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Effect of Angiotensin Converting Enzyme Inhibitor and Benzodiazepine Intake on Bone Loss in Older Japanese

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ABSTRACT

We investigated the effects of several frequently described medication regimens on annual percentage change in bone mineral density (BMD). A longitudinal cohort study (a retrospective analysis) was conducted. Subjects in the Adult Health Study (a prospective cohort study begun in 1958) have been followed through biennial medical examinations in Hiroshima, Japan. Participants were 2,111 subjects (67% women; aged 47-95 years) who were undergoing biennial health examinations from 1994 to 2000. The subjects were examined for the effect of certain drugs on bone mineral change during baseline and one follow-up (4 year later) measurements. Mean annual percentage change in BMD at the femoral neck was −0.38% for men, and −1.14% for women. After adjustment for sex, age, change of weight, alcohol consumption, and smoking status, annual percentage change in BMD decreased by 0.61% among individuals taking angiotensin converting enzyme (ACE) inhibitors continuously in comparison with individuals who had not taken them (p=0.002): also decreased 0.40% among individuals taking benzodiazepines (BZDs) continuously (p=0.034). Our results suggest that careful consideration should be given to the use of ACE inhibitors and BZDs in a cohort of Japanese elderly.

Key words: *Bone loss, Angiotensin converting enzyme inhibitor, Benzodiazepine, Pharmacoepidemiology*

Change in bone mineral density (BMD) is known to be a useful measure for evaluating the risk of bone fractures, and there have been many reports studying various factors that might be associated with change of BMD. While early reports indicate that intake of glucocorticoids[3] and thyroid hormones[29] tends to reduce BMD and that intake of estrogens[9] and thiazide[6] tends to increase it, recent studies have reported that other medications that are not intended for bone therapies could affect bone loss. For example, statins[7], beta-adrenergic blockers (β -blockers)[24], and cyclooxygenase-2-selective non-steroidal anti-inflammatory drugs (COX-2-selective NSAIDs)[5] reportedly tend to increase BMD. Another study reports that low serum vitamin B12 levels may be associated with increased rates of hip bone loss[31]. On the other hand, there are also reports with contrary results[25,30], so there is not yet a unified consensus. In addition, most of these reports are studies based on populations of Caucasians in Western countries. Medications among Caucasians in Western countries are expected to be very different from those among other populations, including Japanese; e.g., the doses and types of medication administered in Japan differ from those in Western countries. Therefore, it is important to find evidence of the effects on BMD, positive or negative, of a variety of drugs and supplements, including those not targeting osteoporosis, such as antihypertensive drugs. The main purpose of this study is to examine the association between BMD change and drug use in a 2,111 cohort of elderly Japanese men and women. This study was a retrospective analysis, and we had no intervention for the effects of medications.

MATERIALS AND METHODS

Study Population

In 1958, the Atomic Bomb Casualty Commission (predecessor to the Radiation Effects Research Foundation) initiated the so-called Adult Health Study (AHS), a cohort follow-up study which relies upon data obtained in the course of biennial health examinations of participants in a fixed cohort to shed light on the many changes in physiological and biochemical functions that might be attributable to atomic bomb radiation exposure[17,36]. The initial AHS cohort consisted of ~15,000 atomic bomb survivors and ~5,000 controls, all of whom were selected from the residents of Hiroshima and Nagasaki on the basis of a supplementary questionnaire that was included in the 1950 national census and a survey of atomic bomb survivors. Participation rates in the study were ~80% throughout the follow-up period. Further information about the expansion of the cohort and details of the health examinations are available elsewhere[1,11].

A total of 2,613 AHS subjects aged between 47 and 95 years underwent physical examination in Hiroshima in the 1994-1995 examination cycle. We then selected 2,111 of these subjects (703 men and 1,408 women) for our analysis. The remaining 73 subjects were excluded either because of a diagnosis suggesting impairment of bone metabolism (such as hyperparathyroidism, renal osteodystrophy, or bilateral oophorectomy) or because they were only available for one examination during the 1994-2000 period. The other remaining 429 subjects were excluded because they were premenopausal or they took drugs that interfered with bone metabolism, including estrogens, glucocorticoids, calcitonins, ipriflavones, bisphosphonates, and thyroid hormones, and the sample sizes of them were too small to attain statistical significance.

