表3　2型糖尿病患者における椎体骨折有無での各因子の比較（つづき）

	男性				女性			
	椎体骨折				椎体骨折			
	なし	あり		p	なし	あり		p
インスリン分泌刺激薬使用者数	27 (32)	23 (44)		0.197	34 (34)	17 (41)		0.573
メトホルミン使用者数	17 (20)	11 (21)		0.999	28 (28)	10 (24)		0.766
ピオグリタゾン使用者数	12 (14)	6 (12)		0.842	8 (8)	6 (15)		0.423
インスリン使用者数	17 (20)	8 (15)		0.630	27 (27)	11 (27)		0.999
糖尿病網膜症あり	30 (35)	18 (35)		0.999	43 (43)	18 (44)		0.718
糖尿病神経障害あり	50 (59)	33 (63)		0.915	64 (65)	39 (95)		0.627
喫煙あり	62 (73)	33 (63)		0.412	4 (4)	2 (5)		0.999
飲酒あり	49 (58)	34 (65)		0.579	10 (10)	2 (5)		0.490

Unpaired t test : $^{*}p<0.05$, $^{**}p<0.01$

（ ）内は%を示す。カテゴリー変数はχ^2検定を用いた。

比（OR）0.53，[95%信頼区間（CI）0.31-0.88]，$p=0.02$）（表4，model 1）。F群ではesRAGEが負に（OR 0.53 [CI 0.33-0.85]，$p<0.01$），pentosidineが正に（OR 1.65 [CI 1.13-2.41]，$p=0.01$），esRAGE/pentosidine比が負に関係した（OR 0.34 [CI 0.19-0.62]，$p<0.01$）。一方，BMDはどの部位もVFと有意な関係を認めなかった。

続いて年齢，BMI，HbA_{1c}および血清Crで調整後において，M群ではesRAGEがVFと有意な負の関係を認めた（OR 0.61 [CI 0.38 - 0.96]，$p=0.03$）（表4，model 2）。さらに骨代謝マーカー，糖尿病罹病期間，糖尿病治療薬，糖尿病合併症の有無，喫煙や飲酒の有無および腰椎BMDで調整を行ったが，VFに対するM群のesRAGEおよびesRAGE/pentosidine比の関係，F群のesRAGE，pentosidineおよびesRAGE/ pentosidineとの有意な関係は保持された（表4，model 3）。

3　考　　察

今回，われわれは性別にかかわらずesRAGEおよびesRAGE/pentosidine比が2DMにおいてVFと関係することを，はじめて臨床的に明らかにした。esRAGEはBMD，骨代謝回転，糖尿病治療薬，糖尿病合併症とは独立してVFに影響する因子と考えられる。esRAGEは細胞間腔でAGEsを含むさまざまなリガンドと結合し，これらのリガンドが細胞表面のRAGEと結合することを防ぐ作用がある[7]。われわれの結果は，リガンドに対しesRAGE量が不足するとRAGEを介して骨に有害な影響を与えることを示唆している。p値を考慮すると，esRAGE単独よりはリガンドとデコイ受容体の比であるesRAGE/pentosidine比が，2DMにおけるVFの相対危険度の評価に際してより有用と考えられた。

おわりに

この研究結果により，2DMではAGE-RAGE系がBMDとは独立して骨強度に影響する因子であり，骨質と関係することが示唆された。2DM

表4　2型糖尿病患者におけるesRAGE値，pentosidine値，esRAGE/pentosidine比，およびBMDと椎体骨折の関係

独立変数	男性		女性	
	OR（95%信頼区間）	p	OR（95%信頼区間）	p
Model 1				
esRAGE	0.79（0.55-1.14）	0.206	0.53（0.33-0.85）	0.009**
Pentosidine	1.39（0.98-1.99）	0.067	1.65（1.13-2.41）	0.010*
esRAGE/pentosidine	0.53（0.31-0.88）	0.015*	0.34（0.19-0.62）	<0.001**
腰椎BMD	0.70（0.49-1.01）	0.059	0.81（0.55-1.21）	0.304
大腿骨頸部BMD	0.85（0.59-1.22）	0.381	0.72（0.48-1.07）	0.099
橈骨遠位端1/3BMD	0.79（0.55-1.12）	0.181	0.70（0.47-1.03）	0.069
Model 2				
esRAGE	0.61（0.38-0.96）	0.032*	0.47（0.27-0.80）	0.006**
Pentosidine	1.34（0.89-2.03）	0.164	1.80（1.08-2.98）	0.023*
esRGAE/pentosidine	0.47（0.25-0.85）	0.013*	0.28（0.13-0.60）	0.001**
Model 3				
esRAGE	0.46（0.25-0.84）	0.012*	0.32（0.16-0.67）	0.002**
Pentosidine	1.49（0.91-2.42）	0.111	1.82（1.05-3.15）	0.034*
esRAGE/pentosidine	0.34（0.15-0.76）	0.009**	0.14（0.04-0.43）	0.001**

Model 1：調整なし。
Model 2：年齢，BMI，HbA_{1c}，血清クレアチニンで調整。
Model 3：年齢，BMI，HbA_{1c}，血清クレアチニン，糖尿病罹病期間，腰椎BMD，糖尿病治療薬，糖尿病合併症，喫煙歴および飲酒歴で調整。
1標準偏差増加あたりのORで示す。
OR：odds ratio，BMI：body mass index，*：$p<0.05$，**：$p<0.01$

ではBMDによる椎体骨折リスク評価が困難であり[3]，esRAGEやesRAGE/pentosidine比が骨質を反映した骨強度のサロゲートマーカーとして利用できる可能性が示唆された。

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ORIGINAL ARTICLE

Baseline atherosclerosis parameter could assess the risk of bone loss during pioglitazone treatment in type 2 diabetes mellitus

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Abstract
Summary We found that serum osteocalcin, femoral bone mineral density (F-BMD), and 1/3R-BMD were decreased during pioglitazone treatment in patients with type 2 diabetes. Moreover, baseline atherosclerosis parameter, serum insulin-like growth factor-I (IGF-I), and urinary N-terminal cross-linked telopeptide of type I collagen (uNTX) values were associated with changes in bone mineral density (BMD). Therefore, these parameters could assess the risk of BMD reduction in patients treated with pioglitazone.
Introduction The aim of this study was to investigate the effects of pioglitazone or metformin on bone mass and atherosclerosis in patients with type 2 diabetes.
Methods A total of 55 Japanese patients were enrolled in this 1-year open-label study and randomized to either pioglitazone (n=22, 15–30 mg/day) or metformin (n=23, 500–750 mg/day) groups. BMD at the lumbar spine, femoral neck (F), and one third of the radius (1/3R), bone markers, and atherosclerosis parameters were measured.
Results In the pioglitazone group, serum osteocalcin significantly decreased at 6 months (p<0.05), although it almost recovered to baseline level at 12 months. F-BMD significantly decreased at 6 months (p<0.05), and 1/3R-BMD significantly decreased at 6 and 12 months (p<0.05), while bone markers or BMD at any site were not changed in the metformin group. Although atherosclerosis parameters were not changed in the pioglitazone group, intima-media thickness (IMT)-mean significantly increased at 6 months (p<0.05) and plaque score significantly increased at 6 and 12 months (p<0.01) in the metformin group. In the pioglitazone group, %changes in F-BMD were significantly and negatively correlated with baseline IMT-Max, IMT-mean, and plaque scores (r=−0.61, p<0.01; r=−0.71, p<0.01; and r=−0.68, p<0.01, respectively), and %changes in 1/3R-BMD were significantly and negatively correlated with baseline uNTX and IMT-Max (r=−0.57, p<0.01 and r=−0.48, p<0.05, respectively) and positively with IGF-I (r=0.45, p<0.05).
Conclusions Baseline IMT, uNTX, and IGF-I could assess the risk of BMD reduction in diabetic patients treated with pioglitazone.

