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 ($P = 0.35$),

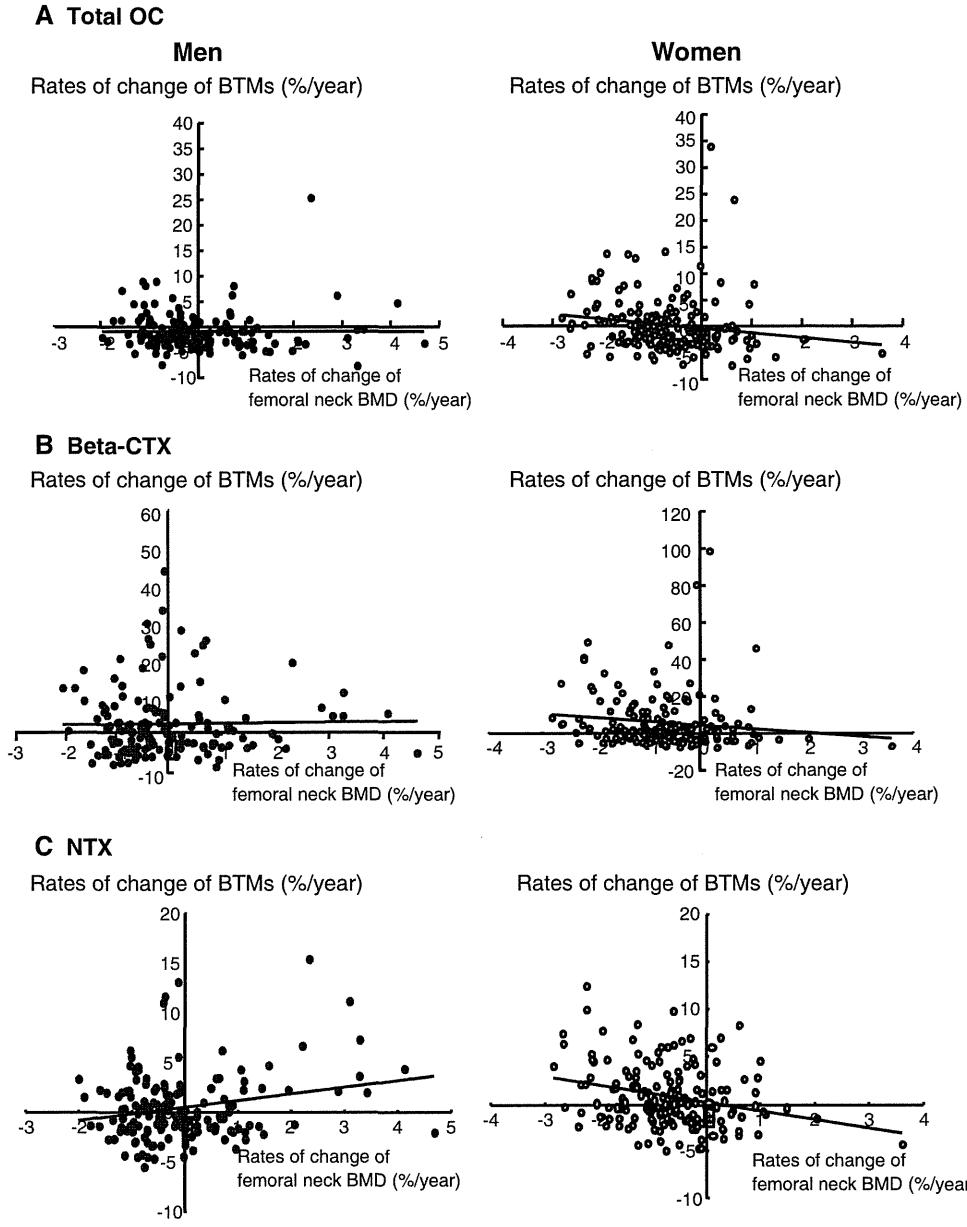

Fig. 1 Association between rates of change of biochemical markers of bone turnover and rates of change of bone mineral densities at the femoral neck. *OC*, total osteocalcin (**a**); *BTM*, biochemical markers of bone turnover; *beta-CTX*, beta-isomerized C-terminal cross-linking telopeptide of type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