Bone Mineral Density

BMD was measured at the femoral neck at each biennial health examination using a dual x-ray absorptiometer (DXA, QDR-2000; Hologic Inc, Waltham, MA, USA). An anthropomorphic spine phantom was scanned daily to calibrate the DXA. There was no significant fluctuation in machine performance during the study period. The precision of the DXA was also carefully monitored over the study period using the anthropomorphic phantom, and was found to be less than 1%.

In our study, annual percentage change in BMD was defined as follows:

$$\text{Annual Percentage Change in BMD (\%)} = \frac{\text{BMD at second exam - BMD at baseline exam}}{\text{BMD at baseline exam} \times \text{follow-up period (yrs.)}} \times 100$$

Other Measurements

Subjects were asked to bring every over-the-counter or prescription medication they were taking, and in interviews by trained nurses were asked how often and how much of these medications they were taking. Subjects known to be taking at least one medication were then categorized into 4 groups for each medication according to "on" vs. "off" status of the use of each drug in the baseline (1994-1995)/second follow-up (1996-2000) surveys: "New" if the status in the baseline/follow-up was "off"/"on", "Continuing" if "on"/"on", "Stop" if "on"/"off", and, "No" if "off/ "off".

Height, body weight[8], and blood pressure measurements[4] were recorded during every survey. Subjects were interviewed by nurses to obtain disease histories, years since menopause, and lifestyle information including smoking status and drinking habits[14,22,23,32]. After examination and interview, every subject consulted a doctor, who ascertained the diagnoses. Diagnosis of hypertension was based on a systolic blood pressure $\geqq$ 140 mm Hg, a diastolic blood pressure $\geqq$ 90 mm Hg, or current treatment with antihypertensive drugs.

Because we did not have data about subjects' physical activity at baseline, we used the data about status of going out in 1996-1997 as a substitute for activity of daily living at the baseline. We asked subjects whether they could go out alone or with a helper, or if it was impossible to go out in 1996-1997.

Since we had previously reported that there was no evidence of an association between atomic bomb radiation dose and BMD in our study cohort[10], we did not include radiation dose as a possible factor.

All subjects gave written informed consent for BMD measurements and all other examination items.

Statistical analyses

Linear regression models were used to examine the relationship of drug use to annual percentage change in BMD at the femoral neck. For the response variable of annual percentage change in BMD at the femoral neck, a preliminary univariate linear regression model was fitted using each drug use as an indicator covariate. The effect of drug use was adjusted by including in each model factors that expected to affect BMD change at the femoral neck, such as sex, age (years), change of weight (kg), diastolic blood pressure (mm Hg), smoking status (non-smoker, ex-smoker, or current smoker), and alcohol consumption (non-drinker, ex-drinker, or current drinker). For adjustment, we examined height, body mass index, systolic blood pressure, baseline disease such as hypertension, diabetes mellitus, cardiovascular disease, coronary heart disease and stroke. Hypertension and cardiovascular disease had associations with

annual percentage change in BMD at the femoral neck in univariate regression models (regression coefficient yes/no (p value); -0.256 (p = 0.01), -0.178 (p = 0.04), respectively), but did not in multivariate models (regression coefficient (p value); -0.146 (p = 0.16), -0.126 (p = 0.20), respectively). Other factors did not have associations.

The medications that appeared to have strong associations ($p<0.10$) with the response variable were calcium channel blockades (CCBs), benzodiazepines (BZDs), and angiotensin converting enzyme (ACE) inhibitors (regression coefficient of continuing/no (p value): -0.244 (p = 0.043), -0.435 (p = 0.020), -0.632 (p = 0.002), respectively). Each of them was negatively associated with annual percentage change in BMD at the femoral neck.

Next, a preliminary multivariate linear regression model was fitted including those medications that exhibited significant associations with the response variable in the univariate linear regressions. Again, the effects of the drugs were modified by sex, age, change of weight, diastolic blood pressure, smoking status, and alcohol consumption. We came to a definite final model with non-significant terms, i.e. CCB use and diastolic blood pressure, removed.

