[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

(p=0.002). However, log(BAP) did not correlate with any parameter.

Simple correlations between bone remodeling and indices of glucose metabolism

Simple correlation analyses were performed between bone markers versus indices of glucose metabolism in men (Table 2) and in postmenopausal women (Table 3). In men, log(OC) significantly and negatively correlated with fasting plasma glucose (FPG) and HbA_{1c} (p=0.004 and p=0.006, respectively). Log(ucOC) significantly and negatively correlated with FPG and HbA_{1c} (p=0.013 and p<0.001). ucOC/OC ratio significantly and negatively correlated with HbA_{1c} (p=0.017). Log(uNTX) significantly and negatively correlated with fasting C-peptide (p=0.012). On the other hand, in postmenopausal women, log(OC) significantly and negatively correlated with HbA_{1c} (p=0.039). Log(ucOC) significantly and negatively correlated with HbA_{1c} (p=0.026). However, log(BAP) did not correlate with any parameter.

Adjusted analyses between bone markers versus adiponectin and body composition parameters

Next, to investigate whether bone markers were related to fat metabolism independent of age, duration of diabetes, body stature, and renal function as well as glucose metabolism, multiple regression analyses adjusted for age, duration of diabetes, body height, weight, serum creatinine, FPG, HbA_{1c}, and fasting C-peptide were performed between the levels of bone markers versus fat mass and serum adiponectin level in men and postmenopausal women (Table 4). In men, log(OC) and log(ucOC) significantly and negatively correlated with %trunk fat and V/S ratio (at least p<0.05). ucOC/OC ratio significantly and positively correlated with log(adiponectin) (p= 0.015). Log(uNTX) significantly and negatively correlated with %trunk fat, visceral fat area, and V/S ratio (p= 0.004, p=0.016, and p=0.032, respectively). In postmenopausal women, log(OC) significantly and negatively correlated with %trunk fat and visceral fat area (p=0.029 and p= 0.034, respectively). Log(OC) and log(uNTX) significantly and positively correlated with log(adiponectin; p<0.001 and p=0.036, respectively). In contrast, log(BAP) did not correlate with any parameter in either men or postmenopausal women.

Adjusted analyses between bone remodeling and indices of glucose metabolism

Next, to investigate whether bone markers were related to glucose metabolism independent of age, duration of diabetes, body stature, and renal function, as well as fat mass and serum adiponectin, multiple regression analyses

Table 4 Adjusted analyses between the values of bone markers versus fat mass or serum adiponectin level in men and postmenopausal women with type 2 diabetes

	Log(OC)		Log(ucOC)		ucOC/OC		Log(BAP)		Log(uNTX)	
	β	p	β	p	β	p	β	p	β	p
Men										
%Fat	−0.19	0.123	−0.07	0.603	−0.01	0.928	−0.25	0.064	−0.24	0.052
%Trunk fat	−0.26	0.004	−0.27	0.003	−0.16	0.108	−0.06	0.552	−0.26	0.004
Visceral fat area	−0.16	0.188	−0.08	0.487	−0.09	0.491	−0.10	0.441	−0.28	0.016
Subcutaneous fat area	−0.01	0.980	0.19	0.271	0.18	0.323	−0.17	0.374	0.03	0.883
Visceral/subcutaneous fat ratio	−0.18	0.024	−0.19	0.017	−0.12	0.171	−0.11	0.215	−0.17	0.032
Log(adiponectin)	−0.07	0.405	0.13	0.114	0.21	0.015	−0.05	0.544	−0.02	0.852
Postmenopausal women										
%Fat	−0.04	0.806	−0.18	0.334	−0.18	0.313	0.18	0.317	−0.15	0.401
%Trunk fat	−0.26	0.029	−0.22	0.075	−0.07	0.573	−0.06	0.660	−0.22	0.075
Visceral fat area	−0.36	0.034	−0.29	0.096	−0.07	0.687	0.13	0.478	−0.20	0.254
Subcutaneous fat area	−0.04	0.854	−0.17	0.459	−0.25	0.250	0.07	0.772	−0.25	0.270
Visceral/subcutaneous fat ratio	−0.18	0.110	−0.12	0.334	0.01	0.904	0.13	0.298	0.03	0.789
Log(adiponectin)	0.41	<0.001	0.17	0.149	−0.08	0.455	0.02	0.884	0.24	0.036