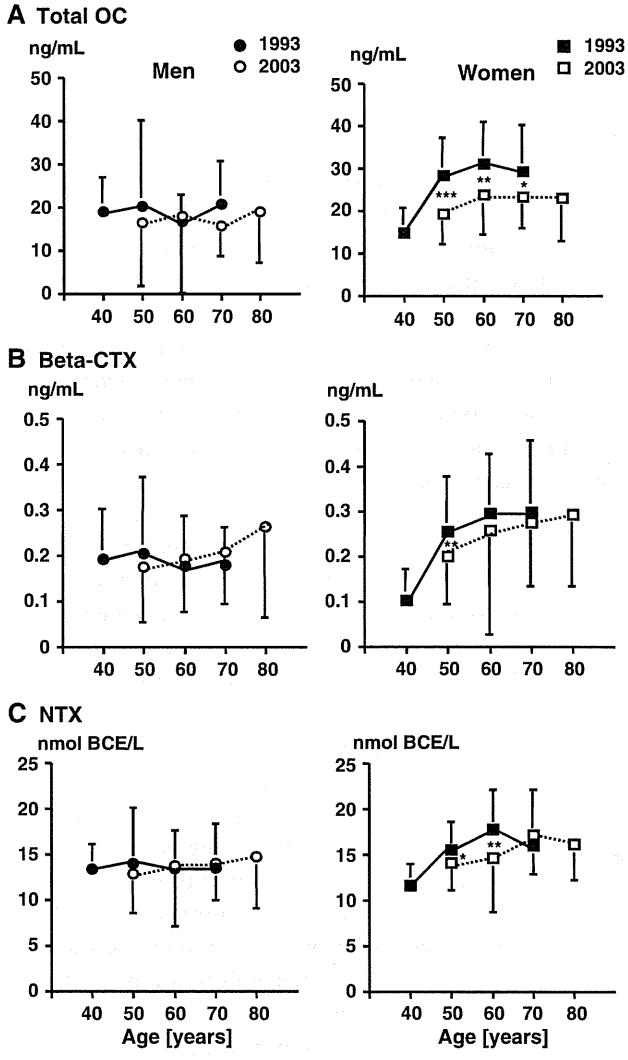

Fig. 2 Changes in serum biochemical markers of bone turnover over 10 years, classified by age strata. **a** Total osteocalcin (*OC*). **b** Beta-isomerized C-terminal cross-linking telopeptide of type I collagen (*beta-CTX*). **c** N-terminal cross-linking telopeptide of type I collagen (*NTX*). *BCE*, 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.

Conflict of interest The authors have no conflicts or disclosures to declare regarding the present manuscript.

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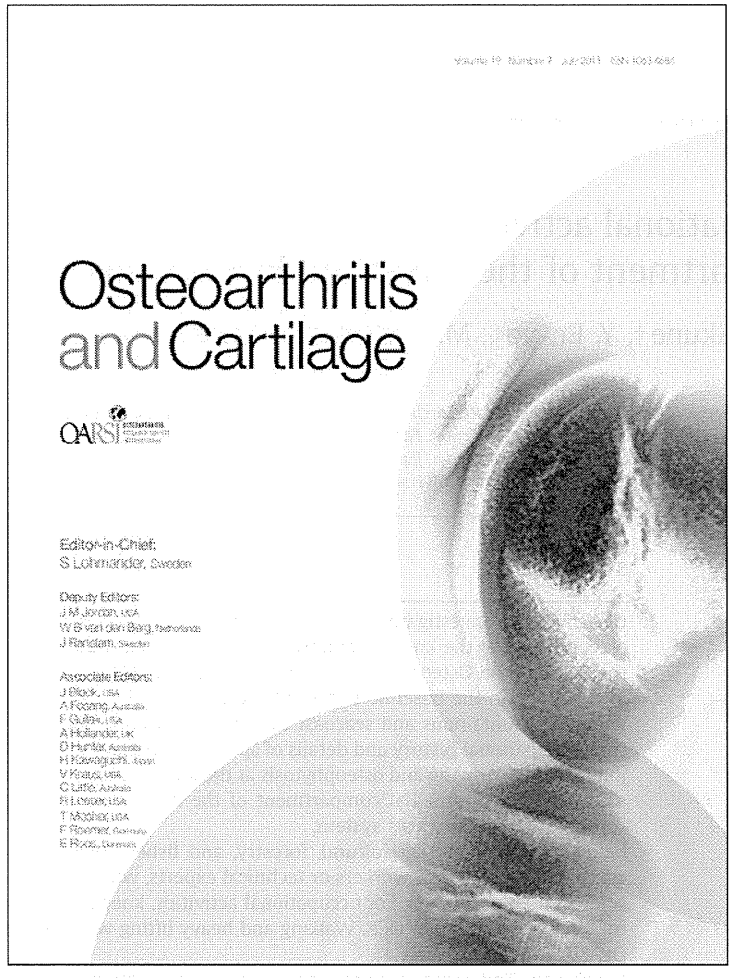

Osteoarthritis and Cartilage 19 (2011) 840–846

Osteoarthritis and Cartilage

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Association of occupational activity with joint space narrowing and osteophytosis in the medial compartment of the knee: the ROAD study (*OAC5914R2*)

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ARTICLE INFO

Article history:
Received 7 September 2010
Accepted 22 March 2011

Keywords:
Osteoarthritis
Knee
Occupation
Cohort
Epidemiology

SUMMARY

Objective: We investigated the association of occupational activity with joint space narrowing and osteophytosis at the knee separately in Japanese subjects using a large-scale population-based cohort of the Research on Osteoarthritis Against Disability (ROAD).

Methods: From the baseline survey of the ROAD study, 1,402 participants (512 men and 890 women) living in mountainous and seacoast communities were analyzed. Information collected included a lifetime occupational history and details of specific workplace physical activities. To estimate the severity of joint space narrowing and osteophytosis at the knee, minimum joint space width (mJSW) and osteophyte area (OPA) in the medial compartment of the knee were measured using a knee osteoarthritis (OA) computer-aided diagnosis system.

Results: For women, agricultural, forestry, and fishery workers had significantly lower mJSW values compared with clerical workers or technical experts, whereas OPA did not differ significantly among job titles in men or women. For occupational activities, kneeling and squatting were associated with lower mJSW as well as higher OPA. Walking and heavy lifting were associated with lower mJSW, but not with OPA.

Conclusion: This cross-sectional study using a population-based cohort suggests that an occupational activity that includes kneeling and squatting appears to have a greater effect on knee OA.

Introduction

Knee osteoarthritis (OA), which causes cartilage degeneration and osteophyte formation at joints in the limbs, is a major public health issue causing chronic disability in the elderly in developed countries[1–3]. The prevalence of knee OA is high in the elderly in Japan[4] and 25,300,000 subjects aged 40 years and older are estimated to experience radiographic knee OA[5]. Further, according to the recent National Livelihood Survey of the Ministry of Health, Labour and Welfare in Japan, OA is ranked fourth among diseases that cause disabilities that subsequently require support with regard to activities of daily living[6].