Keywords Atherosclerosis · Bone mineral density · Metformin · Pioglitazone · Type 2 diabetes mellitus

Introduction

Thiazolidinediones (TZDs) such as pioglitazone and rosiglitazone are peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists and are widely used for the treatment of type 2 diabetes [1, 2]. PPAR-γ is known to be expressed in bone marrow cells. It acts as a molecular switch that regulates the fate of pluripotent mesenchymal stem cells, and PPAR-γ activation induces bone loss characterized by deficient osteoblast function [3, 4]. Clinically, a meta-analysis has shown that long-term use of TZDs causes bone

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mineral density (BMD) reduction and raises fracture risk in women with type 2 diabetes [5]. On the other hand, only a few prospective studies on the adverse effects of TZDs on bone were conducted [6, 7]. To our knowledge, there are no prospective randomized studies with longer duration than a few months in patients with type 2 diabetes. It is also unknown what parameters, if any, could predict the TZDs-induced BMD reduction in the population.

Although experiments with cultured cells indicated that metformin, another drug increasing insulin sensitivity, stimulated the differentiation and mineralization of osteoblasts [8], it is unclear whether or not metformin affects bone markers or BMD in humans.

In this study, we examined the long-term effects of pioglitazone or metformin on bone markers and BMD as well as atherosclerosis parameters up to 12 months in Japanese patients with type 2 diabetes and performed an exploratory analysis to determine if any of the measured parameters could assess the risk of the pioglitazone-induced bone loss in the population.

Research design and methods

Subjects

This study was approved by the ethical review board of our institution and complied with the Helsinki Declaration. From November 1, 2006 to January 1, 2008, patients with type 2 diabetes were enrolled if informed consent was obtained after a detailed explanation of the study purpose and methods. The participants in this study were 55 patients who visited Shimane University Hospital for treatment of type 2 diabetes. All women had been without spontaneous menstrual cycle for more than 1 year. We excluded patients from this study (1) with hepatic or renal dysfunction or nutritional derangements, (2) who had taken TZDs or metformin, and (3) who had taken drugs known to influence bone and calcium metabolism, such as vitamin D, bisphosphonate, or estrogen up until the time of the study. In this 1-year open-label study, participants were randomized to either pioglitazone or metformin groups. Pioglitazone (15–30 mg) was orally administered once daily, and metformin (250 mg) was two or three times after meal (500–750 mg/day). The numbers of patients who had been taking insulin, sulfonylurea, and alpha-glucosidase inhibitors, respectively, were 12, 6, and 3 in the pioglitazone group and 13, 5, and 2 in the metformin group. Chi-square tests showed that there were no significant differences in premedication between the two groups (data not shown). All prescription medications of each patient were not changed during the study. The Brinkmann index was calculated by daily cigarette numbers multiplied by smoking years. All patients suffered no new fractures during this study.

Radiography and biochemical measurements

BMD values of the lumbar spine (L), femoral neck (F), and one third of the radius (1/3R) were measured by dual-energy X-ray absorptiometry (QDR-4500; Hologic, Waltham, MA, USA) as previously described [9, 10].

After overnight fasting, serum and urine were collected. Biochemical markers were measured by standard biochemical methods as previously described [9–12]. Serum osteocalcin and insulin-like growth factor-I (IGF-I) were measured by radioimmunoassay, and urinary N-terminal cross-linked telopeptide of type-I collagen (uNTX) was measured by enzyme-linked immunosorbent assay.

Arterial stiffness measurement and ultrasonographic measurement of carotid intima-media thickness

Brachial ankle pulse wave velocity (baPWV) measurement and B-mode ultrasonographic imaging of the carotid artery were performed using the VaSera VS-1000 (Fukuda Denshi, Tokyo, Japan) and HDI 5000 (Philips, Tokyo, Japan), a high-resolution, real-time ultrasonograph with a 7.5-MHz transducer, respectively, as previously described [11, 12].

Statistical analysis

Data were expressed as mean ± SD. Student's t tests were used for comparison between two groups, paired t tests for comparison of mean values within groups, χ^2 tests for nominal scale, and correlation and multiple regression analysis for the relationships between two parameters. All analyses were carried out using the statistical computer program StatView (Abacus Concepts, Berkeley, CA, USA). A value of $p < 0.05$ was considered to be significant.

Results

Baseline characteristics of patients and comparison of parameters between the pioglitazone and the metformin groups

A total of 55 patients were enrolled, with none of them withdrawing from the study (Table 1). Body mass index (BMI) was significantly lower in the pioglitazone group than in the metformin group. BMD, T score, and Z score at the lumbar spine were significantly higher in the pioglitazone group than in the metformin group.

Table 1 Baseline characteristics of patients and comparison of parameters between the pioglitazone and the metformin groups

Number of subjects (male/female)	Pioglitazone 22 (14/8)	Metoformin 23 (13/10)	*p*
Age (years)	67±10	66±10	0.649
Duration of diabetes (years)	14±9	12±11	0.547
Body height (cm)	161.7±9.6	158.1±9.5	0.213
Body weight (kg)	57.6±8.4	62.2±10.9	0.124
BMI (kg/m^2)	22.0±2.3	24.9±3.7	0.003
Systolic blood pressure (mmHg)	128±15	130±12	0.556
Diastolic blood pressure (mmHg)	80±9	80±11	0.975
Brinkmann index	243±375	192±424	0.670
LDL-C (mg/dl)	107±21	104±24	0.694
HDL-C (mg/dl)	61±20	63±23	0.693
Serum creatinine (mg/dl)	0.77±0.20	0.76±0.20	0.768
Serum albumin (g/dl)	4.1±0.5	4.2±0.4	0.358
Serum calcium (mg/dl)	9.4±0.4	9.4±0.4	0.755
HbA1c (%)	7.9±1.7	7.9±1.3	0.944
IGF-I (ng/ml)	116±14	132±35	0.172
L BMD (g/cm^2)	1.072±0.187	0.911±0.138	0.002
T score	0.3±1.5	−1.0±1.2	0.002
Z score	1.1±0.9	0.1±1.1	0.003
F BMD (g/cm^2)	0.727±0.119	0.681±0.113	0.192
T score	−0.9±0.9	−1.3±0.9	0.238
Z score	0.5±1.1	0.1±1.2	0.204
1/3 BMD (g/cm^2)	0.645±0.098	0.603±0.117	0.201
T score	−1.4±1.4	−2.1±2.0	0.188
Z score	0.6±1.2	−0.2±1.7	0.080
Osteocalcin (ng/ml)	8.0±3.5	7.1±3.1	0.377
uNTX (nMBCE/mM-Cr)	41.6±29.2	39.0±28.4	0.763
Right-baPWV (m/s)	16.5±4.1	15.4±2.5	0.258
Left-baPWV (m/s)	15.9±3.2	15.3±2.7	0.487
IMT-Max (mm)	2.1±0.9	2.3±1.0	0.656
IMT-Mean (mm)	1.26±0.35	1.38±0.48	0.334
Plaque score	6.9±4.1	7.2±5.3	0.837

Data are means ± SD

BMI body mass index, *IGF-I* insulin-like growth factor-I, *LDL* low-density lipoprotein cholesterol, *HDL* high-density lipoprotein cholesterol, *HbA1c* hemoglobin A1c, *L* Lumbar, *F* femoral neck, 1/3R one third of radius, *BMD* bone mineral density, *uNTX* urinary N-terminal cross-linked telopeptide of type-I collagen, *baPWV* branchial ankle pulse wave velocity, *IMT* intima-media thickness

Chronological changes in body weight, glycemic control, insulin-like growth factor-I, BMD, bone markers, and parameters of atherosclerosis

Chronological changes in body weight, glycemic control, IGF-I, BMD, bone markers, and atherosclerosis parameters were shown in Table 2. Body weight and BMI were significantly and consecutively increased at 3, 6, and 12 months in the pioglitazone group, while they were not significantly changed in the metformin group. HbA_{1c} was significantly decreased at 3, 6, and 12 months in both the pioglitazone group and the metformin group, with no significant differences in HbA_{1c} improvements at any time points between the two groups (data not shown).

In the pioglitazone group, F-BMD was significantly decreased at 6 months (%change, −2.41 ± 5.72%), and 1/3R-BMD was significantly decreased at 6 and 12 months (%change, −1.75 ± 2.56% and −1.65 ± 3.31%, respectively). Changes in 1/3R-BMD in the pioglitazone group were significantly different from those in the metformin group at 6 months (p=0.038). Serum osteocalcin level was significantly decreased at 6 months, while uNTX was not significantly changed. In contrast, BMD at any site or bone markers were not significantly changed in the metformin group.