In addition, we fit each model separately for the two sex groups, because annual percentage changes in BMD at the femoral neck level generally differ between men and women.

Statistical analysis software SAS 8.2 (SAS software version 8.2 for regression analysis, SAS Institute, Cary, North Carolina, U.S.) was used for all statistical analyses. Two-sided p values were calculated, with $p < 0.05$ as the cut off point for statistical significance.

RESULTS

The mean interval between the baseline and second survey, i.e. the average observation period, was 4.0 years. Characteristics of the subjects in our cohort are summarized in Table 1. Mean (standard deviation (SD)) age at the time of baseline survey was 62.8 (9.9) years for men and 67.0 (9.0) years for women. Mean (SD) annual percentage change in BMD measured at the femoral neck was −0.38% (1.49) for men and −1.14% (1.95) for women. The numbers of subjects who were taking main medications are shown by sex and groups (No, Stop, New, and Continuing) in Table 2. In our cohort, 35% of all subjects used one or more antihypertensive drugs.

Table 3 contains the estimated regression coefficients of the final full model with non-significant terms removed. In this model, after the adjustment for sex, age, change of weight, smoking status, and alcohol consumption, we found that the BMD at the femoral neck annually decreased by 0.61% of the baseline on average among the continuing users of ACE inhibitors compared with the non-users (p = 0.002), and by 0.40% among the continuing users of BZDs compared with the non-users (p = 0.034). It should be noted that diastolic blood pressure and CCB intake did not show a significant association with annual percentage change in femoral neck BMD (p = 0.113, p = 0.199, respectively) in the multivariate regression model. There were few differences in sex, age, BMD, blood pressure, and other measurements between CCB users and ACE inhibitor users. It should be noted that 44% of ACE inhibitor users also used CCB. On the other hand, 18% of CCB users concurrently used ACE inhibitor.

Since BMD generally changes differently between men and women, it seems more appropriate to fit the models separately for the two sex groups. The results tell us that, while no significant association was found between use of any drug and BMD change for men, there was a significant association for women (Table 4). The average BMD at the femoral neck annually decreased by 0.74% of the baseline among the continuing female users of ACE inhibitors compared with those women who never used them (p=0.002), after the adjustment for age, change of weight, smoking status, and alcohol consumption. The result also suggested that, among the continuing female users of BZDs, BMD decreased by 0.45% of the baseline per year on average compared with non-users (p=0.058).

To validate the effect of ACE inhibitors, an antihypertensive drug, on BMD change, we need to analyse the data for female subjects diagnosed with hypertension. In this cohort, the number of females diagnosed with hypertension was 628 with the mean (SD) age 69.6 (8.6) years, among which the users of ACE inhibiters were 133 with mean age 70.4 (8.2) and the non-users were 495 with mean age 69.3 (8.7). Since the age distributions of these two groups show no major discrepancy, age is unlikely to be a confounder. In this linear regression analysis, BMD at the femoral neck appeared to decrease on average by 0.86% of the baseline per year among the continuing female users of ACE inhibitors in comparison with those who never took them, after adjustments were made for age and change of weight (Table 5). Interestingly, both systolic blood pressure and diastolic blood pressure did not exhibit a significant association with BMD change among hypertensive women.

It was suspected, in the case of users of BZDs and ACE inhibitors, that physical activities would be low, and there was an observable association between drug use and bone loss. Although we did not have data about physical activities in subjects at baseline, we did have data for the next examination cycle (1996-1997), when we interviewed subjects about whether they could go out alone,