Established risk factors for knee OA in Caucasians include older age, female sex, evidence of OA in other joints, obesity, and previous injury or surgery of the knee[7–11]. Evidence is accumulating in Caucasians that the disease is more common in people who have performed heavy physical work[12–17], particularly in those whose jobs have involved kneeling or squatting[18–24]. We also showed that occupational activities that included sitting, standing, walking, climbing, and heavy lifting had a significant association with moderate knee OA, and kneeling and squatting were associated with severe knee OA[25]. However, in our and other studies regarding occupational risks for knee OA, the disease was defined according to the Kellgren–Lawrence (KL) grade[26] or whether subjects had undergone total knee arthroplasty. KL grade is the most conventional system to grade radiographic severity of knee OA, but in this categorical system, joint space narrowing and osteophyte formation are not assessed separately. In addition, because the KL system emphasizes osteophytosis, it is unclear how to handle knee OA with joint space narrowing but no osteophytosis. Further, we have already reported that occupational activities of

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doi:10.1016/j.joca.2011.03.008

kneeling and squatting were significantly associated with KL ≥3 knee OA, but not with KL ≥2 knee OA[25]. Considering the definition of the KL grade[26], this difference may suggest distinct risk factors between osteophytosis and joint space narrowing. However, we cannot clarify whether osteophytosis and joint space narrowing have distinct risk factors, because osteophytosis and joint space narrowing are not separately defined according to the KL grade. In addition, a recent cross-sectional study has shown that osteophytosis was unrelated not only to joint space narrowing on plain radiographs, but also to cartilage loss measured by quantitative magnetic resonance imaging[27]. Furthermore, our study on an experimental mouse model for OA has identified a cartilage-specific molecule, carminerin, that regulates osteophytosis without affecting joint cartilage destruction during OA progression[28,29]. This accumulating evidence has indicated that joint space narrowing and osteophytosis may have distinct etiologic mechanisms and their progression may be neither constant nor proportional. Thus, to examine factors associated with knee OA, these two OA features should be assessed separately. However, to the best of our knowledge, there are no large population-based studies that investigate occupational factors associated with joint space narrowing and osteophyte formation separately.

In the present study, we measured medial minimum joint space width (mJSW) and osteophyte area (OPA) in the large-scale population-based cohort study called the Research on Osteoarthritis Against Disability (ROAD). The purpose of the present study was to investigate the association of job title and occupational activity with joint space narrowing and osteophytosis at the knee separately, and to clarify which kinds of occupational activities were associated with joint space narrowing and osteophytosis. Furthermore, we aimed to clarify whether the association of each occupational activity with joint space width and OPA was different.

Subjects and methods

Subjects

The ROAD study is a nationwide prospective study to establish epidemiologic indexes for evaluation of clinical evidence for the development of a disease-modifying treatment for bone and joint diseases (with OA and osteoporosis as the representative bone and joint diseases) consisting of population-based cohorts in several communities in Japan. As a detailed profile of the ROAD study has been described in detail elsewhere[4,5,30,31], only a brief summary is provided here. To date, we have completed creation of a baseline database including clinical and genetic information on 3,040 inhabitants (1,061 men and 1,979 women) ranging in age from 23 to 95 years (mean, 70.6 years) who were recruited from listings of resident registrations in three communities: an urban region in Itabashi, Tokyo; a mountainous region in Hidakagawa, Wakayama; and a seacoast region in Taiji, Wakayama. All participants provided written informed consent, and the study was conducted with the approval of ethics committees of the University of Tokyo and the Tokyo Metropolitan Institute of Gerontology. Information collected about job title and occupational activity included a lifetime occupational history with details of seven types of specific workplace physical activities: sitting on a chair, kneeling, squatting, standing, walking, climbing, and heavy lifting. Participants were asked whether they engaged in the following activities: sitting on a chair for ≥2 h/day, kneeling for ≥1 h/day, squatting for ≥1 h/day, standing for ≥2 h/day, walking for ≥3 km/day, climbing up slopes or steps for ≥1 h/day, and lifting loads weighing ≥10 kg ≥1 time/week. Information on these activities was obtained for the principal job, defined as the job at which the participant had worked longest. These definitions were chosen to be similar to definitions used in previous studies of occupations and OA[22,23,25]. Anthropometric measurements included height and weight, and body mass index (BMI; weight [kg]/height^2 [m^2]) was calculated. From baseline data of all participants, the present study analyzed 1,402 participants (512 men and 890 women) aged ≥50 years living in mountainous and seacoast cohorts, after excluding 69 subjects with lateral knee OA.

