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Low Serum 25-Hydroxyvitamin D Levels Associated With Falls Among Japanese Community-Dwelling Elderly

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ABSTRACT: Previous studies have shown that low serum 25-hydroxyvitamin D [25(OH)D] level is a risk factor for falls among the elderly in European and North American populations. We used a cross-sectional community-based survey to study the association of serum 25(OH)D level and falls among Japanese community-dwelling elderly. A total of 2957 elderly persons (950 men and 2007 women) 65–92 yr of age who participated in mass health examinations for the prevention of geriatric syndrome for the elderly underwent an interview, blood analysis, and physical performance testing. Experience of falls over the previous year was assessed in an interview. Physical performance tests of handgrip strength, stork standing time with the eyes open, and normal waking speed as risk factors for falls among the elderly were conducted. Serum albumin and 25(OH)D concentrations were analyzed. Mean 25(OH)D concentration was significantly lower in women than in men ($p < 0.001$). Women showed a significant decline of 25(OH)D level with increased age ($p < 0.001$). There was also a significant difference in the prevalence of 25(OH)D insufficiency [25(OH)D level < 20 ng/ml] between the sexes ($p < 0.001$). The rate of falls was significantly higher in the lowest quartile of 25(OH)D level in women ($p = 0.02$) and in women with 25(OH)D insufficiency ($p = 0.001$). Women also showed significant declines in all three fall-related physical performance tests. Multiple logistic regression analysis showed significant and independent associations between 25(OH)D level and experience of falls in women only ($p = 0.01$). Low 25(OH)D level was significantly associated with a high prevalence of falls in Japanese elderly women because of their inferior physical performance. Low serum 25(OH)D levels appear preventable and easily treated; there is an evident need for greater awareness to screen and thus prevent this condition.
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Key words: 25-hydroxyvitamin D, fall, physical performance, community elderly

INTRODUCTION

THE IMPORTANCE OF vitamin D for skeletal health is well known.[1,2] Through the regulation of calcium and phosphorus levels in the blood by promoting their absorption from food in the intestines, vitamin D promotes bone formation and mineralization for the development of a strong skeleton. Vitamin D deficiency, which can result from inadequate intake coupled with inadequate sunlight exposure, plays an important role in the development of osteoporosis because of the induction of a secondary hyperparathyroidism that mobilizes calcium from the bone.

Vitamin D deficiency results not only in impaired bone mineralization, but also in myopathy in the elderly.[3,4] It has also been shown recently to be associated with a decline of muscle strength,[5–8] sarcopenia,[7] and functional limitations and disability,[5,8] and probably because of these phenomena, with falls in the elderly.[9–11] We have studied and reported that concomitant low serum 25-hydroxyvitamin D [25(OH)D] and albumin were associated with decreased objective physical performance among Japanese community-dwelling elderly from a nutritional point of view.[12] However, falls were not taken into account in the previous study.

The aim of this study was to investigate the association between serum 25(OH)D levels and falls, and the associated physical performance among community-dwelling Japanese elderly who in some previous studies have been reported to have stronger muscle strength and lower fall rates than whites.[13,14] We hypothesized that low 25(OH)D levels (1) correlate with poor muscle strength, balance, and walking capability and (2) are consequently associated with the occurrences of falls among community-dwelling Japanese elderly.

MATERIALS AND METHODS

Subjects

The participants were 2957 residents (950 men and 2007 women) ≥65 yr of age living in Itabashi ward in Tokyo, Japan, who had participated in mass health checkups for the community elderly (Otasha-Kenshin) conducted in October/November 2004 and 2005. Otasha-Kenshin, which means "health checkups for successful aging" in Japanese, is a comprehensive mass health examination for commu-

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nity-dwelling elderly that aims to prevent "geriatric syndrome" including falls and fractures, incontinence, poor oral health, mild cognitive impairment, depression, and undernutrition. The overall aim is to prevent the loss of independence and the need for long-term care in later life. Details of Otasha-Kenshin, including participant details, investigation methods, and its contents, have been described in our earlier papers.[15–17] None of the subjects analyzed in this study had a history of malignant diseases, current treatment of vitamin D, chronic renal failure, or other serious diseases affecting vitamin D regulation. All participants were essentially ambulatory, lived independently in their homes, and had sound functional capacity. Participants provided written informed consent to participate in the study, which was approved by the Institutional Review Board and Ethic Committee of the TMIG (Accepted No. 5, July 1, 2004).

Data collection

Interviews were conducted to assess the age, physical activity, and chronic disease conditions of subjects. History of chronic diseases was self-reported and included hypertension, stroke, heart disease, diabetes, and renal failure. Heart disease included angina pectoris, acute myocardial infarction, congestive heart failure, and various arrhythmias. Renal failure was defined as chronic renal failure under treatment including hemodialysis, which can affect the regulation and metabolism of serum vitamin D levels.

We also assessed fall experience over the previous year. A fall was defined as an unintentional change in position resulting in coming to rest at a lower level or on the ground. The subjects were asked about their falls in the same manner as in our previous study.[18] That is, they were asked the question, "Have you experienced any falls during the previous 12 months?" Those who reported one or more falls were asked about the circumstances and consequences of each fall (i.e., the time, reason and place of the fall, the presence or absence of injury, and whether they visited a doctor).

Previous population-based studies have confirmed that physical performance characteristics, including those based on handgrip strength, stork standing time with the eyes open, and normal walking speed, are risk factors for falls among the community elderly in Japan.[18–20] Moreover, these three variables have also been confirmed by a covariance structure model as the essential factors underlying physical performance measures for Japanese elderly living in a community.[21]

Handgrip strength

The peak handgrip force (kg) of each hand was measured by Smedley's hand dynamometer (Yagami, Tokyo, Japan). The test was performed twice, and the higher of the two measurements made on the dominant hand was recorded.

Stork standing

While standing on a square (0.4 × 0.4 m), each subject stood on one foot while watching a point set at eye level 1 m away and tried to maintain this posture. A stopwatch measured the duration in seconds, up to a maximum of 1 min, and the longer of two attempts was recorded.

Normal walking speed

A flat walking path of 11 m was marked with tape at the 3- and 8-m points. A stopwatch measured the time taken to walk 5m, from the time when a foot first touched the ground after the 3-m line to when a foot touched the ground after the 8-m line. The participants were asked to take the test by walking at their normal or preferable speed. The test was repeated and the faster speed recorded.

Because of the possibility of a high correlation among the three physical performance tests, after confirmation by Pearson's correlation coefficient (r), normal walking speed was selected as the representative independent variable for the multiple logistic regression model.

Measurement of serum levels of albumin and 25(OH)D

Blood samples were collected in a nonfasting state and in a sitting position. Analyses were carried out centrally in one laboratory (Special Reference Laboratories, Tokyo, Japan). Serum 25(OH)D levels are commonly used as a measure of vitamin D status,[22–24] and these were measured with an RIT 2 kit (Dia Sorin, Stillwater, MN, USA). The RIT 2 method is based on an antibody specific to 25(OH)D; using this method, the CV was <1%. We summarized the serum 25(OH)D levels of these subjects into quartiles, and used the 25 percentile cut-off to compare groups of subjects with higher and lower 25(OH)D. Lower serum 25(OH)D was defined as 25.0 ng/ml (62.5 nM) or below for men and 21.0 ng/ml (52.5 nM) or below for women. For a definition of vitamin D insufficiency, based on studies performed in the United States and Australia[25,26] showing that a serum 25(OH)D level of at least 15–20 ng/ml is needed to achieve optimum PTH levels, we defined a 25(OH)D level of <20 ng/ml as insufficiency.

Statistical analysis

All data were analyzed with SPSS software for Windows, version 13.0 (SPSS, Chicago, IL, USA); the level of significance was set at 5%.

Means and SDs (for continuous variables) along with proportions (for categorical variables) were calculated for all participants. Differences between men and women were assessed using t-tests for continuous variables and χ^2 tests for categorical data. Differences in serum 25(OH)D levels were analyzed among the four age groups by one-way ANOVA in both sexes. Furthermore, comparisons of fall-related variables by 25(OH)D level were performed using analysis of covariance (ANCOVA) controlled for age in continuous variables, and Mantel-Haenszel χ^2 tests were used to adjust for age in categorical variables in both sexes.

To analyze the association of serum albumin and 25(OH)D level with physical performance (i.e., handgrip strength, stork standing time with the eyes open, and normal walking speed), multiple regression analysis was conducted with age adjustment. To study the association of falls and 25(OH)D levels, logistic regression analysis was

TABLE 1. CHARACTERISTICS OF STUDY PARTICIPANTS

Characteristics	*Male (n = 950)*	*[Min–Max]*	*Female (n = 2007)*	*[Min–Max]*	*p*
Age (yr, mean ± SD)	74.5 ± 5.1	[65–89]	75.4 ± 4.7	[65–92]	<0.001*
Fall experience over the previous year (yes, %)	103 (10.8)		372 (18.5)		<0.001†
Hand grip strength (kg, mean ± SD)	31.4 ± 6.6	[10–52]	18.8 ± 4.6	[1–38]	<0.001*
Stork standing time with eyes open (s, mean ± SD)	37.1 ± 22.5	[1–95]	35.8 ± 23.3	[1–88]	0.152*
Normal walking speed (m/s, mean ± SD)	1.23 ± 0.26	[0.40–2.08]	1.18 ± 0.29	[0.15–2.00]	<0.001*
Serum albumin (g/dl, mean ± SD)	4.35 ± 0.23	[3.4–5.0]	4.31 ± 0.21	[3.3–5.0]	<0.001*
Serum 25(OH)D level (ng/ml, mean ± SD)	28.5 ± 5.0	[8–42]	24.2 ± 4.9	[9–38]	<0.001*
Age group	(n)		(n)		
65–69	(173) 28.4 ± 4.5		(163) 26.8 ± 3.8		
70–74	(314) 28.5 ± 5.3		(763) 24.2 ± 4.6		
75–79	(320) 28.6 ± 4.9		(675) 24.0 ± 5.1		
80+	(143) 28.4 ± 5.5	$p = 0.97$‡	(406) 23.6 ± 5.3	$p < 0.001$‡	
Quartile [cut-off value of 25(OH)D for each percentile]	(ng/ml)		(ng/ml)		
25 percentile	25.0		21.0		
50 percentile	29.0		24.0		
75 percentile	32.0		28.0		
Insufficient (<20 ng/ml, %)	4.8		17.7		<0.001†

* Student's *t*-test for continuous variables between males and females.
† χ^2 test for categorical variables between males and females.
‡ ANOVA in both males and females.

conducted using "fall experience over the previous year" as a dependent variable and other variables [age, physical performance test, serum albumin, and 25(OH)D levels] as independent variables.