In the pioglitazone group, baPWV or intima-media thickness (IMT) were stable and not significantly changed up to 12 months. In contrast, significant deterioration was observed in IMT-mean at 6 months and in plaque scores at 6 and 12 months in the metformin group. Changes in plaque score in the metformin group were significantly greater than those in the pioglitazone group at 6 and 12 months (p=0.030 and p=0.033, respectively).

Table 2 Chronological changes in body weight, glycemic control, insulin-like growth factor-I, BMD, bone markers, and parameters of atherosclerosis

Variables	Baseline	3months	6months	12months
Pioglitazone				
Body weight (kg)	57.6 (8.4)	58.9 (8.4)**	60.2 (8.7)***	61.2 (8.8)***
BMI (kg/m^2)	22.0 (2.3)	22.5 (2.2)**	23.0 (2.3)***	23.4 (2.6)***
HbA1c (%)	7.9 (1.7)	7.4 (1.7)**	7.2 (1.2)**	7.1 (1.2)***
IGF-I (ng/ml)	116 (41)		117 (30)	114 (29)
L BMD (g/cm^2)	1.072 (0.187)		1.078 (0.202)	1.070 (0.187)
F BMD (g/cm^2)	0.727 (0.119)		0.708 (0.115)*	0.715 (0.116)
1/3R BMD (g/cm^2)	0.645 (0.098)		0.634 (0.101)**	0.635 (0.107)*
Osteocalcin (ng/ml)	8.0 (3.5)	7.4 (3.6)	6.8 (3.0)*	7.7 (3.8)
uNTX (nMBCE/mM-Cr)	41.6 (29.2)	37.9 (28.9)	41.7 (32.8)	39.5 (28.3)
Right-baPWV (m/s)	16.5 (4.1)		15.5 (2.8)	15.9 (3.8)
Left-baPWV (m/s)	15.9 (3.2)		15.6 (2.5)	15.5 (2.7)
IMT-Max (mm)	2.1 (0.9)		2.2 (1.2)	2.1 (1.1)
IMT-Mean (mm)	1.26 (0.35)		1.28 (0.40)	1.25 (0.47)
Plaque score	6.9 (4.1)		7.3 (4.7)	6.4 (5.7)
Metoformin				
Body weight (kg)	62.2 (10.9)	61.8 (9.6)	62.3 (10.2)	62.7 (10.8)
BMI (kg/m^2)	24.9 (3.7)	24.7 (3.2)	24.9 (3.4)	25.1 (3.6)
HbA1c (%)	7.9 (1.3)	7.1 (1.0)**	7.1 (1.0)*	7.1 (1.1)*
IGF-I (ng/ml)	132 (35)		135 (37)	136 (38)
L BMD (g/cm^2)	0.911 (0.138)		0.922 (0.154)	0.920 (0.142)
F BMD (g/cm^2)	0.681 (0.113)		0.673 (0.096)	0.666 (0.108)
1/3R BMD (g/cm^2)	0.603 (0.117)		0.609 (0.127)	0.604 (0.121)
Osteocalcin (ng/ml)	7.1 (3.1)	6.8 (2.9)	6.6 (2.6)	7.0 (3.2)
uNTX (nMBCE/mM-Cr)	39.0 (28.4)	34.9 (18.8)	35.0 (16.7)	38.9 (24.5)
Right-baPWV (m/s)	15.4 (2.5)		15.5 (2.4)	15.9 (3.0)
Left-baPWV (m/s)	15.3 (2.7)		15.5 (2.5)	15.9 (3.4)
IMT-Max (mm)	2.3 (1.0)		2.4 (0.9)	2.3 (0.7)
IMT-Mean (mm)	1.38 (0.48)		1.46 (0.41)*	1.44 (0.42)
Plaque score	7.2 (5.3)		9.3 (5.0)***	8.9 (5.0)**

Data are means (SD)

BMI body mass index, *HbA1c* hemoglobin A1c, *IGF-I* insulin-like growth factor-I, *L* lumbar, *F* femoral neck, *1/3R* one third of radius, *BMD* bone mineral density, *uNTX* urinary N-terminal cross-linked telopeptide of type-I collagen, *baPWV* branchial ankle pulse wave velocity, *IMT* intima-media thickness

*$p<0.05$; **$p<0.01$; ***$p<0.001$

Correlations between baseline values of each parameter versus %changes in BMD at each skeletal site in the pioglitazone group

We performed correlation analysis between baseline values of each parameter versus %changes in BMD at each skeletal site at 6 months in order to investigate which parameters could predict BMD reduction in patients treated with pioglitazone. In the pioglitazone group, %changes in F-BMD were significantly and negatively correlated with baseline values of IMT-Max, IMT-mean, and plaque scores ($r=-0.61$, $p=0.002$; $r=-0.71$, $p<0.001$; and $r=-0.68$, $p=0.001$, respectively). %Changes in 1/3R-BMD were significantly and positively correlated with baseline serum IGF-I level ($r=0.45$, $p=0.038$) and negatively correlated with baseline uNTX and IMT-Max ($r=-0.57$, $p=0.006$ and $r=-0.48$, $p=0.024$, respectively).

Multiple regression analyses adjusted for age, gender, BMI, duration of diabetes, initial HbA_{1c}, and serum creatinine showed that %changes in F-BMD were still significantly and negatively correlated with baseline IMT-mean and plaque score in the pioglitazone group ($r=-0.66$, $p=0.019$ and $r=-0.65$, $p=0.027$, respectively) and tended to be negatively correlated with baseline IMT-Max ($r=-0.51$, $p=0.057$). Percent changes in 1/3R-BMD were still significantly and positively correlated with baseline serum IGF-I level ($r=0.68$, $p=0.036$) and negatively correlated with baseline uNTX and IMT-Max ($r=-0.77$, $p=0.007$ and $r=-0.51$, $p=0.014$, respectively).

Discussion

Many clinical studies have shown that osteoporosis is associated with atherosclerosis or the cardiovascular disease [13, 14]. On the other hand, pioglitazone is considered to be unique in its ability to reduce a progression of atherosclerosis and to prevent an occurrence of cardiovascular events

[15]. In this study, we found that baseline atherosclerosis parameters such as IMT and plaque scores were negatively associated with %changes in L-BMD and 1/3R-BMD in the pioglitazone group and that the parameters of atherosclerosis were stable and not changed in the pioglitazone group, while these deteriorated in the metformin group, suggesting that osteoporosis is associated with atherosclerosis in type 2 diabetes and that a precaution against bone loss is necessary if atherosclerosis is found by carotid ultrasonography before pioglitazone administration in patients with type 2 diabetes, although pioglitazone could prevent the progression of atherosclerosis.

IGFs are among the most important regulators of bone cell function due to their anabolic effects on the skeleton [16, 17]. We have previously shown that serum IGF-I level was positively associated with BMD and inversely with the risk of vertebral fractures in postmenopausal women [9, 18]. In the present study, baseline serum IGF-I level was significantly and positively associated with %changes in 1/3R-BMD in the pioglitazone group, suggesting that circulating IGF-I could also alleviate bone loss in the patients treated with pioglitazone and could be clinically useful for assessing its risk.

Previous population-based studies indicated that use of metformin was associated with a significantly decreased risk of fracture in type 2 diabetes [19]. Experiments with cultured cells also showed that metformin stimulated the differentiation of osteoblasts [8]. However, little is known whether or not metformin could affect bone in humans. In this study, metformin did not affect bone markers or BMD for 12 months. Thus, the beneficial effects of metformin on bone, if any, might be due to improved bone quality rather than bone mass, which are not reflected by bone markers or BMD.

This study has several limitations. First, the sample size was not large enough to make definite conclusions. Second, we analyzed only subjects who visited Shimane University Hospital, a tertiary center, for evaluation or treatment of diabetes mellitus and osteoporosis. Third, the present study lacks a parallel placebo or treatment control. Fourth, body weight is known to be positively associated with BMD. In the pioglitazone group, body weight was clearly increased during treatment. Therefore, we may underestimate the detrimental effects of pioglitazone on BMD. Finally, since the capacity of insulin secretion and the degree of obesity in Asian populations are known to be lower than those in Western people [20], our findings might not be universal and not applicable to Western populations.

