthermore, QUS of the calcaneus, which is an easily accessible site rich in trabecular bone, evolving with age in parallel to lumbar vertebra [35], is superior to DXA in patients with vertebral deformities or paravertebral calcifications in the elderly in whom DXA of the spine is less accurate [36]. Despite these limitations, this study, for the first time, investigated the factors that correlated with bone mass or bone resorption evaluated by urinary NTx concentration in postmenopausal women with type 2 diabetes mellitus. Urinary NTx concentration is a marker of ongoing bone resorption, whereas the stiffness index quantitates the present state of osteoporosis. Large prospective trials and intervention studies are needed to better assess the factors that influence bone mass and bone resorption in postmenopausal women with type 2 diabetes mellitus.

In conclusion, serum DHEA-S concentration correlated positively with bone mass, whereas glycemic control, BMI, or duration of diabetes did not correlate with bone mass or urinary NTx concentration in postmenopausal women with type 2 diabetes mellitus.

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