Radiographic assessment

All participants had radiographic examination of both knees using an anterior–posterior view with weight-bearing and foot map positioning. The beam was positioned parallel to the floor with no angle and aimed at the joint space. To visualize the joint space properly and to make the patella centralized over the lower end of the femur, we used fluoroscopic guidance with an anterior–posterior X-ray beam. The images were downloaded into Digital Imaging and Communication in Medicine (DICOM) format files. mJSW in the medial compartment and OPA at the medial tibia were measured by the KOACAD (knee osteoarthritis computer-aided diagnosis) system, and a knee with the lower mJSW was defined as the designated knee of a participant. The KOACAD system has been described in detail elsewhere[32], and is summarized here only briefly. The KOACAD 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 in general clinical practice. This system was programmed to measure mJSW in the medial and lateral compartments and OPA at the medial tibia using digitized knee radiographs. Initially, correction for radiographic magnification was performed based on the image size of a rectangular metal plate. Next, to determine the region of interest (ROI), the center of the tibiofemoral joint was determined as follows. A vertical neighborhood difference filter, that vertically scanned digital images to detect the margins of the tibial and femoral condyles, was applied to identify points with high absolute values for difference of scale, and then the center of all points was calculated, that was defined as the center of the tibiofemoral joint, and a 480 × 200 pixels of rectangle with the center was decided as the ROI (Supplementary Figure). Within the ROI, the outline of the femoral condyle was designated as the upper rim of the joint space by vertical filtering with the 3 × 3 square neighborhood difference filter. The both ends of the upper rim were determined using a Canny's filter to remove the noise associated with lines, and vertical lines from the ends were designated as the outside rims of the joint space. Outlines of anterior and posterior margins of the tibial plateau were drawn similarly to that of the femoral condyle, and the middle line between the two outlines was designated as the lower rim of the joint space [Fig. 1(A)]. A straight regression line for the lower rim outline was then drawn, and the intersection of the lower rim outline and the regression line was designated as the inside rim. Medial and lateral joint space areas were determined as areas surrounded by the upper, lower, inside, and outside rims as defined above. Medial and lateral mJSWs were further determined as the minimum vertical distances in the respective joint space area [Fig. 1(B)]. To measure the OPA, 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 OPA [Fig. 1(C)]. We have previously published reference values of joint space width and OPA by gender and age strata in Japan using the KOACAD system[33].

Statistical analysis

The differences of age, height, weight, BMI, mJSW, and OPA at the designated knee between men and women were examined by

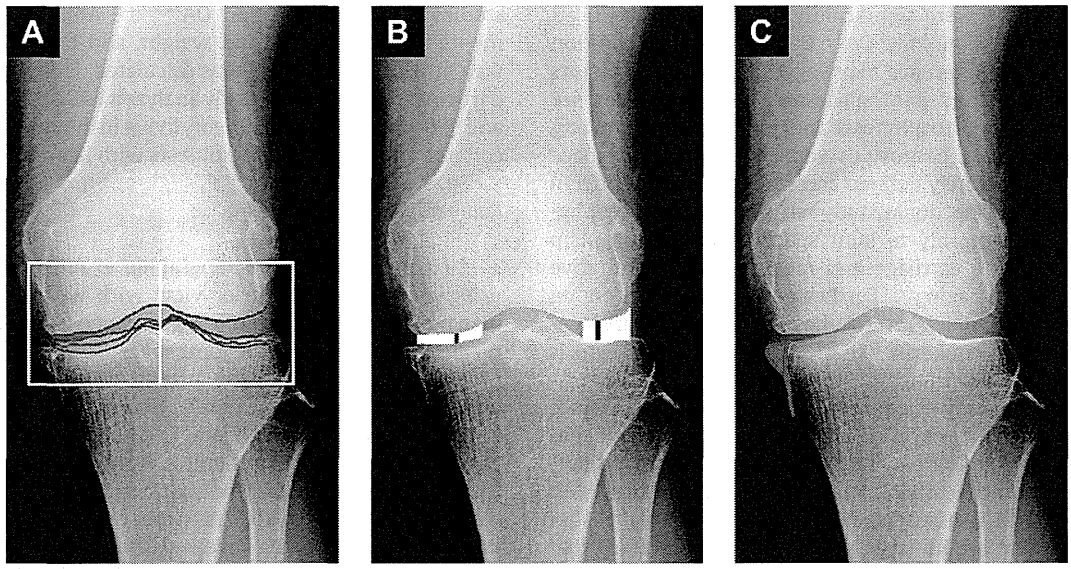

Fig. 1. Schema of image processing by KOACAD (cited from reference number[9]). (A). Outlines of anterior and posterior margins of the tibial plateau. The middle line between the two outlines is defined as the lower rim of the joint space. (B). Medial and lateral mJSWs were defined as the minimum vertical distances in the joint space area. (C). OPA (red area) that is medially prominent over the smoothly extended outline of the tibia.

the non-paired Student's *t*-test. The percentage of each occupational activity was compared between men and women by chi-square test. To determine the association of job title with mJSW and OPA, the Tukey Honestly Significant Differences (HSD) test was used after adjustment for age, gender, and BMI in the overall population and after adjustment for age and BMI in men and women. To determine the association of mJSW and OPA with each occupational activity separately after adjustment for age, gender, and BMI in the overall population, multiple regression analyses were used with age, gender, BMI, and each occupational activity as independent variables in the overall populations. Further, to determine the association of mJSW and OPA with each occupational activity separately after adjustment for age and BMI in men and women, multiple regression analyses were used with age, BMI, and each occupational activity as independent variables. Next, to determine the independent association of occupational activities with mJSW, multiple regression analysis was used with age, gender, BMI, and all significantly associated occupational activities in the overall subjects, and with age, BMI and all significantly associated occupational activities in men and women, as explanatory variables, statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC).

Results

Characteristics of the 1,402 participants aged ≥50 in the mountainous and seacoast cohorts of the ROAD study are shown in Table I. mJSW was significantly lower in women than in men, whereas OPA was significantly higher in women compared with men. OPA was moderately associated with mJSW ($R^2 = 0.21$, $P < 0.05$) by linear regression analysis. When we analyzed the association of height with mJSW, the R^2 was 0.027 and 0.076 in men and women, respectively ($P < 0.05$). With regards to OPA, the R^2 was 0.01 and 0.006 in men and women, respectively ($P < 0.05$).

