CLINICAL STUDY

Relationships between serum adiponectin levels versus bone mineral density, bone metabolic markers, and vertebral fractures in type 2 diabetes mellitus

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Abstract

Background: Although, adiponectin might be associated with bone metabolism, the relationships between serum adiponectin and bone mineral density (BMD) as well as vertebral fracture in type 2 diabetes are still unclear.

Objective and methods: We investigated the relationships between each of serum total and high molecular weight (HMW) adiponectin versus BMD, bone markers, and the presence of vertebral fractures in a total of 231 men and 170 post-menopausal women with type 2 diabetes.

Results: Multiple regression analysis adjusted for age, duration of diabetes, BMI, serum creatinine, and HbA_{1c} showed that serum total adiponectin was negatively correlated with BMD at the total, lumbar spine, and femoral neck ($r = -0.165$, $P < 0.05$; $r = -0.187$, $P < 0.05$; and $r = -0.136$, $P < 0.05$ respectively) and positively with urinary N-terminal cross-linked telopeptide of type-I collagen in men ($r = 0.148$, $P < 0.05$), and that serum HMW adiponectin was negatively correlated with BMD at the lumbar spine ($r = -0.146$, $P < 0.05$). Multivariate logistic regression analysis adjusted for the parameters described above showed that total adiponectin was associated with the presence of vertebral fractures in men (odds ratio (OR) = 1.396, 95% confidential interval (CI) 1.020–1.911 per s.d. increase, $P < 0.05$), and both total and HMW adiponectin were associated with moderate or severe vertebral fractures (OR = 1.709, 95% CI 1.048–2.787 per s.d. increase, $P < 0.05$ and OR = 1.810, 95% CI 1.112–2.946 per s.d. increase, $P < 0.05$ respectively), but not in post-menopausal women.

Conclusions: Serum adiponectin could be associated with BMD and turnover and clinically useful for assessing the risk of vertebral fractures in type 2 diabetic men.

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Introduction

Cumulative evidence has shown 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 that 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, s.c. 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, suggesting that adiponectin could also influence bone metabolism (9, 10).

Several clinical studies have shown that serum adiponectin level was negatively correlated with BMD (11–15), while others showed no significant correlation (16–19) or a positive correlation with BMD (20). On the other hand, it is still unclear whether or not serum adiponectin level is associated with bone fractures, although one cohort study showed no significant correlation between serum adiponectin and fracture risk (21). Thus, the relationships of serum adiponectin with BMD and vertebral fractures need to be clarified further to solve the discrepancy and the lack of data respectively.

Although patients with type 2 diabetes show no apparent bone mass reduction compared with non-diabetic subjects, their fracture risks are known to increase approximately 1.5-fold at the hip, proximal humerus, forearm, and foot (22–25). On the other hand, two large-scale studies have shown that a risk for vertebral fractures was not significantly higher in patients with type 2 diabetes than in those without

diabetes (22, 23). However, we found that lumbar BMD was not associated with the presence of vertebral fractures in the group, suggesting the insensitiveness of BMD to assess their fracture risk (26). Thus, surrogate markers that supplement BMD and detect the fracture presence are required. We have recently shown that serum insulin-like growth factor-1 and pentosidine levels could be clinically useful for assessing the risk of vertebral fractures independent of BMD in post-menopausal women with type 2 diabetes (27, 28). However, we found that these parameters were not effective in the male counterpart, and thus some other biochemical markers are needed to assess the fracture risk in them.

Serum adiponectin may be a potential candidate for assessing the risk of vertebral fractures in diabetic men. Lenchik *et al.* showed that adiponectin exerted an independent negative correlation with BMD in subjects including 86% with type 2 diabetes (11). By contrast, Tamura *et al.* showed that adiponectin was positively correlated with BMD at the radius in diabetic patients (20). The discrepancy between the two studies might be partly because they lumped men and women together, although serum adiponectin concentration is known to be different between the sexes (29, 30).