Table 1. Characteristics of Study Subjects

Sex	Men	Women
Subjects, number	703	1408
At the Time of Baseline Survey:		
Age, mean (SD), years	62.8 (9.9)	67.0 (9.0)
40-49 years, %	14.8	3.5
50-59 years, %	17.5	14.2
60-69 years, %	48.7	44.9
70-79 years, %	12.2	28.2
80 years or over, %	6.8	9.2
Femoral neck BMD, mean (SD), g/cm^2	0.736 (0.115)	0.610 (0.100)
Height, mean (SD), cm	163.4 (6.2)	149.9 (5.8)
Body weight, mean (SD), kg	60.8 (9.0)	52.1 (8.8)
Body mass index, mean (SD), kg/m^2	22.7 (2.9)	23.2 (3.6)
Systolic blood pressure, mean (SD), mm Hg	132.5 (20.9)	132.8 (11.1)
Diastolic blood pressure, mean (SD), mm Hg	80.0 (12.2)	77.2 (11.8)
Non drinker, %	19.3	71.3
Current drinker, %	73.4	27.3
Exit drinker, %	7.3	1.4
Non smoker, %	17.0	86.9
Current smoker, %	50.2	9.7
Exit smoker, %	32.8	3.4
Hypertension, %	46.9	44.6
Diabetes mellitus, %	16.2	11.0
Cardiovascular disease, %	31.3	31.9
Coronary heart disease, %	5.3	6.9
Stroke, %	4.4	3.4
Cancer, %	4.6	6.0
Years after menopause, mean (SD), years	-	18.3 (9.9)
At the Time of Second Survey:		
Femoral neck BMD, mean (SD), g/cm^2	0.725 (0.123)	0.583 (0.107)
Body weight, mean (SD), kg	60.4 (9.1)	51.7 (9.1)
Annual percentage change in femoral neck BMD, mean (SD), %	-0.38 (1.49)	-1.14 (1.95)
Change of weight, mean (SD), kg	-0.27 (2.6)	-0.36 (2.7)

No, number; SD, standard deviation; BMD, bone mineral density.

Table 2. Number of Subjects on Main Medications

Sex	Men				Women			
Used drug	No	Stop	New	Continuing	No	Stop	New	Continuing
Calcium channel blockade	515	19	88	81	998	23	203	184
Vitamin D_3	678	4	17	4	1112	39	165	92
Benzodiazepine	619	7	48	29	1178	31	129	70
Angiotensin converting enzyme inhibitor	630	24	30	19	1218	16	107	67
Calcium	670	12	12	9	1231	40	103	34
Vitamin B_{12}	651	7	31	14	1246	28	103	31
COX-2 selective NSAID*	676	6	18	3	1283	19	86	20
Statin	679	2	14	8	1297	19	68	24
Beta-adrenergic antagonist	663	7	19	14	1326	14	41	27
Thiazide diuretics agent	690	3	4	6	1348	13	23	24
Vitamin K_2	699	0	4	0	1357	2	47	2

*COX-2 selective NSAID: cyclooxygenase-2-selective non-steroidal anti-inflammatory drugs (etodolac, piroxicam, sulindac, and zaltoprofen were categorized in this group).

Table 3. Multivariate Linear Regression with Adjustment for Sex, Age, Change of Weight, Smoking Status, and Alcohol Consumption

Annual Percentage Change in Femoral Neck BMD (R^2=0.1039)	Regression Coefficient	Standard Error	p value
Sex*	-0.682	0.122	<.001
Age (years)	0.120	0.044	.006
Age-square	-0.001	0.0003	.001
Change of weight (kg)	0.092	0.015	<.001
Alcohol consumption (current-/non-drinker)	0.278	0.092	.003
Alcohol consumption (ex-/non-drinker)	0.335	0.118	.005
Smoking status (current-/non-smoker)	-0.368	0.121	.002
Smoking status (ex-/non-smoker)	-0.045	0.073	.535
Angiotensin converting enzyme inhibitor use			
Stop/No	-0.583	0.280	.037
New/No	-0.143	0.160	.374
Continuing/No	-0.607	0.200	.002
Benzodiazepine use			
Stop/No	0.536	0.302	.076
New/No	0.057	0.143	.692
Continuing/No	-0.395	0.186	.034

*: Men=0 and women=1.