RESULTS

The basic characteristics of the subjects, including age, handgrip strength, stork standing with the eyes open, normal walking speed, serum albumin level, and 25(OH)D level, are shown in Table 1. Mean ages were 74.5 ± 5.1 yr in men and 75.4 ± 4.7 yr in women ($p < 0.001$). Concerning fall experience, the numbers (percentage) of individuals who experienced a fall over the previous year were 103 (10.8%) in men and 372 (18.5%) in women. The prevalence of falls was significantly higher in women than men ($\chi^2 = 28.30$, $p < 0.0001$). The number of falls varied from one to five. Sixty-one men (59.2%) and 259 women (69.8%) had experienced only one fall, whereas 42 men and 113 women had recurrent falls of two or more times. The predominant cause of falling was "tripping" in both sexes, followed by "slipping" and "missing a step." The consequences of falling, that is, the conditions of injury, were clearly different between men and women. Although "bruise" (38.7%) and "scratch" (26.1%) were frequent among women, "no injury" accounted for nearly one half (44.7%) of men.

The mean 25(OH)D concentrations were 28.5 ± 5.0 ng/ml in men and 24.2 ± 4.9 ng/ml in women ($p < 0.001$). Only in women was there a significant decline of 25(OH)D concentration with increasing age by ANOVA ($p < 0.001$). Forty-six (4.8%) men and 356 (17.7%) women had a 25(OH)D level of <20 ng/ml (50 nM; $p < 0.001$).

Comparisons of the rate of fall experience over the previous year, average number of falls, physical performance tests, and serum albumin levels are shown in Table 2. Subjects who were judged as not appropriate for the tests because of high blood pressure, heart failure, lumbago, knee pain, etc., were excluded from the physical performance tests. Thus, the total number of subjects who underwent the physical performance tests was 2837 (917 men and 1921 women) in the handgrip strength test, 2519 (792 men and 1727 women) in the stork standing test, and 2044 (455 men and 1589 women) in the normal walking speed test. First, comparisons were conducted between the lowest quartile group (≤25.0 ng/ml in men and ≤21.0 ng/ml in women) and the higher groups. Both hand grip strength and stork standing time were significantly different in men, and all of the measurements were significantly different in women. Furthermore, for women only, the rate of fall experience and average number of falls were significantly higher in the lowest quartile group compared with the other groups ($p = 0.02$ for the rate and $p = 0.021$ for the number). Second, comparisons were conducted between the 25(OH)D insufficiency group (<20 ng/ml) and the normal group (≥20 ng/ml). Hand grip strength and serum albumin level in men, and all measurements except hand grip strength in women, were significantly different between these two groups. Stork standing time, normal walking speed, and serum albumin level were significantly lower in the 25(OH)D insufficiency group. As for rate of fall experience and average number of falls, only women showed that the 25(OH)D insufficiency group had a significantly higher rate ($p = 0.001$) and average number ($p = 0.006$) of falls than the normal group.

Table 3 shows the associations of serum concentrations of albumin and 25(OH)D with physical performance tests by multiple regression models adjusted for age. Serum 25(OH)D level showed significant association with all three variables in the physical performances of both men and women. However, serum albumin level showed significant association only with handgrip strength in both sexes.

Calculations of Pearson's correlation coefficient (r) were

TABLE 2. SERUM 25(OH)D LEVEL AND CHARACTERISTICS FOR MALES AND FEMALES

Characteristics	Male			Female		
	Lower (≦25.0 ng/ml) (n = 249)	*Higher (≧26.0 ng/ml) (n = 701)*	*p*	*Lower (≦21.0 ng/ml) (n = 576)*	*Higher (≧22.0 ng/ml) (n = 1431)*	*p*
Fall experience over the previous year (yes, *n*, %)	27 (10.8)	76 (10.8)	0.938*	129 (22.4)	243 (17.0)	0.020*
Average number of falls (times, mean ± SD)	2.1 ± 2.4	1.7 ± 1.1	0.422†	1.6 ± 1.2	1.4 ± 0.8	0.021[b]
Hand grip strength (kg, mean ± SD)	30.5 ± 6.7	31.7 ± 6.5	0.020[b]	17.9 ± 4.6	19.2 ± 4.6	0.002[b]
Stork standing time with eye open (s, mean ± SD)	34.6 ± 22.5	38.2 ± 22.5	0.046[b]	31.7 ± 23.5	37.7 ± 23.0	<0.001†
Normal walking speed (m/s, mean ± SD)	1.19 ± 0.26	1.25 ± 0.26	0.061†	1.12 ± 0.28	1.21 ± 0.27	<0.001†
Serum albumin (g/dl, mean ± SD)	4.34 ± 0.24	4.35 ± 0.26	0.616†	4.28 ± 0.23	4.33 ± 0.21	<0.001†
	Insufficiency (<20.0 ng/ml) (n = 46)	*Normal (≧20.0 ng/ml) (n = 904)*	*p*	*Insufficiency (<20.0 ng/ml) (n = 356)*	*Normal (≧20.0 ng/ml) (n = 1651)*	*p*
Fall experience over the previous year (yes, *n*, %)	3 (6.5)	100 (11.1)	0.454*	92 (25.8)	280 (17.0)	0.001*
Average number of falls (times, mean ± SD)	2.7 ± 0.6	1.8 ± 1.5	0.338†	1.7 ± 1.3	1.4 ± 1.5	0.006†
Hand grip strength (kg, mean ± SD)	28.5 ± 6.4	31.5 ± 6.5	0.003†	18.1 ± 4.7	19.0 ± 4.6	0.420[b]
Stork standing time with eye open (s, mean ± SD)	31.4 ± 22.9	37.5 ± 22.5	0.124†	29.8 ± 22.9	37.2 ± 23.2	<0.001†
Normal walking speed (m/s, mean ± SD)	1.16 ± 0.79	1.24 ± 0.26	0.138†	1.11 ± 0.29	1.20 ± 0.27	<0.001†
Serum albumin (g/dl, mean ± SD)	4.27 ± 0.26	4.35 ± 0.22	0.027†	4.27 ± 0.23	4.32 ± 0.21	<0.002†

* The Mantel-Haenszel χ^2 test adjusted for age.
† ANCOVA adjusted for age.

TABLE 3. ASSOCIATION OF SERUM ALBUMIN AND 25(OH)D LEVELS WITH PHYSICAL PERFORMANCE FOR MALES AND FEMALES

	Handgrip strength			*Stork standing time*			*Normal walking speed*		
	β	*SE*	*p*	*β*	*SE*	*p*	*β*	*SE*	*p*
Men									
Albumin	0.096	0.852	0.001	0.002	3.644	0.947	0.025	0.053	0.576
25(OH)D	0.067	0.037	0.020	0.075	0.152	0.030	0.111	0.002	0.012
Women									
Albumin	0.109	0.459	<0.001	0.037	2.502	0.106	0.045	0.030	0.051
25(OH)D	0.062	0.020	0.003	0.109	0.105	<0.001	0.143	0.001	<0.001

Values are adjusted for age. *p* values are derived from multiple regression analysis.
β, standardized regression coefficient.

carried out to confirm the correlation among the three physical performance tests. The results showed high and significant intercorrelation for these three variables; correlation coefficients were from 0.23 (handgrip strength and stork standing time) to 0.35 (stork standing time and normal walking speed) in men and from 0.31 (handgrip strength and stork standing time) to 0.47 (stork standing time and normal walking speed) in women, and all were significant at $p < 0.001$. Therefore, we adopted only "normal walking speed" to represent the physical performance tests as well as the independent variable for the final multiple logistic regression model.

Table 4 shows the associations of fall experience over the previous year with normal walking speed, serum albumin, and 25(OH)D levels by multiple logistic regression models with age adjustment. Normal walking speed (unit = 0.1 m/s) showed a significant protective effect against falls in

TABLE 4. MULTIPLE LOGISTIC REGRESSION MODEL OF FACTORS ASSOCIATED WITH FALL EXPERIENCE OVER THE PREVIOUS YEAR

Risk factor	Male			Female		
	OR	*95% CI*	*p*	*OR*	*95% CI*	*p*
Age (yr)	1.02	0.95–1.10	NS	1.02	0.99–1.06	NS
Normal walking speed (0.1 m/s)	0.87	0.77–0.97	0.015	0.92	0.88–0.97	0.001
Albumin (g/dl)	1.69	0.45–6.33	NS	1.60	0.88–2.90	NS
25(OH)D (ng/ml)	1.00	0.95–1.06	NS	0.97	0.94–0.99	0.010

Dependent variable was "fall experience over the previous year" (yes = 1, no = 0).
The unit of normal walking speed was transferred from meters per second to 0.1 m/s in this final multiple logistic regression model.
NS, not significant.

both men (OR = 0.87, 95% CI = 0.77–0.97) and women (OR = 0.92, 95%CI = 0.88–0.97). Serum 25(OH)D level (unit = 1 ng/ml) also had a significant and independent protective effect for falls found only in women (OR = 0.97, 95% CI = 0.94–0.99, $p = 0.01$).

DISCUSSION

Maintenance of physical performance in old age is an important factor not only for a healthy and independent life in the community but also a way to prevent falls that can lead to a marked decline in activities of daily living (ADLs). A national survey in Japan has shown that the annual frequency of falls is >20% in those >65 yr of age and that ~10% of these falls result in fractures.[27]

This study showed that the proportion of people who reported falls in the previous year increased with age and that falls were more common in women than in men.[20] These findings are consistent with the results of other studies of falls among community-dwelling elderly.[28–32] Aoyagi et al.[33] reported that the proportion of falls in the previous year after age standardization for Japanese was about one half of that of whites. Furthermore, the incidence of hip fracture among Japanese elderly was found to be much lower than that reported for whites in North America and Europe.[34] This difference is probably partly the result of the lower fall rate among Japanese, suggesting that both ethnicity (genetics) and lifestyle (environmental) factors may be involved.[14] Recently, some studies have shown that lower serum vitamin D level is a risk factor for falls and fall-associated physical performance among the elderly.[9,10,35] At present, however, there are few studies on the association between serum 25(OH)D level and falls in Japanese community elderly, whose frequency of falls is less than that observed in Europe and the United States.[6–8,13]

