The Journal of Rheumatology

The Journal of Rheumatology Volume 38, no. 5

Association of Knee Osteoarthritis with the Accumulation of Metabolic Risk Factors Such as Overweight, Hypertension, Dyslipidemia, and Impaired Glucose Tolerance in Japanese Men and Women: The ROAD Study

NORIKO YOSHIMURA, SHIGEYUKI MURAKI, HIROYUKI OKA, HIROSHI KAWAGUCHI, KOZO NAKAMURA and TORU AKUNE

J Rheumatol 2011;38;921-930
http://www.jrheum.org/content/38/5/921

Association of Knee Osteoarthritis with the Accumulation of Metabolic Risk Factors Such as Overweight, Hypertension, Dyslipidemia, and Impaired Glucose Tolerance in Japanese Men and Women: The ROAD Study

NORIKO YOSHIMURA, SHIGEYUKI MURAKI, HIROYUKI OKA, HIROSHI KAWAGUCHI, KOZO NAKAMURA, and TORU AKUNE

ABSTRACT. ***Objective.*** To clarify the association of knee osteoarthritis (KOA) with overweight (OW), hypertension (HTN), dyslipidemia (DL), and impaired glucose tolerance (IGT), which are components of metabolic syndrome (MS), in a Japanese population.

Methods. We enrolled 1690 participants (596 men, 1094 women) from the large-scale cohort study Research on Osteoarthritis Against Disability (ROAD), begun in 2005 to clarify epidemiologic features of OA in Japan. KOA was evaluated by the Kellgren-Lawrence grade, minimum joint space width (MJSW), minimum joint space area (JSA), and osteophyte area (OPA). OW, HTN, DL, and IGT were assessed using standard criteria.

Results. The prevalence of KOA in the total population in the age groups ≤ 39, 40–49, 50–59, 60–69, 70–79, and ≥ 80 years was 2.2%, 10.7%, 28.2%, 50.8%, 69.0%, and 80.5%, respectively. Logistic regression analyses after adjustment for age, sex, regional difference, smoking habit, alcohol consumption, physical activities, regular exercise, and history of knee injuries revealed that the OR of KOA significantly increased according to the number of MS components present (1 component: OR 1.21, 95% CI 0.88–1.68, p = 0.237; 2 components: OR 1.89, 95% CI 1.33–2.70, p < 0.001; 3 or more components: OR 2.72, 95% CI 1.77–4.18; p < 0.001). The number of MS components was inversely related to medial MSJW (ß = –0.148, R^2 = 0.21, p < 0.001), medial JSA (women only; ß = –0.096, R^2 = 0.18, p = 0.001), and positively related to OPA (ß = 0.12, R^2 = 0.11, p < 0.001).

Conclusion. The accumulation of MS components is significantly related to presence of KOA. MS prevention may be useful to reduce cardiovascular disease and KOA risk. (First Release Feb 15 2011; J Rheumatol 2011;38:921–30; doi:10.3899/jrheum.100569)

Key Indexing Terms:
EPIDEMIOLOGY METABOLIC SYNDROME KNEE OSTEOARTHRITIS
RISK FACTORS ROAD STUDY

Osteoarthritis (OA), which causes cartilage and disc degeneration and osteophyte formation at joints in the limbs and spine, is a major public health problem in the elderly that affects activities of daily living (ADL) and quality of life, leading to increased morbidity and mortality[1,2,3]. According to the recent National Livelihood Survey by the Ministry of Health, Labour and Welfare in Japan, OA is ranked fourth among diseases that cause disabilities requiring support and longterm care[4].

In the same report, cardiovascular disease (CVD) is

From the Department of Joint Disease Research, and the Department of Clinical Motor System Medicine, 22nd Century Medical and Research Center, Graduate School of Medicine; and the Department of Orthopaedic Surgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan.

Supported by Grants-in-Aid for Scientific Research B20390182 (N. Yoshimura), C20591737 (T. Akune), C20591774 (S. Muraki), and the Young Scientists A18689031 (H. Oka). Collaborating research with NSF 08033011-00262 (Director, N. Yoshimura) from the Ministry of Education, Culture, Sports, Science and Technology, and H17-Men-eki-009 (Director, K. Nakamura), H18-Choujyu-037 (Director, T. Nakamura), and H20-Choujyu-009 (Director, N. Yoshimura) from the Ministry of Health, Labour and Welfare in Japan. This study was also supported by grants from the Japan Osteoporosis Society (N. Yoshimura), Nakatomi Foundation (N. Yoshimura), and research aid from the Japanese Orthopaedic Association (Director, H. Kawaguchi).

N. Yoshimura, MD, PhD, Department of Joint Disease Research, 22nd Century Medical and Research Center, Graduate School of Medicine, The University of Tokyo; S. Muraki, MD, PhD, Department of Clinical Motor System Medicine, 22nd Century Medical and Research Center, Graduate School of Medicine, The University of Tokyo; H. Oka, MD, Department of Joint Disease Research, 22nd Century Medical and Research Center, Graduate School of Medicine, The University of Tokyo; H. Kawaguchi, MD, PhD; K. Nakamura, MD, PhD, Department of Orthopaedic Surgery, Faculty of Medicine, The University of Tokyo; T. Akune, MD, PhD, Department of Clinical Motor System Medicine, 22nd Century Medical and Research Center, Graduate School of Medicine, The University of Tokyo.

Address correspondence to Dr. N. Yoshimura, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: yoshimuran-ort@h.u-tokyo.ac.jp

Accepted for publication December 21, 2010.

ranked first in causing disabilities in the elderly[4]. Most individuals who develop CVD have multiple risk factors[5]. The presence of these risk factors in specific combinations, called metabolic syndrome (MS), is a complex risk factor that predisposes affected individuals to CVD morbidity and mortality. Although various terms have been used to define MS, it is generally thought to consist of a combination of overweight (OW), hypertension (HTN), dyslipidemia (DL), and impaired glucose tolerance (IGT)[6].

Knee OA (KOA) and MS share age and obesity as risk factors[1,7,8,9,10,11]. Many investigators have considered the association of OA with other components of MS. In an early population study, Lawrence first reported that diastolic blood pressure was associated with KOA in women[12]. Regarding DL, Kellgren reported a significant association between women with hand OA and above-average serum cholesterol levels in the 1960s[13]. Cimmino and Cutolo examined the role of glucose and OA, and observed significantly higher levels of plasma glucose in women with OA than in those without OA[14]. Although contradictory findings regarding the association of such metabolic factors with OA have been reported[15,16,17,18,19], Hart, *et al* found that metabolic factors such as blood glucose, hypercholesterolemia, and even treated HTN were associated with the development of KOA. Based on that evidence, they proposed that the etiology of OA had an important systemic and metabolic component[20]. This hypothesis has been supported by data from several population-based studies performed in the United States[21,22]. However, to our knowledge, few population-based studies have demonstrated a dose-response relationship between the severity of KOA and an increasing number of the components of MS. Our first purpose was to clarify the association between the presence of KOA, defined using the Kellgren-Lawrence (KL) scale, and the number of MS components in a Japanese population.

Moreover, in most of these studies that confirmed the association between the presence of KOA and the components of MS, KOA was defined according to KL grade[23]. KL grade is the most conventional system for measuring the radiographic severity of KOA, but does not separately assess joint space narrowing and osteophyte formation. Accumulating evidence has shown that osteophytosis and joint space narrowing have distinct etiologic mechanisms, and their progression is neither constant nor proportional[24,25,26]. Thus, to examine the factors associated with KOA, these 2 OA features should be assessed separately. However, no reports to date have clarified the association of indices of KOA, such as minimum joint space width (MJSW), joint space area (JSA), and osteophyte area (OPA), with the accumulation of the number of components of MS. Our second purpose was to determine whether the accumulation of MS components influenced the values of MJSW, JSA, and OPA.

Further, MS is an emerging epidemic in both men and women worldwide, and with the increase in the global population of Asians, an understanding of the epidemiology of diseases as they relate to Asian populations is required. We have reported that the prevalence of KOA was much higher in a Japanese population than in elderly whites in the United States and Europe, although not largely different from that of African American and Chinese populations[27]. In contrast, the prevalence of MS in East Asian countries including China, Korea, and Japan was reported to be lower than in white populations[28]. In light of the rapid increase in the population of Asian countries, prevention strategies for obesity-related chronic diseases such as MS and KOA should be implemented immediately. Our final aim was to clarify the association between MA components and KOA in people of Asian ethnicity.

MATERIALS AND METHODS

Study population. We used the cohorts established in 2005 for a program called Research on Osteoarthritis Against Disability (ROAD). The ROAD study is a nationwide, prospective study of OA composed of population-based cohorts in several communities in Japan. Details of the cohort profile have been reported[29,30], thus the study population is described here only in brief. We created a baseline database including clinical and genetic information from 3040 residents of Japan (1061 men and 1979 women) with a mean age (SD) of 70.3 (11.0) years [71.0 (10.7) years in men and 69.9 (11.2) years in women]. These subjects were recruited from resident registration listings in 3 communities with different characteristics: an urban region in Itabashi, Tokyo; a mountainous region in Hidakagawa, Wakayama; and a coastal region in Taiji, Wakayama.

We enrolled 1690 Japanese subjects (596 men; 1094 women) residing in the mountainous and coastal areas. Table 1 lists the background characteristics of all the participants. All participants provided written informed consent, and the study was conducted with the approval of the ethics committees of the University of Tokyo. Participants completed an interviewer administered questionnaire of 400 items that included lifestyle information such as occupation, smoking habit, alcohol consumption, family history, medical history, physical activity, reproductive variables, and health-related quality of life. Anthropometric measurements included height, weight, waist length (seaside region only), wrist circumference, bilateral grip strength, and body mass index [BMI; weight (kg)/height (m)^2]. Systolic and diastolic blood pressure (BP) were measured by an experienced public health nurse using a mercury sphygmomanometer. Medical information on systemic, local, and mental health status, including information concerning knee, hip, and lower back pain; swelling and range of motion of the joints; and patellar and Achilles tendon reflex was collected by experienced orthopedic surgeons.

Radiographic assessment. All participants underwent radiographic examination of both knees using an anterior-posterior view with weight-bearing and foot-map positioning. Fluoroscopic guidance with a horizontal anterior-posterior radiograph beam was used to visualize the joint space. Knee radiographs were read by a single experienced orthopedist without knowledge of participants' clinical status, and categorized using the KL grading scale[23]. Regarding the differences in knee OA grades between the 2 sides, among 1681 participants who underwent X-ray examinations of both knees, 1226 (72.9%) individuals had the same KL grades for both knees. For 396 (23.6%) participants, the difference in knee KL grades between the 2 knees was 1, and for the remaining 59 (3.5%) subjects, the KL grades differed by more than 2 grades. In such cases, the higher KL grade was assigned to the participant. The same observer scored 100 randomly selected knee radiographs more than 1 month after the first reading to determine intraobserver variability. The intraobserver variability (0.86) evaluated for KL grade (0–4) was confirmed by kappa analysis to be sufficient for the assessment.

Table 1. Background characteristics of the participants.

	Total	Men	Women
Age, yrs			
≤ 39	45	14	31
40–49	149	44	105
50–59	316	107	209
60–69	482	157	325
70–79	539	220	319
≥ 80	159	54	105
Total, n	1690	596	1094
Mean (SD) selected characteristics			
Age, yrs	65.2 (12.0)	66.3 (11.7)	64.7 (12.1)
Height, cm	155.2 (9.3)	163.4 (7.2)	150.7 (6.9)
Weight, kg	55.6 (10.8)	62.2 (10.9)	52.0 (8.8)
BMI, kg/m^2	23.0 (3.4)	23.2 (3.2)	22.9 (3.5)
Systolic BP, mm Hg	135.1 (20.7)	137.9 (19.6)	133.5 (21.1)
Diastolic BP, mm Hg	74.2 (11.5)	77.0 (11.6)	72.7 (11.2)
Serum levels of HDL cholesterol, mg/dl	60.8 (15.7)	56.1 (15.8)	63.4 (15.0)
Serum levels of HbA1c, %	5.20 (0.74)	5.23 (0.83)	5.19 (0.68)
Prevalence of selected characteristics, %			
Current smoking habit	13.1	29.9	3.8
Current alcohol consumption	39.8	66.7	25.1
Medication for hypertension	32.3	29.5	33.9
Medication for dyslipidemia	6.5	3.0	8.5
Medication for diabetes mellitus (including insulin injection)	5.9	7.7	4.9
Prevalence of each component of metabolic syndrome, %			
Obesity	25.3	26.7	24.6
Hypertension	69.7	74.8	66.9
Dyslipidemia	12.3	13.9	11.4
Impaired glucose tolerance	21.5	24.3	20.0

BMI: body mass index; BP: blood pressure; HDL: high-density lipoprotein; HbA1c: hemoglobin A1c.