There was great diversity in job titles of study participants (Table II). Although a substantial proportion included clerical workers and technical experts, there were many agricultural, forestry, and fishery workers. Among various occupational activities, agricultural, forestry, and fishery workers had the highest rates of kneeling, squatting, standing, walking, climbing, and lifting weights, and the lowest rates for sitting on a chair, whereas clerical workers and technical experts had the lowest rates for the former activities and the highest rates for the latter activity (Fig. 2).

Table I
Characteristics of participants

	Overall	Men	Women
Number of subjects	1,402	512	890
Age, years	68.2 ± 9.2	68.9 ± 9.1	67.7 ± 9.2*
Height, cm	154.4 ± 9.3	162.4 ± 6.9	149.9 ± 7.2*
Weight, kg	55.3 ± 10.5	61.0 ± 10.3	52.0 ± 9.1*
BMI, kg/m^2	23.1 ± 3.4	23.1 ± 3.1	23.1 ± 3.5
mJSW, mm	2.5 ± 1.1	2.9 ± 1.0	2.3 ± 1.1*
OPA, mm^2	3.0 ± 7.9	1.4 ± 4.4	3.9 ± 9.3*

Values are mean ± SD except where indicated.
mJSW, minimum joint space width.
* $P < 0.05$ vs men by non-paired *t* test.

Table II
Number (percentage) of participants with job title and occupational activity reported as the principal job

	Overall	Men	Women
Job titles, *n* (%)			
Clerical workers/technical experts	350 (25.0)	164 (32.0)	186 (20.9)
Agricultural/forestry/fishery workers	299 (21.3)	158 (30.9)	141 (15.8)
Factory/construction workers	148 (10.6)	67 (13.1)	81 (9.1)
Shop assistants/managers	124 (8.8)	24 (4.7)	100 (11.2)
Housekeepers	118 (8.4)	0 (0.0)	118 (13.3)
Teachers	80 (5.7)	40 (7.8)	40 (4.5)
Dressmakers	46 (3.3)	1 (0.2)	45 (5.1)
Clinical workers	40 (2.9)	1 (0.2)	39 (4.4)
Hairdressers	17 (1.2)	6 (1.2)	11 (1.2)
Others (cook, taxi driver, etc.)	70 (5.0)	21 (4.1)	49 (5.5)
No answer	110 (7.8)	30 (5.9)	80 (9.0)
Occupational activities, n (%)			
Sitting on a chair ≥2 h/day	629 (44.9)	247 (48.2)	382 (42.9)
Kneeling ≥1 h/day	280 (20.0)	92 (18.0)	188 (21.1)
Squatting ≥1 h/day	368 (26.2)	127 (24.8)	241 (27.1)
Standing ≥2 h/day	1,179 (84.0)	439 (85.7)	740 (83.1)
Walking ≥3 km/day	638 (45.5)	255 (49.8)	383 (43.0)
Climbing ≥1 h/day	325 (23.2)	175 (34.2)	150 (16.9)*
Lifting weights ≥10 kg ≥1 time/week	750 (53.5)	336 (65.6)	414 (46.5)*

* $P < 0.05$ vs men by chi-square test.

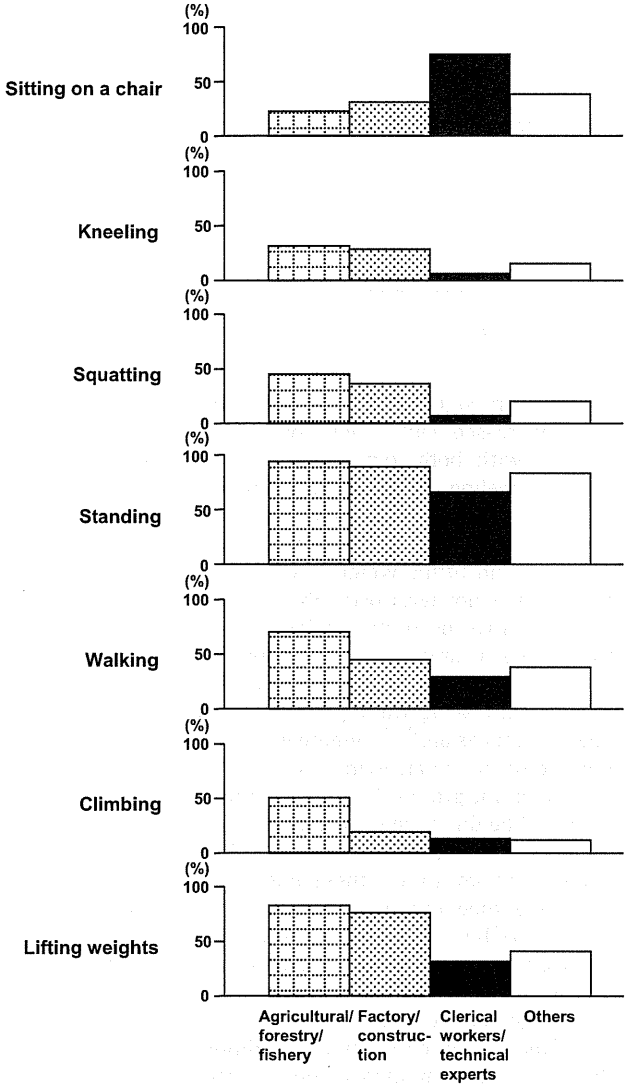

Fig. 2. Percentage of participants engaged in each occupational activity: sitting on a chair ≥2 h/day, kneeling ≥1 h/day, squatting ≥1 h/day, standing ≥2 h/day, walking ≥3 km/day, climbing ≥1 h/day, or lifting weights ≥10 kg ≥1 time/week among agricultural, forestry, and fishery workers; factory and construction workers; clerical workers and technical experts; and others.