In this study, we found that there were significant sex differences of 25(OH)D level on average and in a pattern of decline along with aging; namely, women had significant lower serum 25(OH)D levels at any age group and showed remarkable declines with aging. One of the reasons for this sex difference may be general inactivity and lower intake of vitamin D from daily food among Japanese elderly women compared with men. One Japanese national survey showed that, compared with 39.1% of men, 32.6% of women engage in physical activity for at least 30 min two or more times a week.[36] Our previous study also reported that women had significantly lower rates of regular sports activity than men (13.8% versus 21.5%).[37] Furthermore, we recently reported that one of the significant predictors for cessation of regular activity was "female sex" as well as "smoking" and "slow walking speed" from a population-based, 2-yr follow-up study.[38] The national survey also showed that women took smaller amounts of vitamin D than men (8.6 ± 9.0 versus 8.9 ± 10.0 μg/d on average). In particular, for elderly respondents ≥70 yr of age, the average intake of vitamin D in women (8.6 ± 9.0 μg/d) was much less than that in men (10.3 ± 10.1 μg/d).[36] These factors of physical inactivity and lower intake of daily vitamin D in elderly women may have caused their observed higher frequency of 25(OH)D insufficiency compared with men. It is known that the main source of vitamin D in humans is considered to be through the skin, where vitamin D is produced during exposure to UVB sunlight.[39,40] In our study, to avoid any seasonal variation of serum 25(OH)D, data collection was carried out only during autumn (October/November), which meant that serum 25(OH)D levels would be almost stable and at an average throughout the year among a normal Japanese population.[41]

The range of serum 25(OH)D levels was 8–42 ng/ml in men and 9–38 ng/ml in women. Although it is still uncertain what an optimal 25(OH)D level is, it has been suggested that the range of 25(OH)D levels should be 32–100 ng/ml, with a lower limit somewhere between 15 and 36 ng/ml.[41–43] In this study, we defined a 25(OH)D level of <20 ng/ml as insufficiency. From this, we judged that the prevalence of 25(OH)D insufficiency was significantly predominant in women (17.7%) compared with men (4.8%; $p < 0.001$). Studies concerning the prevalence of 25(OH)D insufficiency in various populations have been challenged because of the lack of standardization of assays and different cut-off points.[44] A comparison of serum 25(OH)D level between hip fracture patients and nonhip fracture controls in Japan showed that average serum 25(OH)D concentrations were significantly different: 17.8 ng/ml in hip fracture patients and 25.8 ng/ml in nonhip fracture controls.[45] Furthermore, 62% of the hip fracture patients ($N = 50$) had 25(OH)D insufficiency, defined as having a serum 25(OH)D concentration <20 ng/ml. In this context, the elderly whose 25(OH)D levels were <20 ng/ml can be considered to be in insufficiency and at high risk of a hip fracture, which indeed has increased sharply during last two decades in Japan.[46]

In comparisons of serum 25(OH)D levels and fall-associated variables between the group of participants in the lowest quartile and the groups of the three other higher quartiles of serum 25(OH)D level, all variables but normal walking speed in men were significantly lower in the lower 25(OH)D group than in the higher group. Our findings are consistent with the results from a Swedish population-based study of 986 community-living elderly women[47] that showed that the lower 25(OH)D group was significantly correlated with inferior gait speed, inferior balance test, and lower knee extension/flexion strength results, all of which are fall-associated variables.

The rate of fall experience over the previous year by serum 25(OH)D level was significantly different only in women [i.e., 22.4% in the lower 25(OH)D group and 17.0% in the higher group ($p = 0.02$) and also 25.8% in the insufficiency group and 17.0% in the normal group ($p = 0.001$), respectively]. The average numbers of falls were also significantly different between the lower and higher groups ($p = 0.021$) and between the insufficient and normal groups ($p = 0.006$), respectively. This finding that lower serum 25(OH)D level or 25(OH)D insufficiency status is associated with falls among elderly women is consistent with many previous studies.[3,4,11] However, there is a controversy about which type of vitamin D [i.e., 25(OH)D or $1,25(OH)_2D_3$] is associated with fall risk. Faulkner et al.,[48] who examined the relationship of vitamin D supplementation and the serum concentration of vitamin D metabolites

with falls in older white, community-dwelling women (n = 389) in the Unites States, reported that only the higher serum $1,25(OH)_2D_3$ concentration was associated with a lower fall risk but that 25(OH)D concentration was not associated with falls. In our study, as one of the study limitations, serum $1,25(OH)_2D_3$ was not assessed in the participants undergoing the mass health examination.

Further analysis on the association of serum albumin and 25(OH)D levels with the fall-associated variables in this study showed that only serum 25(OH)D level had a significant association with all three fall-associated variables in both sexes. On the other hand, serum albumin had a significant association only with handgrip strength in both sexes. The mechanism connecting serum albumin and muscle mass or power is not clear.[49,50] However, serum albumin concentration may be a marker of the protein status of an individual, with lower values indicating a diminished protein reserve and stimulated catabolic processes leading to muscle break down and also muscle strength decline.[50] Thus, low serum albumin, even within a normal range, is independently associated with weaker muscle strength and future decline in older men and women.[51,52]

Serum 25(OH)D concentration is an important determinant of muscle mass and sarcopenia. In an observational study of community-dwelling elderly in the Netherlands, incident sarcopenia, defined as a minimum of 40% decline in muscle strength and 3% decline in muscle mass, was found to be twice as likely among 25(OH)D-deficient elderly [25(OH)D level < 10 ng/ml] than among elderly with a 25(OH)D level of >20 ng/ml.[7] With respect to the role of vitamin D in muscle strength, the majority of the actions of vitamin D are mediated through $1,25(OH)_2D_3$ binding to nuclear vitamin D receptor (VDR) that can directly modulate the transcription of the gene possessing a functional binding site for VDR in its regulatory region.[8,53] Therefore, muscle strength seems to be influenced by the VDR genotype in the muscle cell. With the use of specific restriction endonucleases, several VDR polymorphisms have been determined. In nonobese, older women, a 23% difference in quadriceps strength and a 7% difference in grip strength between two homozygote types of a restriction site have been found.[54] The action of vitamin D is affected by allelic variance of the VDR. A genomic study on VDR polymorphism has shown that Japanese women had much lower frequency of homozygote BB (1.4%) than white women (16.7%).[55] In this context, if the VDR polymorphism affects not only the BMD but also muscle strength of elderly women, Japanese women in general may have an advantage with respect to their lower frequency of falls and associated hip fractures.

As for the association of serum 25(OH)D level and fall experience over the previous year by multiple logistic regression models, even after the adjustment of other fall-associated variables, serum 25(OH)D level was independently associated with falls in women as well as normal walking speed in this study. A considerable number of population-based studies have also been conducted on walking ability in relation to the occurrence of falls in the elderly.[56–60] Increased body sway, uneven distances, and uneven timing during walking were identified as risk factors for falls.[61,62] The authors have previously reported that both fall experience over the previous year and a decline in walking speed were very strong predicators of the occurrence of frequent falls in a 5-yr follow-up cohort study among Japanese community-living elderly.[18] Muscle function or strength as the single most important component for walking ability has been consistently identified as a risk factor for hip fractures as a consequence of falls in the elderly. Subclinical 25(OH)D insufficiency is also considered to be an important risk factor for hip fractures in elderly people in both white[63,64] and Japanese[45] populations. We have suggested that elderly women with lower 25(OH)D levels and with a significant decline in their fall-associated variables tend to decline in walking capability and be more vulnerable to falls even in elderly populations of Japanese women, whose fall rate has been reported to be low.

A relevant issue regarding the role of 25(OH)D in physical performance is that vitamin D supplementation has been reported to be significantly effective in maintaining or improving physical performance and preventing falls among the elderly. In a recent randomized and multiple-dose study, Broe et al.[65] reported that a high dose of vitamin D (800 IU/d) reduced the risk of falls dramatically by 72% lower (adjusted-incidence rate ratio) than participants taking a placebo over the same 5-mo period in a nursing home. However, the question of which type of vitamin D supplementation (i.e., either cholecalciferol or calciferol) is more effective at reducing falls among community-living elderly is still controversial. The association of vitamin D supplementation with better physical performance related to the risk of falls and/or reduced falls is unclear at present. For example, a meta-analysis of five randomized controlled trials provided some evidence that vitamin D supplementation might reduce falls,[10] whereas the results of a second meta-analysis of four randomized controlled trials of vitamin D found no such evidence.[66]

Our findings suggesting that there are significant relationships between serum 25(OH)D and fall-associated physical performance and with falls themselves could provide guidance about how to prevent falls and fractures, particularly hip fractures resulting from falls among community-living elderly. Two such countermeasures are to improve muscle strength, especially in the lower extremities, and to enhance balance ability.[67,68] For example, from a randomized controlled exercise intervention trial for Japanese community-dwelling elderly, we have proven that a moderate exercise intervention program in addition to a home-based program significantly improved fall-associated variables and, consequently, decreased the incidence of falls for 1.5 yr after the intervention.[67]

Another countermeasure would be to maintain a high level of serum 25(OH)D by adequate intake of foods containing vitamin D, supplementation, and exposure to sunlight. Incidentally, fish consumption seems to play an important role in maintaining adequate vitamin D nutrition among elderly Japanese.[69] In general, these countermeasures seem to be consistent with the traditional Japanese lifestyle. Our earlier study using risk factor analysis of hip fractures in elderly Japanese showed that such a traditional

Japanese lifestyle, including living on Japanese tatami mats and eating fish daily, was strongly associated with a decrease in the risk of hip fractures.[14] Living on Japanese tatami mats, including futon-style bedding, seems have great benefits for preventing falls and hip fractures by continuously strengthening the muscles of the hip girdle and lower extremities by sitting, squatting, and frequent standing over the course of many years. Furthermore, consumption of dark-meat fish, which is rich in vitamin D, also seems to be beneficial for maintaining adequate 25(OH)D levels in the elderly, especially in the winter season.[69]

Before detailing our final conclusions, some limitations of our study must be considered. (1) The subjects analyzed were not selected randomly from the study population; as well, they were relatively healthy elderly persons who were able to travel from their homes to the health checkup venue. As a result, elderly persons with lower physical functional capacity were excluded. (2) Plasma $1,25(OH)_2D_3$, albumin-corrected calcium, and PTH, which would provide information on the extent of any primary vitamin D deficiency,[8,48,70] and creatine clearance that may affect the metabolism of vitamin D through the kidney,[71] were not assessed in this study. It is well known that increased secretion of PTH is associated with decreased serum 25(OH)D levels, which may commonly occur in the elderly. However, according to a survey on the nutritional status of vitamin D among Japanese community-dwelling elderly, Nakamura et al.[72] reported that only 1.8% of the subjects had elevated intact PTH levels. (3) We did not analyze the genotype of the VDR that could influence muscle strength and, likely, the fall rate as well. (4) This study was cross-sectional and therefore did not provide cause/effect relationships, although we showed a significant correlation between physical performance and serum 25(OH)D levels in Japanese community-dwelling elderly. Therefore, a longitudinal follow-up study and controlled clinical trials would seem necessary to confirm the role of serum 25(OH)D in falls and its association with the physical performance of the elderly.

In conclusion, our findings showed that a lower serum 25(OH)D level was significantly associated with fall experience over the previous year and with fall-associated variables in Japanese women whose fall rate has been reported to be about one half that of white women. This indicates that serum 25(OH)D level has a common and positive relationship with the occurrence of falls in elderly women, and probably beyond any genetic background represented by VDR phenotype differences and anthropometric and nutritional differences.