Further, to evaluate the KOA severity using quantitative measurements, the medial and lateral MJSW, medial and lateral JSA, and OPA were measured separately, using a KOA computer-assisted diagnostic system (KOACAD). The KOACAD was programmed to measure MJSW and JSA in the medial and lateral compartments, OPA at the medial tibia, and femorotibial angle (FTA) using digitized knee radiographs. Initially, correction for radiographic magnification was performed on the basis of the image size of a rectangular metal plate.

Next, to determine the region of interest (ROI) including the tibiofemoral joint space, a vertical neighborhood difference filter was applied to identify points with high absolute values for difference of scales. The centers of all points were then calculated, and the ROI was selected. Within the ROI, the outline of the femoral condyle was designated as the upper rim of the joint space. The 2 ends were determined, and vertical lines from the ends were designated as the outside rims of the joint space. Outlines of the anterior and posterior margins of the tibial plateau were drawn similarly to that of the femoral condyle, and the middle line between the 2 outlines was designated as the lower rim of the joint space. A straight regression line for the lower rim outline was then drawn, and the intersection of the lower rim outline and the regression line were designated as the inside rims. Medial and lateral JSA were determined as areas surrounded by the upper, lower, inside, and outside rims. Medial and lateral MJSW were further determined as the minimum vertical distances in the respective JSA. To measure osteophyte area and FTA, medial and lateral outlines of the femur and tibia were drawn. Inflection points for these outlines were then calculated. The medial outline of the tibia from the inflection point was drawn upward to the joint level, and the area that was medially prominent over the smoothly extended outline was designated as the osteophyte area. For FTA, a middle line between the medial and lateral outlines of the femur from the top of the image to the inflection points was drawn, and the straight regression line was determined as the axis of the femur. Similarly, the straight regression line of the middle line of the tibia from the bottom to the inflection points was designated as the axis of the tibia. The lateral angle between the 2 axes lines was calculated as FTA. In general clinical practice, this system can quantify the major features of knee OA on standard radiographs and allows objective, accurate, simple, and easy assessment of the structural severity of knee OA without any manual operation.

Regarding the relationship between the measurements of KOA, we have confirmed the correlation values were more than 0.5 between medial JSA and medial MJSW, and between lateral JSA and lateral MJSW, indicating that these are confounding factors for each other. Osteophyte area was not significantly associated with either medial JSA or medial MJSW. Further, JSA and MJSW on the lateral side were positively correlated with those on the medial side. These measurements showed good correlation between KL grades ($p < 0.0001$)[31].

Blood examination. All blood and urine samples were extracted between 9:00 AM and 3:00 PM. Some samples were extracted under fasting conditions. After centrifugation of blood samples, sera were immediately placed in dry ice and transferred to a deep freezer within 24 hours. These samples were stored at –80°C until assayed.

For the samples of participants in the baseline study, the following items were measured: blood counts, hemoglobin, hemoglobin A1c (HbA1c), blood sugar, total protein, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltranspeptidase, high-density lipoprotein (HDL) cholesterol, total cholesterol, triglycerides (TG), blood urea nitrogen, uric acid, and creatinine. These analyses were performed at the same laboratory within 24 hours after the extraction (Osaka Kessei Research Laboratories Inc., Osaka, Japan).

Definition of MS components. This definition was based mainly on the criteria of the Examination Committee of Criteria for Metabolic Syndrome in Japan[32]. According to these criteria, an abdominal circumference ≥ 85 cm in men and ≥ 90 cm in women is a necessary condition for MS. HTN was diagnosed as systolic BP ≥ 130 mm Hg and/or diastolic BP ≥ 85 mm Hg, DL as serum TG level ≥ 150 mg/dl and/or serum HDL cholesterol level < 40 mg/dl, and IGT as fasting serum glucose ≥ 110 mg/dl. Because there has been considerable debate regarding the measurement of abdominal circumference[33,34], we decided to use BMI ≥ 25 instead as an indicator of overweight, based on the criteria of the Japan Society for the Study of Obesity[33]. Also, because not all blood samples were obtained under fasting conditions, we did not use participants' data concerning serum levels of glucose and TG, because of their large variation depending on hours after eating. Instead, we used a serum HDL cholesterol level < 40 mg/dl to indicate DL, and serum HbA1c level ≥ 5.5% to indicate IGT. These are indices used in the National Health and Nutrition Survey in Japan, and they were adopted as criteria for MS in this national screening based on the difficulty of collecting the samples under fasting conditions[35]. Further, subjects being treated with medication for HTN, DL, or diabetes mellitus were regarded as having the respective disorder.

Statistical analysis. All statistical analyses were performed using Stata statistical software (Stata Corp., College Station, TX, USA). Differences in proportion were compared by the chi-squared test. Differences in continuous values were tested for significance using ANOVA for comparisons among multiple groups, and Scheffe's least significant difference test for pairs of groups. Significant items were selected, and multiple regression and logistic regression analyses were performed by adjusting selected variables. Various confounding factors were used for the adjustment for each multivariate analysis.

RESULTS

Study population. Table 1 shows selected characteristics of the participants including age, height, weight, BMI, systolic

and diastolic BP, and serum levels of HDL cholesterol and HbA1c, classified by sex. Two-thirds of the 1690 participants were women, and their mean age was 1.5 years younger than that of the men ($p = 0.0098$).

Height, weight, and BMI were significantly lower in women than in men (height, $p < 0.0001$; weight, $p < 0.0001$; BMI, $p = 0.049$). Both measurements of systolic BP and diastolic BP were significantly higher in men than in women (systolic BP and diastolic BP, $p < 0.0001$). However, there was no significant difference in serum levels of HbA1c between men and women ($p = 0.2472$). The serum level of HDL cholesterol was significantly lower in men than in women ($p < 0.0001$).

Table 1 also shows the proportion of subjects who smoked (regularly or more than once a month) and consumed alcohol (drinking regularly or more than once a month); medication use; and the prevalence of OW, HTN, DL, and IGT. Smoking and drinking were significantly more common in men than in women ($p < 0.001$). In the total population, the component of MS with the highest prevalence was HTN, followed by OW, IGT, and DL. The prevalence of HTN and IGT was significantly higher in men than in women (HTN, $p = 0.001$; IGT, $p = 0.039$).

Prevalence of KOA and its association with components for MS. The prevalence of KOA in the total population in the age groups ≤ 39, 40–49, 50–59, 60–69, 70–79, and ≥ 80 years was 2.2%, 10.7%, 28.2%, 50.8%, 69.0%, and 80.5%, respectively. KOA prevalence tended to be higher with increasing age in both the sexes. The prevalence of KOA was significantly higher in women than in men ($p < 0.001$). Table 2 shows the mean values of each component of MS compared between the absence and presence of KOA. In the overall population, mean values of age, BMI, systolic BP, and HbA1c were significantly higher, and HDL cholesterol significantly lower, in subjects with KOA than in those without KOA. This tendency was much more pronounced in women than in men.

Logistic regression analysis was performed using the presence of KOA as an objective variable and OW, HTN, DL, and IGT each as explanatory variables, after adjusting for age and sex. In the overall population, the analysis revealed that only OW was significantly positively associated with KOA (OR 2.33, 95% CI 1.79–3.04, $p < 0.001$). Logistic regression analysis using the same objective and explanatory factors and stratified according to sex indicated that only HTN was positively associated with KOA in men (OR 1.61, 95% CI 1.03–2.53, $p = 0.038$), and only OW in women (OR 3.48, 95% CI 2.42–5.01, $p < 0.001$).

Table 3 shows the prevalence of potential associated lifestyle factors for KOA classified by the absence or presence of KOA. In the overall population, significantly associated factors for KOA included residential area, smoking habit, alcohol consumption, bicycling regularly as a factor of physical activity, and regular exercises. These factors should be taken into consideration as confounders for the following multivariate analysis.

Then, logistic regression analysis was repeated using the presence of KOA as an objective variable and OW, HTN, DL, and IGT each as explanatory variables, after adjusting for age, sex, regional difference, smoking habit, alcohol consumption, physical activities including regular bicycling in the past 12 months, regular exercises such as football, tennis, baseball, and golf; and history of knee injuries. The analysis revealed that OW and HTN were significantly positively associated with KOA (OW: OR 2.74, 95% CI 1.07–3.62, $p < 0.001$; HTN: OR 1.43, 95% CI 1.09–1.86, $p < 0.001$). Logistic regression analysis using the same objective and explanatory factors and stratified according to sex indicated that OW and HTN were positively associated with KOA in men (OW: OR 1.76, 95% CI 1.13–2.74, $p < 0.05$; HTN: OR 1.77, 95% CI 1.11–2.84, $p < 0.05$), and only OW in women (OR 3.63, 95% CI 2.51–5.25, $p < 0.001$). These results suggest that all components of MS were not equally associated with the presence of KOA.

Then, to clarify the association between all the components of MS and KOA, logistic regression analysis was repeated using the presence of KOA as an objective variable and all components for MS, such as OW, HTN, DL, and IGT, as explanatory variables, after adjustment for age, sex, regional difference, smoking habit, alcohol consumption, physical activities, regular exercises, and history of knee injuries. In the overall population, the analysis revealed that

Table 2. Mean (SD) of each component of metabolic syndrome in the absence or presence of knee osteoarthritis (KOA).

	Total			Men			Women		
	KOA–	KOA+	p	KOA–	KOA+	p	KOA–	KOA+	p
Age, yrs	59.8 (12.1)	70.5 (9.1)	0.0001	62.5 (12.1)	71.5 (8.8)	0.0001	57.8 (11.8)	70.3 (9.1)	0.0001
BMI, kg/m^2	22.4 (3.2)	23.5 (3.4)	0.0001	23.0 (3.2)	23.5 (3.2)	0.0931	22.0 (3.1)	23.6 (3.6)	0.0001
Systolic BP, mm Hg	130.7 (19.9)	139.3 (20.7)	0.0001	134.5 (18.9)	142.5 (19.6)	0.0001	127.9 (20.0)	138.0 (21.0)	0.0001
Diastolic BP, mm Hg	74.2 (11.2)	74.2 (11.8)	0.9890	77.1 (11.6)	76.8 (11.5)	0.6970	72.1 (10.4)	73.1 (11.8)	0.1380
Serum levels of HDL cholesterol, mg/dl	62.8 (16.6)	58.9 (14.5)	0.0001	57.5 (16.2)	54.1 (15.0)	0.0095	6.6 (15.8)	60.8 (13.9)	0.0001
Serum levels of HbA1c, %	5.13 (0.68)	5.26 (0.78)	0.0003	5.22 (0.83)	5.23 (0.80)	0.9409	5.07 (0.53)	5.28 (0.77)	0.0001

BMI: body mass index; BP: blood pressure; HDL: high-density lipoprotein; HbA1c: hemoglobin A1c.

Table 3. Prevalence (%) of portential associated factors for knee osteoarthritis (KOA) classified by the absence or presence of KOA.