Table 4. Multivariate Linear Regression with Adjustment for Age, Change of Weight, Smoking Status, and Alcohol Consumption (Women)

Annual Percentage Change in Femoral Neck BMD (R^2=0.0756)	Regression Coefficient	Standard Error	p value
Age (years)	0.222	0.063	<.001
Age-square	-0.002	0.0005	<.001
Change of weight (kg)	0.094	0.019	<.001
Alcohol consumption (current-/non-drinker)	0.298	0.118	.012
Alcohol consumption (ex-/non-drinker)	0.468	0.230	.042
Smoking status (current-/non-smoker)	-0.343	0.178	.054
Smoking status (ex-/non-smoker)	-0.239	0.142	.092
Angiotensin converting enzyme inhibitor use			
Stop/No	-0.443	0.472	.348
New/No	-0.136	0.191	.482
Continuing/No	-0.743	0.243	.002
Benzodiazepine use			
Stop/No	0.303	0.365	.406
New/No	0.065	0.179	.716
Continuing/No	-0.449	0.236	.058

Table 5. Multivariate Linear Regression with Adjustment for Age, and Change of Weight (Hypertensive Women)

Annual Percentage Change in Femoral Neck BMD (R^2=0.1048)	Regression Coefficient	Standard Error	p value
Age (years)	0.235	0.104	.024
Age-square	-0.002	0.0007	.010
Change of weight (kg)	0.166	0.030	<.001
Angiotensin converting enzyme inhibitor use			
Stop/No	-0.517	0.538	.337
New/No	0.074	0.256	.772
Continuing/No	-0.855	0.300	.005

Table 6. Multivariate Linear Regression with Adjustment for Sex, Age, Change of Weight, Smoking Status, and Alcohol Consumption (Ambulatory subjects: N = 1932)

Annual Percentage Change in Femoral Neck BMD (R^2=0.0978)	Regression Coefficient	Standard Error	p value
Sex*	-0.716	0.121	<.001
Age (years)	0.159	0.045	.001
Age-square	-0.001	0.0003	<.001
Change of weight (kg)	0.073	0.015	<.001
Alcohol consumption (current-/non-drinker)	0.200	0.092	.030
Alcohol consumption (ex-/non-drinker)	0.270	0.120	.025
Smoking status (current-/non-smoker)	-0.357	0.120	.003
Smoking status (ex-/non-smoker)	-0.023	0.073	.754
Angiotensin converting enzyme inhibitor use			
Stop/No	-0.653	0.287	.023
New/No	-0.071	0.161	.660
Continuing/No	-0.554	0.202	.006
Benzodiazepine use			
Stop/No	0.349	0.308	.257
New/No	0.149	0.143	.300
Continuing/No	-0.271	0.193	.160

*: Men=0 and women=1

or with a helper, or if it was impossible to go out. Assuming that these data at 1996-1997 were so similar that they could be substituted for physical activities at 1994-1995, we examined the relation between these data, drug use, and bone loss.

In this cohort, 1932 subjects answered that they could go out alone, 56 subjects needed helpers, 16 subjects found it impossible to go out, and 107 subjects did not answer. We did not find the relation between bone loss and these categories. We then analysed the procedure described previously concerning subjects who could go out alone (Table 6). It should be noted that BZD intake did not show a significant association with annual percentage change in femoral neck BMD (regression coefficient of continuing/no (p value); -0.271 (p = 0.160)) in the multivariate regression model.

DISCUSSION

The main purpose of the present study was to investigate the associations between BMD change and the use of several drugs in a cohort of 2,111 Japanese subjects who underwent one baseline measurement and one follow-up measurement (4 year later) of BMD. This study was motivated by the fact that, while most earlier studies investigating factors associated with BMD change have been for populations in the United States and Europe, few were found for other populations including the Japanese. Although this is a retrospective study, the result suggests that loss of BMD at the femoral neck might be attributed to ACE inhibitor and BZD intake among older Japanese. Other factors that appeared to be associated with BMD change were sex, age, change of weight, alcohol consumption, and smoking status.