Muscle weakness or sarcopenia, frailty, and falls, all of which can be frequent among the elderly and therefore are often categorized as geriatric syndrome, have a major impact on the elderly in terms of both morbidity and mortality. The connections between these items related to geriatric syndrome and 25(OH)D has been well established by many population-based epidemiological studies including this one. Such geriatric syndromes could be prevented by both exercise interventions and adequate levels of serum 25(OH)D; these would help maintain good physical performance and functional capacity for a high quality of life among community-dwelling elderly.

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REFERENCES

1. Riggs BL 2003 Role of the vitamin-D-endocrine system in the pathophysiology of postmenopausal osteoporosis. J Cell Biochem **88:**209–215.
2. Lips P 2001 Vitamin D deficiency and secondary hyperparathyroidism in the elderly: Consequences for bone loss and fractures and therapeutic implications. Endocr Rev **22:**477–501.
3. Bischoff HA, Stahelin HB, Urscheler N, Ehrsam R, Vonthein R, Perrig-Chiello P, Tyndall A, Theiler R 1999 Muscle strength in the elderly: Its relation to vitamin D metabolites. Arch Phys Med Rehabil **80:**54–58.
4. Mowe M, Haug E, Bohmer T 1999 Low serum, calcidiol concentration in older adults with reduced muscular function. J Am Geriatr Soc **47:**220–226.
5. Verhaar HJ, Samson M, Lansen PA, de Vreede PL, Macnen JW, Duursma SA 2001 Muscle strength, functional mobility and vitamin D in older women. Aging Clin Exp Res **12:**455–460.
6. Janssen H, Samson MM, Verhaar HJ 2002 Vitamin D deficiency, muscle function, and falls in elderly people. Am J Clin Nutr **75:**611–615.
7. Visser M, Deeg D, Lips P 2003 Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): The Longitudinal Ageing Study Amsterdam. J Clin Endocrinol Metab **88:**5766–5772.
8. Campbell PMF, Allain TJ 2006 Muscle strength and vitamin D in older people. Gerontology **52:**335–338.
9. Dhesi JK, Bearne LM, Moniz C, Hurley MV, Jackson SH, Swift CG, Allain TJ 2002 Neuromuscular and psychomotor function in elderly subjects who fall and the relationship with vitamin D status. J Bone Miner Res **17:**891–897.
10. Bischoff HA, Stahelin HB, Dick W, Akos R, Knecht M, Salis C, Nebiker M, Theiler R, Pfeifer M, Begerow B, Lew RA, Conzelmann M 2003 Effects of vitamin D and calcium supplementation on falls: A randomized controlled trial. J Bone Miner Res **18:**343–351.
11. Stein MS, Wark JD, Scherer SC, Walton SL, Chick P, Di Carlantonio M, Zajac JD, Flicker L 1999 Falls relate to vitamin D and parathyroid hormone in an Australian nursing home and hostel. J Am Geriatr Soc **47:**1195–1201.
12. Kwon J, Suzuki T, Yoshida H, Kim H, Yoshida Y, Iwasa H 2007 Concomitant lower serum albumin and vitamin D levels are associated with decreased objective physical performance among Japanese community-dwelling elderly. Gerontology **53:**322–328.
13. Lipsitz LA, Nakajima I, Gagnon M, Hirayama T, Connelly CM, Izumo H, Hirayama T 1994 Muscle strength and fall rates among residents of Japanese and American nursing homes: An international cross-cultural study. J Am Geriatr Soc **42:**953–959.
14. Suzuki T, Yoshida H, Hashimoto T, Yoshimura N, Fujiwara S, Fukunaga M, Nakamura T, Yoh K, Inoue T, Hosoi T, Orimo H 1997 Case-control study of risk factor for hip fracture in Japanese elderly by Mediterranean Osteoporosis Study (MEDOS) questionnaire. Bone **21:**461–467.
15. Suzuki T, Iwasa H, Yoshida H, Kim H, Watanabe S, Yukawa H 2003 Comprehensive health examination ('Otasha-Kenshin') for the prevention of geriatric syndromes and a bedridden state in the community elderly. 1. Differences in characteristics between participants and non-participants (in Japanese with English summary). Jpn J Public Health **50:**39–48.

16. Iwasa H, Suzuki T, Yoshida H, Kim H, Yoshida Y 2003 Cognitive function as the factor determining higher level competence in community-dwelling elderly (In Japanese with English summary). Jpn J Public Health **50**:950–958.
17. Kwon J, Suzuki T, Kim H, Yoshida H, Yoshida Y, Furuna T 2005 Health status and physical fitness undernourished community-dwelling elderly people. (In Japanese with English summary). Jpn J Phys Fitness Sports Med **54**:99–106.
18. Suzuki T, Sugiura M, Furuna T, Nishizawa S, Yoshida H, Ishizaki T, Kim H, Yukawa H 1999 Association of physical performance and falls among the community elderly in Japan in a 5 year follow-up study (in Japanese with English summary). Jpn J Geriatr **36**:472–478.
19. Obuchi S, Shibata H, Suzuki T, Yasumura S 1994 Relationship between walking ability and risk of falls in community-dwelling elderly in Japan. J Phys Ther Sci **6**:39–44.
20. Yasumura S, Haga H, Suzuki T, Shibata H 1994 Rate of falls: The correlates among elderly people living in an urban community in Japan. Age Ageing **23**:323–327.
21. Nagasaki H, Itoh H, Furuna T 1995 The structure underlying physical performance measures for older adults in the community. Aging Clin Exp Res **7**:451–458.
22. Thomas MK, Demay MB 2000 Vitamin D deficiency and disorders of vitamin D metabolism. Endocrinol Metab Clin North Am **29**:611–627.
23. Lips P, Chapuy MC, Dawson-Hughes B, Pols HA, Holick MF 1999 An international comparison of serum 25-hydroxyvitamin D measurements. Osteoporos Int **9**:394–397.
24. Nakamura K, Nashimoto M, Yamamoto M 2000 Summer/winter differences in the serum 25-hydroxyvitamin D3 and parathyroid hormone kevels of Japanese women. Int J Biometeorol **44**:186–189.
25. Malabanan A, Veronikis IE, Holick MF 1998 Redefining vitamin D insufficiency. Lancet **351**:805–806.
26. Need AG, Horowitz M, Morris HA, Nordin BC 2000 Vitamin D status: Effects on parathyroid hormone and 1.25-dihydroxyvitamin D in postmenopansal women. Am J Clin Nutr **71**:1577–1581.
27. Shibata H (ed.) 1997 An Overall Survey on Falls and Fractures of Community Elderly. Report on the Studies by National Science Research Grants. No. 07307007. Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan.
28. Blake AJ, Morgan K, Bendall MJ, Dallosso H, Ebrahim SB, Aric TH, Fentem PH, Bassey EJ 1988 Falls by elderly people at home: Prevalence and associated factors. Age Ageing **17**:365–372.
29. Wickham C, Cooper C, Margetts BM, Barker DJ 1989 Muscle strength, activity, housing and the risk of falls in elderly people. Age Ageing **18**:47–51.
30. Prudham D, Evans JG 1981 Factors associated with falls in the elderly: A community study. Age Ageing **10**:141–146.
31. Winner SJ, Morgan CA, Evans JG 1989 Perimenopausal risk of falling and incidence of distal forearm fracture. BMJ **298**:1486–1488.
32. O'Loughlin JL, Robitaille Y, Boivin JF, Suissa S 1993 Incidence of and risk factors for falls and injurious falls among the community-dwelling elderly. Am J Epidemiol **137**:342–354.
33. Aoyagi K, Ross P, Davis JW, Wasnich RD, Hayashi T, Takemoto T 1998 Falls among community-dwelling elderly in Japan. J Bone Miner Res **13**:1468–1474.
34. Schwartz AV, Kelsey JL, Maggi S, Tuttleman M, Ho SC, Jonsson PV, Poor G, Sisson JA, Xu L, Matkin CC, Nelson LM, Heyse SP 1999 International variation in the incidence of hip fractures: Cross-national project on osteoporosis for the WHO program for research on aging. Osteoporos Int **9**:242–253.
35. Pfeifer M, Begerow B, Minne HW, Abrams C, Nachtigall D, Hansen C 2000 Effects of a short-term vitamin D and calcium supplementation on body sway and secondary hyperparathyroidism in elderly women. J Bone Miner Res **15**:1113–1118.
36. Ministry of Health Labour and Welfare 2006 The National Health and Nutrition Survey, Japan, 2003. Tokyo, Ministry Health, Labour and Welfare, Tokyo, Japan
37. Kim H, Suzuki T, Iwasa H, Yoshida H 2003 A prospective study of the effects of regular sports practice on mortality among the elderly in a rural community in Japan: An 8-year follow-up study. Geriatr Gerontol Int **3**:S15–S20.
38. Shimada H, Lord S, Yoshida H, Kim H, Suzuki T 2007 Predictors of cessation of regular leisure-time physical activity in community-dwelling elderly people. Gerontology **53**:293–297.
39. Vieth R 2000 Problems with direct 25-hydroxyvitamin D assays. And the target amount of vitamin D nutrition desirable for patients with osteoporosis. Osteoporos Int **11**:635–636.
40. Holick MF 2004 Sunlight and vitamin D for bone health status and ramifications of vitamin deficiency. Am J Clin Nutr **80**(Suppl):1706S–1709S.
41. Ono Y, Suzuki A, Kotake M, Zang X, Nishiwaki K, Ishikawa Y, Imamura S, Nagata M, Takamoto S, Itoh M 2005 Seasonal changes of serum 25-hydroxynitamin D and intact parathyroid hormone level in a normal Japanese population. J Bone Miner Metab **23**:147–151.
42. Hollis BW 2005 Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: Implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr **135**:317–322.
43. Vieth R 1999 Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr **69**:842–856.
44. Binkly N, Krueger D, Cowgill CS 2004 Assay variation confounds the diagnosis of hypovitamin D: A call for standardization. J Clin Endocrinol Metab **89**:3152–3157.
45. Sakuma M, Endo N, Oinuma T, Hayami T, Endo E, Yazawa T, Watanabe K, Watanabe S 2006 Vitamin D and intact PTH status in patients with hip fracture. Osteoporos Int **17**:1608–1614.
46. Orimo H, Hashimoto T, Sakata K, Yoshimura N, Hosoi T, Suzuki T 2000 Trends in the incidence of hip fracture in Japan, 1987-1997: The third nationwide survey. J Bone Miner Metab **18**:126–131.
47. Gerdhem P, Ringsberg KAM, Obrant KJ, Akesson K 2005 Association between 25-hydroxyvitamin D level, physical activity, muscle strength and fractures in the prospective population-based OPRA study of elderly women. Osteoporos Int **16**:1425–1431.
48. Faulkner KA, Cauley JA, Zmuda JM, Landsittel DP, Newman AB, Studenski SA, Redfern MS, Ensrud KE, Fink HA, Lane NE, Nevitt MC 2006 Higher 1.25-dihydroxyvitamin D_3 concentrations associated with lower fall rates in older community-dwelling women. Osteoporos Int **17**:1318–1328.
49. Baumgartner RN, Koehler KM, Romero L, Garry PJ 1996 Serum albumin is associated with skeletal muscle in elderly men and women. Am J Clin Nutr **64**:552–558.
50. Visser M, Kritchevsky S, Newman A, Goodpaster B, Tylavasky F, Nevitt M, Harris T 2005 Lower serum albumin concentration and change in muscle mass: The health, aging and body composition study. Am J Clin Nutr **82**:531–537.
51. Kwon J, Suzuki T, Kim H, Yoshida H, Kumagai S, Yoshida Y, Furuna T, Sugiura M 2005 Health status and physical fitness of undernourished community-dwelling elderly people (in Japanese with English summary). Jpn J Phys Fitness Sports Med **54**:99–106.
52. Schalk BW, Deeg DJ, Penninx BW, Bouter L, Visser M 2005 Serum albumin and muscle strength: A longitudinal study in older men and women. J Am Geriatr Soc **53**:1331–1338.
53. Bischoff HA, Borchers M, Gudat F, Deurmueller U, Theiler R, Staehelin HB, Dick W 2001 In situ detection of the 1,25-dihydroxyvitamin D3 receptor in human skeletal muscle tissue. Histochem J **33**:19–24.
54. Janssen H, Samson M, Verhaar H 2002 Vitamin D deficiency, muscle function, and falls in elderly people. Am J Clin Nutr **75**:611–615.
55. Tokita A, Matsumoto H, Morrison NA, Tawa T, Miura Y, Irimoto M, Yamamori S, Watanabe T, Kuwabara Y, Yabuta K, Eisman JA 1997 Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. J Bone Miner Res **12**:494–495.
56. Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace

RB 1995 Lower extremity function in persons over the age of 70 year as a predictor of subsequent disability. N Engl J Med **332:**556–561.
57. Suzuki T, Yoshida H, Kim H, Yukawa H, Furuna T, Shinkai S, Ishizaki T, Wtanabe S, Shibata H 2003 Walking speed as a good predictor for maintenance of I-ADL among the rural community elderly in Japan: A 5-year follow-up study from TMIG-LISA. Geriatr Gerontol Int **3:**S6–S14.
58. Buchner DM, Cress ME, Esselman PC, Margherita AT, de Lateur BJ, Cambell AJ, Wagner EH 1996 Factors associated with changes in gait speed in older adults. J Gerontol A Biol Sci Med Sci **51:**M297–M302.
59. Gibbs J, Hughes S, Dunlop D, Singer R, Chang RW 1996 Predictors of change in walking velocity in older adults. J Am Geriatr Soc **44:**126–132.
60. Kwon J, Suzuki T, Yoshida H, Kim H, Yoshida Y, Iwasa H, Furuna T 2007 Association between change in bone mineral density and decline in usual walking speed in elderly community dwelling Japanese women during 2 year of follow-up. J Am Geriatr Soc **55:**240–244.
61. Tinetti ME, Speechly M, Ginter SF 1988 Risk factors for falls among elderly persons living in the community. N Engl J Med **319:**1701–1707.
62. Topper AK, Maki BE, Hokkiday PJ 1993 Are activity-based assessments of balance and gait in the elderly predictive of risk of falling and/or type of fall? J Am Geriatr Soc **41:**479–487.
63. LeBoff MS, Kohlmeier L, Hurwitz S, Franklin J, Wright J, Glowacki J 1999 Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. JAMA **281:**1505–1511.
64. Nuti R, Martini G, Valenti R, Gambera D, Gennari L, Salvadori S, Avanzati A 2004 Vitamin D status and bone turnover in women with acute hip fracture. Clin Orthop **422:**208–213.
65. Broe KE, Chen TC, Weinberg J, Bischoff-Ferrari HA, Holick MF, Kiel DP 2007 A higher dose of vitamin D reduces the risk of falls in nursing home residents: A randomized, multiple-dose study. J Am Geriatr Soc **55:**234–239.
66. Latham NK, Anderson CS, Reid IR 2003 Effects of Vitamin D supplementation on strength, physical performance, and falls in older persons: A systematic review. J Am Geriatr Soc **51:**1219–1226.
67. Suzuki T, Kim H, Yoshida H, Ishizaki T 2004 Randomized controlled trial of exercise intervention for the prevention of falls in community-dwelling elderly Japanese women. J Bone Miner Metab **22:**602–611.
68. Rubenstein LZ, Josephson KR, Trueblook PR, Loys S, Harker JO, Pietruszka FM, Robbins AS 2000 Effects of group exercise program on strength, morbility, and falls among fall-prone elderly men. J Gerontol A Biol Sci Med Sci **55:**M317–M321.
69. Nakamura K 2006 Vitamin D insufficiency in Japanese populations: From the viewpoint of the prevention of osteoporosis. J Bone Miner Metab **24:**1–6.
70. Nakamura K, Nishiwaki T, Ueno K, Yamamoto M 2005 Serum 25-hydroxylvitamin D and parathyroid hormone levels of non-institutionalized elderly Japanese requiring care. J Bone Miner Metab **23:**488–494.
71. Dukas LC, Schacht E, Mazor Z, Stahelin HB 2005 A new significant and independent risk factor for falls in elderly men and women: A low creatinine clearance of less than 65 ml/min. Osteoporos Int **16:**332–338.
72. Nakamura K, Nashimoto M, Hori Y, Yamamoto M 2000 Serum parathyroid hormone in healthy female Japanese in relation to serum 25-hydroxy vitamin D. Int J Vitamin Nutr Res **70:**287–292.

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Combination of Obesity with Hyperglycemia is a Risk Factor for the Presence of Vertebral Fractures in Type 2 Diabetic Men

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Abstract Although patients with type 2 diabetes show no bone mineral density (BMD) reduction, fracture risks are known to increase. It is unclear why the patients have an increased risk of fracture despite sufficient BMD. We investigated the relationships of body mass index (BMI), HbA_{1c}, and urinary C-peptide (uC-peptide) versus BMD, bone metabolic markers, serum adiponectin, and prevalent vertebral fracture (VF). A total of 163 Japanese type 2 diabetic men were consecutively recruited, and radiographic and biochemical data were collected. BMI was positively correlated with BMD at the whole body, lumbar spine, and femoral neck ($P < 0.05$) and negatively correlated with osteocalcin and urinary N-terminal cross-linked telopeptide of type-I collagen (uNTX) ($P < 0.01$). HbA_{1c} was negatively correlated with osteocalcin ($P < 0.01$) but not BMD at any site. Subjects were classified into four groups based on BMI and HbA_{1c} (group LL BMI < 24 and $HbA_{1c} < 9$, group LH BMI < 24 and $HbA_{1c} \geqq 9$, group HL BMI $\geqq 24$ and $HbA_{1c} < 9$, group HH BMI $\geqq 24$ and $HbA_{1c} \geqq 9$). Serum adiponectin, osteocalcin, and uNTX were lower and the incidence of VF was higher despite sufficient BMD in the HH group. Multivariate logistic regression analysis adjusted for age, duration of diabetes, uC-peptide, and estimated glomerular filtration rate showed that the HH group was associated with the presence of a VF and multiple VFs (odds ratio [OR] = 3.056, 95% confidence interval [CI] 1.031–9.056, $P = 0.0439$, and OR = 5.415, 95% CI 1.126–26.040, $P = 0.0350$, respectively). Combination of obesity with hyperglycemia was a risk factor for VF despite sufficient BMD in diabetic men.

Keywords Type 2 diabetes mellitus · Body mass index · HbA_{1c} · Vertebral fracture · Bone turnover

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The number of patients with diabetes mellitus and osteoporosis is rapidly increasing in industrialized countries where Western-style aging societies are prevalent. The relationship between diabetes and osteoporotic fractures is becoming increasingly recognized [1]. Both vertebral and hip fractures are most important osteoporotic fractures because they frequently occur and enhance the mortality of elderly people as high as six- to ninefold [2, 3]. The mortality increase is more prominent in men than in women [2], and absolute risk of subsequent fracture after an initial one in men is higher than or similar to that in women [4]. Therefore, it is no less important to predict the risk of vertebral and hip fractures in diabetic subjects than in their nondiabetic counterparts, especially in men.

Previous studies have shown that type 1 diabetes is associated with a decrease in bone mineral density (BMD) and an increased risk of osteoporotic hip and other fractures [5, 6]. In contrast, although patients with type 2 diabetes show no BMD reduction, fracture risks are known

to increase approximately up to 1.5-fold at the hip, proximal humerus, forearm, and foot not only in women [6–8] but also in men [6, 7]. However, it is unclear why patients with type 2 diabetes have an increased risk of fracture despite normal BMD. Hyperglycemia in type 2 diabetes might be associated with factors that influence bone strength and quality independent of BMD. Several experiments using cultured osteoblasts showed that high glucose and/or increased advanced glycemic end products (AGEs) impaired mineralization or osteocalcin production in the cells [9, 10], suggesting that hyperglycemia may cause diminished bone formation.

Body weight is known to impact bone turnover and to be positively correlated with BMD, and higher weight/body mass index (BMI) therefore reduce risks for vertebral and hip fractures in nondiabetic subjects [11, 12]. Previous studies have shown that BMD is closely related to body weight [13] and that change in spinal BMD is related to baseline BMI [14]. On the other hand, type 2 diabetes is caused by obesity-induced insulin resistance and is therefore strongly associated with obesity, and some studies have indicated that patients with the disease also have high BMD equivalent to nondiabetic obese counterparts [1]. However, it is unclear whether or not obese patients with type 2 diabetes would also be at reduced risk for fractures as seen in nondiabetics.

Insulin stimulates proliferation of osteoblasts [15] and increases indices of bone formation such as insulin-like growth factor-I [16] and bone morphogenetic protein [17] when administered locally over bone [18]. Previous studies have shown that circulating insulin concentration is the principal determinant of BMD at the femoral neck and lumbar spine [19]. Although obese diabetic patients have insulin resistance that blunts the hypoglycemic effect of the hormone and causes its compensatory oversecretion, other hormonal actions are kept intact, and increased circulating insulin may exert anabolic actions on bone [20]. A previous study has shown that insulin resistance, which was estimated from an intravenous glucose tolerance test, was significantly and positively correlated with BMD [21]. Thus, high BMD is a very consistent finding across a wide range of hyperinsulinemic states, including obesity and type 2 diabetes [22]. However, it is unclear whether or not this beneficial effect of hyperinsulinemia on BMD also prevents fractures in patients with type 2 diabetes.