	Total			Men			Women		
	KOA–	KOA+	p	KOA–	KOA+	p	KOA–	KOA+	p
Residing in coastal area	65.6	32.1	0.000	60.8	26.7	0.000	69.0	34.3	0.000
Current smoking	16.7	9.5	0.000	34.7	23.5	0.012	3.92	3.53	0.060
Current alcohol drinking	46.2	33.4	0.000	68.1	65.3	0.475	30.8	20.2	0.000
Bicycling every day in the past 12 mo	52.6	59.3	0.006	55.1	55.1	0.998	50.8	61.0	0.001
Regular exercise such as football, tennis, baseball, and golf	18.3	10.6	0.000	34.9	30.0	0.209	6.53	2.51	0.001
Past injury of either knee	2.4	2.8	0.560	1.4	4.1	0.046	3.1	2.4	0.466

OW was significantly positively associated with KOA (OR 2.65, 95% CI 1.98–3.54, p < 0.001). Logistic regression analysis using the same objective and explanatory factors and stratified according to sex indicated that, in both sexes, OW was the only factor that was significantly associated with KOA (men: OR 1.64, 95% CI 1.04–2.59, p < 0.05; women: OR 3.64, 95% CI 2.48–5.34, p < 0.001), while in men, there was weak but not significant association between HTN and KOA (OR 1.61, 95% CI 0.99–2.60, p = 0.053). These results suggest that obesity, among the various components for MS, was most significantly correlated to KOA.

Prevalence of KOA and its association with the number of components for MS. Table 4 shows the prevalence of KOA classified by the number of components for MS: the prevalence of KOA tended to increase with the increase in the number of MS components (p for trend < 0.001) in the total population. However, the prevalence of KOA in men and women did not tend to increase monotonically. Thus, in men, the prevalence of KOA in the groups with 2 MS components was lower than that in the groups with 1 component. Similarly, in women, the prevalence of KOA in the group with 2 MS components was higher than that in the group with 3 or more components.

To clarify the effect of the accumulation of MS components on the presence of KOA, logistic regression analysis was performed using the presence of KOA as the objective variable and the MS components (OW, HTN, DL, and IGT) present as explanatory variables after adjustment for age and sex. Compared to the reference condition (no MS components), increasing the number of components of MS significantly increased the OR for the presence of KOA (vs no component; 1 component: OR 1.18, 95% CI 0.87–1.61, p = 0.273; 2 components: OR 1.74, 95% CI 1.25–2.44, p = 0.001; more than 3 components: OR 2.15, 95% CI 1.44–3.23; p < 0.001). Again, the same analysis was also performed stratified by sex. In men, although no dose-response effects of the accumulation of MS components on KOA were observed when the number of the components was 1 or 2, the accumulation of 3 or more components of MS tended to be significantly associated with a higher OR of KOA (vs no component; 1 component: OR 1.94, 95% CI 1.11–3.39, p = 0.021; 2 components: OR 1.61, 95% CI 0.89–2.91, p = 0.117; more than 3 components: OR 2.96, 95% CI 1.5–5.85, p = 0.002). In contrast, in women, no significant difference was observed between the presence of no components and 1 component; however, 2 or more components of MS increased the risk of KOA significantly (vs no component; 1 component: OR 0.89, 95% CI 0.61–1.29, p = 0.527; 2 components: OR 1.94, 95% CI 1.27–2.96, p = 0.002; more than 3 components: OR 1.71, 95% CI 1.01–2.87, p = 0.044).

Table 4. Prevalence (%) of knee osteoarthritis, classified by the number of components of metabolic syndrome (MS). MS components consisted of obesity, hypertension, dyslipidemia, and impaired glucose tolerance.

No. MS Components	Total	Men	Women
0	32.5	24.8	35.4
1	49.9	44.8	52.9
2	60.5	42.7	71.8
≥ 3	62.2	51.3	69.4

Logistic regression analysis was performed using the presence of KOA as the objective variable and the number of MS components present (OW, HTN, DL, and IGT) as explanatory variables, after adjustment for age, sex, regional difference, smoking habit, alcohol consumption, physical activities, regular exercises, and history of knee injuries. Figure 1 shows the OR of the association between accumulation of components of MS and presence of KOA. Compared to the reference condition (no components of MS), increasing the number of components of MS significantly increased the OR for the presence of KOA (vs no component; 1 component: OR 1.21, 95% CI 0.88–1.68, p = 0.237; 2 components: OR 1.89, 95% CI 1.33–2.70, p < 0.001; > 3 components: OR 2.72, 95% CI 1.77–4.18, p < 0.001). Again, the same analysis was also performed stratified by sex. In men, although no dose-response effects of the accumulation of MS components on KOA were observed when the number of the components was 1 or 2, the accumulation of 3 or more components of MS tended to be significantly associated with a higher OR of KOA (vs no com-

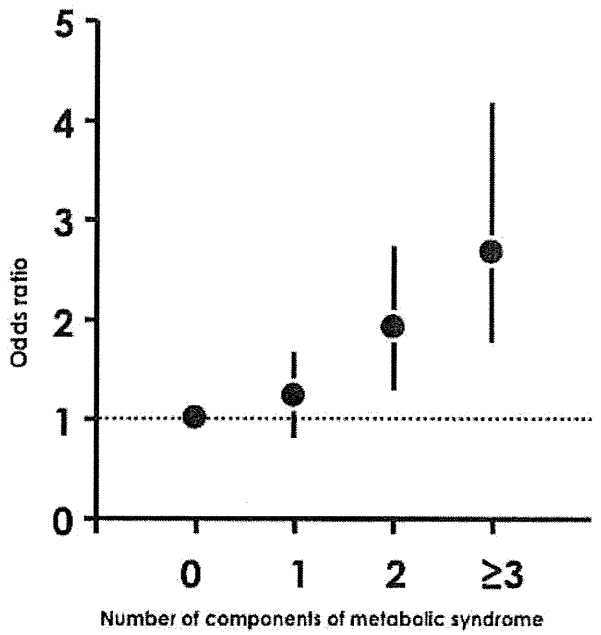

Figure 1. Odds ratios of the association between the number of components of metabolic syndrome and the presence of knee osteoarthritis, compared to no components present.

ponent; 1 component: OR 2.07, 95% CI 1.15–3.74, p = 0.016; 2 components: OR 1.68, 95% CI 0.89–3.17, p = 0.110; more than 3 components: OR 3.88, 95% CI 1.87–80.6, p < 0.001). In contrast, in women, no significant difference was observed between the presence of no component and 1 component; however, 2 or more components of MS increased the OR of KOA significantly (vs no component; 1 component: OR 0.88, 95% CI 0.59–1.32, p = 0.541; 2 components: OR 2.13, 95% CI 1.36–3.34, p = 0.001; > 3 components: OR 2.17, 95% CI 1.25–3.77, p = 0.006).

Joint space narrowing and areas of osteophytes in the knee, and their association with components of MS. Tables 5A and 5B show the mean measurements of indices for KOA, medial MJSW (mm), lateral MJSW (mm), medial JSA (mm^2), lateral JSA (mm^2), and OPA (mm^2), classified by the number of components of MS. The values of medial MJSW tended to be significantly lower, and those of OPA significantly higher, with the increasing number of components of MS. The values of medial JSA in women belonging to the group with no component of MS were significantly higher than in those belonging to the groups with 1, 2, 3, or more components of MS, but no such tendency was observed in men. There was no relationship between the values of lateral MJSW, lateral JSA, and the number of components of MS.

Multiple regression analysis was performed using values of medial MJSW as the objective variable and the number of components of MS present as explanatory variables, after adjustment for age, sex, regional difference, smoking habit, alcohol consumption, physical activities, regular exercises, and history of knee injuries. In the overall population, we found that the number of components of MS was inversely related to the values of medial MSJW (ß = –0.148, R^2 = 0.21, p < 0.001). An analysis performed using the same objective and explanatory factors and stratified by sex showed the same tendency in both men and women (men: ß = –0.152, R^2 = 0.14, p < 0.001; women: ß = –0.149, R^2 = 0.18, p < 0.001).

Multiple regression analysis was then performed using OPA values as the objective variable and the number of components of MS present as explanatory variables, after adjustment for age, sex, regional difference, smoking habit, alcohol consumption, physical activities, regular exercises, and history of knee injuries. The analysis revealed that the number of components of MS was positively related to OPA values (ß = 0.12, R^2 = 0.11, p < 0.001). An analysis performed using the same objective and explanatory factors and stratified by sex showed the same tendency in both men and women (men: ß = 0.15, R^2 = 0.08, p < 0.001; women: ß = 0.11, R^2 = 0.11, p < 0.001).

In women, multiple regression analysis was performed using values of medial JSA as the objective variable and the number of components of MS present as explanatory variables, after adjustment for age, regional difference, smoking habit, alcohol consumption, physical activities, regular exercises, and history of knee injuries. The analysis revealed that the number of components of MS was inversely related to the values of medial JSA in women (ß = –0.096, R^2 = 0.18, p = 0.001).

DISCUSSION

We found that an increase in the number of components of MS was significantly associated with the presence of KOA diagnosed by using the KL scale in Japanese men and women. We also clarified that the values of medial MJSW and OPA in men and women, and medial JSA in women as features of KOA, were significantly associated with the increase in the number of MS components.

KOA and MS share age and OW as risk factors[1,7,8,9,10,11]. We have already reported that higher BMI was associated with radiographic KOA based on an analysis using the same population evaluated in our study[36], and it was also clarified that OW was the strongest factor that influenced the prevalence of KOA.

Regarding the association between clustering of metabolic factors and KOA, Hart, *et al* found that metabolic factors including blood glucose, hypercholesterolemia, and HTN were associated with both unilateral and bilateral KOA and were independent of OW[20]. Sowers, *et al*[21] defined the presence of ≥ 2 of the following criteria as cardiometabolic clustering: low levels of HDL cholesterol, elevated levels of low-density lipoprotein cholesterol, TG, BP, C-reactive protein, waist/hip ratio, glucose levels, and dia-

Table 5A. Mean (SD) of medial and lateral minimum joint space width (MJSW) classified by the number of components of metabolic syndrome (MS). MS components consisted of obesity, hypertension, dyslipidemia, and impaired glucose tolerance.

No. MS Components	Medial MJSW, mm			Lateral MJSW, mm		
	Total	Men	Women	Total	Men	Women
0	2.98 (0.81)	3.33 (0.66)	2.85 (0.82)	4.00 (1.18)	4.37 (1.13)	3.86 (1.17)
1	2.69 (1.01)[a]	3.05 (0.97)	2.49 (0.98)[a]	3.96 (1.13)	4.43 (1.05)	3.70 (1.08)
2	2.43 (1.19)[ab]	2.87 (1.10)[a]	2.15 (1.17)[ab]	3.85 (1.19)	4.15 (1.10)	3.66 (1.22)
≥ 3	2.42 (1.22)[ab]	2.73 (1.24)[a]	2.22 (1.17)[a]	4.06 (1.27)	4.26 (1.29)	3.93 (1.24)

[a] Significantly different from values obtained in the absence of components ($p < 0.05$). [b] Significantly different from values obtained with 1 component ($p < 0.05$).

Table 5B. Mean (SD) of medial and lateral joint space area (JSA) and area of osteophytosis (OPA), classified by number of components of metabolic syndrome (MS). MS components consisted of obesity, hypertension, dyslipidemia, and impaired glucose tolerance.

No. MS Components	Medial JSA, mm^2			Lateral JSA, mm^2			OPA, mm^2		
	Total	Men	Women	Total	Men	Women	Total	Men	Women
0	96.3 (27.6)	111.4 (25.6)	98.8 (26.2)	111.0 (33.2)	132.2 (34.2)	103.3 (29.2)	1.81 (6.42)	0.93 (2.97)	2.13 (7.26)
1	90.2 (31.7)[a]	104.0 (30.7)	82.3 (29.6)[a]	111.0 (32.4)	131.2 (30.5)	99.5 (27.5)	3.06 (7.89)	1.33 (4.26)	4.05 (9.21)
2	85.2 (36.7)[a]	101.1 (34.3)	75.0 (34.6)[ab]	111.7 (32.2)	128.9 (29.6)	100.6 (28.8)	5.34 (11.25)[ab]	2.45 (5.36)	7.18 (13.44)[ab]
≥ 3	88.2 (39.3)	102.0 (40.1)	79.1 (36.0)[a]	118.2 (35.3)	132.5 (34.7)	108.8 (32.5)[b]	6.26 (9.59)[ab]	3.82 (8.70)[ab]	7.86 (9.85)[ab]

[a] Significantly different from values obtained in the absence of components ($p < 0.05$). [b] Significantly different from values obtained with 1 component ($p < 0.05$).

betes mellitus, and assessed the association between cardiometabolic clustering and KOA. They found that KOA was significantly more frequent in obese women with cardiometabolic clustering compared with those without it[21]. Using data from the National Health and Nutrition Examination Survey III (NHANES III), Singh, *et al* suggested that adults with OA in the United States have a high prevalence of CVD risk factors[19], and Puenpatom and Victor demonstrated that each of the 5 cardiovascular risk factors that comprise MS, HTN, abdominal OW, hyperglycemia, elevated TG, and low HDL cholesterol, was more prevalent in the population with OA than in the population without OA[22]. However, to our knowledge, few population-based studies have shown a dose-response relationship between the presence of KOA and the accumulation of the number of MS components.