Multiple regression analysis was performed between bone markers versus fat mass, glucose metabolism, or serum adiponectin adjusted for age, duration of diabetes, body height, weight, serum creatinine, fasting plasma glucose, HbA_{1c}, and fasting C-peptide

Log logarithm, *OC* osteocalcin, *ucOC* undercarboxylated osteocalcin, *BAP* bone-specific alkaline phosphatase, *uNTX* urinary N-terminal cross-linked telopeptide of type-I collagen, β standard partial regression coefficient, p probability value

adjusted for age, duration of diabetes, body height, weight, serum creatinine, %fat, %trunk fat, visceral and subcutaneous fat area, V/S ratio, and log(adiponectin) were performed between the levels of bone markers versus FPG, HbA_{1c}, and fasting C-peptide in men and postmenopausal women (Table 5). In men, log(OC) and log(ucOC) significantly and negatively correlated with FPG ($p=0.003$ and $p<0.001$), and log(ucOC) significantly and negatively correlated with HbA_{1c} ($p=0.017$). In postmenopausal women, log(OC) significantly and negatively correlated with FPG ($p=0.045$). In contrast, ucOC/OC ratio, log (BAP), and log(uNTX) did not correlate with any parameter in either men or postmenopausal women.

We also performed simple correlations and adjusted analyses between bone remodeling and parameters for fat/glucose metabolism after excluding patients using insulin. The results were almost similar, although some of them were not significant because the statistical power was lost due to the reduction in the study population (data not shown).

Discussion

In this study, we found that the serum ucOC value was negatively correlated with %trunk fat and V/S ratio and that ucOC/OC ratio positively correlated with serum adiponectin level in men. In addition, ucOC level was negatively correlated with FPG and HbA_{1c} in men. These findings suggest that ucOC is associated with fat mass, especially visceral fat, as well as with glucose level in diabetic men. Taken together, the present study seems to be the first clinical one suggesting that not only OC but also ucOC is associated with lipid/glucose metabolism in type 2 diabetes.

Recent studies have shown that fat mass could influence bone metabolism independent of body weight through adipocytokines, which are secreted from adipocytes [9, 11, 25]. On the other hand, recent two animal studies have shown that OC derived from osteoblasts function as a hormone regulating glucose metabolism and fat mass [14, 15]. Lee et al. showed that mice deficient of *ESP*, a model of gain of osteocalcin bioactivity, displayed decreased fat mass, and increased serum adiponectin levels [14]. In contrast, they showed that OC-deficient mice displayed increased fat mass and decreased serum adiponectin levels [14]. Ferron et al. showed that recombinant ucOC injection decreased fat mass and increased the expression of adiponectin from white fat in wild-type mice [15]. They have also shown that ucOC regulated β cell gene expression and affected plasma glucose levels in vivo [14, 15]. These experiments suggest that bone metabolism and lipid/glucose metabolism may have a common pathogenetic basis between them, which seems to accord with our clinical findings.

