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Baseline serum total adiponectin level is positively associated with changes in bone mineral density after 1-year treatment of type 2 diabetes mellitus

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Abstract

Although recent clinical studies have shown that serum adiponectin level was negatively associated with bone mineral density (BMD), serum adiponectin action on bone metabolism in humans is still unclear. We investigated the relationships between serum levels of total and high–molecular weight (HMW) adiponectin and its ratio (HMW-total ratio) vs chronological changes in BMD at the lumbar spine, femoral neck (FN), and one third of the radius after 1-year treatment of type 2 diabetes mellitus in 32 Japanese patients. Serum total adiponectin, but not HMW adiponectin or HMW-total ratio, was significantly and positively correlated with percentage change in FN-BMD ($r = 0.35$, $P < .05$). Multiple regression analysis adjusted for age, duration of diabetes, sex, body height, body weight, waist circumference, serum creatinine, and hemoglobin A_{1c} showed that serum total adiponectin was still significantly and positively correlated with percentage change in FN-BMD ($r = 0.65$, $P < .01$). On the other hand, no significant relationships were found between serum levels of hemoglobin A_{1c}, pentosidine, bone formation markers (bone-specific alkaline phosphatase and osteocalcin), or a bone resorption marker (urinary N-terminal cross-linked telopeptide of type-I collagen) vs percentage change in BMD at any site. These findings suggest that serum total adiponectin could be clinically useful for predicting BMD change during treatment of type 2 diabetes mellitus. Adiponectin might protect against BMD reduction in patients with type 2 diabetes mellitus.

1. Introduction

Adiponectin, one of the adipocytokines, has recently attracted widespread attention, especially in diabetes field, because of its beneficial antidiabetic and antiatherosclerotic effects in the regulation of energy homeostasis and insulin sensitivity [1,2]. It is specifically and highly expressed in visceral and subcutaneous fat as well as bone marrow fat depots [3]. Several in vivo and in vitro experiments have shown that adiponectin has a stimulatory action on osteoblastogenesis and bone formation [4-7]. We have previously shown that osteoblasts have an adiponectin receptor and that the proliferation, differentiation, and mineralization of the cells are enhanced by adiponectin [4]. Moreover, a previous animal study has shown that overexpression of adiponectin enhanced bone formation and inhibited bone resorption, resulting in an increase in bone mineral density (BMD) [7]. These findings suggest that adiponectin could positively influence bone metabolism. On the other hand, several clinical studies including ours have shown that serum adiponectin level was negatively correlated with BMD [8-13], suggesting that serum adiponectin might have a negative impact on BMD. Therefore, further studies are needed to clarify the reason of discrepancy between basic experiments and clinical studies as well as the role of adiponectin in bone metabolism in humans.

Although patients with type 2 diabetes mellitus show no apparent bone mass reduction compared with nondiabetic subjects, their fracture risks are known to increase approximately 1.5-fold at the hip, proximal humerus, forearm, and foot [14]. However, little is known about the mechanism of diabetes-related bone fragility and the factors involved in bone abnormality in patients with type 2 diabetes mellitus. We have previously shown that hypoadiponectinemia

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caused by hyperglycemia and obesity might be partly associated with low bone turnover, which is thought to cause diabetes-related bone fragility [15] and resultant vertebral fractures [16]. We have also recently indicated that baseline serum adiponectin level was positively associated with an increase in serum osteocalcin (OC), an osteoblast-specific bone formation marker, during glycemic control in type 2 diabetes mellitus [17]. Moreover, recent animal studies have shown that serum OC has a beneficial effect on glucose and fat metabolism [18,19]. Indeed, in cross-sectional and longitudinal clinical studies, we and others indicated that serum OC level was negatively associated with plasma glucose level, fat mass, as well as atherosclerotic markers in patients with type 2 diabetes mellitus [20] and nondiabetic subjects [21,22]. Taken together, these findings suggest that bone metabolism and glucose/fat metabolism are associated with each other and that serum adiponectin may be a useful marker for assessing not only glucose/fat metabolism but also bone metabolism in type 2 diabetes mellitus.

To our knowledge, there are no longitudinal studies that investigate the relationships between serum adiponectin levels and chronological changes in BMD in patients with type 2 diabetes mellitus. In this study, to address this issue, we measured serum levels of total and high–molecular weight (HMW) adiponectins in Japanese patients with type 2 diabetes mellitus and investigated the relationship of each molecular isoform of adiponectin with chronological changes in BMD after 1-year treatment of diabetes.