In our study, the logistic regression analysis revealed that only OW was significantly associated with KOA, and other components were not significant, using the presence of KOA as an objective variable and all components for MS, such as OW, HTN, DL, and IGT as explanatory variables and after adjustment for potential confounders. However, we found that the higher the number of components of MS, the greater the OR of the presence of KOA. This result indicates that, even if the effect of each component of MS on KOA may be weak, accumulation of the number of components may significantly worsen KOA.

In addition, we found that medial MJSW values in men and women, and medial JSA values in women tended to be significantly lower with the increase in the number of components of MS. In contrast, OPA values became significantly higher with the increase in the number of components of MS. Regarding the association between JSW and KOA, Sowers, *et al* used statistical models that included variables representing obesity, cardiometabolic status, and lateral and medial JSW differences to show that a 1-mm increase in the difference between lateral and medial JSW was associated with 2.1 times greater odds of having KOA, and subjects who were obese with cardiometabolic clustering had 4.5 times greater odds of having KOA[21]. However, no other reports have addressed direct associations between indices of KOA, such as MJSW, JSA, and OPA values, with the accumulation of the number of components of MS. In our study, we confirmed that the accumulation of the number of MS components present influenced the values of both MJSW, JSA (women only), and OPA, which determine the features and severity of KOA.

Regarding the association of clustering of components for MS and KOA, a few hypotheses have been suggested. Hart, *et al* attributed the effect of excess endogenous estrogens to the aromatization of estrone in fat tissue[20]. Regarding the endogenous secreted products, Sowers, *et al* suggested that leptin and adiponectin levels influenced the development of OA[21]. They stated that leptin concentrations

in the synovial fluid of patients with OA correlated with their BMI, and levels of adiponectin are low in obese individuals and in those with CVD. Another hypothesis states that atherosclerotic change may play a role in the development of OA. Kornaat, *et al* reported the association between increased popliteal artery vessel wall thickness and generalized OA[37]. It has been hypothesized that atherosclerotic changes and obesity-associated metabolic changes in the subchondral bone are associated with OA[37,38]. In obese subjects, metabolic changes in the striated muscles induced by the interaction of insulin resistance and systemic inflammation might lead to fatigue and muscle weakness, which influences the balance between damage and repair mechanisms leading to OA[37,39]. In our study, we could not substantiate these hypotheses because of the lack of relevant measurements. However, in the followup study, we will obtain the ankle brachial pressure index and pulse wave velocity of the ROAD subjects, and thus we will further the evidence regarding the association between arteriosclerosis and KOA.

In our study, a sexual dimorphism pattern was shown in prevalence of KOA (women > men) and components of MS such as values of BMI (men > women), BP (men > women), and HDL cholesterol (women > men). Regarding KOA, being female is well known as a strong risk factor, according to our previous survey and other studies[27,40,41,42,43,44], possibly implicating an involvement of muscle strength to compensate for the mechanical stress, since women are known to have less muscle strength than men[45]. Sex differences in the prevalence of MS might be partly explained by endogenous sex steroids. As mentioned, Hart, *et al* attributed the effect of excess endogenous estrogens to the aromatization of estrone in fat tissue[20]. Recent systematic review and metaanalysis of observational studies concluded that there is a sex-dependent association between levels of testosterone and occurrence of MS[46]. In addition, the difference in prevalence of associated confounding factors may influence the effect of sex difference on the occurrence of MS. In our study, there are sex differences in lifestyle-related factors, which might influence the occurrence of MS. For example, the proportions of smokers and alcohol consumers are both significantly higher in men than in women (both $p < 0.001$). Regarding the physical activities, the proportion of men who exercised regularly was significantly higher than that of women ($p < 0.001$). Therefore, for the statistical analyses, we adjusted not only for age and sex, but also for such potentially confounding factors to show the association between components of MS and KOA.

With regard to ethnic differences in MS, Hoang, *et al* reviewed epidemiological studies and reported that the prevalence of MS in East Asians was lower than that in whites[28]. However, the prevalence of MS may increase rapidly. Nestel reported a dramatic increase in the prevalence of MS in a cohort from Beijing, from 9% to 21%, between 1992 and 2002[47]. In addition, as reported, the prevalence of KOA in Japanese as well as Chinese cohorts is significantly higher than in whites[27,36]. In light of the rapidly increasing population in Asian countries, prevention strategies for obesity-related chronic diseases, such as MS and KOA, should be implemented immediately. In our study, we clarified that components of MS and their accumulation were associated with KOA in Asian subjects. Based on these findings, the prevention of MS may be useful in the prevention of not only CVD, but also KOA, in both Asian and Western countries, and may lead to a reduction in the number of patients who have a disability arising from joint disorders.

There are several limitations in our study. First, although the ROAD study includes a large number of participants, these participants may not be truly representative of the general population. To confirm whether the participants of the ROAD study are representative of the Japanese population, we compared anthropometric measurements and the frequencies of smoking and alcohol consumption between study participants and the general Japanese population, and no significant differences were found, except that male ROAD study participants aged 70–74 years were significantly smaller in terms of body structure than the overall Japanese population ($p < 0.05$)[29]. This difference should be considered when evaluating the potential risk factors for men aged 70–74 years; factors such as body build, particularly heavy weight, are known to be associated with the presence of MS and KOA. Thus, our results might represent an underestimation. Second, this was a cross-sectional study, and the causal relationship between metabolic factors and KOA remains unclear. Metabolic factors may have changed recently or been longstanding; this can only be ascertained by a longitudinal study that clarifies the incidence and/or progression rates of KOA in the same cohort. The first such followup of the ROAD cohort is in progress; it intends to clarify the causal relationships between musculoskeletal diseases and MS for early prevention of the disabilities. Third, we categorized MS by using the criteria defined by the Examination Committee of Criteria for Metabolic Syndrome in Japan[29], except for the definition of overweight. We used BMI ≥ 25 as the criterion for OW status, as defined by the Japan Society for the Study of Obesity[30]. In addition, since the blood samples obtained were not always from participants under fasting conditions, we used serum HDL cholesterol level < 40 mg/dl to indicate DL, and serum HbA1c level ≥ 5.5% to indicate IGT, which are indices used by the National Health and Nutrition Survey in Japan[32]. These differences in the definition of MS may skew the true association between MS and KOA. However, our aim was to determine how the accumulation of MS components was related to KOA, and we believe the indices we used for OW, HTN, DL, and IGT accurately reflected the participants' physical condition.

Our study evaluated a large-scale population from the

ROAD study and revealed that the presence of KOA was significantly associated with increases in the number of components of MS. Additionally, the number of components of MS was inversely related to medial MSJW values and positively related to OPA values. The prevention of MS may be useful for both CVD and KOA in Asian populations. Further investigations, along with continued longitudinal surveys in the ROAD study, will elucidate the components of MS and occurrence or progress of KOA.

REFERENCES

1. Sharma L, Kapoor D. Epidemiology of osteoarthritis. In: Moskowitz RW, Altman RD, Hochberg MC, Buckwalter JA, Goldberg VM, editors. Osteoarthritis: diagnosis and medical/surgical management. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2007:3-26.
2. Guccione AA, Felson DT, Anderson JJ, Anthony JM, Zhang Y, Wilson PW, et al. The effects of specific medical conditions on the functional limitations of elders in the Framingham Study. Am J Public Health 1994;84:351-8.
3. Felson DT, Zhang Y. An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. Arthritis Rheum 1998;41:1343-55.
4. Ministry of Health, Labour and Welfare, Japan. The outline of the results of National Livelihood Survey 2007 [Japanese]. [Internet. Accessed January 7, 2011.] Available from: http://www.mhlw.go.jp/toukei/list/20-19-1.html
5. Dahlöf B. Cardiovascular disease risk factors: epidemiology and risk assessment. Am J Cardiol 2010;105:3A-9A.
6. Day C. Metabolic syndrome, or what you will: definitions and epidemiology. Diab Vasc Dis Res 2007;4:32-8.
7. Felson DT, Anderson JJ, Naimark A, Walker WM, Meenan RF. Obesity and knee osteoarthritis: the Framingham Study. Ann Intern Med 1988;109:18-24.
8. Hart DJ, Spector TD. The relationship of obesity, fat distribution and osteoarthritis in the general population: the Chingford Study. J Rheumatol 1993;20:331-5.
9. Van Saase JL, Vandenbroucke JP, Van Romunde LK, Valkenburg HA. Osteoarthritis and obesity in the general population. A relationship calling for an explanation. J Rheumatol 1998; 15:1152-8.
10. Magliano M. Obesity and arthritis. Menopause Int 2008;14:149-54.
11. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, et al. OARSI recommendations for the management of hip and knee osteoarthritis. Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage 2008;16:137-62.
12. Lawrence JS. Hypertension in relation to musculoskeletal disorders. Ann Rheum Dis 1975;34:451-6.
13. Kellgren JH. Osteoarthritis in patients and populations. BMJ 1961;1:1-6.
14. Cimmino MA, Cutolo M. Plasma glucose concentration in symptomatic osteoarthritis: a clinical and epidemiological survey. Clin Exp Rheumatol 1990;8:251-7.
15. Davis MA, Ettinger WH, Neuhaus JM. The role of metabolic factors and blood pressure in the association of obesity with osteoarthritis of the knee. J Rheumatol 1988;15:1827-32.
16. Cooper C, McAlindon T, Snow S, Vines K, Young P, Kirwan J, et al. Mechanical and constitutional risk factors for symptomatic knee osteoarthritis: differences between medial tibiofemoral and patellofemoral disease. J Rheumatol 1994;21:307-13.
17. Martin K, Lethbridge-Cejku M, Muller DC, Elahi D, Andres R, Tobin JD, et al. Metabolic correlates of obesity and radiographic features of knee osteoarthritis: data from the Baltimore Longitudinal Study of Aging. J Rheumatol 1997;24:702-7.
18. Stürmer T, Brenner H, Brenner RE, Günther KP. Non-insulin dependent diabetes mellitus (NIDDM) and patterns of osteoarthritis. The Ulm osteoarthritis study. Scand J Rheumatol 2001;30:169-71.
19. Singh G, Miller JD, Lee FH, Pettitt D, Russell MW. Prevalence of cardiovascular disease risk factors among US adults with self-reported osteoarthritis: data from the Third National Health and Nutrition Examination Survey. Am J Manag Care 2002;8:S383-91.
20. Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford study. J Rheumatol 1995;22:1118-23.
21. Sowers M, Karvonen-Gutierrez CA, Palmieri-Smith R, Jacobson JA, Jiang Y, Ashton-Miller JA. Knee osteoarthritis in obese women with cardiometabolic clustering. Arthritis Rheum 2009;61:1328-36.
22. Puenpatom RA, Victor TW. Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. Postgrad Med 2009;121:9-20.
23. Kellgren JH, Lawrence LS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494-502.
24. Jones G, Ding C, Scott F, Glisson M, Cicuttini F. Early radiographic osteoarthritis is associated with substantial changes in cartilage volume and tibial bone surface area in both males and females. Osteoarthritis Cartilage 2004;12:169-74.
25. Yamada T, Kawano H, Koshizuka Y, Fukuda T, Yoshimura K, Kamekura S, et al. Carminerin contributes to chondrocyte calcification during endochondral ossification. Nat Med 2006;12:665-70.
26. Kamekura S, Kawasaki Y, Hoshi K, Shimoaka T, Chikuda H, Maruyama Z, et al. Contribution of runt-related transcription factor 2 to the pathogenesis of osteoarthritis in mice after induction of knee joint instability. Arthritis Rheum 2006;54:2462-70.
27. Muraki S, Oka H, Akune T, Mabuchi A, En-yo Y, Yoshida M, et al. Prevalence of radiographic knee osteoarthritis and its association with knee pain in the elderly of Japanese population-based cohorts: the ROAD study. Osteoarthritis Cartilage 2009;17:1137-43.
28. Hoang KC, Le TV, Wong ND. The metabolic syndrome in East Asians. J Cardiometab Syndr 2007;2:276-82.
29. Yoshimura N, Muraki S, Oka H, Mabuchi A, En-Yo Y, Yoshida M, et al. Prevalence of knee osteoarthritis, lumbar spondylosis, and osteoporosis in Japanese men and women: the Research on Osteoarthritis/Osteoporosis Against Disability Study. J Bone Miner Metab 2009;27:620-8.
30. Yoshimura N, Muraki S, Oka H, Kawaguchi H, Nakamura K, Akune T. Cohort profile: Research on Osteoarthritis/Osteoporosis Against Disability Study. Int J Epidemiol 2010;39:988-95.
31. Oka H, Muraki S, Akune T, Mabuchi A, Suzuki T, Yoshida H, et al. Fully automatic quantification of knee osteoarthritis severity on standard radiographs. Osteoarthritis Cartilage 2008;16:1300-6.
32. The Examination Committee of Criteria for Metabolic Syndrome. The definition and criteria of metabolic syndrome [Japanese]. J Jpn Soc Intern Med 2005;94:794-809.
33. Examination Committee of Criteria for 'Obesity Disease' in Japan; Japan Society for the Study of Obesity. New criteria for 'obesity disease' in Japan. Circ J 2002;66:987-92.
34. Shibata K, Suzuki S, Sato J, Ohsawa I, Goto S, Hashiguchi M, et al. Abdominal circumference should not be a required criterion for the diagnosis of metabolic syndrome. Environ Health Prev Med 2010;15:229-35.
35. Ministry of Health, Labour and Welfare. The outline of the results of National Health and Nutrition Survey 2008 [Japanese]. [Internet. Accessed January 7, 2011.] Available from: http://www.mhlw.go.jp/houdou/2009/11/dl/h1109-1b.pdf
36. Muraki S, Akune T, Oka H, Mabuchi A, En-yo Y, Yoshida M, et al. Association of occupational activity with radiographic knee