Recently, the difference in molecular weight of adiponectin is known to be important for its function. Adiponectin exists in the circulation as a trimer (low molecular weight (LMW)), a hexamer (medium molecular weight (MMW)), and a high molecular weight (HMW) form. Previous study showed that only HMW adiponectin could induce activation of AMP-activated protein kinase (AMPK) in hepatocytes, while both total and HMW adiponectin could activate AMPK in myocytes (30). On the other hand, some investigators have indicated that LMW and HMW adiponectin activated different signal transduction pathways via changes in its oligomerization state (31). Thus, biological activities among these isoforms of adiponectin are still unclear, especially in bone cells.

In this study, to address these issues, we measured serum total and HMW adiponectins in Japanese men and post-menopausal women with type 2 diabetes, and investigated the relationship of each of the hormonal levels to BMD, bone metabolic markers, and the presence of vertebral fractures separately in each sex.

Subjects and methods

Subjects

The subjects in this study were 231 men and 170 post-menopausal women with type 2 diabetes (age; mean 59.6 and 66.7 respectively). We consecutively recruited subjects who visited Shimane University Hospital for an education, evaluation, or treatment of diabetes. All women had been without spontaneous menses for more

than 1 year. Nobody had hepatic or renal dysfunction, disturbance of physical activity, or nutritional derangements that might cause changes in bone metabolism. Forty-one, 69, 24, and 34 men, as well as 45, 52, 35, and 20 women had been taking insulin treatment, sulfonylurea, metformin, and α -glucosidase inhibitor respectively. Subjects treated with thiazolidinedione were excluded in this study. All subjects were free of drugs known to influence bone and calcium metabolism like vitamin D, bisphosphonate, and estrogen replacement therapy until the time of the present study. Sixty-three (27%) men and 58 (34%) women had diabetic retinopathy, while 132 (57%) men and 97 (57%) women had diabetic neuropathy. One hundred and three (45%) men and 6 (4%) women were current smokers, and 79 (34%) men and 3 (2%) women consumed over 1 U/day of alcohol. This study was cross-sectional and approved by the ethical review board of our institution and complied with the Helsinki declaration. All subjects agreed to participate in the study and gave informed consent.

Radiography

Lateral X-ray films of the thoracic and lumbar spine were taken at the same week of the serum collection. The anterior, central, and posterior heights of each of the 13 vertebral bodies from Th4-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 >20% compared with the height of the nearest uncompressed vertebral body (32). Vertebral fractures were classified as follows; mild, a reduction of 20–25%; moderate, 25–40%; severe, more than 40%. None of the subjects had a history of serious trauma.

BMD and biochemical measurements

BMD values of the total (T), 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 s.d.

After overnight fasting, serum and first-void urine samples were collected. Biochemical markers were measured by standard biochemical methods. Hemoglobin A_{1c} (HbA_{1c}) was determined by HPLC. Osteocalcin and urinary N-terminal cross-linked telopeptide of type-I collagen (uNTX) were measured by RIA and ELISA respectively, as previously described (33, 34). Serum HMW adiponectin levels were

measured by an ELISA kit (Fujirebio, Tokyo, Japan) as indicated by the manufacturer. In brief, 96 wells of a microtiter plate were coated with anti-HMW adiponectin MAB. One hundred μ l of serum samples diluted 1:441 was placed in each of the 96 wells. The MAB conjugated with HRP was used as the detecting antibody. Contents of wells were incubated for 30 min with tetramethylbenzidine. After the reaction was stopped, the absorbance was measured at 450 nm. The coefficient of variation of measurements of HMW adiponectin was 2.0%. Serum total adiponectin levels were measured by another ELISA kit (Otsuka Pharmaceuticals, Tokyo, Japan) as indicated by the manufacturer. In brief, after boiling serum samples in SDS buffer for 5 min to convert all adiponectin to a monomeric form, samples were analyzed with the ELISA system to determine total adiponectin in serum. The coefficient of variation of measurements of total adiponectin was 3.1%.