Among those medications that might have an effect on BMD, some drugs such as glucocorticoids, thyroid hormones, estrogens, thiazide diuretics, and vitamin K_2 were not commonly prescribed in Japan at the time of survey, and thus we were unable to examine their associations with BMD change due to the small sample sizes. Although intake of statins, β-blockers, COX-2-selective NSAIDs, or vitamin B_{12} was occasionally observed in our cohort, no significant association was found for any of these drugs with BMD change. Vitamin D_3 and calcium supplements were frequently used in our cohort but did not show significant associations with BMD change. This is in agreement with some earlier studies that report that these drugs are effective not to increase but rather to maintain BMD[2,18].

There are some papers indicating that women diagnosed with hypertension express low BMD compared with normotensive women[4,35] In preclinical studies, there is some discussion about a possibility of the rennin-angiotensin system (RAS) being associated with regulation of bone resorption. Hagiwara et al. reported that angiotensin II controlled differentiation of bone blast cells, and consequently bone formation was contained[13]. Hatton et al demonstrated that angiotensin II might be a stimulator of osteoclastic bone resorption[15].

The synthetic pathway of angiotensin II, which was well known in terms of high blood pressure, is called RAS. Angiotensinogen, which is secreted from the liver and used for the formation of angiotensin II, is converted to angiotensin I by renin secreted from the kidney. Angiotensin I is then converted to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II is the major bioactive product of RAS. Angiotensin II has a variety of effects on the body. As with most other capillary beds in the body, constriction of efferent arterioles increases arterioler resistance, raising systemic arterial blood pressure and decreasing blood flow. Angiotensin II promotes secretion of aldosterone, which causes blood vessel contraction through specific receptors of the organism, and promotes maintenance of Na-level and discharge of K.

ACE acts not only on RAS but also simultaneously on the bradykinin system (BKS), and stimulates degradation of bradykinin (BK), converting it into an inactive substance. Therefore, ACE inhibitors also work on BKS, and maintain BK as high concentrations; at the same time, they work on RAS and suppress generation of angiotensin II. As mentioned above, although angiotensin II controls differentiation of bone blast cells, and bone formation is controlled as a result, some papers have reported that BK also stimulated bone resorption and reduced BMD[12,19,20]. From such reports, we consider that use of ACE inhibitors may increase BMD through reduced concentration of angiotensin II. On the other hand, BMD may be decreased through increased BK concentration.

Recently, in an experiment using rats, one group reported that angiotensin receptor blocker (ARB) restrained bone resorption[28], although ARB was designed as an antihypertensive drug without BK effect. The above results, however, seem to be consistent with the interpretation that use of ACE inhibitors may increase BMD through reduced concentration of angiotensin II, and may decrease BMD because of increased BK concentration on the other hand.

Another paper reported that ACE inhibitors might have possible benefits in treating not only hypertension but also osteoporosis among older Chinese[21]. A cross-sectional study of 3,887 Chinese men (n = 1958) and women (n = 1929), aged 65 years and over, was used to explore the association between ACE inhibitor use and BMD. The study consisted of a comparison of BMDs between users and nonusers of ACE inhibitors. However, mean weight in users was heavier than

that in nonusers. Also the rates of concomitant administration of ACE inhibitor, thiazide and statin were higher than those in nonusers. Although these effective factors were adjusted in multiple linear regression analysis, weight, thiazide and statin use have strong effects on BMD[6-8]. The existence of bias might be suspected. Furthermore, the use of antihypertensive medications in China was different from that in Japan. Women and men were more frequently prescribed beta-blockers and calcium supplements in China than patients in Japan. But women were less frequently prescribed ACE inhibitors and CCB in China than women in Japan. We would like to know the duration of antihypertensive treatment use there.

Additionally, one paper demonstrated that treatment with beta-blockers, ACE inhibitors, and CCBs was associated with reduced fracture risk[26]. That research represented the results of a large, nationwide, population-based pharmaco-epidemiological case-control study. The study subjects consisted of 124,655 cases that had experienced a fracture and 373,962 age- and gender-matched controls. The study found that treatment with ACE inhibitors was associated with reduced fracture risk. But the category of ACE inhibitors in the study also included ARBs. The mean age of cases was 43 years. The cases included hip fractures, forearm fractures, and spinal fractures. The cases tended to have a higher frequency of comorbidity. As no information on patients' compliance was available in the study, a prescription was used as a proxy for actual use of a drug. In addition, adjustment for differences in body weight was not possible.