In conclusion, this exploratory study suggests that pioglitazone compared with metformin is a negative regulator for bone but useful for preventing a progression of atherosclerosis in type 2 diabetes and that baseline values of atherosclerosis parameters, uNTX, and serum IGF-I could assess the risk of BMD reduction in patients treated with pioglitazone. Thus, a further large investigation is warranted to confirm these findings.

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Conflicts of interest None.

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ORIGINAL ARTICLE

Relationship between treatments with insulin and oral hypoglycemic agents versus the presence of vertebral fractures in type 2 diabetes mellitus

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Abstract Although previous studies indicated that hypoglycemic agents could affect bone metabolism, little is known about whether these agents are associated with the risks of osteoporotic fracture in Japanese patents with type 2 diabetes. We examined whether treatments of diabetes, such as insulin administration, sulfonylurea, thiazolidinedione, and metformin, were associated with the presence of vertebral fractures in 494 men and 344 postmenopausal women with type 2 diabetes. We analyzed the relationships between each treatment versus bone turnover markers, bone mineral density (BMD), and the presence of prevalent vertebral fractures. Multiple logistic regression analysis adjusted for age, duration of diabetes, body mass index, serum creatinine, serum C-peptide, and HbA_{1c} showed that, in postmenopausal women, treatments with insulin administration or thiazolidinedione were significantly and positively associated with the presence of vertebral fractures [odds ratio (OR) = 2.27, $P = 0.012$ and OR = 3.38, $P = 0.038$, respectively], whereas treatment with sulfonylurea was significantly and inversely associated with vertebral fractures (OR = 0.48, $P = 0.018$). These relationships were still significant after additional adjustment for lumbar BMD. In contrast, no significant relationships between treatments with any agent and the presence of vertebral fractures were found in men. These findings suggest that postmenopausal women treated with insulin or thiazolidinedione have a high risk of vertebral fractures independent of age, body stature, blood glucose level, insulin secretion, or BMD whereas treatment with sulfonylurea is associated with a decreased risk.

Keywords Thiazolidinedione · Insulin · Sulfonylurea · Type 2 diabetes mellitus · Vertebral fracture

Introduction

The number of patients with diabetes mellitus and osteoporosis is rapidly increasing in industrialized countries where Western-style aging societies are prevalent. Recently, the relationship between diabetes and osteoporotic fractures is becoming increasingly recognized [1]. Both vertebral and hip fractures are very important osteoporotic fractures because they frequently occur and increase the mortality of elderly people as much as six- to ninefold [2, 3]. Although patients with type 2 diabetes do not show bone mineral density (BMD) reduction, fracture risks are known to increase at the hip, proximal humerus, forearm, and foot [4–6], as well as the vertebrae [7]. Therefore, the etiology and treatment of diabetes-related bone disease have recently attracted widespread attention.

The effects of hypoglycemia agents on bone metabolism have recently been discussed. Thiazolidinediones (TZDs) such as pioglitazone and rosiglitazone are peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists and are widely used for treatment of patients with type 2 diabetes. PPAR-γ is also expressed in bone marrow cells, and it acts as a molecular switch that regulates the fate of pluripotent mesenchymal stem cells, which are able to differentiate into adipocytes or osteoblasts. Previous in vitro studies have shown that TZDs stimulate the differentiation into adipocytes in preference over osteoblasts [8, 9]. Haploinsufficiency of the PPAR-γ gene in mice induces a high

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bone density phenotype characterized by increased bone formation [10, 11], whereas treatment of rodents with PPAR-γ agonists induces bone loss characterized by deficient osteoblast function [11–13]. Clinically, a recent meta-analysis has shown that long-term TZDs use causes BMD reduction as well as greater risks of fracture in women with type 2 diabetes, but not in men [14].

Insulin induces a wide variety of growth and metabolic responses and plays important roles in the anabolic regulation of bone metabolism [15, 16]. Patients with insulin deficiency show a decreased BMD and high fracture risk [4], and insulin treatment improved the disturbances in calcium metabolism [17, 18]. However, other studies have indicated that insulin-treated diabetes was associated with an increased risk of fractures [19, 20]. In contrast, recent epidemiologic studies have shown that fracture rate was decreased in patients treated with sulfonylurea [21] and metformin [20, 21]. These studies were performed in Caucasian subjects, and thus little is known about whether these agents are associated with the risks of osteoporotic fracture in Asian patents with type 2 diabetes. The capacity of insulin secretion and degree of obesity in Asians are known to be different from those of Western people [22, 23].

In this study, to examine this issue, we investigated the relationships between treatments of diabetes such as TZDs, insulin administration, sulfonylurea, and metformin versus BMD and bone turnover markers as well as the presence of prevalent vertebral fractures in Japanese men and postmenopausal women with type 2 diabetes.

Subjects and methods

Subjects

The subjects in this study were a total of 838 Japanese patients with type 2 diabetes (494 men: mean age, 60.1 years; 344 postmenopausal women: mean age, 67.2 years). We consecutively recruited subjects who visited Shimane University Hospital for education, evaluation, or treatment of diabetes. Subjects agreed to participate in this study and gave informed consent. This study was approved by the institutional review board of our institution. No patient had hepatic or renal dysfunction or nutritional derangements that might cause changes in bone metabolism. Of the patients, 93, 163, 31, and 62 men, as well as 98, 118, 20, and 64 postmenopausal women, had been taking insulin treatment, sulfonylurea, TZDs, and metformin, respectively; 220 men and 117 postmenopausal women had not previously been receiving any medications for diabetes. All subjects were free of drugs known to influence bone and calcium metabolism, such as vitamin D and bisphosphonates, up to the time of the present study.

Radiography

Lateral X-ray films of the thoracic and lumbar spine were taken at the same week as the serum collection. The anterior, central, and posterior heights of each of the 13 vertebral bodies from T4 to L4 were measured. A vertebral fracture was diagnosed if at least one of three height measurements along the length of the same vertebrae had decreased by more than 20% compared to the height of the nearest uncompressed vertebral body [24]. None of the subjects had a history of serious trauma.

BMD values of the lumbar spine (L), femoral neck (F), and one-third of the radius (1/3R) were measured by dual-energy X-ray absorptiometry (QDR-4500; Hologic, Waltham, MA, USA). The same operator tested all the subjects during the study to eliminate operator discrepancies. The coefficients of variation (precision) of measurements of L-, F-, and 1/3R-BMD by our methods were 0.9%, 1.7%, and 1.9%, respectively. Z score indicates deviation from the normal age- and sex-matched mean in standard deviation (SD).

Biochemical measurements

After overnight fasting, serum and first-void urine samples were collected. Biochemical markers were measured by standard biochemical methods, as previously described [25, 26]. HbA_{1c} was determined by high performance liquid chromatography (HPLC). Bone-specific alkaline phosphatase (BAP) and osteocalcin were measured by enzyme immunoassay and radioimmunoassay (RIA), respectively. Serum C-peptide and urinary N-terminal cross-linked telopeptide of type I collagen (uNTX) were measured by enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Data were expressed as mean $\pm$ SD. Student's t tests were used for comparison between two groups and χ^2 tests for nominal scale. Multiple logistic regression analysis was performed after being adjusted for age, duration of diabetes, body mass index (BMI), serum creatinine, serum C-peptide, and HbA_{1c}, as well as BMD. All analysis was performed using the statistical computer program StatView (Abacus Concepts, Berkeley, CA, USA). $P < 0.05$ was considered to be significant.

Results

Baseline characteristics of subjects

Demographic and biochemical parameters and BMD were compared between men and postmenopausal women with

type 2 diabetes (Table 1). Patient age, duration of diabetes, BAP, osteocalcin, and uNTX were significantly lower in men than in postmenopausal women ($P < 0.01$). On the other hand, body height, body weight, serum creatinine, absolute BMD, T score, and Z score at each site, except for Z score at the lumbar spine, were significantly higher in men than in postmenopausal women ($P < 0.05$).

Comparison of bone turnover markers and BMD between patients treated with and without each medication

We compared bone turnover markers and BMD values at each site between patients treated with and without each drug. There were no significant differences in BMD values at any site or bone formation markers (BAP and osteocalcin) between patients treated with insulin administration, sulfonylurea, TZDs, or metformin and those not so treated (data not shown). uNTX in postmenopausal women treated with sulfonylurea or metformin was significantly lower than that in those without either medication (sulfonylurea: 48.3 ±21.1 vs. 57.0 ± 36.2, $P = 0.038$; metformin: 45.6 ± 18.6 vs. 56.4 ± 34.6, $P = 0.030$).