Mean ± standard deviation (SD) values of mJSW (mm) in agricultural, forestry, or fishery workers; factory or construction workers; clerical workers or technical experts; and other workers were 2.4 ± 1.2, 2.5 ± 1.1, 2.8 ± 1.0, and 2.4 ± 1.1, respectively. Tukey HSD test after adjustment for age, gender, and BMI showed that there were no significant associations between job titles and mJSW. Further, because characteristics such as height, weight, mJSW and OPA differ significantly between men and women, we conducted separate analyses of the association of job title with mJSW and OPA in men and women. mJSW (mm) in agricultural, forestry, or fishery workers; factory or construction workers; clerical workers or technical experts; and other workers were 2.7 ± 1.1, 2.9 ± 0.9, 3.0 ± 0.9, and 2.9 ± 1.0 in men and 2.0 ± 1.1, 2.2 ± 1.1, 2.6 ± 0.9, and 2.3 ± 1.1 in women respectively. There were no associations in men, but for women, agricultural, forestry, or fishery workers had significantly lower mJSW than clerical workers or technical experts using Tukey HSD test without adjustment. To adjust for body size, we used Tukey HSD test after adjustment for height, and the results were similar ($P < 0.05$). Further, after adjustment for age and BMI, the results were also similar ($P < 0.05$). Mean ± SD values of OPA (mm^2) in agricultural, forestry, or fishery workers; factory or construction workers; clerical workers or technical experts; and other workers were 2.9 ± 6.5, 2.9 ± 6.8, 1.6 ± 4.0 and 3.9 ± 10.2, respectively. Tukey HSD test after adjustment for age and BMI showed no significant association between job titles and OPA in either men or women.

Tables III and IV show the mean values of mJSW and OPA according to occupational activity. SD for OPA was quite a large in the present study, because the range was 0–121.5 mm^2 and 1,055 (75.2%) had no osteophytes. Sitting was associated with higher mJSW and lower OPA by linear regression analysis without adjustment; after adjustment for age, gender, and BMI, the significance disappeared. Kneeling and squatting were significantly associated with lower mJSW as well as higher OPA. Walking and lifting weights were significantly associated with lower mJSW, but not with OPA. When we analyzed the association of occupational activities with mJSW and OPA in men and women separately, the results in women were similar to results in the overall population, but there were few factors associated with mJSW or OPA in men (Supplementary Tables I and II).

To determine independent associations of the significant occupational factors shown in Table III with mJSW, multiple regression analysis was performed with age, gender, BMI, and the significant occupational factors as independent variables. Because chi-square test showed that squatting was strongly associated with kneeling (odds ratio 139.5, $P < 0.0001$), we used kneeling when both squatting and kneeling were significantly associated KOACAD parameters. Squatting, kneeling, walking, and lifting weights were significantly associated with mJSW by the abovementioned analysis (Table III); thus, when we used age, gender, BMI, kneeling, walking, and lifting weights as independent variables, multiple regression analysis showed that kneeling was independently associated with mJSW (regression coefficient −0.17, 95% confidence interval [CI] −0.30 to −0.04, $P = 0.01$), and lifting weights tended to be independently associated with mJSW (regression coefficient −0.11, 95% CI −0.22 to 0.002, $P = 0.055$), but walking was not

Table III
mJSW according to occupational activity

	Occupational activity		Crude regression coefficient (95% CI)	*P* value	Adjusted regression coefficient* (95% CI)	*P* value
	No	Yes				
Sitting on a chair ≥2 h/day	2.4 ± 1.2	2.6 ± 1.0	0.22 (0.11–0.33)	0.0002	0.08 (−0.02 to 0.19)	0.117
Standing ≥2 h/day	2.6 ± 1.1	2.3 ± 1.2	−0.33 (−0.47 to −0.19)	<0.0001	−0.21 (−0.34 to −0.09)	0.0009
Kneeling ≥1 h/day	2.6 ± 1.0	2.3 ± 1.1	−0.36 (−0.49 to −0.23)	<0.0001	−0.24 (−0.35 to −0.12)	<0.0001
Squatting ≥1 h/day	2.7 ± 0.9	2.5 ± 1.1	−0.19 (−0.34 to −0.03)	0.016	−0.06 (−0.21 to 0.08)	0.364
Walking ≥3 km/day	2.6 ± 1.0	2.4 ± 1.2	−0.20 (−0.32 to −0.09)	0.0005	−0.11 (−0.21 to −0.002)	0.046
Climbing ≥1 h/day	2.5 ± 1.0	2.5 ± 1.2	−0.06 (−0.20 to 0.07)	0.038	−0.02 (−0.15 to 0.11)	0.733
Lifting weights ≥10 kg ≥ once/week	2.6 ± 1.0	2.5 ± 1.1	−0.10 (−0.21 to 0.01)	0.08	−0.16 (−0.26 to −0.06)	0.003

Values are mean ± SD.
* Adjusted regression coefficient was calculated using multiple regression analysis after adjustment for age, gender, and BMI.