2. Subjects and methods

2.1. Subjects

This study was longitudinal and approved by the ethical review board of our institution, and complied with the Helsinki declaration. We recruited subjects who visited Shimane University Hospital for an education, evaluation, or treatment of diabetes if informed consent was obtained after a detailed explanation of the study purpose and methods. The subjects in this study were a total of 32 patients (18 men and 14 postmenopausal women) with type 2 diabetes mellitus (mean age, 63.7 years). Baseline characteristics are shown in Table 1. 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. Fifteen, 7, 2, and 1 patients had been taking insulin treatment, sulfonylurea, metformin, and α-glucosidase inhibitor, respectively. Subjects treated with thiazolidinedione were excluded in this study because thiazolidinedione could affect serum adiponectin levels and BMD. 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. All patients had no new fractures during this study.

Table 1
Baseline characteristics

Subjects (male/female)	32 (18/14)
Age (y)	63.7 ± 9.7
Duration of diabetes (y)	12.1 ± 10.4
Body height (cm)	159.0 ± 8.8
Body weight (kg)	61.6 ± 10.7
BMI (kg/m^2)	24.4 ± 3.9
Waist circumference (cm)	86.4 ± 12.2
Serum creatinine (mg/dL)	0.72 ± 0.19
HbA_{1c} (%)	7.7 ± 1.5
Pentosidine (μg/L)	41.0 ± 24.4
Total adiponectin (μg/mL)	8.1 ± 5.5
HMW adiponectin (μg/mL)	7.5 ± 6.7
HMW-total adiponectin ratio	0.90 ± 0.55
BAP (U/L)	28.4 ± 8.7
OC (ng/mL)	6.9 ± 2.8
uNTX (nmol BCE/mmol Cr)	38.7 ± 25.5

Reference range: serum creatinine, 0.44 to 1.23; HbA1c; 4.3 to 5.8; pentosidine, 9.15 to 43.1; total adiponectin, 4.1 to 18.9; BAP, 9.6 to 35.4; OC, 2.5 to 13.0; uNTX, male: 13.0 to 66.2, female: 14.3 to 89.0. BMI indicates body mass index. BCE indicates bone collagen equivalents; Cr indicates creatinine.

2.2. BMD measurements

The BMD values of the lumbar spine (L), femoral neck (FN), and one third of the radius (1/3R) were measured at baseline and 1 year in all patients by dual-energy x-ray absorptiometry (QDR-4500; Hologic, Waltham, MA). The same operator tested all of 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).

2.3. Biochemical measurements

Biochemical markers including adiponectin and bone turnover markers were measured at baseline in all patients. 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 high-performance liquid chromatography. Serum OC and bone-specific alkaline phosphatase (BAP) were measured by radioimmunoassay, and serum pentosidine and urinary N-terminal cross-linked telopeptide of type-I collagen (uNTX) were measured by enzyme-linked immunosorbent assay (ELISA), as previously described [13,16,17,23].

Serum HMW adiponectin levels were measured by an ELISA kit (Fujirebio, Tokyo, Japan), as previously described [13]. In brief, 96 wells of a microtiter plate were coated with anti-HMW adiponectin monoclonal antibody. One hundred microliters of serum samples diluted 1:441 was placed in each of the 96 wells. The monoclonal antibody conjugated with horseradish peroxidase was used as the detecting antibody. Contents of wells were incubated for 30 minutes with tetramethylbenzipine. 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 previously described [13,16,17]. In brief, after boiling serum samples in sodium dodecyl sulfate buffer for 5 minutes 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%.

2.4. Statistical analysis

Data were expressed as mean ± SD. Because serum total and HMW adiponectin levels as well as bone turnover markers (BAP, OC, and uNTX) showed markedly skewed distributions, logarithmic transformation (log) of these values were carried out before performing correlation and multiple regression analysis. Statistical significance between baseline and 1 year was determined using the Wilcoxon signed rank tests. We analyzed the relationships between baseline characteristics including serum adiponectin levels and percentage changes in BMD at each skeletal site by correlation and multiple regression analysis. All analysis was performed using the statistical computer program StatView (Abacus Concepts, Berkeley, CA). $P < .05$ was considered to be significant.

3. Results

3.1. Baseline BMD and chronological changes in BMD at each skeletal site

Chronological changes in BMD at each site were shown in Table 2. Bone mineral density at each site was not significantly changed after 1 year when evaluated as a whole subjects, although the absolute value, *T*- and *Z*-scores of FN-BMD tended to be decreased.

3.2. Relationship between serum total or HMW adiponectin vs chronological changes in BMD at each skeletal site

Simple correlation analyses showed that each individual percentage change in FN-BMD was significantly and positively correlated with their baseline log(total adiponectin) ($r = 0.35$, $P = .047$) and that each individual percentage change in 1/3R-BMD was significantly and positively correlated with body height and body weight ($r = 0.57$, $P = .001$ and $r = 0.53$, $P = .002$, respectively) (Table 3). On the other hand, HbA_{1c}, pentosidine, HMW adiponectin, and bone turnover markers were not significantly associated with percentage change in BMD at any skeletal site.