In this study, to examine these issues, we selected serum HbA_{1c}, BMI, and urinary C-peptide (uC-peptide) as markers for hyperglycemia, obesity, and residual insulin secretion, respectively, in Japanese men with type 2 diabetes. We investigated the relationship between each of these parameters and BMD, bone metabolic markers, serum adiponectin levels, and the presence of vertebral fractures. We found that combination of obesity with hyperglycemia predisposes type 2 diabetic men to vertebral fractures in spite of normal BMD, possibly through low bone turnover and resultant bone fragility.

Subjects and Methods

Subjects

The subjects in this study were 163 Japanese men with type 2 diabetes aged 25–83 years (mean 57.7). We consecutively recruited subjects who visited Shimane University Hospital for education, evaluation, or treatment of diabetes. Subjects agreed to participate in the study and gave informed consent. This study was approved by the institutional review board of our institution. Nobody had hepatic or renal dysfunction or nutritional derangements that might cause changes in bone metabolism. We excluded patients with anemia (hemoglobin < 12.0 g/dL) because HbA_{1c} values might be influenced by anemia. Twenty-six patients had received insulin treatment and 61 patients had taken oral hypoglycemic agents (sulfonylurea, 55; metformin, 23; alpha-glucosidase inhibitor, 18). All subjects were free of drugs known to influence bone and calcium metabolism like vitamin D and bisphosphonate as well as thiazolidinedione until the time of the present study.

Radiography

Lateral X-ray films of the thoracic and lumbar spine were taken at the same week as serum collection. The anterior, central, and posterior heights of each of the 13 vertebral bodies from Th4 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 >20% compared to the height of the nearest uncompressed vertebral body [23]. Multiple vertebral fractures were identified as two or more vertebral fractures. None of the subjects had a history of serious trauma.

BMD and Biochemical Measurements

BMD values of the whole body (W), 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). The same operator tested all of the subjects during the study to eliminate operator discrepancies. The coefficients of variation (precision) of measurements of the lumbar spine, femoral neck, and mid-radius 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).

After overnight fasting, serum was collected. Biochemical markers were measured by standard biochemical

methods. Estimated glomerular filtration rate (eGFR) was calculated using the equation reported in the clinical practice guidelines for diagnosis and treatment of chronic kidney disease [24] as follows: $0.741 \times 175 \times \text{age}^{-0.203} \times \text{serum creatinine}^{-1.154}$. HbA_{1c} (normal range 4.3–5.8%) was determined by high-performance liquid chromatography (HPLC), as previously described [25–27]. Bone-specific alkaline phosphatase (BAP) in serum and C-peptide in urine pooled for 24 hours (uC-peptide) were measured by enzyme immunoassay (EIA) and chemiluminescent EIA (CLEIA), respectively. Serum osteocalcin and urinary N-terminal cross-linked telopeptide of type-I collagen (uNTX) were measured by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). Total adiponectin and high–molecular weight (HMW) adiponectin were measured by ELISA kits (Otsuka Pharmaceuticals, Tokyo, Japan, and Fujirebio, Tokyo, Japan, respectively) as indicated by the manufacturers. The coefficients of variation of measurements of total adiponectin and HMW adiponectin by each ELISA kit were 3.1% and 2.0%, respectively.

Statistical Analysis

Data were expressed as mean ± SD. Because serum total and HMW adiponectin showed a markedly skewed distribution, logarithmic (log) transformation of these values was carried out before performing correlation and regression analysis. Statistical significance between two groups was determined using the Mann–Whitney *U*-test. Simple, multiple, and logistic regression analyses were performed using the statistical computer program StatView (Abacus Concepts, Berkely, CA). $P < 0.05$ was considered significant.

Results

Baseline Characteristics of Subjects and Comparison of Parameters Among Those with and without Vertebral Fractures

Baseline characteristics of subjects are shown in Table 1. We compared these parameters among subjects with a vertebral fracture, with multiple vertebral fractures, and without vertebral fractures and found no significant differences among them (data not shown).

Relationship of BMI, HbA_{1c}, and uC-Peptide Versus BMD, Bone Metabolic Markers, and Serum Adiponectin Levels

We selected BMI, HbA_{1c}, and uC-peptide as markers for obesity, hyperglycemia, and endogenous insulin secretion, respectively. Since our simple regression analysis showed that BMI, HbA_{1c}, and uC-peptide were affected by age and renal function (data not shown), multiple regression analyses were performed with BMI, HbA_{1c}, and uC-peptide adjusted for age, duration of diabetes, and eGFR as independent variables versus BMD at each skeletal site, bone metabolic markers, or serum adiponectin levels as dependent variables (Table 2). BMI was significantly and positively correlated with W-, L-, and F-BMD ($P = 0.0070$, $P = 0.0128$, and $P < 0.0001$, respectively) but not with 1/3R-BMD. BMI was also significantly and negatively correlated with osteocalcin, uNTX, and log(total adiponectin) ($P = 0.0070$, $P = 0.0002$, and $P = 0.0359$, respectively) and tended to be negatively correlated with log(HMW adiponectin). HbA_{1c} was significantly and negatively correlated with osteocalcin ($P = 0.0022$) but not with BMD at any site, any other bone metabolic markers, or serum adiponectin levels. On the other hand, uC-peptide was not correlated with BMD at any site or any bone metabolic markers, except for a negative correlation with log(total adiponectin).

Table 1 Baseline characteristics of subjects

Number of subjects	163
Age (years)	57.7 ± 12.8
Diabetes duration (years)	9.8 ± 8.7
Body weight (kg)	65.2 ± 13.4
Height (cm)	165.6 ± 7.0
BMI (kg/m^2)	23.7 ± 3.9
FPG (mg/dL)	176 ± 64
HbA_{1c} (%)	9.4 ± 2.8
Creatinine (mg/dL)	0.74 ± 0.12
eGFR (mL/min/1.73 m^2)	83.9 ± 17.6
uC-peptide (μg/day)	68.7 ± 48.8
BAP (U/L)	26.7 ± 10.5
Osteocalcin (ng/mL)	5.0 ± 2.2
uNTX (nMBCE/mM-Cr)	35.1 ± 17.6
Total adiponectin (μg/mL)	5.08 ± 2.97
HMW adiponectin (μg/mL)	5.00 ± 4.00
W-BMD (g/cm^2)	1.081 ± 0.106
L2–L4 BMD (g/cm^2)	1.043 ± 0.175
T score	−0.03 ± 1.46
Z score	0.44 ± 1.06
F-BMD (g/cm^2)	0.781 ± 0.122
T score	−0.65 ± 0.96
Z score	0.25 ± 0.99
1/3R-BMD (g/cm^2)	0.726 ± 0.067
T score	−1.70 ± 1.20
Z score	−0.80 ± 1.05
Number of subjects with vertebral fracture	39
Number of subjects with multiple vertebral fractures	14

FPG, fasting plasma glucose

Table 2 Correlations of BMI, HbA_{1c}, and urinary CPR versus BMD at each site, bone metabolic markers, or serum adiponectin levels

	BMI		HbA1c		uC-peptide	
	r	*P*	*r*	*P*	*r*	*P*
W-BMD (g/cm^2)	0.237	0.0070	0.018	0.8354	0.032	0.7132
L2–L4 BMD (g/cm^2)	0.215	0.0128	−0.078	0.3577	0.070	0.4126
F-BMD (g/cm^2)	0.379	<0.0001	−0.079	0.2898	0.085	0.2621
1/3R-BMD (g/cm^2)	0.068	0.4113	0.115	0.1657	0.004	0.9609
BAP (U/L)	0.072	0.4186	0.036	0.6775	0.065	0.4512
Osteocalcin (ng/mL)	−0.250	0.0070	−0.285	0.0022	−0.001	0.9951
uNTX (nMBCE/mM-Cr)	−0.314	0.0002	−0.013	0.8702	−0.034	0.6687
Log(total adiponectin)	−0.211	0.0359	0.099	0.3345	−0.305	0.0034
Log(HMW adiponectin)	−0.192	0.0649	0.098	0.3572	−0.161	0.1279

Multivariate regression analysis was performed between BMI, HbA1c, and uC-peptide adjusted for age, duration of diabetes, and eGFR as independent variables and BMD at each skeletal site, bone marker, or serum adiponectin levels as dependent variables

Comparison of Various Variables Among Four Groups Classified by BMI and HbA_{1c}

Next, since these results showed that BMI and HbA_{1c}, but not uC-peptide, were significantly and independently associated with serum osteocalcin levels, we compared demographic and biochemical parameters including bone metabolic markers and serum adiponectin concentrations as well as BMD values at each skeletal site among four groups classified by BMI and HbA_{1c} (group LL BMI < 24 and $HbA_{1c} < 9$, group LH BMI < 24 and $HbA_{1c} \geqq 9$, group HL BMI $\geqq 24$ and $HbA_{1c} < 9$, group HH BMI $\geqq 24$ and $HbA_{1c} \geqq 9$) (Table 3). There were no significant differences in age or duration of diabetes among the groups. Absolute values and Z scores of L-BMD in group HL and F-BMD in groups HL and HH were significantly higher than those in group LH (at least $P < 0.05$). Absolute values of L-BMD in group HL and absolute values and Z score of F BMD in group HH were significantly higher than those in group LL (at least $P < 0.05$). Serum osteocalcin level in group HH was significantly lower than that in group LL ($P < 0.01$). Moreover, uNTX in groups HL and HH were significantly lower than those in groups LL and LH (at least $P < 0.05$). Serum levels of total and HMW adiponectins in group HH were lower than those in group LH ($P < 0.05$), and HMW adiponectin in group HL was lower than that in group LH ($P < 0.05$). The prevalence of vertebral fractures was highest in group HH despite sufficient BMD.

Multivariate logistic regression analysis was performed with the presence of a vertebral fracture or multiple vertebral fractures as a dependent variable and each group adjusted for age, duration of diabetes, uC-peptide, and eGFR as independent variables (Table 4). Group HH was significantly associated with the presence of a vertebral fracture and multiple vertebral fractures ($P = 0.0440$ and $P = 0.0354$, respectively).

Discussion

In this study, combination of obesity with poor glycemic control in men with type 2 diabetes was significantly associated with the presence of vertebral fractures in spite of sufficient BMD. Multiple regression analysis showed that BMI was negatively correlated with serum osteocalcin and uNTX levels and that HbA_{1c} was negatively correlated with serum osteocalcin level. Moreover, these bone metabolic markers were lower in the group with higher BMI and higher HbA_{1c} (HH group), suggesting that low bone turnover, especially suppression of bone formation, may cause bone fragility that is not defined by BMD and may be linked to vertebral fractures in the obese and hyperglycemic group.