Table IV
OPA according to occupational activity

	Occupational activity		Crude regression coefficient (95% CI)	P value	Adjusted regression coefficient* (95% CI)	P value
	No	Yes				
Sitting on a chair ≥2 h/day	3.5 ± 8.8	2.4 ± 6.7	−1.06 (−1.89 to −0.22)	0.013	−0.39 (−1.19 to 0.41)	0.339
Kneeling ≥1 h/day	2.5 ± 6.9	4.8 ± 10.9	2.25 (1.22 to 3.29)	<0.0001	1.62 (0.65–2.60)	0.0011
Squatting ≥1 h/day	2.5 ± 6.8	4.3 ± 10.5	1.72 (0.78 to 2.66)	0.0004	1.03 (0.13–1.92)	0.025
Standing ≥2 h/day	2.1 ± 5.6	3.2 ± 8.3	1.02 (−0.12 to 2.16)	0.079	0.25 (−0.84 to 1.33)	0.657
Walking ≥3 km/day	3.0 ± 8.7	3.0 ± 7.0	0.05(−0.79 to 0.88)	0.912	−0.56 (−1.37 to −0.24)	0.170
Climbing ≥1 h/day	3.1 ± 8.4	2.7 ± 6.2	−0.39 (−1.38 to 0.59)	0.434	−0.78 (−1.76 to 0.20)	0.119
Lifting weights ≥10 kg ≥ once/week	3.0 ± 8.1	3.0 ± 7.8	0.04 (−0.79 to 0.88)	0.920	0.20 (−0.60 to 1.00)	0.624

Values are mean ± SD.
* Adjusted regression coefficient was calculated using multiple regression analysis after adjustment for age, gender, and BMI.

(regression coefficient −0.055, 95% CI −0.164 to 0.054, $P=0.32$). Further, when we analyzed the independent associations of occupational activities with mJSW in women in the same way, kneeling was independently associated with mJSW (regression coefficient −0.20, 95% CI −0.36 to −0.03, $P=0.02$), and walking tended to be independently associated with mJSW (regression coefficient −0.13, 95% CI −0.27 to 0.005, $P=0.058$), but lifting weights were not (regression coefficient −0.09, 95% CI −0.23 to 0.05, $P=0.22$).

Discussion

The present study is the first epidemiologic study using a large-scale, population-based cohort to determine the association of job title and occupational activity with joint space narrowing and osteophytosis separately. These variables were estimated not by categorical grade but by continuous values such as mJSW and OPA at the knee. In the present study, kneeling, squatting, walking, and heavy lifting were significantly associated with mJSW. For OPA, kneeling and squatting were significantly associated with higher OPA, whereas other activities were not.

Although agricultural, forestry, and fishery workers have been historically among the first to be identified in relation to knee OA in Caucasians[34,35], no studies have focused on mJSW or OPA separately. The present study is the first to examine the association of characteristic features of knee OA such as mJSW and OPA separately with job title, and clarified that, among women, agricultural, forestry, and fishery workers had significantly lower mJSW compared with clerical workers and technical experts. As other authors have hypothesized, the combination of intense exposure to heavy labor of varied nature and repeated local stresses, especially at a young age, could contribute to some systemic mechanism in the development of OA[36]. This argument would support the implementation of preventive measures as a priority to reduce the intensity of physical labor in this sector—particularly for young female farm workers. In contrast, there were no associations between job titles and mJSW in men. Because men are known to have greater muscle strength than women at all ages[37], and muscle strength has a protective effect on knee OA[38,39], it might be that the greater muscle strength obscures the harmful effects of agricultural, forestry, and fishery work, leading to lower risk for knee OA in men.

For kneeling and squatting, studies in Caucasians have suggested that these occupational activities, and job titles that require them, are associated with knee OA[19–24], whereas our previous study showed that these activities were significantly associated with severe knee OA. However, in all previous studies, knee OA was diagnosed by KL grade or whether subjects had undergone total knee arthroplasty. The present study was the first to clarify the association of kneeling and squatting with joint space narrowing and osteophytosis separately. In addition, these variables were not estimated using a categorical method but rather with continuous values such as mJSW and OPA. This study clarified that kneeling and squatting were significantly associated with decreased mJSW as well as increased OPA. There were no occupational activities associated with both joint space narrowing and osteophytosis except for kneeling and squatting; in addition, kneeling had a larger impact on mJSW than lifting weights. Thus, these occupational activities must be strongly associated with knee OA.

Walking and lifting weights were associated with joint space narrowing but not with osteophytosis in the present study. This discrepancy may be partly explained by the high prevalence of osteophytosis in Japan. In fact, our previous study[4] showed that KL = 2 OA, which consists of definite osteophytosis but no definite joint space narrowing, was high in subjects in Japan compared with studies in Caucasians[40,41], whereas KL = 3 OA, which consists of definite joint space narrowing, did not differ significantly between these two ethnic groups. The higher prevalence of osteophytosis in Japan could be due to lifestyle factors, because the Japanese traditional lifestyle includes sitting on the heels on a mat and using Japanese-style lavatories; these positions may cause mechanical stress to the knee joint and possibly lead to acceleration of osteophytosis. The burden on the knee associated with walking and lifting weights may be weaker compared with the burden associated with kneeling and squatting; thus, the association between osteophytosis and occupational activities of walking and lifting weights may be obscured by the traditional Japanese lifestyle. In addition, the separate associations of walking and lifting weights with joint space narrowing and osteophytosis suggest that these two features of knee OA may have distinct etiological mechanisms. In fact, a recent cross-sectional study has shown that osteophytosis was unrelated not only to joint space narrowing on plain radiographs, but also to cartilage loss measured by quantitative magnetic resonance imaging[27]. The present study also showed that mJSW and OPA were significantly correlated, but each predicted only 21% of the variation in the other. Furthermore, our study on an experimental mouse model for OA has identified a cartilage-specific molecule, carminerin, that regulates osteophytosis without affecting joint cartilage destruction during OA progression[28,29].