Statistical analysis

Data were expressed as mean $\pm$ s.d. Because serum total and HMW adiponectin levels showed markedly skewed distributions, logarithmic transformation (log) of these values were carried out before performing correlation and regression analysis. Statistical significance between two groups was determined using Student's *t*-test. Simple, multiple, and logistic regression analysis were

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

Table 1 compares the male and post-menopausal female diabetic patients with respect to demographic and biochemical parameters and BMD. Patient age, serum total, and HMW adiponectins, osteocalcin, and uNTX were significantly lower in the males than in the females ($P < 0.0001$). On the other hand, body height, body weight, creatinine, absolute BMD at each site were significantly higher in the males than in the females ($P < 0.0001$).

Relationship between each of serum total and HMW adiponectin levels versus BMD at each skeletal site and bone metabolic markers

Our simple regression analysis showed that serum total and HMW adiponectin levels were significantly affected by age and body stature (Table 2). Thus, multiple regression analyses were performed between each of the serum adiponectin levels versus BMD at each skeletal site and bone metabolic markers adjusted for age, body mass index (BMI), as well as duration of diabetes, serum

Table 1 Baseline characteristics of subjects.

	Men	Postmenopausal women	P
Number of subjects	231	170	
Age (years)	59.6 $\pm$ 13.3	66.7 $\pm$ 10.2	<0.0001
Diabetes duration (years)	10.7 $\pm$ 9.0	12.3 $\pm$ 9.9	0.0619
Body height (cm)	165.9 $\pm$ 6.7	150.4 $\pm$ 6.0	<0.0001
Body weight (kg)	65.4 $\pm$ 15.8	55.4 $\pm$ 11.0	<0.0001
BMI (kg/m ²)	23.6 $\pm$ 4.7	24.5 $\pm$ 4.5	0.0563
HbA1c (%)	8.8 $\pm$ 2.6	8.4 $\pm$ 2.4	0.6918
Creatinine (mg/dl)	0.80 $\pm$ 0.18	0.64 $\pm$ 0.17	<0.0001
Total adiponectin (μ g/ml)	6.31 $\pm$ 4.10	8.80 $\pm$ 5.85	<0.0001
HMW adiponectin (μ g/ml)	6.26 $\pm$ 5.61	9.08 $\pm$ 7.32	<0.0001
Total BMD (g/cm ²)	1.080 $\pm$ 0.113	0.926 $\pm$ 0.114	<0.0001
L2-4 BMD (g/cm ²)	1.044 $\pm$ 0.189	0.881 $\pm$ 0.190	<0.0001
T score	-0.03 $\pm$ 1.58	-1.12 $\pm$ 1.71	<0.0001
Z score	0.48 $\pm$ 1.16	0.62 $\pm$ 1.22	0.2724
F BMD (g/cm ²)	0.786 $\pm$ 0.133	0.643 $\pm$ 0.131	<0.0001
T score	-0.62 $\pm$ 1.05	-1.33 $\pm$ 1.20	<0.0001
Z score	0.33 $\pm$ 1.09	0.50 $\pm$ 1.22	0.1507
1/3R BMD (g/cm ²)	0.712 $\pm$ 0.069	0.533 $\pm$ 0.090	<0.0001
T score	-1.53 $\pm$ 1.30	-2.49 $\pm$ 1.72	<0.0001
Z score	-0.59 $\pm$ 1.15	0.63 $\pm$ 1.50	<0.0001
Osteocalcin (ng/ml)	5.0 $\pm$ 2.4	7.2 $\pm$ 2.9	<0.0001
uNTX (nMBCE/mM-Cr)	32.6 $\pm$ 17.3	54.9 $\pm$ 33.9	<0.0001
Vertebral fracture	80 (34.6%)	52 (30.6%)	0.4567
Vertebral fracture (moderate or severe)	19 (8.2%)	21 (12.4%)	0.2321
Osteopenia	52 (22.5%)	69 (40.5%)	0.5635
Osteoporosis	11 (4.8%)	31 (18.2%)	0.1140

BMI, body mass index; FPG, fasting plasma glucose; HbA_{1c}, hemoglobin A_{1c}; HMW, high molecular weight; BMD, bone mineral density; L, lumbar; F, femoral neck; 1/3R, one-third of the radius; uNTX, urinary N-terminal cross-linked telopeptide of type-I collagen.