osteoarthritis and lumbar spondylosis in elderly patients of population-based cohorts: a large-scale population-based study. Arthritis Rheum 2009;61:779-86.
37. Kornaat PR, Sharma R, van der Geest RJ, Lamb HJ, Kloppenburg M, Hellio le Graverand MP, et al. Positive association between increased popliteal artery vessel wall thickness and generalized osteoarthritis: is OA also part of the metabolic syndrome? Skeletal Radiol 2009;38:1147-51.
38. Conaghan PG, Vanharanta H, Dieppe PA. Is progressive osteoarthritis an atheromatous vascular disease? Ann Rheum Dis 2005;64:1539-41.
39. Rojas-Rodríguez J, Escobar-Linares LE, Garcia-Carrasco M, Escárcega RO, Fuentes-Alexandro S, Zamora-Ustaran A. The relationship between the metabolic syndrome and energy-utilization deficit in the pathogenesis of obesity-induced osteoarthritis. Med Hypotheses 2007;69:860-8.
40. Kellgren JH, Lawrence JS. Osteo-arthrosis and disk degeneration in an urban population. Ann Rheum Dis 1958;17:388-97.
41. Felson DT, Naimark A, Anderson J, Kazis L, Castelli W, Meenan RF. The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. Arthritis Rheum 1987;30:914-8.
42. Anderson JJ, Felson DT. Factors associated with osteoarthritis of the knee in the first national Health and Nutrition Examination Survey (HANES I). Evidence for an association with overweight, race, and physical demands of work. Am J Epidemiol 1988;128:179-89.
43. van Saase JL, van Romunde LK, Cats A, Vandenbroucke JP, Valkenburg HA. Epidemiology of osteoarthritis: Zoetermeer survey. Comparison of radiological osteoarthritis in a Dutch population with that in 10 other populations. Ann Rheum Dis 1989;48:271-80.
44. Dillon CF, Rasch EK, Gu Q, Hirsch R. Prevalence of knee osteoarthritis in the United States: arthritis data from the Third National Health and Nutrition Examination Survey 1991-94. J Rheumatol 2006;33:2271-9.
45. Sinaki M, Nwaogwugwu NC, Phillips BE, Mokri MP. Effect of gender, age, and anthropometry on axial and appendicular muscle strength. Am J Phys Med Rehabil 2001;80:330-8.
46. Brand JS, van der Tweel I, Grobbee DE, Emmelot-Vonk MH, van der Schouw YT. Testosterone, sex hormone-binding globulin and the metabolic syndrome: a systematic review and meta-analysis of observational studies. Int J Epidemiol 2010 Sep 24 [E-pub ahead of print].
47. Nestel P. Nutritional aspects in the causation and management of the metabolic syndrome. Endocrinol Metab Clin North Am 2004;33:483-92.

J Bone Miner Metab (2011) 29:699–708
DOI 10.1007/s00774-011-0266-8

ORIGINAL ARTICLE

Changes in serum levels of biochemical markers of bone turnover during 10 years among Japanese men and women: associated factors and birth-cohort effect. The Taiji Study

Noriko Yoshimura · Shigeyuki Muraki · Hiroyuki Oka · Hiroshi Kawaguchi · Kozo Nakamura · Toru Akune

Received: 9 September 2010 / Accepted: 3 March 2011 / Published online: 6 April 2011

Abstract We aimed to clarify changes in biochemical markers of bone turnover (BTMs) over 10 years, associations with changes in bone mineral density (BMD), and birth-cohort effects in a Japanese community. We randomly selected 400 individuals (age, 40–79 years; 50 of each gender and age stratum) from a list of registered residents in 1993. We measured BMD of the spine and hip, and serum concentrations of total osteocalcin (OC), beta-C-terminal cross-linking telopeptide of type I collagen (beta-CTX), and N-terminal cross-linking telopeptide of type I collagen (NTX), in 1993 and 2003. Of the 400 subjects, 322 (153 men, 169 women) completed the 10-year follow-up. Mean change rates (standard deviation) for serum total OC, beta-CTX, and NTX over 10 years were −1.00 (3.74)%/year, 5.10 (22.48)%/year, and 0.40 (3.41)%/year, respectively, in men, and 0.02 (5.32)%/year, 5.53 (14.54)%/year, and 0.62 (3.26)%/year, respectively, in women. Change rates of BTMs were higher for women in their forties than for women in their fifties to seventies ($P < 0.05$), and higher in the menstrual transition group than in pre- and postmenopausal groups ($P < 0.001$). Changes in levels of BTMs over 10 years in women were significantly associated with change rates of BMDs at L2–L4 and total hip after adjusting for potential confounders. A significant birth-cohort effect was observed among women in their fifties. We concluded that change rates of BTMs during the 10 years were influenced by menstrual transition, age, and sex and associated with bone loss at L2–L4 and total hip.

Keywords Biochemical markers of bone turnover · Bone loss · Menstrual transition · Birth-cohort effect

N. Yoshimura (✉) · H. Oka
Department of Joint Disease Research, 22nd Century Medical and Research Center, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: yoshimuran-ort@h.u-tokyo.ac.jp

S. Muraki · T. Akune
Department of Clinical Motor System Medicine, 22nd Century Medical and Research Center, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

H. Kawaguchi · K. Nakamura
Department of Orthopaedic Surgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan

Introduction

In Japan, about 10 million patients are estimated to have osteoporosis (OP) [1], and osteoporotic fractures are ranked fifth among the diseases responsible for causing disabilities requiring support [2]. Moreover, the number of cases of hip fracture has increased steeply in the past 20 years with the rapid aging of the population [3]. Early detection of OP to reduce the risk of osteoporotic fractures is therefore an urgent issue in terms of maintenance of quality of life in the elderly and containment of medical costs required for their care.

Biochemical markers of bone turnover (BTMs) are widely used in clinical situations to evaluate the efficacy of treatments for OP [4–6]. Several epidemiological studies have shown that BTMs can predict bone loss in women [7–10], but few reports appear to have examined trends in BTMs during more than one decade. In addition, few reports have clarified associations between changes in levels of BTMs and bone loss, particularly in men [11, 12].

Moreover, observation over the course of a decade enables comparison of values at the same age strata among different birth cohorts. We have already identified a birth-cohort effect in values of bone mineral density (BMD) using data from 1990 and 2000 in another cohort established in Wakayama Prefecture, a mountainous area in Japan [13]. In that study, values of bone mineral density (BMD) for women in their fifties and for men in their sixties were significantly higher for the younger than for the older birth cohort [13].

We established a cohort comprising men and women in a rural area in Japan and followed this cohort for 10 years. The present study was performed for the purpose of clarifying three issues: (1) changes in BTMs over 10 years in men and women as classified by age and menstrual transition; (2) associations between increases in BTMs and bone loss over 10 years in both men and women; and (3) effects of birth cohort on BTMs among general inhabitants, namely, whether differences in BTMs exist between birth cohorts for a given age stratum in both men and women.

Materials and methods

Cohort profile and eligible participants

The survey was performed in the Japanese town of Taiji. The Taiji cohort has been profiled in detail elsewhere [14–16] and so is only described briefly here. Taiji is located in the southern coastal area of Wakayama Prefecture, Japan. A list of all inhabitants born between 1913 and 1952, and therefore between 40 and 79 years old in 1993, was compiled on the basis of resident registrations as of the end of 1992. A cohort of 2,261 inhabitants (1,028 men, 1,233 women) was identified, and all members of the cohort completed a self-administered, 125-item questionnaire addressing lifestyle factors such as dietary habits, smoking habits, alcohol consumption, and physical exercise (whole cohort).

From this total cohort, 50 men and 50 women from each of four age groups (total, 400 participants) between 40 and 79 years by decade of birth year (1913–1922, 1923–1932, 1933–1942, and 1943–1952) were selected randomly and underwent BMD measurement in 1993. At this time, blood samples were taken from all participants (BMD cohort, baseline study). Background data including physical characteristics and mean BMD values for all 400 participants at baseline are shown in Table 1.

Among the 400 participants, 21 individuals (4 men, 17 women) had been diagnosed with osteoporosis in the past, but none had been treated using bisphosphonates, raloxifene, or calcitonin. Among the female participants, of 100 female participants in their forties and fifties at baseline, 41 women (41.0%) were premenopausal with regular periods, 14 (14.0%) were premenopausal with irregular periods, and the remaining 45 (45.0%) were postmenopausal.

Among the 400 participants at baseline, 322 (80.5%; 153 men, 169 women) participated in the examination held after 10 years. Loss of 78 participants at the 10-year follow-up was explained as follows: 52 participants had died (33 men, 19 women); 14 participants had moved (6 men, 8 women); 6 participants were ill (4 men, 2 women); 3 participants refused to participate (2 men, 1 woman); and 3 participants were away from the area at the time of follow-up (2 men, 1 woman).

During the 10 years, 37 new fragile fractures (10 men, 27 women), including 5 spinal fractures (1 man, 4 women), were reported in the interviewer-administered questionnaire surveys.

All examinations were performed with the full consent of participants. These study protocols were approved by the ethics committees of both Wakayama Medical University and the University of Tokyo.

BMD measurements

Baseline BMD was measured in 1993 using dual-energy X-ray absorptiometry (DXA) (QDR 1000; Hologic, Bedford, MA, USA). At baseline, all 400 participants (200 men, 200 women) underwent measurement of BMD from anteroposterior images of lumbar vertebrae L2–L4 and the proximal femur (femoral neck, Ward's triangle, trochanter, and total hip). These measurements were repeated on the same participants after 10 years.