Comparison of demographic and biochemical parameters between patients with and without vertebral fractures

We compared various parameters including HbA_{1c}, serum C-peptide, bone turnover markers, and BMD values at each site between patients with and without vertebral fractures (Table 2). Men and postmenopausal women with vertebral fractures were significantly older ($P < 0.001$), shorter in height ($P < 0.001$), and had lower absolute BMD at each site (at least $P < 0.05$) than their counterparts without vertebral fractures. Body weight in men with vertebral fractures was significantly lower than that in men without fractures ($P = 0.027$). Duration of diabetes, serum creatinine, and uNTX in postmenopausal women with vertebral fractures were significantly higher than those in postmenopausal women without fractures ($P = 0.003$, $P = 0.003$, and $P = 0.035$, respectively). On the other hand, no significant differences in the levels of fasting plasma

Table 1 Baseline characteristics of subjects

	Men $n = 494$	Postmenopausal women $n = 344$	P
Age (years)	60.1 ± 13.2	67.2 ± 9.7	<0.001
Diabetes duration (years)	10.7 ± 9.0	12.4 ± 9.7	0.009
Body height (cm)	165.3 ± 7.0	150.4 ± 5.8	<0.001
Body weight (kg)	65.5 ± 14.8	54.7 ± 10.8	<0.001
BMI (kg/m^2)	23.9 ± 4.4	24.1 ± 4.3	0.339
Serum creatinine (mg/dl)	0.79 ± 0.18	0.64 ± 0.18	<0.001
FPG (mg/dl)	167 ± 61	166 ± 62	0.822
HbA_{1c} (%)	8.8 ± 2.4	8.7 ± 2.3	0.911
Serum C-peptide (ng/ml)	1.8 ± 1.2	1.7 ± 0.9	0.356
L2–L4 BMD (g/cm^2)	1.033 ± 0.184	0.872 ± 0.177	<0.001
T score	−0.12 ± 1.55	−1.25 ± 1.59	<0.001
Z score	0.42 ± 1.12	0.55 ± 1.15	0.105
FN BMD (g/cm^2)	0.770 ± 0.126	0.637 ± 0.127	<0.001
T score	−0.72 ± 1.00	−1.39 ± 1.16	<0.001
Z score	0.25 ± 1.01	0.43 ± 1.21	0.034
1/3R BMD (g/cm^2)	0.706 ± 0.074	0.532 ± 0.088	<0.001
T score	−1. 46 ± 1.49	−2.53 ± 1.70	<0.001
Z score	−0.44 ± 1.37	0.61 ± 1.48	<0.001
BAP (U/l)	26.1 ± 9.9	31.5 ± 11.9	<0.001
Osteocalcin (ng/ml)	5.0 ± 2.4	7.0 ± 3.1	<0.001
uNTX (nMBCE/mM-Cr)	34.2 ± 22.0	54.2 ± 32.3	<0.001
Vertebral fracture	166 (33.6%)	103 (29.9%)	0.264*

Data are mean ± SD. P values were calculated using Student's t test or *χ^2 test

BMI body mass index, *FPG* fasting plasma glucose, *HbA_{1c}* hemoglobin A_{1c}, *BMD* bone mineral density, *L* lumbar spine, *FN* femoral neck, *1/3R* one-third of the radius, *BAP* bone-specific alkaline phosphatase, *uNTX* urinary N-terminal cross-linked telopeptide of type I collagen

glucose (FPG), HbA_{1c}, C-peptide, BAP, osteocalcin, or Z score at any skeletal site were observed between subjects with and without vertebral fractures in either sex.

Relationships between treatments of diabetes and the presence of vertebral fractures

Next, we performed χ^2 tests between patients with and without vertebral fractures to examine whether treatments with each medication were associated with the presence of vertebral fractures (see Table 2). Postmenopausal women treated with insulin administration or TZDs were significantly associated with an increased risk of vertebral fractures ($P = 0.005$ and $P = 0.045$, respectively), whereas postmenopausal women without any medication were significantly associated with a decreased risk ($P = 0.032$). On the other hand, no significant relationships were found between any treatment and the presence of vertebral fractures in men.

Next, multiple logistic regression analyses were performed between each treatment of diabetes versus the presence of vertebral fractures adjusted for age, duration of diabetes, BMI, serum creatinine, serum C-peptide, and HbA_{1c} (Table 3), because we found these confounders were significantly different between those with and without medication (data not shown). The risk of vertebral fractures was significantly higher in postmenopausal women treated with insulin or TZDs independent of age, body stature, blood glucose level, or insulin secretion [odds ratio (OR) = 2.27, $P = 0.012$ and OR = 3.38, $P = 0.038$, respectively], while treatment with sulfonylurea was associated with a decreased risk (OR = 0.48, $P = 0.018$). These observations were still significant after additional adjustments for lumbar bone mineral density (L-BMD) (OR = 2.20, $P = 0.020$; OR = 3.51, $P = 0.036$; and OR = 0.51, $P = 0.029$, respectively). On the other hand, no significant relationships between diabetes treatment and the presence of vertebral fractures were found in men.

Table 2 Comparison of demographic and biochemical parameters between subjects with and without vertebral fractures

	Men			Postmenopausal women		
Vertebral fracture	Yes $n = 166$	No $n = 328$	P	Yes $n = 103$	No $n = 241$	P
Age (years)	64.0 ± 12.2	58.1 ± 13.2	<0.001	72.1 ± 8.8	65.0 ± 9.3	<0.001
Diabetes duration (years)	11.7 ± 8.7	10.1 ± 9.1	0.075	14.8 ± 9.9	11.4 ± 9.5	0.003
Body height (cm)	163.7 ± 7.2	166.0 ± 6.7	<0.001	148.6 ± 5.9	151.2 ± 5.6	<0.001
Body weight (kg)	63.4 ± 12.8	66.5 ± 15.7	0.027	53.4 ± 12.2	55.3 ± 10.2	0.134
BMI (kg/m^2)	23.5 ± 3.7	24.0 ± 4.7	0.261	24.1 ± 4.9	24.2 ± 4.0	0.896
Serum creatinine (mg/dl)	0.80 ± 0.20	0.78 ± 0.17	0.379	0.68 ± 0.21	0.62 ± 0.16	0.003
FPG (mg/dl)	160 ± 57	171 ± 63	0.064	164 ± 61	168 ± 63	0.619
HbA_{1c} (%)	8.5 ± 2.1	8.9 ± 2.5	0.114	8.6 ± 2.4	8.8 ± 2.2	0.532
Serum C-peptide (ng ml)	1.7 ± 1.1	1.9 ± 1.2	0.299	1.7 ± 0.9	1.8 ± 0.8	0.680
L2–L4 BMD (g/cm^2)	1.005 ± 0.171	1.048 ± 0.190	0.017	0.817 ± 0.191	0.895 ± 0.166	<0.001
Z score	−0.30 ± 1.02	0.48 ± 1.16	0.106	0.41 ± 1.19	0.61 ± 1.13	0.140
FN BMD (g/cm^2)	0.749 ± 0.114	0.782 ± 0.131	0.010	0.601 ± 0.123	0.655 ± 0.126	0.002
Z score	0.17 ± 0.90	0.29 ± 1.07	0.277	0.31 ± 1.24	0.49 ± 1.19	0.252
1/3R BMD (g/cm^2)	0.691 ± 0.077	0.715 ± 0.071	0.002	0.507 ± 0.086	0.543 ± 0.087	0.003
Z score	−0.56 ± 1.44	−0.37 ± 1.33	0.198	0.55 ± 1.39	0.63 ± 1.52	0.695
BAP (U/l)	27.2 ± 10.9	25.5 ± 9.3	0.087	32.8 ± 13.3	30.9 ± 11.1	0.214
Osteocalcin (ng/ml)	5.0 ± 2.6	4.9 ± 2.3	0.822	6.9 ± 3.2	7.0 ± 3.0	0.673
uNTX (nMBCE/mM-Cr)	36.0 ± 19.3	33.2 ± 23.2	0.231	60.1 ± 40.9	51.2 ± 26.6	0.035
No medication	66 (39.8%)	154 (47.0%)	0.129	28 (27.2%)	89 (36.9%)	0.032
Insulin	33 (19.9%)	60 (18.3%)	0.670	40 (38.8%)	58 (24.1%)	0.005
Sulfonylurea	56 (33.7%)	107 (32.6%)	0.804	33 (32.0%)	85 (35.3%)	0.563
Thiazolidinedione	13 (7.8%)	18 (5.5%)	0.310	10 (9.7%)	10 (4.2%)	0.045
Metformin	18 (10.8%)	44 (13.4%)	0.415	17 (16.5%)	47 (19.5%)	0.513