Table 2 The correlations between serum adiponectin versus bone mineral density, bone metabolic marker, or other variables.

	Men				Postmenopausal women			
	Log (total adiponectin)		Log (HMW adiponectin)		Log (total adiponectin)		Log (HMW adiponectin)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age	0.292	<0.0001	0.202	0.0021	0.323	<0.0001	0.264	0.0005
Duration of diabetes	0.203	0.0022	0.214	0.0012	0.310	0.0001	0.288	0.0003
Body height	−0.143	0.0295	−0.074	0.2650	−0.139	0.0711	−0.108	0.1613
Body weight	−0.409	<0.0001	−0.229	0.0005	−0.344	<0.0001	−0.322	<0.0001
BMI	−0.432	<0.0001	−0.247	0.0001	−0.314	<0.0001	−0.306	<0.0001
HbA _{1c}	−0.102	0.1223	−0.053	0.4251	0.077	0.3194	0.138	0.0743
Creatinine	0.081	0.2190	0.053	0.4231	0.120	0.1178	−0.034	0.6591
Total BMD	−0.301	<0.0001	−0.173	0.0145	−0.264	0.0012	−0.194	0.0195
L2-4 BMD	−0.220	0.0009	−0.180	0.0072	−0.205	0.0072	−0.128	0.0954
Z score	−0.163	0.0150	−0.112	0.0949	−0.085	0.2748	−0.034	0.6601
F BMD	−0.326	<0.0001	−0.209	0.0019	−0.293	0.0002	−0.297	0.0001
Z score	−0.211	0.0017	−0.138	0.0411	−0.184	0.0193	−0.225	0.0041
1/3R BMD	−0.252	0.0002	−0.136	0.0477	−0.286	0.0003	−0.203	0.0110
Z score	−0.041	0.5551	−0.051	0.4629	−0.099	0.2176	−0.054	0.5052
Osteocalcin	0.183	0.0083	0.084	0.2300	0.196	0.0156	0.051	0.5351
uNTX	0.189	0.0045	0.107	0.1124	0.113	0.1540	0.177	0.0241

BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}; BMD, bone mineral density; L, lumbar; F, femoral neck; 1/3R, one-third of the radius; uNTX, urinary N-terminal cross-linked telopeptide of type-I collagen; HMW, high molecular weight.

creatinine, and HbA_{1c} (Table 3). In men, log (total adiponectin) was significantly and negatively correlated with T-, L-, and F-BMD ($P < 0.05$) and positively correlated with uNTX ($P < 0.05$), while log (HMW adiponectin) was only significantly and negatively correlated with L-BMD ($P < 0.05$). On the other hand, in post-menopausal women, neither log (total adiponectin) nor log (HMW adiponectin) were correlated with BMD at any site or any bone metabolic markers, except that log(total adiponectin) was significantly and positively correlated with osteocalcin ($P < 0.01$).

Comparison of serum adiponectin levels and other variables between patients with and without vertebral fractures

Next, we compared serum total and HMW adiponectin levels and other parameters between patients with and

without vertebral fractures or with moderate or severe vertebral fractures (Table 4). The male and post-menopausal female patients with vertebral fractures or with moderate or severe vertebral fractures were significantly older ($P < 0.05$), shorter in height ($P < 0.05$), lower in absolute values of T-BMD ($P < 0.05$), and L-BMD ($P < 0.05$) than their counterparts without fractures. The post-menopausal female patients with vertebral fractures or with moderate or severe vertebral fractures had significantly lower absolute F-BMD and 1/3R-BMD than those without fractures ($P < 0.05$). The post-menopausal female patients with moderate or severe vertebral fractures had significantly lower Z score of F-BMD than those without fractures ($P < 0.05$). Serum total adiponectin level was significantly higher in men and post-menopausal women with vertebral fractures or with moderate or severe vertebral fractures than in those

Table 3 The correlations between serum adiponectin versus bone mineral density or bone metabolic marker.