To control the precision of DXA, the equipment was checked at every examination in 1993 and 2003 using the same phantom, and values for BMD of the phantom under DXA were regulated to 1.030 ± 0.016 g/cm^2 (1.5%) during all examinations. All BMD measurements were performed by the same medical doctor (N.Y.). To clarify the coefficient of variation (CV) for BMD measurements from DXA scans by the investigator, the same phantom was measured seven times in 1 day, then once a day at the same time every day for 5 days, and once a week at the same time and same day of the week for 4 weeks. CVs of intraday, interday, and interweek variability for this investigator were 0.13%, 0.39%, and 0.42%, respectively [17].

Measurements of BTMs

Blood examinations both at baseline and at the 10-year follow-up were performed in June. All blood samples were

Table 1 Summary characteristics for participants at baseline classified by age and gender

Birth cohort	Age group (years)	*n*	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	L2–L4 BMD (g/cm^2)	Femoral neck BMD (g/cm^2)	Total hip BMD (g/cm^2)
Men									
1943–52	40–49	50	44.2 (2.6)	168.8 (5.2)	69.0 (10.4)	24.2 (3.2)	1.05 (0.15)	0.86 (0.09)	1.00 (0.12)
1933–42	50–59	50	54.8 (2.7)	165.6 (5.0)[a]	63.5 (9.4)[a]	23.1 (2.9)	0.98 (0.17)	0.80 (0.13)[a]	0.94 (0.14)
1923–32	60–69	50	64.6 (2.5)	163.0 (4.8)[a]	62.9 (9.6)[a]	23.6 (3.2)	1.04 (0.21)	0.77 (0.11)[a]	0.92 (0.12)[a]
1913–22	70–79	50	74.0 (2.7)	160.7 (5.4)[a,b]	57.5 (8.3)[a,b,c]	22.2 (2.8)	0.97 (0.19)	0.71 (0.08)[a,b,c]	0.83 (0.09)[a,b,c]
1913–52	40–79	200	59.4 (11.4)	164.5 (5.9)	63.2 (10.2)	23.3 (3.1)	1.01 (0.18)	0.79 (0.12)	0.92 (0.13)
Women									
1943–52	40–49	50	44.0 (2.8)	154.4 (5.0)	54.1 (8.3)	22.7 (3.1)	1.07 (0.14)	0.79 (0.10)	0.90 (0.11)
1933–42	50–59	50	55.8 (2.8)	154.9 (5.3)	59.4 (10.0)[a]	24.8 (4.0)[a]	0.92 (0.16)[a]	0.70 (0.11)[a]	0.81 (0.12)[a]
1923–32	60–69	50	64.8 (2.6)	151.1 (4.6)[a,b]	52.1 (9.1)[b]	22.8 (3.5)[b]	0.78 (0.17)[a,b]	0.62 (0.09)[a,b]	0.71 (0.10)[a,b]
1913–22	70–79	50	74.4 (2.8)	147.7 (5.4)[a,b,c]	48.4 (8.2)[a,b]	22.2 (3.4)[b]	0.77 (0.12)[a,b]	0.59 (0.10)[a,b]	0.66 (0.12)[a,b]
1913–52	40–79	200	59.7 (11.6)	152.0 (5.8)	53.5 (9.7)	23.1 (3.6)	0.89 (0.19)	0.68 (0.13)	0.77 (0.14)

Values are given as mean with standard deviation in parentheses

BMI body mass index, *BMD* bone mineral density, *n* number of participants

[a] Significantly different ($P < 0.05$) from values of participants in their forties

[b] Significantly different ($P < 0.05$) from values of participants in their fifties

[c] Significantly different ($P < 0.05$) from values of participants in their sixties

obtained between 0900 and 1500 in both surveys. Subjects who provided consent to participate in the blood examination were randomly allocated a specific time to undergo sampling, with times set at 15-min intervals between 0900 and 1500. Participant samplings could not be adjusted according to time after eating.

After centrifugation of blood samples, serum samples were immediately placed in dry ice and transferred to a deep freezer within 24 h. These samples were kept at −80°C until assay. BTMs collected in 1993 were measured after 7 years, when the methods for measurement of novel BTMs were introduced. Samples collected in 2003 were used for measurement within 1 year. Samples in 1993 and 2003 were measured using the same assay.

From the serum samples of participants in the baseline study, total osteocalcin (OC) was measured as a marker of bone formation. OC level was measured using an electrochemiluminescent immunoassay (ECLIA) (Elecsys N-MID Osteocalcin; Roche Diagnostics, Mannheim, Germany) [18]. Intraassay CV was 0.5% and sensitivity was 0.5 ng/ml. To monitor bone resorption, a beta-isomerized C-terminal cross-linking telopeptide of type I collagen (beta-CTX) and an N-terminal cross-linking telopeptide of type I collagen (NTX) were used. Serum beta-CTX was measured using an ECLIA (Elecsys beta-CrossLaps; Roche Diagnostics). Intraassay CV was 2.0% and sensitivity was 0.01 ng/ml [18]. Serum NTX was measured using an enzyme-linked immunosorbent assay (Osteomark NTX serum; Ostex International, Seattle, WA, USA) [19, 20]. Intraassay CV was 4.6% and sensitivity was 3.2 nM bone collagen equivalents (BCE/l).

Statistical analysis

All statistical analyses were performed using STATA statistical software (College Station, TX, USA). Differences in values of BMDs, BTMs, and change rates of BMDs and BTMs were tested for significance using analysis of variance (ANOVA) for comparisons among multiple groups and Scheffe's least significant difference test for pairs of groups. Correlation coefficients were estimated to identify associations between changes in levels of BTMs and BMD over 10 years. After controlling for the potential confounders listed in the Results section, multivariate regression analysis was performed using rates of change for BMDs at each site such as L2–L4, femoral neck, and total hip as an objective factor, and rates of change for each BTM such as total OC, beta-CTX, and NTX as explanatory factors and standardized partial regression coefficients were estimated. To address cohort effects on the values of BTMs, BTM levels of subjects in their fifties, sixties, and seventies in 1993 were compared to those of subjects in their fifties, sixties, and seventies in 2003 using a nonpaired *t* test.

Table 2 Annual change rate (%/year) in bone mineral density (BMD) over 10 years, classified by age and gender

Birth cohort	Age group (years)	*n*	L2–L4 BMD	Femoral neck BMD	Total hip BMD
Men					
1943–1952	40–49	43	−0.02 (0.59)	−0.15 (1.09)	−0.11 (0.71)
1933–1942	50–59	46	0.16 (0.8)	−0.30 (0.70)*	−0.30 (0.50)*
1923–1932	60–69	41	0.23 (0.94)	0.03 (1.25)	−0.36 (0.81)*
1913–1922	70–79	23	−0.15 (0.90)	0.66 (1.62)[b]	−0.24 (1.30)
1913–1952	40–79	153	0.08 (0.81)	−0.03 (1.17)	−0.26 (0.80)*
Women					
1943–1952	40–49	47	−1.14 (1.01)*	−0.86 (0.96)*	−0.68 (0.84)*
1933–1942	50–59	47	−0.79 (1.18)*	−0.64 (0.94)*	−0.70 (0.84)*
1923–1932	60–69	44	−0.32 (0.78)*[a]	−0.57 (0.89)*	−0.73 (0.80)*
1913–1922	70–79	31	−0.60 (0.92)*	−0.61 (1.08)*	−0.90 (0.68)*
1913–1952	40–79	169	−0.73 (1.03)*	−0.68 (0.96)*	−0.74 (0.80)*

Values are given as mean with standard deviation in parentheses

BMD bone mineral density; *n* number of subjects

* Significantly different ($P < 0.01$) for changes over 10 years

[a] Significantly different ($P < 0.05$) from values of subjects in their forties

[b] Significantly different ($P < 0.05$) from values of subjects in their fifties

Results

Eligible participants and changes in BMD over 10 years

Over the 10 years, 322 of the 400 participants at baseline (80.4%; 153 men, 169 women) completed baseline and follow-up measurements. Among these, one man in his sixties declined to undergo baseline blood examination for BTMs. Evaluations of changes in BTMs were thus performed using the remaining 321 subjects (80.3%; 152 men, 169 women).

Rates of change for BMD during the 10-year period, classified by age and gender, are shown in Table 2. For men, BMD at L2–L4 in their fifties and sixties had increased slightly by the 10-year follow-up but had decreased slightly in their forties and seventies. BMD at the femoral neck had decreased in their forties and fifties and had increased in their seventies. BMD at the total hip had decreased in all age strata. These changes were significant at the femoral neck for men in their fifties ($P < 0.01$) and at the total hip for men in their fifties and sixties ($P < 0.01$). No significant differences were apparent between age strata except at the femoral neck between men in their fifties and those in their seventies ($P < 0.05$).

For women, BMD at the lumbar spine L2–L4, femoral neck, and total hip had decreased in all age strata over the 10 years, similar to findings in men at the total hip. These changes were significant ($P < 0.01$). However, no significant differences in rates of change were seen across age strata, with the exception of women in their forties and sixties at L2–L4.

Mean levels at baseline and comparative changes over 10 years in BTMs

Age–gender distributions of mean BTM levels at the initial survey are shown in Table 3. No significant difference was seen among the age groups for BTM levels in men, whereas significant differences were seen for each marker between women in their forties and women in their fifties to seventies ($P < 0.05$ each). Table 3 also shows the changes in serum total OC, beta-CTX, and NTX over 10 years in men and women. In men, in general, levels of serum total OC significantly decreased ($P < 0.05$) and those of beta-CTX significantly increased ($P < 0.05$), but no significant difference was identified in the rate of change for BTMs among any age strata. Serum levels of total OC, beta-CTX, and NTX for women in their forties were significantly lower than those of women in their fifties to seventies ($P < 0.05$) and change rates over 10 years for women in their forties were significantly higher ($P < 0.05$).

Rates of change of BTMs and BMDs over 10 years were compared by menstrual status over 10 years (Table 4). Among 94 female subjects in their forties and fifties, 52 women (55.3%) were premenopausal at baseline. During

Table 3 Mean values at baseline and annual change rate (%/year) of biochemical markers of bone turnover (BTMs) over 10 years, classified by age and gender

Birth cohort	Age group (years)	Total OC (ng/ml)		Beta-CTX (ng/ml)		NTX (nmol BCE/l)	
		Baseline	Change rate (%/year)	Baseline	Change rate (%/year)	Baseline	Change rate (%/year)
Men							
1943–1952	40–49	18.8 (7.5)	−1.54 (4.80)*	0.190 (0.107)	1.66 (8.00)	13.3 (2.8)	−0.04 (3.44)
1933–1942	50–59	19.7 (18.3)	−1.21 (2.41)*	0.197 (0.162)	4.48 (17.33)	14.0 (6.0)	−0.04 (3.19)
1923–1932	60–69	16.4 (6.1)	−0.20 (3.93)	0.174 (0.107)	4.82 (10.72)*	13.6 (4.1)	0.53 (3.32)
1913–1922	70–79	18.9 (8.1)	−0.95 (3.45)	0.187 (0.099)	13.35 (49.33)	13.5 (3.4)	1.88 (3.76)*
1913–1952	40–79	18.5 (11.1)	−1.00 (3.74)*	0.187 (0.121)	5.10 (22.48)*	13.6 (4.2)	0.40 (3.41)
Women							
1943–1952	40–49	14.9 (5.7)	4.24 (6.94)*	0.103 (0.066)	16.84 (20.55)*	11.6 (2.3)	2.63 (3.29)*
1933–1942	50–59	28.1 (8.8)[a]	−1.08 (3.83)[a]	0.255 (0.121)[a]	1.35 (8.77)[a]	15.4 (3.3)[a]	−0.23 (3.59)[a]
1923–1032	60–69	32.6 (12.4)[a]	−2.06 (2.99)*,[a]	0.301 (0.136)[a]	1.28 (8.71)[a]	17.8 (4.4)[a,b]	−0.25 (2.23)[a]
1913–1022	70–79	28.3 (10.3)[a]	−1.73 (3.21)*,[a]	0.275 (0.153)[a]	1.13 (6.77)[a]	16.0 (3.2)[a]	0.11 (2.66)[a]
1913–1052	40–79	26.0 (11.6)	0.02 (5.32)	0.234 (0.145)	5.53 (14.54)*	15.2 (4.0)	0.62 (3.26)*