Data are means ± SD. P values were calculated using Student's t test or χ^2 test

BMI body mass index, *HbA_{1c}* hemoglobin A_{1c}, *BMD* bone mineral density, *L* lumbar spine, *FN* femoral neck, *1/3R* one-third of the radius, *BAP* bone-specific alkaline phosphatase, *uNTX* urinary N-terminal cross-linked telopeptide of type I collagen

Table 3 Association between diabetes treatments and the presence of vertebral fractures in postmenopausal women with type 2 diabetes

	Presence of vertebral fractures					
	Men		Postmenopausal women		Postmenopausal women[a]	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
No medication	1.03 (0.64–1.65)	0.899	0.93 (0.43–1.84)	0.830	0.85 (0.42–1.72)	0.651
Insulin	0.94 (0.54–1.63)	0.813	2.27 (1.20–4.28)	0.012	2.20 (1.13–4.27)	0.020
Sulfonylurea	0.91 (0.58–1.41)	0.657	0.48 (0.27–0.88)	0.018	0.51 (0.27–0.93)	0.029
Thiazolidinedione	1.09 (0.48–2.46)	0.840	3.38 (1.07–10.71)	0.038	3.51 (1.09–11.38)	0.036
Metformin	0.57 (0.30–1.09)	0.092	0.74 (0.37–1.48)	0.392	0.75 (0.37–1.52)	0.421

Multivariate logistic regression analysis was performed with the presence of vertebral fractures as a dependent variable and each treatment of diabetes adjusted for age, duration of diabetes, BMI, serum creatinine, serum C-peptide, and HbA_{1c} as independent variables

OR odds ratio, *CI* confidence interval

[a] Additionally adjusted for lumbar (L-)BMD

Discussion

In this study, treatment with TZDs or insulin administration was significantly and positively associated with the presence of prevalent vertebral fractures, whereas treatment with sulfonylurea was significantly and inversely associated with vertebral fractures in diabetic postmenopausal women, but not in men. These findings suggest that postmenopausal patients treated with TZDs or insulin administration have an increased risk of vertebral fractures whereas postmenopausal patients treated with sulfonylurea have a lower risk. Thus, TZDs or insulin use requires precaution against vertebral fractures in not only Western postmenopausal women but also Asian postmenopausal women with type 2 diabetes.

Accumulating evidence indicates that TZDs have negative impact on bone metabolism. Grey et al. [27] have shown that 14-week rosiglitazone treatment decreased bone formation markers, osteocalcin, procollagen type I N-terminal propeptide (PINP), and femoral (F)-BMD in healthy postmenopausal women. Schwartz et al. [28] have reported that long-term use of TZDs caused reduction of whole-body BMD and L-BMD in older diabetic women, as shown by a 4-year observational cohort study. Recently, a meta-analysis has revealed that L- and F-BMD were significantly reduced, and that risk of fractures was significantly increased, in women exposed to TZDs, but not in men [14]. Moreover, a previous clinical trial showed that risk of fractures in the bones of the extremities (foot, hand, and proximal humerus) was significantly increased, whereas there was no increased risk of clinical spine or hip fractures [29, 30], suggesting a negative impact on cortical bone. However, little is known about whether the fracture rate in vertebrae, which contain a relatively higher proportion of trabecular bone, is increased. In this study, although we found no differences in BMD or bone markers between patients treated with and without TZDs, for the first time we found that TZDs use was significantly associated with an increased risk of prevalent vertebral fractures, the most frequent osteoporotic fracture, in Japanese postmenopausal women with type 2 diabetes. This finding suggests that TZDs might have a negative impact on bone metabolism in Asian people as well, whose capacity of insulin secretion and degree of insulin resistance are different from those of Caucasian subjects [22, 23]. Moreover, we found a significant relationship independent of L-BMD between TZDs use and vertebral fractures, suggesting that TZDs induce deterioration of bone quality regardless of bone mass. Thus, BMD measurement may not be sensitive enough to assess the risk of bone fragility in patients treated with TZDs, and further studies are needed to explore new markers that substitute for the insensitivity of BMD.

Insulin administration and sulfonylurea are widely used for treatment of patients with type 2 diabetes. These agents are known to improve glycemic control by increasing insulin concentration in the circulation as well as its action in the liver and muscle. Although circulating insulin is considered to stimulate osteoblastogenesis and to enhance bone formation [15, 16], the effects of insulin administration and sulfonylurea on bone metabolism seem to be just the opposite. Previous studies have indicated that insulin-treated diabetes was associated with an increased risk of fractures [19, 20]. Melton et al. [20] showed that fracture risks were increased in diabetic patients with insulin treatment. In contrast, Vestergaard et al. [21] reported that sulfonylurea use was associated with a significant trend toward decreased risk of any fractures. In this study, treatment with insulin administration was associated with an increased risk of vertebral fractures whereas treatment with sulfonylurea was associated with a decreased risk in postmenopausal women after adjustment with confounding factors, although Student's *t* test showed no significant difference (see

Table 2). Thus, the present findings are consistent with the previous ones [19–21], suggesting that the influence of diabetic medication on bone is similar regardless of race or ethnic group. However, the mechanism is still unclear. Because patients with insulin administration commonly have a long duration of diabetes and diabetic complications, there is a possibility that these factors could affect the presence of vertebral fractures. On the other hand, Ma et al. [31] showed that sulfonylurea induced the proliferation and differentiation of rat osteoblasts. Although treatment with insulin administration improves blood glucose by exogenous insulin, sulfonylurea decreases blood glucose via stimulation of endogenous insulin secretion. Because residual insulin secretion is needed for hepatic expression and generation of insulin-like growth factor I (IGF-I) [32, 33], which has been reported to be associated with vertebral fractures in postmenopausal women with type 2 diabetes [26], sulfonylurea might have a beneficial effect through the enhancement of IGF-I secretion.

Previous population-based studies indicated that use of metformin was associated with a significantly decreased risk of fractures in type 2 diabetes [20, 21]. Experiments with cultured cells also showed that metformin stimulated the differentiation and mineralization of osteoblasts [34, 35]. In this study, treatment with metformin was not significantly associated with a decreased risk of vertebral fractures in men or postmenopausal women. This discrepancy might occur because the present study examined only prevalent vertebral fractures and did not include other fractures such as hip fracture.

This study has several limitations in addition to not examining nonvertebral fractures. First, the sample size is not large enough compared with other community-based studies. Second, we analyzed only subjects who visited Shimane University Hospital, a tertiary center, for evaluation or treatment of diabetes mellitus and osteoporosis. Therefore, the patients enrolled in this study might have relatively severe states of the disorders and might not be representative of Japanese diabetic patients. Third, the conclusions of this study are weakened by its cross-sectional design.

In conclusion, we found that postmenopausal women with type 2 diabetes treated with insulin administration or TZDs had an increased risk of vertebral fractures, whereas treatment with sulfonylurea was associated with a decreased risk in the Japanese population. Thus, we should be cautious about the increased risk of vertebral fractures in postmenopausal diabetic patients treated with insulin administration or TZDs.

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Conflict of interest statement The authors have no conflict of interest to disclose.