	Men				Postmenopausal women			
	Log (total adiponectin)		Log (HMW adiponectin)		Log (total adiponectin)		Log (HMW adiponectin)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Total BMD	−0.165	0.0356	−0.058	0.4293	−0.118	0.1741	−0.056	0.5156
L2-4 BMD	−0.187	0.0133	−0.146	0.0382	−0.071	0.4028	−0.016	0.8517
F BMD	−0.136	0.0480	−0.086	0.1763	−0.096	0.2216	−0.150	0.0557
1/3R BMD	−0.129	0.0715	−0.040	0.5475	−0.098	0.2237	−0.038	0.6383
Osteocalcin	0.078	0.3020	0.025	0.7264	0.269	0.0033	0.105	0.2554
uNTX	0.148	0.0489	0.074	0.2920	0.053	0.5293	0.075	0.3789

Multiple regression analysis was performed between adiponectin versus BMD at each skeletal site and bone markers adjusted for age, duration of diabetes, BMI, creatinine, and HbA_{1c}. BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}; BMD, bone mineral density; L, lumbar; F, femoral neck; 1/3R, one-third of the radius; uNTX, urinary N-terminal cross-linked telopeptide of type-I collagen; HMW, high molecular weight

Table 4 Comparison of demographic and biochemical parameters including serum total and high molecular weight adiponectins between subjects with and without vertebral fractures.

	Men					Postmenopausal women				
	No vertebral fracture	Vertebral fracture	Moderate or severe Vertebral fracture		<i>P</i>	No vertebral fracture	Vertebral fracture	Moderate or severe Vertebral fracture		<i>P</i>
		<i>P</i>		<i>P</i>			<i>P</i>		<i>P</i>	
Number of patients	151	80		19		118	52		21	
Age	57.8±13.3	63.0±12.7	0.0052	65.1±10.0	0.0240	64.7±9.8	71.3±9.8	<0.0001	74.2±10.3	0.0001
Duration of diabetes	10.2±9.2	11.4±8.7	0.3421	15.7±10.3	0.0199	11.5±9.6	14.2±10.3	0.1172	16.0±10.3	0.0717
Body height	167.1±6.5	163.8±6.7	0.0004	163.8±5.2	0.0380	151.3±5.7	148.3±6.0	0.0029	145.3±6.1	<0.0001
Body weight	67.0±17.3	62.3±12.0	0.0339	63.1±7.6	0.3352	56.2±10.8	53.6±11.5	0.1545	51.1±13.8	0.0658
BMI	23.9±5.2	23.1±3.6	0.2795	23.5±2.7	0.7684	24.5±4.3	24.3±5.0	0.8000	24.2±6.0	0.7546
HbA1c	8.9±2.8	8.8±2.2	0.7644	8.5±1.9	0.5694	8.8±2.3	8.8±2.6	0.9720	8.8±2.5	0.9919
Creatinine	0.79±0.18	0.80±0.19	0.8403	0.83±0.23	0.4664	0.63±0.17	0.68±0.17	0.0709	0.65±0.14	0.5123
Total adiponectin	5.84±3.78	7.19±4.55	0.0165	8.39±4.50	0.0073	8.04±4.98	10.53±7.22	0.0101	10.82±7.51	0.0353
HMW adiponectin	5.88±4.86	6.97±6.77	0.1581	9.72±7.73	0.0030	8.38±6.80	10.67±8.24	0.0599	10.67±8.71	0.1839
Total BMD	1.094±0.115	1.053±0.103	0.0137	1.030±0.103	0.0317	0.944±0.113	0.882±0.103	0.0021	0.853±0.093	0.0035
L2-4 BMD	1.063±0.203	1.009±0.154	0.0432	0.966±0.153	0.0464	0.902±0.172	0.832±0.219	0.0246	0.767±0.272	0.0037
Z score	0.56±1.26	0.33±0.93	0.1444	0.07±0.87	0.1004	0.70±1.14	0.45±1.40	0.2234	0.11±1.84	0.0556
F BMD	0.798±0.137	0.764±0.122	0.0764	0.745±0.107	0.1104	0.661±0.124	0.603±0.138	0.0085	0.537±0.162	0.0003
Z score	0.38±1.17	0.25±0.92	0.4152	0.19±0.91	0.5097	0.58±1.18	0.31±1.31	0.2010	-0.16±1.70	0.0224
1/3R BMD	0.716±0.072	0.704±0.063	0.2191	0.702±0.069	0.4166	0.544±0.090	0.509±0.087	0.0292	0.497±0.077	0.0432
Z score	-0.56±1.18	-0.64±1.09	0.6528	-0.47±0.99	0.7548	0.64±1.57	0.61±1.33	0.9230	0.77±1.42	0.7471
Osteocalcin	5.1±2.5	4.8±2.3	0.4558	5.3±3.0	0.7760	7.1±3.0	7.2±2.8	0.8527	6.5±2.4	0.3940
uNTX	32.4±18.0	33.0±16.2	0.7975	34.5±20.1	0.6411	52.4±28.4	60.5±43.5	0.1568	60.3±42.5	0.2945

BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}; HMW, high molecular weight; BMD, bone mineral density; L, lumbar; F, femoral neck; 1/3R, one-third of the radius; uNTX, urinary N-terminal cross-linked telopeptide of type-I collagen.

without fractures ($P < 0.05$). Serum HMW adiponectin was significantly higher in men with moderate or severe vertebral fractures than in those without fractures ($P < 0.01$). No difference was found in serum osteocalcin or uNTX between those with and without fractures.

When multivariate logistic regression analysis was performed with the presence of vertebral fractures as a dependent variable and serum total and HMW adiponectin levels, bone markers, and absolute BMD values at each site adjusted for age, BMI, duration of diabetes, serum creatinine, and HbA_{1c} as independent variables (Table 5), total adiponectin in men was selected as an index affecting the presence of vertebral fractures ($P < 0.05$), as well as T-BMD and L-BMD ($P < 0.05$). Moreover, serum total and HMW adiponectin levels as well as T-BMD and L-BMD were associated with the presence of moderate or severe vertebral fractures in men ($P < 0.05$) (Table 5). By contrast, no parameters were selected in post-menopausal women.

Discussion

In this study, we found that serum total adiponectin level was negatively correlated with T-, L-, and F-BMD and positively with uNTX, and serum HMW adiponectin level was negatively correlated with L-BMD in diabetic men, while it was positively correlated with serum osteocalcin level, but not with uNTX or BMD at any site in post-menopausal women. Logistic regression analysis showed that total and HMW adiponectin were significantly and positively associated with the presence of vertebral fractures in type 2 diabetic men, suggesting that they are not only correlated with BMD or uNTX but

also are useful markers for assessing the risk of vertebral fractures specifically in diabetic males.

Several studies investigated the relationship between serum adiponectin and BMD in subjects without diabetes. In non-diabetic men, Oh *et al.* showed that serum adiponectin level had no significant correlation with BMD in 80 adults (19). By contrast, Peng *et al.* showed that the hormonal level was significantly and negatively correlated with T-, L-, and F-BMD in 232 men (15). Moreover, Michaëlsson *et al.* recently showed that a negative association between adiponectin and BMD was found in two cohorts, one recruited 441 men and another 507 men (21). Our finding was consistent with the latter two reports. In non-diabetic women, serum adiponectin level was reported to be negatively correlated with BMD (12–14), while other studies showed no significant correlation (16–18). Our finding that the hormone level was not significantly correlated with BMD at any site in diabetic women seems to be in accordance with the latter observation. By contrast, few studies were performed in diabetic subjects with regard to the relationship between serum adiponectin level and BMD. Lenchik *et al.* showed that after adjusting for age, gender, race, smoking, and diabetes status, serum adiponectin was inversely associated with BMD in 38 women and 42 men (86% with type 2 diabetes) (11). Tamura *et al.* showed that there were a significant positive correlation between serum adiponectin level and Z score at R-BMD, but not at L- or F-BMD in 40 Japanese patients (28 men and 12 women) with type 2 diabetes (20). Although they investigated men and women together, some adiponectin variability is suggested to be sex related: serum total and HMW adiponectins have been reported to be higher in post-menopausal women