Values are given as mean with standard deviation in parentheses

BTMs, biochemical markers of bone turnover; OC, osteocalcin; beta-CTX, beta-isomerized C-terminal cross-linking telopeptide of type I collagen; NTX, N-terminal cross-linking telopeptide of type I collagen; BCE, bone collagen equivalents

* Significantly different ($P < 0.05$) for changes over 10 years

[a] Significantly different ($P < 0.05$) from values of subjects in their forties

[b] Significantly different ($P < 0.05$) from values of subjects in their fifties

Table 4 Mean values at baseline and annual change rate of BTMs over 10 years, classified by menstrual status in women

Menstrual status over 10 years	*n*	Age (years)	Total OC (ng/ml)		Beta-CTX (ng/ml)		NTX (nmol BCE/l)	
			Baseline	Change rate (%/year)	Baseline	Change rate (%/year)	Baseline	Change rate (%/year)
Premenopause	12	41.3 (1.3)	13.7 (5.3)	0.96 (7.53)	0.101 (0.059)	9.63 (24.49)	11.8 (2.5)	0.83 (3.13)
Transition to menopause	40	46.3 (3.6)	16.7 (7.3)[b]	4.40 (6.49)*,[b]	0.120 (0.090)[b]	15.16 (18.21)*,[b]	11.7 (2.3)[b]	2.76 (3.30)*,[b]
Postmenopause	117	63.9 (7.9)	29.5 (9.6)[a]	−1.57 (3.50)*	0.281 (0.136)[a]	1.86 (9.69)*	16.6 (3.7)[a]	−0.13 (2.94)
Total	169	58.1 (11.1)	25.4 (10.9)	0.02 (5.32)	0.231 (0.144)	5.53 (14.54)*	15.1 (4.1)	0.62 (3.26)*

Values are given as mean with standard deviation in parentheses

BMD, bone mineral density; BTMs, biochemical markers of bone turnover; *n*, number of subjects; OC, osteocalcin; beta-CTX, beta-isomerized C-terminal cross-linking telopeptide of type I collagen; NTX, N-terminal cross-linking telopeptide of type I collagen; BCE, bone collagen equivalents

* Significantly different ($P < 0.05$) for changes over 10 years

[a] Significantly different ($P < 0.001$) from values of participants in the premenopausal group

[b] Significantly different ($P < 0.001$) from values of participants in the postmenopausal group

the 10-year observation, 12 (12.8%) remained premenopausal, but 40 (42.6%) progressed into menopause. Table 4 shows that rates of change for all BTMs over the 10 years were significantly increased in the group with transition into menopause ($P < 0.05$). Change rates were significantly higher in women with transition into menopause compared to postmenopausal women ($P < 0.001$).

Association between changes in BTMs and changes in BMD over 10 years

Associations between changes in BTMs and changes in BMD were analyzed. Correlation coefficients of changes to L2–L4 BMD and changes to OC, beta-CTX, and NTX were –0.12 ($P = 0.16$), 0.04 ($P = 0.66$), and –0.08 ($P = 0.35$),

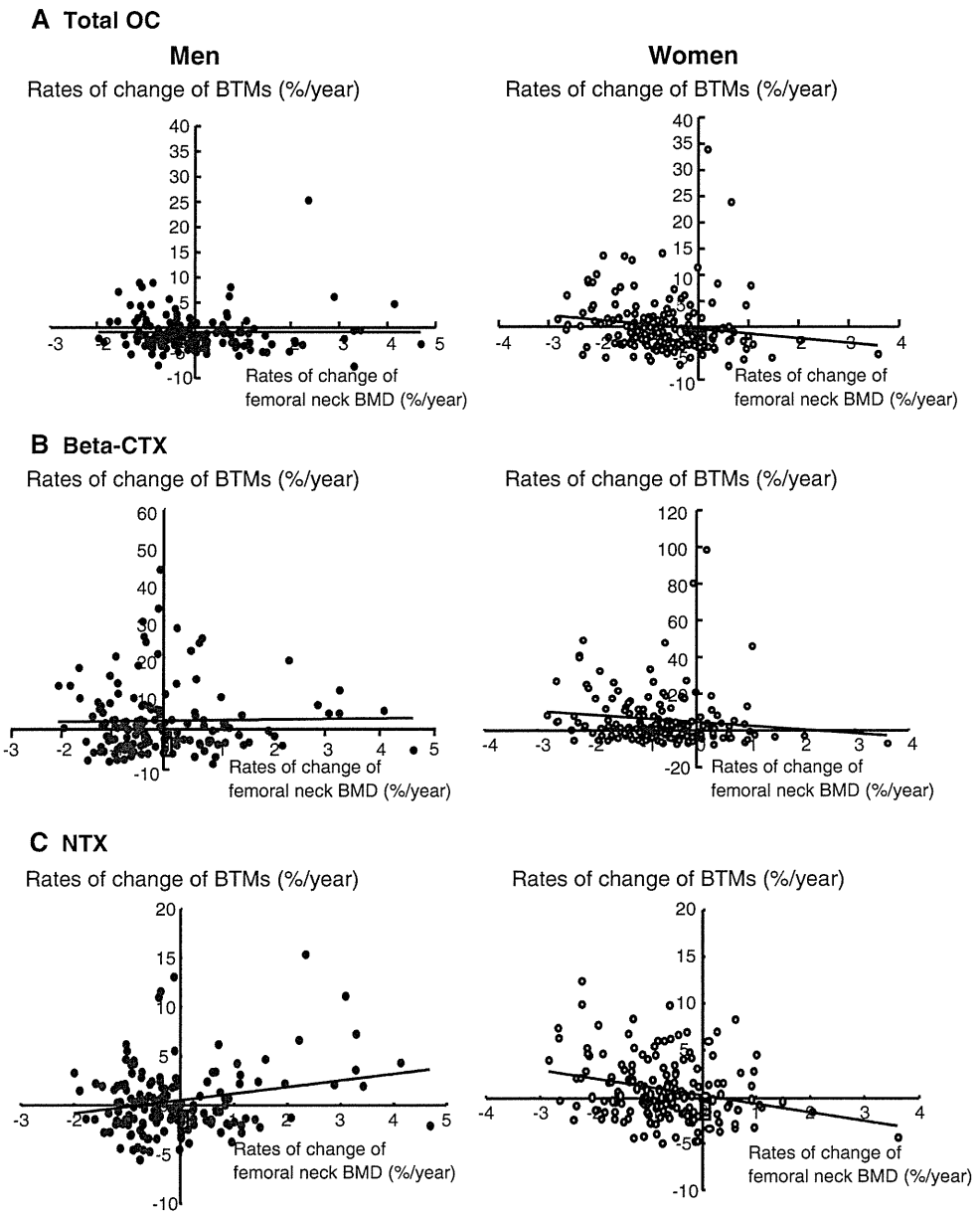

Fig. 1 Association between rates of change of biochemical markers of bone turnover and rates of change of bone mineral densities at the femoral neck. *OC*, total osteocalcin (**a**); *BTM*, biochemical markers of bone turnover; *beta-CTX*, beta-isomerized C-terminal cross-linking telopeptide of type I collagen (**b**); *NTX*, N-terminal cross-linking telopeptide of type I collagen (**c**)

respectively, in men, and –0.20 ($P = 0.01$), –0.16 ($P = 0.04$), and –0.29 ($P = 0.0002$), respectively, in women. Correlation coefficients of changes to femoral neck BMD and changes to OC, beta-CTX, and NTX were –0.003 ($P = 0.98$), 0.19 ($P = 0.02$), and 0.23 ($P = 0.004$), respectively, in men, and –0.15 ($P = 0.04$), –0.13 ($P = 0.09$), and –0.27 ($P = 0.0005$), respectively, in women. Correlation coefficients of changes to total hip BMD and changes to OC, beta-CTX, and NTX were –0.16 ($P = 0.05$), –0.07 ($P = 0.39$), and –0.02 ($P = 0.86$), respectively, in men and –0.19 ($P = 0.01$), –0.12 ($P = 0.12$), and –0.28 ($P = 0.0002$), respectively, in women. These findings indicate that increased BMD at the femoral neck in men correlated significantly with increased serum levels of beta-CTX and NTX. By contrast, decreased BMD at all sites (that is, L2–L4, femoral neck, and total hip) in women was significantly related to increased serum levels of BTMs. Figure 1 shows scatter plots for changes in total OC, beta-CTX, and NTX and changes to BMD at the femoral neck in both men and women. At the femoral neck, the direction of association between changes of bone resorption markers and BMDs differed between men and women, although the direction of association of changes to BTMs and BMD in both men and women were similar at L2–L4 and total hip.

To clarify associations between changes in BTM and BMD after adjusting for confounders, multivariate regression analysis was performed. Regarding the change of values of BMD at L2–L4, multivariate regression analysis

Table 5 Standardized partial regression coefficient (β) of changes of BTMs for annual change rate for BMD

BTMs	L2–L4 BMD		Femoral neck BMD		Total hip BMD	
	β	P	β	P	β	P
Men						
Total OC	−0.12	0.139	0.06	0.455	−0.16	0.056
Beta-CTX	0.03	0.747	−0.04	0.632	0.11	0.166
NTX	−0.08	0.323	0.01	0.875	−0.01	0.938
Women						
Total OC	−0.18	0.024	−0.16	0.068	−0.31	<0.001
Beta-CTX	−0.09	0.269	−0.06	0.457	−0.18	0.027
NTX	−0.21	0.006	−0.06	0.495	−0.34	<0.001

Standardized partial regression coefficients were obtained after adjustment for age and body mass index

BMD, bone mineral density; BTMs, biochemical markers of bone turnover; *n*, number of subjects; OC, osteocalcin; beta-CTX, beta-isomerized C-terminal cross-linking telopeptide of type I collagen; NTX, N-terminal cross-linking telopeptide of type I collagen

was performed using change rates of L2–L4 BMD as an objective factor and change rates of each BTM as an explanatory factor after controlling for age, body mass index (BMI), occurrence of clinical vertebral fractures over 10 years in both men and women, and menstrual status over 10 years (0, premenopausal; 1, transition to menopause; 2, menopausal) in women. Furthermore, with regard to the proximal hip, including the femoral neck and total hip, multivariate regression analysis was performed after controlling for age and BMI in both men and women and menstrual status over 10 years in women. Table 5 shows the standardized partial regression coefficient of change rates of BTMs for annual change rates for BMD. For men, there was no significant association of changes of BTMs and changes of BMDs at any of the sites. By contrast, for women, although no significant association was seen between changes of BMD at the femoral neck and changes in BTM, change rates of total OC and NTX were significantly associated with change rates of L2–L4 BMD, and change rates of total OC, beta-CTX, and NTX were significantly associated with change rates of BMD at the total hip (Table 5).