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ORIGINAL ARTICLE

Serum undercarboxylated osteocalcin was inversely associated with plasma glucose level and fat mass in type 2 diabetes mellitus

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Abstract
Summary Although recent animal studies have shown that undercarboxylated osteocalcin acts as a hormone regulating glucose metabolism and fat mass, little is known about the relationships in humans. We reported here for the first time that undercarboxylated osteocalcin were associated with glucose/fat metabolism in patients with type 2 diabetes.
Introduction Recent studies have shown that undercarboxylated osteocalcin (ucOC) acts as a hormone regulating glucose metabolism and fat mass. We investigated the relationship between ucOC as well as other bone turnover markers [serum OC, bone-specific alkaline phosphatase (BAP), and urinary N-terminal cross-linked telopeptide of type-I collagen] versus serum levels of glucose, fasting serum C-peptide, and adiponectin as well as the amount of fat mass in type 2 diabetes.
Methods A total of 180 men and 109 postmenopausal women were consecutively recruited, and radiographic and biochemical characteristics were collected. Fat mass was measured by dual X-ray absorptiometry (DXA) and computed tomography (CT).
Results In men, ucOC negatively correlated with percent trunk fat (%trunk fat; by DXA) and visceral/subcutaneous fat ratio (by CT) as well as fasting plasma glucose and HbA_{1c} (at least $p<0.05$). Multiple regression analysis showed that these associations were still significant independent of age, duration of diabetes, body stature, and renal function as well as glucose or fat metabolism, whereas BAP, another bone formation marker, did not correlate with any variable. On the other hand, although ucOC also negatively correlated with %fat and %trunk fat as well as HbA_{1c} (at least $p<0.05$) in postmenopausal women, we found no significant association in multiple regression analysis.
Conclusions These findings suggest that ucOC is associated with plasma glucose level and fat mass in men with type 2 diabetes.

Keywords Glucose metabolism · Osteocalcin · Type 2 diabetes mellitus · Undercarboxylated osteocalcin · Visceral fat

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Introduction

Cumulative evidence shows that there is a positive correlation between bone mineral density (BMD) and fat mass, suggesting that body fat and bone mass are related to

each other [1–3]. Several studies on adipocyte function have revealed that not only is adipose tissue an energy-storing organ but it also secretes a variety of biologically active molecules, which are named adipocytokines [4]. Adiponectin is one of the adipocytokines specifically and highly expressed in visceral, subcutaneous, and bone marrow fat depots [5]. It is also abundantly present in plasma [6] and has been proposed to play important roles in the regulation of energy homeostasis and insulin sensitivity [7, 8]. We and other researchers have shown that osteoblasts have an adiponectin receptor and that the proliferation, differentiation, and mineralization of osteoblastic cells are enhanced by adiponectin [9, 10]. We also clinically found that serum adiponectin was associated with BMD, bone turnover, and the presence of vertebral fractures in patients with type 2 diabetes [11]. These findings suggest that fat mass as well as serum adiponectin are involved in not only glucose/lipid metabolism but also bone metabolism.

Osteocalcin (OC), one of the osteoblast-specific secreted proteins, has several hormonal features and is secreted in the general circulation from osteoblastic cells [12, 13]. Recent animal studies have shown that undercarboxylated OC (ucOC) action is related to not only bone metabolism but also glucose metabolism and fat mass [14, 15]. Lee et al. showed that osteocalcin functions as a hormone that regulates glucose metabolism and fat mass in genetically modified mouse [14]. Moreover, Ferron et al. showed that recombinant ucOC administration regulated gene expression in β cells and adipocytes (including adiponectin expression) and affected the development of metabolic diseases, obesity, and type 2 diabetes in wild-type mice [15]. Several clinical studies including ours [16–19] have recently shown that serum OC level was associated with glucose and total adiponectin levels, fat mass, as well as atherosclerosis parameters in humans. We have recently shown that serum OC level was negatively correlated with plasma glucose level and atherosclerosis parameters in patients with type 2 diabetes [16]. Kindblom et al. have shown that OC level was inversely related to plasma glucose level and fat mass in elderly non-diabetic persons [17]. Fernandez-Real et al. have shown that serum OC level was associated with insulin sensitivity in non-diabetes subjects [18]. Pittas et al. have shown that serum OC concentration was inversely associated with fasting plasma glucose (FPG), fasting insulin, homeostasis model assessment for insulin resistance, high-sensitivity C-reactive protein, IL-6, body mass index (BMI), and body fat in cross-sectional analyses. They also found that OC levels were associated with change in FPG in prospective analyses [19]. These experimental and clinical findings suggest that bone metabolism and glucose/fat metabolism are associated with each other through the action of ucOC or OC. However, to our knowledge, there were no clinical reports to investigate the relationships between ucOC and glucose, fat mass, or adiponectin in humans.

In this study, to address this issue, we measured ucOC as well as other bone turnover markers [OC, ucOC, bone-specific alkaline phosphatase (BAP), and urinary N-terminal cross-linked telopeptide of type-I collagen (uNTX)], diabetes-related parameters (FPG, HbA_{1c}, and fasting C-peptide), serum adiponectin, body fat composition by dual X-ray absorptiometry (DXA), and abdominal fat area by computed tomography (CT) in Japanese men and postmenopausal women with type 2 diabetes, and investigated whether or not these bone markers and glucose/lipid metabolism-related parameters are associated with each other.

Methods

Subjects

The participants in this study were 180 men and 109 postmenopausal women with type 2 diabetes (age range, 21–85 and 50–87 years; mean, 59.1 years and 65.2 years, respectively). We consecutively recruited patients who visited Shimane University Hospital for education, evaluation, or treatment of diabetes. One hundred twelve men and 85 postmenopausal women were enrolled from the cohort of our previous study [16]. All women had been without spontaneous menses for more than 1 year. Nobody had hepatic or renal dysfunction or nutritional derangements. The numbers of patients who had been taking insulin, sulfonylurea, metformin, and alpha-glucosidase inhibitors, respectively, were 26, 58, 29, and 21 men, and 29, 45, 26, and 18 women. Patients treated with thiazolidinedione were excluded from this study. All patients were free of drugs known to influence bone and calcium metabolism, such as vitamin D, bisphosphonate, or estrogen, up until the time of the study. This study was cross-sectional in design, approved by the ethical review board of our institution, and complied with the Helsinki Declaration. All patients agreed to participate in the study and provided informed consent.

Radiography

Fat mass was measured by DXA (QDR-4500; Hologic, Waltham, MA) using whole-body absorptiometry software and each value was expressed in kilograms. Percent fat mass (%fat) was calculated by dividing each absolute value of body composition by total body mass. Percent trunk fat (%trunk fat) was calculated by dividing trunk fat mass by total fat mass. The coefficient of variation (precision) of measurements of fat mass was 2.0% [20].

Abdominal adipose tissue was measured using commercially available CT (Toshiba medical systems, Tokyo, Japan), which determined adipose tissue area electronically by setting the attenuation values for the region of interest within a range of −150 and −50 Hounsfield units. Visceral fat area and subcutaneous fat area were determined separately with the use of a trace function, which manually defined the boundary between the visceral and subcutaneous fat with a cursor.

Biochemical measurements

After overnight fasting, blood and urine samples were collected. Biochemical markers were measured by standard biochemical methods. Hemoglobin A_{1c} (HbA_{1c}) was determined by high-performance liquid chromatography. Bone markers and adiponectin were measured as previously described [11, 21–24]. OC and BAP were measured by radioimmunoassay and enzyme immunoassay, respectively [21, 22]. ucOC was measured by electrochemiluminescence immunoassay [23, 24]. We calculated ucOC/OC ratio and used it as one of parameters. Serum C-peptide and uNTX were measured by enzyme-linked immunosorbent assay (ELISA). Serum total adiponectin was measured by an ELISA kit (Otsuka Pharmaceuticals, Tokyo, Japan) [11, 24].

Statistical analysis

Baseline data of subjects were expressed as mean ± SD. Since OC, ucOC, BAP, and uNTX, as well as adiponectin showed a markedly skewed distribution, logarithmic (log) transformation of these values was carried out before performing correlation analysis and multiple regression analysis. Statistical significance between two groups was determined using a Mann–Whitney U-test. All analysis was performed using the statistical computer program StatView (Abacus Concepts, Berkeley, CA). $P<0.05$ was considered to be significant.

Results

Baseline characteristics of patients and comparison of parameters between men and postmenopausal women

The baseline characteristics of the patients are shown in Table 1. We compared these parameters between men and postmenopausal women. Body height, body weight, visceral fat area, visceral/subcutaneous fat ratio (V/S ratio), and serum creatinine were significantly higher in men than in women (p values<0.05). On the other hand, age, %fat, subcutaneous fat area, adiponectin, BAP, OC, ucOC, and uNTX were significantly lower in men than in women (p values<0.05).