Table 5 Associations between the presence of vertebral fractures and serum adiponectin.

	Men		Postmenopausal women	
	OR (95% CI)	P	OR (95% CI)	P
Presence of vertebral fractures				
Total adiponectin	1.396 (1.020–1.911)	0.0371	1.341 (0.916–1.964)	0.1310
HMW adiponectin	1.199 (0.895–1.608)	0.2235	1.214 (0.828–1.780)	0.3208
Total BMD	0.700 (0.501–0.979)	0.0372	0.743 (0.469–1.178)	0.2061
L2-4 BMD	0.709 (0.522–0.963)	0.0280	0.835 (0.553–1.262)	0.3922
F BMD	0.831 (0.593–1.166)	0.2846	0.826 (0.523–1.305)	0.4136
1/3R BMD	1.011 (0.728–1.403)	0.9496	1.013 (0.631–1.623)	0.9588
Osteocalcin	0.844 (0.606–1.176)	0.3163	0.957 (0.626–1.464)	0.8405
uNTX	0.998 (0.743–1.342)	0.9908	1.233 (0.831–1.829)	0.2976
Presence of moderate or severe vertebral fractures				
Total adiponectin	1.709 (1.048–2.787)	0.0316	1.395 (0.824–2.360)	0.2150
HMW adiponectin	1.810 (1.112–2.946)	0.0169	1.150 (0.661–2.001)	0.6208
Total BMD	0.527 (0.296–0.938)	0.0295	0.473 (0.198–1.129)	0.0918
L2-4 BMD	0.527 (0.303–0.916)	0.0233	0.538 (0.259–1.114)	0.0952
F BMD	0.719 (0.410–1.259)	0.2479	0.551 (0.262–1.157)	0.1152
1/3R BMD	1.165 (0.692–1.962)	0.5655	0.950 (0.434–2.082)	0.8984
Osteocalcin	1.096 (0.666–1.804)	0.7173	0.762 (0.389–1.494)	0.4287
uNTX	1.115 (0.682–1.823)	0.6641	1.288 (0.692–2.396)	0.4244

Multivariate logistic regression analysis was performed with the presence of vertebral fractures as a dependent variable and each level of adiponectin adjusted for age, duration of diabetes, BMI, creatinine, and HbA_{1c} as independent variables. HMW, high molecular weight; BMD, bone mineral density; L, lumbar; F, femoral neck; 1/3R, one-third of the radius; uNTX, urinary N-terminal cross-linked telopeptide of type-I collagen; OR, odds ratio; CI, confidential intervals. Unit of change; standard deviation per increase.

than in men (29, 30). Therefore, it would be more suitable to perform clinical studies on adiponectin after separating between men and women in order to avoid such sex-related differences. In the present study, we investigated correlation between adiponectin and BMD in a larger population of each gender. Our findings of significant negative correlation between total and HMW adiponectin and BMD in the diabetic males seem to accord with those of Lenchik *et al.*

To our knowledge, the present study is the first one that investigated the association between the difference in molecular sizes of adiponectin versus BMD, bone metabolic markers, and the presence of vertebral fractures. We found that serum total adiponectin level was associated with BMD, uNTX, and the presence of vertebral fractures more potently than HMW adiponectin, while both serum total and HMW adiponectin were associated with the presence of moderate or severe vertebral fractures. However, little is known about the distribution and function of each adiponectin isoform in the bone microenvironment, and further studies are needed to clarify the significance of adiponectin molecular sizes in bone metabolism.