Comparison of mean BTM levels in given age strata classified by birth cohort

The BTM levels of subjects in their fifties, sixties, and seventies in 1993 were compared to those in their fifties, sixties, and seventies in 2003 (Fig. 2). No significant differences in mean values of BTMs were identified in the same age strata or in different birth cohorts in men. By contrast, the BTM levels of female subjects in 1993 tended

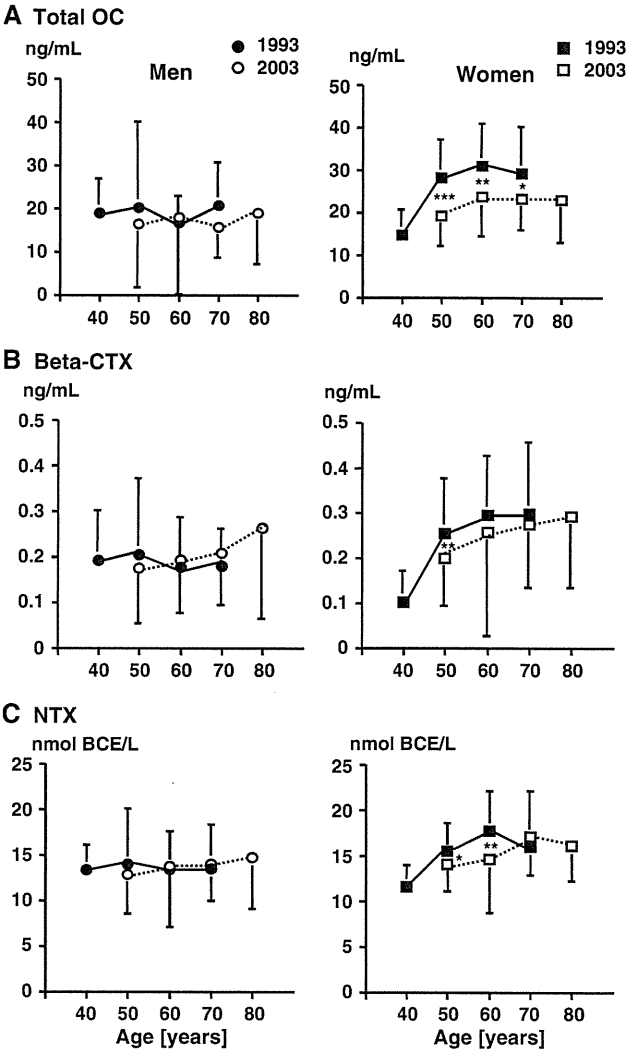

Fig. 2 Changes in serum biochemical markers of bone turnover over 10 years, classified by age strata. **a** Total osteocalcin (*OC*). **b** Beta-isomerized C-terminal cross-linking telopeptide of type I collagen (*beta-CTX*). **c** N-terminal cross-linking telopeptide of type I collagen (*NTX*). *BCE*, bone collagen equivalents. Significantly different from values of participants in the same age strata between different birth-cohorts in 1993 and 2003 (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$)

to be higher than those in 2003 for the same age strata (Fig. 2). This result suggests an effect of birth cohort for serum levels of BTMs in women, particularly those in their fifties, but not in men. That is, BTM levels were significantly lower for women in their fifties in 2003 compared to those in their fifties in 1993.

Discussion

In this 10-year follow-up study, we clarified changes to levels of BTMs in men and women from a rural community

in Japan. Change rates of BTMs over 10 years were influenced by menstrual transition, age, and sex. Increases in both bone formation and bone resorption markers are associated with decreases in BMD at L2–L4 and the total hip in women after controlling for confounding factors. In terms of birth-cohort effect, values of BTMs for participants in 2003 were significantly lower than those in 1993 when compared between the same age strata in women.

We have already reported the age–sex distribution of values of BTMs, such as intact OC, alkaline phosphatase, C-terminal propeptide of type I procollagen, C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase, urinary pyridinoline cross-links of collagen, and deoxypyridinoline cross-links of collagen using the same population as the present study [11]. That report showed that levels of all the aforementioned BTMs were significantly lower in the 40–49 age group than in each of the 50–59, 60–69, and 70–79 age groups in women, whereas no significant differences were apparent among age groups in men [11]. Following the previous study, we clarified changes of BTMs in each age group in the present study, with values of BTMs starting to increase in women in their forties, then stabilizing (beta-CTX, NTX) or mildly decreasing (total OC) among older age groups. The rate of decrease of BTMs was greatest in the menopausal transient group compared to the groups remaining premenopausal or postmenopausal. Although the number of subjects in each category of menstrual status was limited, these results suggest that the onset of menopause in their forties causes dramatic changes in bone metabolism in women. With regard to estrogen and changes of BTMs, Ebeling et al. [21] and Sowers et al. [22] reported that levels of BTMs increased before menopause as a consequence of declining concentrations of serum estradiol (E_2) and increasing concentrations of follicle-stimulating hormone. We have already reported that serum levels of total E_2 were associated with decreased BMD over 3 years among premenopausal women [23].

In terms of the effects of BTM changes on changes in BMD over 10 years, the present study revealed that increases in BTMs over 10 years in women, even for bone formation markers or bone resorption markers, are associated with decreased BMD at L2–L4 and total hip. This association remains after controlling for confounding factors. No previous reports appear to have clarified associations between changes in levels of BTMs and bone loss for one decade. The present study revealed that a higher rise in values of BTMs, particularly total OC and NTX, was associated with faster BMD loss in women. These associations were observed over a reasonably long time period. However, these findings were identified at L2–L4 and total hip, but not at the femoral neck. Although reasons for site-specific differences in the association between BTMs and BMD remain uncertain, we have already reported that bone loss rate differs depending on the site involved in another cohort study [13]. We have also reported that characteristics differ between fast bone loss at the lumbar spine and femoral neck [24]. One reason for these site-specific differences might be that fixing the position for BMD examination using DXA was more difficult for the femoral neck than for L2–L4 or total hip, and as a result, the CV tended to be higher there than at other sites [17]. Changes that increase BMD, such as osteophytosis or sclerotic changes, are also observed most frequently at the lumbar spine, which might be another reason for the site-specific differences. We were unable to perform X-ray examinations of participants in the present study. We thus could not control the influence of degenerative changes and fractures on lumbar L2–L4 BMD. Regarding fractures, we analyzed past clinical vertebral fractures as a confounder, but this was not sufficient. However, these changes seem to increase the BMD, so our results in terms of changes to BMD in the present study may be overestimated. Considering the CV and effect of degenerative changes, measurement for the total hip might be the proper site for observation of BMD change over the long term.

The present study also found evidence of differences in BTM values for a given age stratum between different birth cohorts in women. Data on levels of BTMs in 1993 and 2003 showed that accelerated bone remodeling seemed to improve for women in their fifties to seventies in younger cohorts. However, those results were affected by potential confounders such as differences in age, anthropometric measurements, and menstrual status. We then compared the aforementioned factors between women in their fifties to seventies in 1993 and in 2003. Mean age (SD) for groups in 1993 and 2003 was 65.0 (8.1) years and 64.6 (8.9) years, and mean BMI (SD) in 1993 and 2003 was 23.2 (3.8) kg/m^2 and 23.5 (3.9) kg/m^2. No significant differences were identified between birth cohorts. The proportion of women in menopause in their fifties to seventies was 94.7% in 1993 and 91.3% in 2003. No significant difference was seen between birth cohorts ($P = 0.26$). Even if analysis was focused on women in their fifties, no significant differences were apparent ($P = 0.25$). Although other confounders resulting from differences in generation might have influenced the cohort effect, we conclude that a birth-cohort effect was seen on bone metabolism in middle-aged and elderly women in the present cohort. Our results are consistent with findings we have reported elsewhere that community-dwelling inhabitants in later birth cohorts show higher BMD in middle age, using another cohort established in a mountainous area [13]. The results are also consistent with the findings of Fujiwara et al. [25], who assessed the effects of birth cohort on the incidence of vertebral fracture in Hiroshima and found that incidence

decreased with successive birth decades. Thus, given all these findings, levels of BTMs appear significantly lower, levels of BMD appear significantly higher, and the incidence of vertebral fractures is lower in women from younger birth cohorts in Japan compared to those from older birth cohorts. These results suggest that the problem of osteoporosis might be less severe than has previously been predicted for the future in Japan.

The present study shows several limitations. The primary weakness involved the methods of sample collection. First, not all samples of participants were extracted at a fixed time (e.g., morning) under fixed conditions (e.g., fasting). Samples in this study were extracted between 0900 and 1500, rather than at a fixed time. Circadian variability is known to affect BTM levels [4]. Hannon and Eastell [26] reviewed the circadian variability of BTMs, noting that serum levels of OC peaked between 0200 and 0400 and reached a nadir between 1200 and 1600, whereas serum CTX levels peaked between 0130 and 0430, reaching a nadir between 1100 and 1400. We could not find any reports on circadian rhythms for serum NTX, but Delmas et al. [4] stated that most BTM levels increased at night, peaked between 0200 and 0800, then decreased rapidly to a nadir between 1300 and 2300. Based on these reports, the timing of sample collection was based on when BTM levels were supposed to be reaching a nadir. The present results might thus have underestimated levels of BTMs compared to collection at a fixed time in the morning. Although adjustment for the time after eating is important, particularly for measurements of serum CTX, we could not collect samples under absolutely controlled conditions. Delmas et al. [4] reported that fasting diminishes the rhythm of serum CTX-I, particularly with regard to the rapid decrease in the morning. Because we could not control the timing for collecting blood samples and fasting, we might not have accurately evaluated interindividual changes in BTMs. However, all participants in examinations in both 1993 and 2003 were allocated randomly to a specific sampling time and the allocated time was associated with eating behaviors. Random noise resulting from variability in sampling time and eating status might thus have occurred with relatively equal probability in both 1993 and 2003. Comparison of BTM levels between cohorts, rather than individuals, in 1993 and 2003 thus appears valid.

Second, long-term storage might have influenced BTM levels. In this study, serum samples were immediately placed in dry ice and transferred within 24 h to a deep freezer kept at −80°C. BTMs in the present study were measured utilizing baseline samples after 7 years, given that methods to identify these BTMs were unavailable in 1993. Storage for 7 years might therefore have influenced BTM levels, even at −80°C. No data are available regarding the influence of such long-term storage, although Seibel [27] stated that BTMs in sera would be stable with a storage temperature of −70°C. Hannon and Eastell [26] reported that long-term CVs for OC, serum NTX-I, and serum CTX-I were 27.3% at 9 months, 24.0% at 3 years, and 13.1% at 1 year. The CV for 7-year storage might well be higher than these results. If so, levels of BTMs collected in 1993 and measured in 2000 would have been systematically greater than those obtained in the present study, underestimating differences between 1993 and 2003. Changes over 10 years would thus have been greater and the effects of birth cohort even more pronounced.

Another limitation involves withdrawal bias. Although we completed the 10-year follow-up with a high participation rate, 80.4%, the dropout rate among men in their seventies was rather high (54.0%). This high dropout rate might have resulted in a withdrawal bias, meaning that healthier survivors would have skewed the results of long-term observation. Increases in femoral neck BMD might have been skewed by any such withdrawal bias. However, the main reasons for dropout among men in their seventies were death (52%) and illness (31%), which seem unavoidable. We think that this represents an inherent limitation of all longitudinal follow-up studies. The possibility of withdrawal bias should be considered when interpreting the data.

In conclusion, the present study found that change rates of BTMs were higher for women in their forties than for women in their fifties to seventies ($P < 0.05$) and were higher in the menstrual transition group than in the pre- and postmenopausal groups ($P < 0.001$). Changes in BTMs during the 10 years showed significant associations between bone loss at L2–L4 and total hip in women, after adjusting for confounders. Levels of all BTMs in women in their fifties were significantly lower than in younger birth cohorts.

Acknowledgments This work was supported by the following Grants-in-Aid for Scientific Research: B20390182 (Noriko Yoshimura), C20591737 (Toru Akune), C20591774 (Shigeyuki Muraki), Young Scientists A18689031 (Hiroyuki Oka), and Collaborating Research with NSF 08033011-00262 (Director, Noriko Yoshimura) from the Ministry of Education, Culture, Sports, Science and Technology; H17-Men-eki-009 (Director, Kozo Nakamura), H18-Choujyu-037 (Director, Toshitaka Nakamura), and H20-Choujyu-009 (Director, Noriko Yoshimura) from the Ministry of Health, Labour and Welfare in Japan. This study was also supported by grants from the Japan Osteoporosis Society (Noriko Yoshimura), Nakatomi Foundation (Noriko Yoshimura), and research aid from the Japanese Orthopaedic Association (JOA-Subsidized Science Project Research 2006-1, Director, Hiroshi Kawaguchi). The sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of this report. The authors wish to thank Mrs. Tamako Tsutsumi, Mrs. Kanami Maeda, and other members of the Public Office in Taiji Town and members of the Public Health Center in Shingu City for their assistance in locating and scheduling participants for examinations.