Table 1 Baseline characteristics of subjects

	Men	Postmenopausal women	p values
Number of subjects	180	109	
Age (years)	59.1±12.8	67.2±9.3	<0.001
Duration of diabetes (years)	9.7±8.8	10.8±9.5	0.338
Body height (cm)	166.0±7.6	149.9±5.5	<0.001
Body weight (kg)	67.2±16.5	54.8±11.5	<0.001
BMI (kg/m^2)	24.2±4.9	24.4±4.7	0.830
%Fat (%)	20.2±5.4	29.6±6.6	<0.001
%Trunk fat (%)	50.8±6.1	51.2±6.9	0.673
Visceral fat area (cm^2)	119.3±61.4	103.6±55.6	0.046
Subcutaneous fat area (cm^2)	124.7±82.4	181.0±92.5	<0.001
Visceral/subcutaneous fat ratio	1.06±0.49	0.60±0.25	<0.001
FPG (mg/dl)	171±69	169±54	0.872
HbA1c (%)	9.0±2.5	9.1±2.2	0.687
Fasting C-peptide (ng/ml)	1.8±0.9	1.7±0.8	0.885
Serum creatinine (mg/dl)	0.74±0.13	0.59±0.13	<0.001
Serum adiponectin (μg/ml)	5.84±3.67	7.66±4.93	<0.001
BAP (IU/l)	25.9±8.0	32.7±12.2	<0.001
OC (ng/ml)	4.4±1.9	7.0±3.0	<0.001
ucOC (ng/ml)	2.5±1.6	4.2±3.0	<0.001
uNTX (nMBCE/mM-Cr)	32.0±15.1	55.3±33.7	<0.001

BMI body mass index, *FPG* fasting plasma glucose, *HbA_{1c}* hemoglobin A_{1c}, *BAP* bone-specific alkaline phosphatase, *OC* osteocalcin, *ucOC* undercarboxylated osteocalcin, *uNTX* urinary N-terminal cross-linked telopeptide of type-I collagen, *p* probability value

Simple correlations between bone remodeling, adiponectin, and body composition parameters

Simple correlation analyses were also performed between bone markers versus adiponectin and body composition parameters in men (Table 2) and in postmenopausal women (Table 3). In men, log(OC) significantly and negatively correlated with body mass index (BMI), %fat, %trunk fat, visceral and subcutaneous fat area (at least $p<0.05$), and positively correlated with serum creatinine ($p=0.014$). Log (ucOC) significantly and negatively correlated with %trunk fat and V/S ratio ($p=0.008$ and $p=0.036$, respectively), and positively correlated with body height ($p=0.019$). ucOC/OC ratio significantly and positively correlated with body height, weight, BMI, and visceral and subcutaneous fat area (at least $p<0.05$). Log(BAP) significantly and negatively correlated with serum creatinine and V/S ratio ($p=0.026$ and $p=0.042$, respectively). Log(uNTX) significantly and negatively correlated with body weight, BMI, %fat, %trunk fat, visceral and subcutaneous fat area, and V/S ratio (at least $p<0.05$). On the other hand, in postmenopausal women, log(OC) significantly and negatively correlated

Table 2 The correlations between the values of bone markers versus fat mass, serum adiponectin, or glucose metabolism-related parameters in men with type 2 diabetes

	Log(OC)		Log(ucOC)		ucOC/OC		Log(BAP)		Log(uNTX)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age	0.04	0.586	−0.13	0.096	−0.14	0.072	−0.13	0.086	−0.03	0.702
Duration of diabetes	−0.05	0.544	0.02	0.811	−0.14	0.073	−0.06	0.465	−0.10	0.181
Body height	0.00	0.986	0.18	0.019	0.18	0.017	−0.03	0.712	−0.05	0.485
Body weight	−0.15	0.052	0.08	0.280	0.24	0.002	0.09	0.220	−0.19	0.010
Body mass index	−0.17	0.023	0.02	0.807	0.20	0.008	0.11	0.145	−0.21	0.005
Serum creatinine	0.18	0.014	0.15	0.051	0.03	0.725	−0.17	0.026	−0.30	<0.001
%Fat	−0.24	0.004	−0.09	0.287	0.11	0.182	−0.03	0.740	−0.31	<0.001
%Trunk fat	−0.29	<0.001	−0.22	0.008	0.00	0.960	0.00	0.999	−0.35	<0.001
Visceral fat area	−0.20	0.013	0.01	0.935	0.18	0.028	−0.02	0.823	−0.34	<0.001
Subcutaneous fat area	−0.17	0.033	0.08	0.332	0.25	0.002	0.07	0.384	−0.18	0.026
Visceral/subcutaneous fat ratio	−0.14	0.086	−0.17	0.036	−0.10	0.223	−0.16	0.042	−0.18	0.026
Log(total adiponectin)	0.00	0.959	0.09	0.252	0.10	0.211	−0.11	0.181	0.00	0.992
Fasting plasma glucose	−0.22	0.004	−0.19	0.013	−0.06	0.439	0.04	0.576	−0.05	0.529
HbA_{1c}	−0.21	0.006	−0.27	<0.001	−0.18	0.017	0.12	0.100	0.10	0.184
Fasting C-peptide	−0.12	0.134	−0.01	0.914	0.12	0.136	0.11	0.161	−0.19	0.012

OC osteocalcin, *ucOC* undercarboxylated osteocalcin, *BAP* bone-specific alkaline phosphatase, *Log* logarithm, HbA_{1c} hemoglobin, A_{1c}, *r* correlation coefficient, *p* probability value

with %trunk fat and visceral fat area (p=0.001 and p=0.048, respectively) and positively correlated with serum creatinine and log(adiponectin) (p=0.005 and p<0.001, respectively). Log(ucOC) significantly and negatively correlated with %fat and %trunk fat (p=0.049 and p=0.002, respectively). ucOC/OC significantly and negatively correlated with serum creatinine (p=0.033). Log(uNTX) significantly and negatively correlated with %fat, %trunk fat, and visceral and subcutaneous fat area (at least p<0.05) and positively correlated with log(adiponectin)

Table 3 The correlations between the values of bone markers versus fat mass, serum adiponectin, or glucose metabolism-related parameters in postmenopausal women with type 2 diabetes

	Log(OC)		Log(ucOC)		ucOC/OC		Log(BAP)		Log(uNTX)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age	0.09	0.362	0.08	0.381	0.01	0.892	−0.01	0.933	0.11	0.233
Duration of diabetes	0.13	0.176	0.00	0.992	−0.19	0.056	0.05	0.626	0.07	0.508
Body height	0.09	0.332	−0.06	0.534	−0.14	0.144	−0.01	0.955	−0.06	0.539
Body weight	−0.14	0.135	−0.15	0.109	−0.06	0.556	−0.12	0.194	−0.18	0.056
Body mass index	−0.18	0.055	−0.14	0.146	−0.01	0.928	−0.13	0.180	−0.18	0.058
Serum creatinine	0.26	0.005	0.02	0.840	−0.20	0.033	0.03	0.740	−0.11	0.247
%Fat	−0.20	0.065	−0.21	0.049	−0.13	0.227	−0.09	0.402	−0.26	0.015
%Trunk fat	−0.36	0.001	−0.34	0.002	0.19	0.086	−0.13	0.230	−0.39	<0.001
Visceral fat area	−0.21	0.048	−0.17	0.107	−0.05	0.627	0.01	0.916	−0.27	0.011
Subcutaneous fat area	−0.17	0.116	−0.10	0.330	−0.01	0.895	−0.08	0.480	−0.23	0.028
Visceral/subcutaneous fat ratio	−0.06	0.556	−0.09	0.412	−0.06	0.556	0.16	0.133	−0.04	0.747
Log(total adiponectin)	0.38	<0.001	0.16	0.099	−0.10	0.337	0.04	0.687	0.30	0.002
Fasting plasma glucose	−0.10	0.298	−0.18	0.063	−0.12	0.197	−0.12	0.215	0.08	0.387
HbA_{1c}	−0.20	0.039	−0.21	0.026	−0.08	0.405	−0.05	0.588	0.01	0.959
Fasting C-peptide	−0.08	0.421	−0.10	0.284	−0.07	0.457	−0.03	0.791	−0.16	0.106

OC osteocalcin, *ucOC* undercarboxylated osteocalcin, *BAP* bone-specific alkaline phosphatase, *Log* logarithm, HbA_{1c} hemoglobin A_{1c}, *r* correlation coefficient, *p* probability value