Several studies have indicated that hyperglycemia induces a low turnover of bone by evoking osteoblast dysfunction and suppressing serum OC levels [26, 27]. We and others have also shown that serum OC, a marker for mature osteoblasts, was increased, whereas BAP, a marker for immature osteoblasts, was decreased after treatments of diabetes [24, 28]. Our recent in vitro study has shown that advanced glycation end-products in combination with high glucose-decreased OC expression and mineralization in osteoblastic MC3T3-E1 cells [29]. On the other hand, studies on bone resorption status in diabetes are limited, and the results are conflicting [24, 28, 30]. Our previous clinical findings showed that glucose levels were not

Table 5 Adjusted analyses between the values of bone markers versus glucose metabolism-related parameters in men and postmenopausal women with type 2 diabetes

	Log(OC)		Log(ucOC)		ucOC/OC		Log(BAP)		Log(uNTX)	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Men										
Fasting plasma glucose	−0.28	0.003	−0.32	<0.001	−0.17	0.063	0.04	0.661	−0.04	0.684
HbA_{1c}	−0.17	0.085	−0.22	0.017	−0.10	0.282	−0.02	0.841	−0.14	0.121
Fasting C-peptide	−0.10	0.387	−0.15	0.191	−0.12	0.321	0.01	0.927	−0.09	0.454
Postmenopausal women										
Fasting plasma glucose	−0.23	0.045	−0.22	0.089	−0.08	0.534	−0.09	0.487	−0.06	0.676
HbA_{1c}	−0.18	0.153	−0.18	0.220	−0.03	0.843	−0.20	0.170	0.07	0.649
Fasting C-peptide	0.06	0.711	-0.03	0.849	-0.12	0.495	0.00	0.988	-0.08	0.638

Multiple regression analysis was performed between bone markers versus fat mass, glucose metabolism, or serum adiponectin adjusted for age, duration of diabetes, body height, weight, serum creatinine, %fat, %trunk fat, visceral fat area, subcutaneous fat area, visceral/subcutaneous fat ratio, and log(adiponectin)

Log logarithm, *OC* osteocalcin, *ucOC* undercarboxylated osteocalcin, *BAP* bone-specific alkaline phosphatase, *uNTX* urinary N-terminal cross-linked telopeptide of type-I collagen, *HbA_{1c}* hemoglobin A_{1c}, *β* standard partial regression coefficient, *p* probability value

significantly associated with uNTX [31] and that this bone resorption marker was not changed during glycemic control [24]. In this study, we found that the values of OC and ucOC, but not BAP or uNTX, were associated with glucose levels. These findings suggest that mature osteoblast function is specifically affected by glucose metabolism in type 2 diabetes.

Previous studies on non-diabetic mice and humans showed that serum OC could modulate insulin sensitivity and secretion [14, 15, 18, 19]. However, Shea et al. recently reported that serum ucOC was not associated with the parameters of insulin resistance in non-diabetic subjects [32]. We found that there was no significant correlation between OC or ucOC versus fasting C-peptide, which is a surrogate marker for endogenous insulin secretion, in diabetic patients. Thus, the association between OC or ucOC versus glucose levels in this study seems to be not because OC-modulated insulin sensitivity or secretion, but because high glucose levels suppressed osteoblast function and OC expression. The lack of associations between OC or ucOC versus fasting C-peptide in this study might be partly explained by the existence of diabetes, or alternatively, by the fact that our patients have received several treatments that affect insulin secretion, including sulfonylureas and exogenous insulin. We are unable to completely exclude the effects of these drugs when interpreting insulin secretion or sensitivity in our study population.