Next, multiple regression analyses were performed between baseline serum adiponectin levels or bone turnover markers vs percentage change in BMD at each skeletal site adjusted for age, duration of diabetes, sex, body height, body weight, waist circumference, serum creatinine, and HbA_{1c} (Table 4). Percentage change in FN-BMD was still significantly and positively correlated with log(total

Table 2
Chronological changes in BMD at each site

	Baseline	After 1 y	*P*
L-BMD (g/cm^2)	0.955 ± 0.163	0.956 ± 0.163	.823
T-score	−0.67 ± 1.40	−0.67 ± 1.40	.767
Z-score	0.44 ± 1.18	0.47 ± 1.15	.762
FN-BMD (g/cm^2)	0.711 ± 0.111	0.696 ± 0.094	.082
T-score	−1.00 ± 0.90	−1.13 ± 0.71	.095
Z-score	0.33 ± 0.98	0.21 ± 0.85	.178
1/3R-BMD (g/cm^2)	0.625 ± 0.113	0.629 ± 0.120	.754
T-score	−1.64 ± 1.88	−1.63 ± 1.80	.957
Z-score	0.26 ± 1.61	0.26 ± 1.50	.937

Statistical significance was determined using the Wilcoxon signed rank test.

Table 3
Simple correlations between changes in BMD at each site vs baseline values including serum adiponectin levels and bone turnover markers

Variables	% Changes					
	L-BMD		FN-BMD		1/3R-BMD	
	r	*P*	*r*	*P*	*r*	*P*
Age	0.09	.616	0.22	.230	−0.16	.378
Duration of diabetes	0.23	.201	0.23	.214	0.13	.493
Body height	0.01	.964	0.20	.282	0.57	.001
Body weight	−0.16	.391	0.12	.502	0.53	.002
BMI	−0.20	.270	−0.01	.939	0.13	.479
Waist circumference	−0.26	.190	−0.20	.321	0.13	.516
Serum creatinine	0.04	.835	0.08	.651	0.10	.593
HbA_{1c}	−0.26	.159	−0.20	.279	−0.09	.609
Pentosidine	0.04	.831	0.00	.985	0.05	.775
Log(total adiponectin)	0.15	.418	0.35	.047	−0.13	.471
Log(HMW adiponectin)	−0.04	.826	0.30	.098	−0.02	.909
Log(HMW-total ratio)	−0.22	.237	0.08	.653	0.10	.573
Log(BAP)	−0.22	.231	−0.01	.965	−0.18	.331
Log(OC)	−0.05	.779	0.08	.675	0.03	.876
Log(uNTX)	−0.11	.544	0.01	.976	−0.18	.329

Numbers in each cell describe a correlation coefficient. Logarithmic transformation of adiponectin and bone turnover markers was carried out.

Table 4
Multiple regression analyses between changes in BMD at each site vs baseline serum adiponectin levels and bone turnover markers

Variables	% Changes					
	L-BMD		FN-BMD		1/3R-BMD	
	r	*P*	r	*P*	r	*P*
HbA_{1c}	−0.14	.531	−0.10	.663	−0.07	.797
Pentosidine	0.05	.837	−0.02	.919	0.07	.697
Log(total adiponectin)	0.29	.296	0.65	.009	−0.12	.575
Log(HMW adiponectin)	−0.05	.834	0.38	.114	0.02	.937
Log(HMW-total ratio)	−0.26	.262	0.02	.931	0.10	.579
Log(BAP)	−0.10	.721	0.22	.417	−0.19	.356
Log(OC)	0.06	.868	0.22	.495	−0.01	.970
Log(uNTX)	0.10	.975	0.03	.911	−0.30	.154

Numbers in each cell describe a correlation coefficient. Logarithmic transformation of adiponectin and bone turnover markers was carried out. Multiple regression analysis was performed between BMD and bone markers vs serum adiponectin adjusted for age, duration of diabetes, sex, body height, weight, waist circumference, serum creatinine, and HbA_{1c}.

adiponectin) (r = 0.65, P = .009), although percentage change in 1/3R-BMD was not significantly correlated with body height and body weight (data not shown). On the other hand, HbA_{1c}, pentosidine, HMW adiponectin, and bone turnover marker were not significantly associated with percentage change in BMD at any skeletal site.

4. Discussion

In this study, we found that serum total adiponectin level was significantly and positively associated with percentage change in FN-BMD in patients with type 2 diabetes mellitus after adjustment for age, duration of diabetes, sex, body height, body weight, waist circumference, serum creatinine, and HbA_{1c}. On the other hand, HbA_{1c}, pentosidine, and any bone turnover marker were not associated with percentage change in BMD at any site. These findings suggest that serum total adiponectin is a useful marker for predicting BMD change during treatment of type 2 diabetes mellitus.