Other than these papers, few have reported on the relationship between ACE inhibitor use and osteoporosis incidence. The actual mechanism behind ACE-inhibitor effects on BMD is not entirely understood, but we assume that use of ACE inhibitors decreases angiotensin II, resulting in increased BMD, and increases BK, resulting in decreased BMD. In our human observation study, however, we were only able to observe decreased BMD. This suggests that the effect of BK is greater than that of angiotensin II on BMD at the femoral neck in Japanese elderly. By contrast, angiotensin II may have a greater effect on BMD than BK in Chinese elderly. We do not know, however, whether the different results are due to race, differences in medication, differences of acting sites such as osteoblasts or osteoclasts, or other factors.

These considerations do not contradict the report that ARB increased BMD in Japanese. In the future, we would like to investigate the use of ARBs and consider the association of such use with BMD in our cohort.

ACE inhibitors and CCBs were the two most frequently used antihypertensive drugs in our cohort. Each of them appeared to be significantly associated with bone loss in the univariate linear regression adjusted for sex, age, change of weight, smoking status, and alcohol consumption. This observation made us suspect that antihypertensive drug rather than hypertension[4,33] might affects on BMD. The direct effect of hypertension on BMD, adjusted for possible effects of antihypertensive drugs, could be examined by comparing two groups of hypertensive and non-hypertensive subjects with equal drug use histories. However, this was impossible in this study because those antihypertensive drugs were used only by hypertensive subjects. We, therefore, examined the effect of ACE inhibitors on BMD among hypertensive subjects. In this analysis, a significant association was still found to exist between ACE inhibitor intake and loss of BMD, which indicates that use of ACE inhibitors might tend to accelerate bone loss among hypertensive women.

ARBs are now the first choice among medicines for about half of hypertension patients in Japan[34]. This is because ARBs have the same antihypertensive effects as ACE inhibitors and fewer side effects. However, the effects of ARBs on myocardial infarction are considered to be less serious than those of ACE inhibitors[16]. ACE inhibitors are still making clinical contributions. Other than the above reports, few papers have been published about the association between human BMD and ACE inhibitors. We therefore eagerly await elucidation of the specific roles that angiotensins and ACE inhibitors play in bone metabolism.

The effect of BZD intake, which appeared to be associated with BMD change in this study, may not be direct, since BZD users tend to have lower physical activities[27], which likely leads to larger decreases in BMD through the confounding effect of the physical activity. It would be possible to assess more accurately the direct effect of BZDs on BMD if the physical activity data were available for each individual. As we did not have the data for physical activity at baseline, we had no alternative but to assess that for 1996-1997. It should be noted that BZD intake did not show a significant association with annual percentage change in femoral neck with the adjustment for physical activity in 1996-1997. On the other hand, after the adjustment for physical activity in 1996-1997, there were still negative associations between ACE inhibitor use and BMD change. Our cohort consisted of outpatients, and therefore patients with severe cardiovascular diseases were excluded.

Our study had some obvious limitations. First, we could examine only a few frequently prescribed drugs in our cohort, and thus could not certify the effects of other medications, such as estrogen and glucocorticoid, which are more frequently prescribed and known to have associations with

BMD among Caucasians. Second, this study is a retrospective analysis, and we had no intervention for the effects of medications. Third, we had no data about the subjects' physical activities at the baseline survey. We did not know whether subjects had habits of exercise or not. In addition, although our analyses are adjusted by sex, age, weight, weight change, smoking status, and alcohol consumption, it is not clear if other confounders needed to be taken into account.

In conclusion, our study suggests that careful consideration should be given to use of ACE inhibitors and BZDs, which may be responsible for reducing BMD in elderly patients. Although the mechanisms underlying the effects of ACE inhibitors and BZDs on BMD are not well understood at this time, it is certainly conceivable that angiotensins are involved. It is important to design and conduct statistically valid, randomised clinical trials to ensure that the cause-effect relationship between medications such as ACE inhibitors and BZDs and BMD can be properly ascertained.

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