This study has several limitations in addition to not excluding subjects who underwent insulin or sulfonylurea treatments. First, the sample size was not large enough to make definite conclusions. Second, the subjects in this study included a wide range of ages. Therefore, we cannot exclude the possibility that there are age-related differences between bone metabolism and glucose/fat metabolism, although the multiple regression analyses were performed adjusted for age. Third, we analyzed only subjects who visited Shimane University Hospital, a tertiary center, for evaluation or treatment of diabetes mellitus and osteoporosis. Therefore, the study results only apply to diabetic subjects, and the patients enrolled in this study might have relatively severe states of the disorders and might not be representative of other Japanese men and postmenopausal women with the disorders. Fourth, although many of the variables evaluated in terms of correlation with OC are internally correlated with each other (for example, % fat mass, %trunk fat, BMI, and weight), the statistical significance (p value<0.05) was not adjusted for multiple testing. Fifth, measurement of ucOC could be affected not only by vitamin K status, but also by the total amount of OC in the sample [33]. Therefore, the difficulties of measuring ucOC might affect the results in the present study. Finally, the conclusions of this study are weakened by its cross-sectional design and the absence of age-matched healthy controls. In addition, we could not reproduce the association of the serum ucOC level with fat mass and glucose level in the postmenopausal women. These sex-related differences might depend on the background data such as age, fat mass, serum adiponectin, and serum ucOC level (Table 1). Therefore, further studies are needed to clarify the mechanism responsible for the lack of this association in women.

In conclusion, we found that ucOC and ucOC/OC ratio were associated with visceral fat mass and serum adiponectin levels as well as with plasma glucose level in men with type 2 diabetes. These findings support the recent observation that bone metabolism and its markers, OC and ucOC, link to glucose/fat metabolism.

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2. 慢性合併症―その他

骨病変

Bone Disorders

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2000年の米国国立衛生研究所（National Institutes of Health；NIH）のコンセンサス会議において，骨粗鬆症は骨強度が低下して骨折しやすくなる骨格疾患と定義された．古くより長期に血糖コントロール不良の糖尿病患者には骨粗鬆症が多いことが知られていたが，近年メタ解析により，1型糖尿病ならびに2型糖尿病のいずれも大腿骨頸部骨折リスクが高いことが示され，糖尿病では骨粗鬆症のリスクが高いことが改めて確認された．

病期・分類

糖尿病は原発性骨粗鬆症の診断基準（2000年度改訂版）において，続発性骨粗鬆症に分類される．上述のメタ解析では糖尿病の病型を問わずDXA（dual-energy X-ray absorptiometry）法によるBMD（bone mineral density；骨密度）で予測される以上に大腿骨頸部骨折リスクが高かった[1]．対照群と異なり椎体骨折もBMD非依存性に骨折リスクが増加する[2]．骨強度は「BMD」と「骨質」の要因から構成されることから，糖尿病患者では骨質低下により骨強度が低下しやすいと考えられている．

一方，一部の糖尿病治療薬が骨折に影響することが指摘されている．大規模調査において，インスリン分泌刺激薬やメトホルミンは有意な骨折リスクの増加の報告はなく，インスリン治療者の骨折リスクについては意見が分かれている．一方1年以上のチアゾリジン誘導体服用者では，性別にかかわらず，腰椎および大腿骨頸部のBMDが低下し，女性では全骨折リスクが増加することがメタ解析で明らかとなった．若年者では足部や下肢長幹骨骨折のような非脆弱性骨折が増加し，高齢者では大腿骨頸部骨折および椎体骨折が増加することが示されている．同薬はPPARγ（peroxisome proliferator-activated receptor γ；核内受容体型転写因子）を介して骨芽細胞の分化を低下させ，骨形成が低下して骨強度が低下すると考えられている．

検査・診断

現在のところ糖尿病を含め続発性骨粗鬆症に対する個別の骨粗鬆症の診断基準は存在しない．原発性骨粗鬆症の診断基準（表1）に従い，低骨密度に起因する骨強度の低下による骨折リスクの有無を判定する．すなわち低骨量が原因で軽微な外力によって発生した非外傷性骨折（脆弱性骨折）歴の有無と，BMDまたは胸腰椎2方向の脊椎エックス線像による骨粗鬆化判定の2項目によって診断する．4cm以上の身長の短縮は椎体骨折の可能性が高いので，エックス線撮影を行い椎体骨折の判定基準に従い椎体骨折の有無を判別する．