The present study showed that HbA_{1c} level was not correlated with BMD and was not different between patients with or without vertebral fractures. In contrast, HbA_{1c} level was negatively correlated with serum osteocalcin level but not with BAP. Although a few studies demonstrated that glycemic control estimated by HbA_{1c} was associated with BMD or bone fractures [5, 8], meta-analysis of accumulating studies has shown that HbA_{1c} level was not significantly associated with BMD or fracture incidence [6]. In previous studies, we have also shown that HbA_{1c} levels in postmenopausal women with type 2 diabetes were not significantly different between those with and without vertebral fractures [25–27]. On the other hand, several studies have indicated that hyperglycemia induced a low turnover bone with osteoblast dysfunction and caused suppression of the serum osteocalcin level [28, 29]. Gerdhem et al. [29] showed that serum osteocalcin level, but not BAP, was lower in diabetic women after correction for covariance of body weight and serum creatinine. Okazaki et al. [30] showed that serum osteocalcin level was low before treatment and elevated after treatment of diabetes, while BAP was reduced. Previous in vitro studies have shown that chronic hyperglycemia increased the activity and expression of alkaline phosphatase while it decreased osteocalcin expression and cellular calcium uptake [31], explaining the discrepancy in serum levels of osteocalcin and BAP in the clinical studies.

Table 3 Comparison of various variables among the four groups classified by BMI and HbA_{1c}

	LL	LH	HL	HH
Number of subjects	43	47	35	38
Age (years)	60.9 ± 11.1	56.3 ± 10.5	57.9 ± 15.1	55.7 ± 14.6
Diabetes duration (years)	10.3 ± 8.6	10.2 ± 8.9	8.1 ± 7.8	10.3 ± 9.6
BMI	21.4 ± 1.7	20.8 ± 1.9	27.2 ± 2.8***,†††	26.7 ± 3.5***,†††
FPG	138 ± 35	224 ± 61***,‡‡‡	123 ± 25	210 ± 54***,‡‡‡
HbA_{1c}	7.4 ± 1.0	11.6 ± 2.3***,‡‡‡	7.1 ± 1.2	11.1 ± 2.4***,‡‡‡
uC-peptide	61.5 ± 61.1	61.5 ± 41.1	77.9 ± 48.4	78.1 ± 40.5
Creatinine	0.76 ± 0.12	0.69 ± 0.11**	0.80 ± 0.12†††	0.74 ± 0.12†,‡
eGFR	80.3 ± 16.5	91.8 ± 17.9**	76.9 ± 15.4††	84.8 ± 17.1‡
BAP	27.6 ± 15.1	27.3 ± 8.3	24.6 ± 7.4	27.1 ± 9.0
Osteocalcin	5.8 ± 2.2	5.1 ± 2.7	5.0 ± 1.8	4.1 ± 1.6**
uNTX	41.5 ± 24.7	38.5 ± 15.6	27.8 ± 10.7**,††	30.8 ± 12.2*,†
Total adiponectin	5.68 ± 3.87	5.78 ± 2.99	4.58 ± 2.50	4.04 ± 1.62†
HMW adiponectin	5.43 ± 4.57	6.58 ± 4.93	3.98 ± 2.35†	3.61 ± 2.46†
W-BMD	1.058 ± 0.113	1.064 ± 0.092	1.104 ± 0.117	1.106 ± 0.099
L2–L4 BMD	1.025 ± 0.190	1.005 ± 0.160	1.119 ± 0.169*,††	1.037 ± 0.163
Z score	0.37 ± 1.11	0.20 ± 1.07	0.86 ± 0.98†	0.41 ± 0.99
F-BMD	0.762 ± 0.129	0.735 ± 0.097	0.805 ± 0.122†	0.838 ± 0.118**,†††
Z score	0.20 ± 1.0	−0.20 ± 0.94	0.52 ± 0.88††	0.64 ± 0.90*,†††
1/3R-BMD	0.706 ± 0.066	0.730 ± 0.060	0.724 ± 0.067	0.740 ± 0.075
Z score	−0.99 ± 1.09	−0.80 ± 0.95	−0.76 ± 1.11	−0.67 ± 1.13
Vertebral fracture	8 (18.6%)	10 (21.3%)	8 (22.9%)	13 (34.2%)
Multiple vertebral fractures	3 (7.0%)	4 (8.5%)	0 (0.0%)	7 (18.4%)

FPG, fasting plasma glucose

Group LL BMI < 24 and HbA_{1c} < 9, group LH BMI < 24 and HbA_{1c} ≧ 9, group HL BMI ≧ 24 and HbA_{1c} < 9, group HH BMI ≧ 24 and HbA_{1c} ≧ 9

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus LL; † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ versus LH; ‡ $P < 0.05$, ‡‡ $P < 0.05$, ‡‡‡ $P < 0.001$ versus HL

Table 4 Associations between the presence of vertebral fractures and the four groups classified by BMI and HbA_{1c} in men with type 2 diabetes

	OR (95% CI)	*P*
Presence of a vertebral fracture		
Group LH	1.362 (0.443–4.189)	0.5898
Group HL	1.457 (0.463–4.586)	0.5197
Group HH	3.051 (1.030–9.035)	0.0440
Presence of multiple vertebral fractures		
Group LH	1.848 (0.343–9.964)	0.4748
Group HL	1.722 (0.303–9.794)	0.5398
Group HH	5.353 (1.121–25.557)	0.0354

Multiple logistic regression analysis was performed with the presence of either a vertebral fracture or multiple vertebral fractures as a dependent variable and each of four groups classified by BMI and HbA_{1c} adjusted for age, diabetes duration, uC-peptide, and eGFR as independent variables

The OR of each group was calculated by comparison with group LL

Obesity is known to suppress both bone formation and resorption and to be positively associated with BMD. A large clinical study on Danish postmenopausal women showed that subjects in the highest tertiles of percentage of body fat or BMI had up to 12% higher BMD at baseline and a more than twofold lower 2-year bone loss compared with women in the lowest tertiles [32]. Women with a higher percentage of body fat or BMI had lower baseline levels of uNTX and serum osteocalcin [32]. On the other hand, weight loss over 6–8 months resulted in increases in indices of bone resorption marker expression, deoxypyridinoline and hydroxyproline, of up to 50% [33], suggesting that body weight directly influenced bone resorption and bone turnover. The present findings also showed that BMI was strongly and negatively correlated with serum osteocalcin and uNTX and strongly and positively correlated with BMD. These findings suggest that obesity induces low bone turnover through suppression of both osteoblastic and osteoclastic activities. Combination of hyperglycemia with

obesity could enhance the suppression of serum osteocalcin level and may accelerate reduction in bone formation rate.

Adipocytokines, which are secreted from adipocytes, are known to influence bone metabolism. Adiponectin is one of the adipocytokines specifically and highly expressed in visceral, subcutaneous, and bone marrow fat depots [34]. Several studies have demonstrated that circulating adiponectin concentrations are decreased in patients with obesity, diabetes, and cardiovascular disease [35] and inversely associated with obesity [35, 36] as well as insulin-resistance parameters [36]. Recently, we and other researchers have shown that cultured osteoblasts have an adiponectin receptor and that their proliferation, differentiation, and mineralization are enhanced by adiponectin, showing that adiponectin could stimulate bone formation [37]. In animal studies, Oshima et al. [38] showed that adiponectin adenovirus–treated mice increased trabecular bone mass in vivo and that administration of adiponectin enhanced bone formation and suppressed bone resorption in vitro. Moreover, in a clinical study, Richards et al. [39] showed that serum adiponectin was positively correlated with serum osteocalcin in a large population of women. Thus, adiponectin appears to enhance osteoblastogenesis and bone formation, and reduction in its plasma concentration induced by combination of obesity with hyperglycemia may cause suppressed bone formation. We actually observed that serum adiponectin concentrations were lowest in the group with higher BMI and higher HbA_{1c} (HH group), suggesting that serum hypoadiponectinemia might be involved in the present findings of low turnover bone in patients with obesity and hyperglycemia.

Although circulating insulin is considered to stimulate osteoblastogenesis and enhance bone formation, the present study showed that uC-peptide, as a surrogate marker for residual insulin secretion, was not significantly associated with BMD, bone metabolic markers, or serum adiponectin levels in men with type 2 diabetes. We also found that its level was not different between patients with or without vertebral fractures. These findings are consistent with our previous ones in women with type 2 diabetes, in which there were no associations between serum fasting C-peptide and BMD, bone metabolic markers, or vertebral fractures [24–26]. However, subjects in these studies had received several treatments including insulin administration. Therefore, we should be careful when making conclusions about the relationship between capacity of residual insulin secretion and bone metabolism.

Previous studies showed that hyperglycemia and AGEs could impair parathyroid hormone (PTH) secretion [40–42] and that vitamin D metabolism may also be disturbed by hyperglycemia and insulin deficiency [43–45]. Thus, impaired PTH and vitamin D metabolism might be involved in low turnover bone in diabetes, although we did not measure serum PTH and vitamin D levels in this study. Further studies are needed to investigate whether these hormone levels are associated with bone fractures in type 2 diabetes.

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 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 with the disorders. Consequently, assessment of larger numbers of patients is necessary to determine the usefulness of BMI and HbA_{1c} for predicting the risk of vertebral fractures. Third, the subjects in this study were only Japanese. The capacity of insulin secretion and the degree of obesity in Asians are known to be different from those of Western people [46]. Therefore, we need to investigate whether or not our findings are universal. Finally, longitudinal studies are needed to investigate whether the treatments of both obesity and diabetes can improve bone fragility in type 2 diabetes.

In conclusion, we found that BMI was negatively associated with serum osteocalcin and uNTX and positively associated with BMD, while HbA_{1c} level was negatively correlated with serum osteocalcin but not with BMD in diabetic men. Although BMI or HbA_{1c} per se are not associated with the presence of vertebral fractures, the combination of higher values of both parameters was a risk factor for vertebral fractures in spite of sufficient BMD. Reduced bone turnover and resultant impairment in bone quality in patients with obesity and hyperglycemia may be involved in this observation.