In vitro studies on cultured cells have shown that adiponectin stimulated the differentiation and mineralization of osteoblastic cells via mitogen-activated protein kinase (MAPK) and AMP-activated protein kinase (AMPK) signaling pathway [4-7] and induced osteoblastic differentiation of mesenchymal progenitor cells via enhancing bone morphogenetic protein–2 expression [24]. Luo et al [25] have shown that adiponectin regulated bone turnover via enhancing the receptor activator of nuclear factor-κB ligand expression and suppressing its decoy receptor, osteoprotegerin, in human osteoblasts. These in vitro findings suggest that adiponectin may stimulate osteoblast differentiation and bone formation as well as bone turnover. On the other hand, in vivo animal studies present controversial results about adiponectin action on bone [7,26-28]. Oshima et al [7] showed that adiponectin-overexpressing mice displayed an increase in trabecular bone mass and that adiponectin inhibited the activity of osteoclasts and bone resorption. In contrast, Shinoda et al [26] found no bone abnormality in either adiponectin-deficient mice or adiponectin-overexpressing mice. Shinoda et al [26] and Williams et al [27] also showed that adiponectin had no effect on osteoclastogenesis in adiponectin-deficient mice and in RAW-264.7 cells, an osteoclastic cell line, respectively. Luo et al [25] indicated that human osteoclast precursor cells did not have adiponectin receptors and that adiponectin had no direct effect on the differentiation of the cells. In clinical studies, accumulating cross-sectional ones showed that serum adiponectin was negatively associated with BMD and positively associated with bone turnover markers in healthy subjects [8-11] as well as in patients with type 2 diabetes mellitus [12,13]. One longitudinal study by Jurimae et al [28] showed that baseline serum adiponectin level was associated with decrease in total and L-BMD after 1 year in 35 healthy postmenopausal women. Thus, both clinical and experimental studies suggest that adiponectin may accelerate bone turnover and that hyperadiponectinemia may lead to bone loss in healthy subjects. In contrast, we found that baseline serum adiponectin level was positively correlated with chronological changes in BMD in patients with type 2 diabetes mellitus in this study. The discrepancy between the longitudinal studies by Jurimae et al and ours might be explained by the absence or presence of diabetes in respective study populations. Bone fragility in patients with type 2 diabetes mellitus is thought to be caused by osteoblast dysfunction and subsequent low bone turnover [15]. Thus, high serum adiponectin level could improve low bone turnover–associated bone abnormality seen in diabetic patients by enhancing osteoblast differentiation and bone turnover, which might result in BMD increase during treatment of diabetes.

To our knowledge, the present study is the first one that investigated the association between the different molecular isoforms of adiponectin and changes in BMD. We have previously shown that serum total adiponectin level was associated with BMD and the presence of vertebral fractures in type 2 diabetes mellitus more potently than HMW adiponectin [13]. In this study, serum total adiponectin level, but not HMW adiponectin, was associated with changes in BMD. These findings suggest that total adiponectin may be more involved in bone metabolism than HMW adiponectin. However, little is known about the distribution and function of each adiponectin isoform in the bone microenvironment. Further studies are needed to clarify the significance of adiponectin molecular sizes in bone metabolism.

This study has some limitations. First, the sample size was not large enough to make definite conclusions. Second, we found that baseline serum adiponectin level was significantly associated with changes in FN-BMD, but not L- and 1/3R-BMD. In this study, L-BMD and 1/3-BMD were not changed after 1-year treatment, although FN-BMD tended to be decreased (Table 2). Lumbar spine BMD might be affected by several artifacts such as aortic calcification, osteophyte, and vertebral deformity seen in diabetic patients. Because midshaft radius contains a relatively higher proportion of cortical bone, which is not changed for a short time, 1/3R-BMD might have no significant change after 1-year treatment of diabetes. Third, almost 50% of subjects received insulin treatment before starting this study. Therefore, we cannot totally exclude the possibility that the treatment of diabetes affects bone metabolism. We found that the association of baseline serum total adiponectin with percentage change in FN-BMD was almost the same, however, if we analyzed the data after the additional adjustment with insulin treatment (r = 0.63, P =.012). Finally, we did not assess fracture occurrence in this study. Thus, the usefulness of serum adiponectin levels for predicting incident fracture risk in type 2 diabetes mellitus is unknown.

In conclusion, the present study showed for the first time that baseline serum total adiponectin level was positively associated with changes in FN-BMD after 1-year treatment

of patients with type 2 diabetes mellitus. These findings suggest that serum total adiponectin may have a positive impact on bone metabolism and that the baseline hormonal level may be useful for predicting BMD change during treatment of type 2 diabetes mellitus.

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