一般に糖尿病患者，とりわけ男性は椎体の骨棘や腹部大動脈の石灰化がBMD計測値に影響するため，腰椎に加えて大腿骨近位部のBMD測定を行い骨量低下の有無の判断をすることが望ましい．「脆弱性骨折」がない場合，①BMD値がYAM（young adult mean；若年成人平均値）の70%未満であれば「骨粗鬆症」，②70～80%ならば「骨量減少」と診断する．「脆弱性骨折」を有する場合には，BMD値がYAMの70%未満でなくとも骨粗鬆症と診断される．

一方，既述したように糖尿病では骨質の影

表1　原発性骨粗鬆症の診断基準

低骨量をきたす骨粗鬆症以外の疾患または続発性骨粗鬆症を認めず，骨評価の結果が下記の条件を満たす場合，原発性骨粗鬆症と診断する．

I. 脆弱性骨折[注1]あり			
II. 脆弱性骨折なし			
	骨密度値[注2] YAM：若年成人平均値（20～44歳）	脊椎エックス線像での骨粗鬆化[注3]	従来の骨萎縮度判定基準
正常	YAMの80%以上	なし	骨萎縮なし
骨量減少	YAMの70～80%	疑いあり	骨萎縮度I度
骨粗鬆症	YAMの70%未満	あり	骨萎縮度II度以上

注1）脆弱性骨折：低骨量（骨密度がYAMの80%未満，あるいは脊椎エックス線像で骨粗鬆化がある場合）が原因で，軽微な外力によって発生した非外傷性骨折．骨折部位は脊椎，大腿骨頸部，橈骨遠位端，その他．

注2）骨密度は原則として腰椎骨密度とする．ただし，高齢者において，脊椎変型などのために腰椎骨密度の測定が適当でないと判断される場合には，大腿骨頸部骨密度とする．これらの測定が困難な場合は，橈骨，第二中手骨，踵骨の骨密度を用いる．

注3）脊椎エックス線像での骨粗鬆化の評価は，従来の骨萎縮度判定基準を参考にして行う．

（折茂　肇他：日本骨代謝学会雑誌 2001；18：76-82より引用）

響を強く受ける．骨質は構造特性と材質特性から構成されるが，AGEs（advanced glycation end-products；終末糖化物質）が後者と関係することが報告されている．AGEsの1つであるペントシジンは，動物実験により骨基質のコラーゲン架橋に影響し，骨の材質特性を劣化させ骨強度を低下させることが知られている．近年ペントシジンの増加が閉経後2型糖尿病女性の椎体骨折リスクと有意に相関することが見出され，ペントシジンが材質特性を反映した骨質マーカーとなりうる可能性が示唆されている．しかしペントシジンの測定は保険診療では認められておらず，日常診療で利用できる骨質評価法の確立は今後の課題である．

治療・予後

糖尿病患者の骨粗鬆症治療では骨質改善作用のあるものが適していると考えられる．高ホモシステイン血症による骨量低下のない骨質低下モデルにおいて，ラロキシフェンは骨内のペントシジン含有量を低下させ骨強度が増すことが示されており，糖尿病患者の骨折防止効果が期待される．しかし糖尿病患者を対象にした，現在の限られた骨粗鬆症治療薬の臨床成績からは，対照群と同等のBMD増加効果があるビスホスホネートが糖尿病患者の骨粗鬆症治療において第一選択になるものと思われる．

糖尿病治療薬の選択も重要である．米国糖尿病協会および欧州糖尿病学会は2型糖尿病患者治療において，ピオグリタゾンを骨量減少のない患者に対して処方するよう推奨した[3]．私見ではあるが，原発性骨粗鬆症において骨密度とは独立した骨折の危険因子として同定されている，高齢，既存骨折，喫煙，飲酒，ステロイド使用歴，骨折家族歴，運動不足および易転倒性の有無を考慮して糖尿病治療薬を選択すると，骨折リスクの増加を防ぎうると思われる．

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Ⅳ. その他話題の糖尿病合併症

骨粗鬆症

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