References

1. Barrett-Connor E, Holbrook TL (1992) Sex differences in osteoporosis in older adults with non-insulin-dependent diabetes mellitus. JAMA 268:3333–3337
2. Center JR, Nguyen TV, Schneider D, Sambrook PN, Eisman JA (1999) Mortality after all major types of osteoporotic fracture in men and women an observational study. Lancet 353:878–882
3. Cauley JA, Thompson DE, Ensrud KC, Scott JC, Black D (2000) Risk of mortality following clinical fractures. Osteoporos Int 11:556–561
4. Center JR, Bliuc D, Nguyen TV, Eisman JA (2007) Risk of subsequent fracture after low-trauma fracture in men and women. JAMA 297:387–394
5. Ivers RQ, Cumming RG, Mitchell P, Peduto AJ (2001) Diabetes and risk of fracture: the Blue Mountains Eye Study. Diabetes Care 24:1198–1203
6. Vestergaard P (2007) Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes-a meta-analysis. Osteoporos Int 18:427–444

7. Lipscombe LL, Jamal SA, Booth GL, Hawker GA (2007) The risk of hip fractures in older individuals with diabetes: a population-based study. Diabetes Care 30:835–841
8. Strotmeyer ES, Cauley JA, Schwartz AV, Nevitt MC, Resnick HE, Bauer DC, Tylavsky FA, de Rekeneire N, Harris TB, Newman AB (2005) Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: the health, aging, and body composition study. Arch Intern Med 165:1612–1617
9. Ogawa N, Yamaguchi T, Yano S, Yamauchi M, Yamamoto M, Sugimoto T (2007) The combination of high glucose and advanced glycation end-products (AGEs) inhibits the mineralization of osteoblastic MC3T3-E1 cells through glucose-induced increase in the receptor for AGEs. Horm Metab Res 39:871–875
10. Inaba M, Terada M, Koyama H, Yoshida O, Ishimura E, Kawagishi T, Okuno Y, Nishizawa Y, Otani S, Morii H (1995) Influence of high glucose on 1,25-dihydroxyvitamin D3-induced effect on human osteoblast-like MG-63 cells. J Bone Miner Res 10:1050–1056
11. Ensrud KE, Lipschutz RC, Cauley JA, Seeley D, Nevitt MC, Scott J, Orwoll ES, Genant HK, Cummings SR (1997) Body size and hip fracture risk in older women: a prospective study. Study of Osteoporotic Fractures Research Group. Am J Med 103: 274–280
12. Johnell O, O'Neill T, Felsenberg D, Kanis J, Cooper C, Silman AJ (1997) Anthropometric measurements and vertebral deformities. European Vertebral Osteoporosis Study (EVOS) Group. Am J Epidemiol 146:287–293
13. Felson DT, Zhang Y, Hannan MT, Anderson JJ (1993) Effects of weight and body mass index on bone mineral density in men and women: the Framingham Study. J Bone Miner Res 8:567–573
14. Tremollieres FA, Pouilles JM, Ribot C (1993) Vertebral postmenopausal bone loss is reduced in overweight women: a longitudinal study in 155 early postmenopausal women. J Clin Endocrinol Metab 77:683–686
15. Hickman J, McElduff A (1989) Insulin promotes growth of the cultured rat osteosarcoma cell line UMR-106–01: an osteoblast-like cell. Endocrinology 124:701–706
16. McCarthy TL, Centrella M, Canalis E (1989) Insulin-like growth factor (IGF) and bone. Connect Tissue Res 20:277–282
17. Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, Boyce B, Zhao M, Gutierrez G (1999) Stimulation of bone formation in vitro and in rodents by statins. Science 286:1946–1949
18. Cornish J, Callon KE, Reid IR (1996) Insulin increases histomorphometric indices of bone formation in vivo. Calcif Tissue Int 59:492–495
19. Reid IR, Evans MC, Cooper GJ, Ames RW, Stapleton J (1993) Circulating insulin levels are related to bone density in normal postmenopausal women. Am J Physiol Endocrinol Metab 265:E655–E659
20. Reid IR (2002) Relationships among body mass, its components, and bone. Bone 31:547–555
21. Abrahamsen B, Rohold A, Henriksen JE, Beck-Nielsen H (2000) Correlations between insulin sensitivity and bone mineral density in nondiabetic men. Diabet Med 17:124–129
22. Leidig-Bruckner G, Ziegler R (2001) Diabetes mellitus a risk for osteoporosis? Exp Clin Endocrinol Diabetes 109:S493–S514
23. Genant HK, Jergas M, Palermo L, Nevitt M, Valentin RS, Black D, Cummings SR (1996) Comparison of semiquantitative visual and quantitative morphometric assessment of prevalent and incident vertebral fractures in osteoporosis. The Study of Osteoporotic Fractures Research Group. J Bone Miner Res 11:984–996
24. Japanese Society of Nephrology (2007) Clinical practice guidelines for diagnosis and treatment of chronic kidney disease [in Japanese]. Jpn J Nephrol 49:755–870
25. Yamamoto M, Yamaguchi T, Yamauchi M, Kaji H, Sugimoto T (2007) Bone mineral density is not sensitive enough to assess the risk of vertebral fractures in type 2 diabetic women. Calcif Tissue Int 80:353–358
26. Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Yano S, Sugimoto T (2007) Serum insulin-like growth factor-I is associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes mellitus. Osteoporos Int 18:1675–1681
27. Yamamoto M, Yamaguchi T, Yamauchi M, Yano S, Sugimoto T (2008) Serum pentosidine levels are positively associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes. J Clin Endocrinol Metab 93:1013–1019
28. Verhaeghe J, Suiker AM, Nyomba BL, Visser WJ, Einhorn TA, Dequeker J, Bouillon R (1989) Bone mineral homeostasis in spontaneously diabetic BB rats. II. Impaired bone turnover and decreased osteocalcin synthesis. Endocrinology 124:573–582
29. Gerdhem P, Isaksson A, Akesson K, Obrant KJ (2005) Increased bone density and decreased bone turnover, but no evident alteration of fracture susceptibility in elderly women with diabetes mellitus. Osteoporos Int 16:1506–1512
30. Okazaki R, Totsuka Y, Hamano K, Ajima M, Miura M, Hirota Y, Hata K, Fukumoto S, Matsumoto T (1997) Metabolic improvement of poorly controlled noninsulin-dependent diabetes mellitus decreases bone turnover. J Clin Endocrinol Metab 82:2915–2920
31. Botolin S, McCabe LR (2006) Chronic hyperglycemia modulates osteoblast gene expression through osmotic and non-osmotic pathways. J Cell Biochem 99:411–424
32. Ravn P, Cizza G, Bjarnason NH, Thompson D, Daley M, Wasnich RD, McClung M, Hosking D, Yates AJ, Christiansen C (1999) Low body mass index is an important risk factor for low bone mass and increased bone loss in early postmenopausal women. Early Postmenopausal Intervention Cohort (EPIC) study group. J Bone Miner Res 14:1622–1627
33. Ricci TA, Heymsfield SB, Pierson RN, Stahl T, Chowdhury HA, Shapses SA (2001) Moderate energy restriction increases bone resorption in obese postmenopausal women. Am J Clin Nutr 73:347–352
34. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA (2001) Hypoadiponectemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 86:1930–1935
35. Matsubara M, Maruoka S, Katayose S (2002) Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. Eur J Endocrinol 147:173–180
36. Ryan AS, Berman DM, Nicklas BJ, Shiha M, Gingerich RL, Meneilly GS, Egan JM, Elahi D (2003) Plasma adiponectin and leptin levels, body composition, and glucose utilization in adult women with wide ranges of age and obesity. Diabetes Care 26:2383–2388
37. Kanazawa I, Yamaguchi T, Yano S, Yamauchi M, Yamamoto M, Sugimoto T (2008) Adiponectin and AMP kinase activator stimulate proliferation, differentiation, and mineralization of osteoblastic MC3T3-E1 cells. BMC Cell Biol 8:51–62
38. Oshima K, Nampei A, Matsuda M, Iwaki M, Fukuhara A, Hashimoto J, Yoshikawa H, Shimomura I (2005) Adiponectin increases bone mass by suppressing osteoclast and activating osteoblast. Biochem Biophys Res Commun 331:520–526
39. Richards JB, Valdes AM, Burling K, Perks UC, Spector TD (2007) Serum adiponectin and bone mineral density in women. J Clin Endocrinol Metab 92:1517–1523
40. Sugimoto T, Ritter C, Morrissey J, Hayes C, Slatopolsky E (1990) Effects of high concentrations of glucose on PTH secretion in parathyroid cells. Kidney Int 37:1522–1527

41. Kawagishi T, Morii H, Nakatsuka K, Sasao K, Kawasaki K, Miki T, Nishizawa Y (1991) Parathyroid hormone secretion in diabetes mellitus. Contrib Nephrol 90:217–222
42. Yamamoto T, Ozono K, Miyauchi A, Kasayama S, Kojima Y, Shima M, Okada S (2001) Role of advanced glycation end products in adynamic bone disease in patients with diabetic nephropathy. Am J Kidney Dis 38:S161–S164
43. Ikeda K, Matsumoto T, Morita K, Yamato H, Takahashi H, Ezawa I, Ogata E (1987) The role of insulin in the stimulation of renal 1,25-dihydroxyvitamin D synthesis by parathyroid hormone in rats. Endocrinology 121:1721–1726
44. Pietschmann P, Schernthaner G, Woloszczuk W (1988) Serum osteocalcin levels in diabetes mellitus: analysis of the type of diabetes and microvascular complications. Diabetologia 31:892–895
45. Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E (1995) Serum 25-hydroxyvitamin D_3 levels decreased in impaired glucose tolerance and diabetes mellitus. Diabetes Res Clin Pract 27:181–188
46. Fujimoto WY (1996) Overview of non-insulin-dependent diabetes mellitus (NIDDM) in different population groups. Diabet Med 13:S7–S10

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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 also it 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

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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-I 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 of 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 μl of serum samples diluted 1:441 was placed in each of the 96 wells. The MABconjugated with HRP was used as the detecting antibody. Contents of wells were incubated for 30 min 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 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 ± 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 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	
Number of subjects	231	170	*P*
Age (years)	59.6±13.3	66.7±10.2	<0.0001
Diabetes duration (years)	10.7±9.0	12.3±9.9	0.0619
Body height (cm)	165.9±6.7	150.4±6.0	<0.0001
Body weight (kg)	65.4±15.8	55.4±11.0	<0.0001
BMI (kg/m^2)	23.6±4.7	24.5±4.5	0.0563
HbA1c (%)	8.8±2.6	8.4±2.4	0.6918
Creatinine (mg/dl)	0.80±0.18	0.64±0.17	<0.0001
Total adiponectin (μg/ml)	6.31±4.10	8.80±5.85	<0.0001
HMW adiponectin (μg/ml)	6.26±5.61	9.08±7.32	<0.0001
Total BMD (g/cm^2)	1.080±0.113	0.926±0.114	<0.0001
L2-4 BMD (g/cm^2)	1.044±0.189	0.881±0.190	<0.0001
T score	−0.03±1.58	−1.12±1.71	<0.0001
Z score	0.48±1.16	0.62±1.22	0.2724
F Neck BMD (g/cm^2)	0.786±0.133	0.643±0.131	<0.0001
T score	−0.62±1.05	−1.33±1.20	<0.0001
Z score	0.33±1.09	0.50±1.22	0.1507
1/3R BMD (g/cm^2)	0.712±0.069	0.533±0.090	<0.0001
T score	−1.53±1.30	−2.49±1.72	<0.0001
Z score	−0.59±1.15	0.63±1.50	<0.0001
Osteocalcin (ng/ml)	5.0±2.4	7.2±2.9	<0.0001
uNTX (nMBCE/mM-Cr)	32.6±17.3	54.9±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.