Adiponectin has recently attracted widespread attention, especially in the diabetes field, due to their beneficial anti-diabetic and anti-atherosclerotic effects. We and other researchers have also shown that adiponectin stimulates osteoblastogenesis and bone formation in cultured osteoblasts (9, 10, 35). Luo *et al.* have shown that adiponectin regulated bone turnover via enhancing the receptor activator of nuclear factor- κ B ligand (RANKL) expression and suppressing its decoy receptor, osteoprotegerin (OPG) (36). Thus, agents that are able to increase circulating adiponectin may improve not only energy metabolism or atherosclerosis but also bone metabolism. Indeed, in clinical studies, several researchers documented the significant relationship between serum adiponectin and bone metabolic markers in normal subjects. Peng *et al.* showed that serum adiponectin was positively correlated with BAP and uNTX in 232 men after adjustment for age and fat mass (15). Richard *et al.* showed that serum adiponectin was positively associated with osteocalcin in 1208 women after adjustment for age, BMI, central fat mass, insulin levels, smoking, menopause, and HRT status (12). The present study also showed that total adiponectin level was positively associated with uNTX and osteocalcin in the diabetic males and post-menopausal females respectively. These clinical observations seem to accord with a recent *in vitro* study reporting that osteocalcin increases adiponectin expression in adipocytes (37). Taken together, both experimental and clinical studies suggest that adiponectin could accelerate bone turnover and might improve low bone turnover-associated bone fragility that is typically seen in diabetic patients (38).

The present study indicated a negative correlation between serum adiponectin level and BMD in diabetic

men. This finding seems a little contradictory, given that we and other researchers have shown the stimulatory action of adiponectin on osteoblastogenesis and bone formation by *in vivo* and *in vitro* experiments (9, 10, 35). One possible explanation is that serum adiponectin level in subjects with osteoporosis reactively elevates through its up-regulated synthesis and secretion, in order to protect bone from osteopenia. This explanation is supported by the observation that serum OPG level is also negatively correlated with BMD, although it acts as a decoy receptor for RANKL and protects bone from osteopenia through inhibiting osteoclastic activities (39).

This study has some 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 the 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 men and post-menopausal women with the disorders. Consequently, assessment of larger numbers of patients is necessary to determine the usefulness of serum adiponectin levels for predicting the risk of vertebral fractures. Third, vertebral fracture rates in the present populations (34.6% in male and 30.6% in female) seem to be higher than those observed in Western counterparts. However, we found the similar fracture rate (31.6%) in 193 non-diabetic post-menopausal women in a previous study (40), and comparison of vertebral fracture rates between one Japanese and two European cohorts show that Japanese have a higher fracture rate than Europeans (41–43). Fourth, BMI in the present populations (mean; 23.6 in male and 24.5 in female) were lower than those observed in Western people. It is because the capacity of insulin secretion and the degree of obesity in Asian populations are known to be different from Western people (44). Therefore, further studies are needed to examine whether or not our findings are also seen in Western populations. Fifth, the significant difference in age between men and women found in this study could reduce the significance of comparisons because adiponectin is markedly influenced by age. Finally, a previous genetic study has shown that low serum adiponectin levels might be influenced by genetic factors (45), and thus it is possible that genes for adiponectin may predetermine its serum levels independent of bone status, and the hormone levels may not reflect the bone microenvironment. On the contrary, strengths of our study are that the number of subjects was relatively larger than those of previous studies in type 2 diabetes, and that we measured both the total and HMW adiponectins and examined their relationship with bone parameters in separate genders. We also for the first time showed the association between serum adiponectin levels and the presence of vertebral fractures.

In conclusion, the present study showed that serum adiponectin was associated with BMD, uNTX, and the presence of vertebral fractures in men, and that serum adiponectin was positively associated with serum osteocalcin in post-menopausal women. These findings suggest that serum adiponectin was involved in bone metabolism and that the hormonal level may be as efficient as BMD in assessing the risk of vertebral fractures in diabetic males.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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