In the present study, we found gender differences regarding the association of occupational activities with mJSW. In women, kneeling, squatting, walking, and lifting weights were significantly associated with mJSW, whereas in men, only squatting was significantly associated with mJSW. This difference may be partly explained by muscle strength in men. Because men are known to have greater muscle strength than women at all ages, and muscle strength has a protective effect on knee OA[37–39], it might be that the greater muscle strength obscures the harmful effects of occupational activities on knees in men.

Our technique to measure mJSW is a little different from many other methods (Ref) in that the tibia margin is defined using both the tibial plateau (bright band) and the rim, whereas other methods use the tibial plateau alone[42,43]. However, our preparatory examination showed higher reproducibility in "the middle line between

the anterior and posterior margins of the tibial plateau"[32]. In fact, in our previous study[32], to decide the ideal algorithms for the measurements, we initially evaluated the reproducibility of "the tibial plateau alone" and "'the middle line between the anterior and posterior margins of the tibial plateau" by an intraclass coefficient of correlation (ICC) on radiographs of 20 individuals taken at a 2-week intervals with various knee flexion angles (0, 10, 20, and 30°) and X-ray beam angulations (0, 5, 10, and 15°). Results showed higher reproducibility in "the middle line between the anterior and posterior margins of the tibial plateau" at each condition.

There were several limitations to the present study. First, this is a cross-sectional study on factors associated with knee OA, so a causal association with occupational activity could not be determined. However, information collected included a lifetime occupational history and details of specific workplace physical activities; therefore, ample evidence on the background of joint space narrowing and osteophytosis at the knee could be obtained. Second, a rotation of the knee could cause a large error, especially in OPA, which could hide associations of independent variables with this metric. However, the patella was centralized over the lower end of the femur with the aid of fluoroscopy when we took X-rays; thus the rotational error is likely to be small and have minimal effects on the results of the present study.

In conclusion, the present cross-sectional study using a large-scale population from the ROAD study revealed distinct risk factors of occupational activities for joint space narrowing and osteophytosis in Japanese subjects. Other occupational activities of kneeling and squatting were associated with joint space narrowing as well as osteophytosis. Walking and heavy lifting were associated with joint space narrowing, but not with osteophytosis. Further studies, along with longitudinal data from the ROAD study, will elucidate the environmental background of OA and help clarify clinical evidence for the development of disease-modifying treatments.

Author contributions

All authors have made substantial contributions to all three of sections (1), (2) and (3) below;

(1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data

(2) drafting the article or revising it critically for important intellectual content

(3) final approval of the version to be submitted.

Competing interest

There are no competing interest.

Acknowledgments

This study was supported by Grants-in-Aid for Scientific Research (S19109007, B20390182, C20591737, C20591774), for Young Scientists (A18689031), and for Exploratory Research (19659305) from the Japanese Ministry of Education, Culture, Sports, Science and Technology, H17-Men-eki-009, H18-Choujyu-037, H20-Choujyu-009, H21-Chouju-Wakate-011 and H22-Chouju-Wakate-007 from the Ministry of Health, Labor and Welfare, Research Aid from the Japanese Orthopaedic Association (JOA-Subsidized Science Project Research 2006-1), and Grant No. 166 from the Japan Orthopaedics and Traumatology Foundation.

The authors thank Mrs Tomoko Takijiri and other members of the Public Office in Hidakagawa Town; and Mrs Tamako Tsutsumi, Mrs Kanami Maeda, and other members of the Public Office in Taiji Town, for their assistance in the location and scheduling of participants for examinations.

Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.joca.2011.03.008.

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Mod Rheumatol (2011) 21:608–620
DOI 10.1007/s10165-011-0455-2

ORIGINAL ARTICLE

Biochemical markers of bone turnover as predictors of osteoporosis and osteoporotic fractures in men and women: 10-year follow-up of the Taiji cohort

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Received: 24 December 2010 / Accepted: 17 March 2011 / Published online: 22 April 2011

Abstract We aimed to assess the capacity of biochemical markers of bone turnover (BTMs) to predict bone loss, osteoporosis (OP), and osteoporotic fractures. We randomly selected 400 individuals (age 40–79 years in 1993; 50 of each gender and age stratum) from a list of registered residents. In the years 1993, 1996, 2000, and 2003, bone mineral density (BMD) of the spine and hip were measured by dual-energy X-ray absorptiometry. The BTMs assessed at baseline were serum intact osteocalcin (OC), total OC, bone-specific alkaline phosphatase, C-terminal propeptide of type I procollagen, N-terminal propeptide of type I procollagen (PINP), C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase, C-terminal cross-linking telopeptide of type I collagen (beta-CTX), N-terminal cross-linking telopeptide of type I collagen (NTX), urinary pyridinoline, and deoxypyridinoline (DPD). For 307 completers, multivariate analysis after adjusting for confounders revealed that serum PINP levels in men [hazard ratio (HR) 2.80, $P < 0.05$] and serum PINP (HR 1.65, $P < 0.05$), beta-CTX (HR 1.80, $P < 0.001$), NTX (HR 1.96, $P < 0.01$), and urinary DPD levels (HR 1.40, $P < 0.05$) in women were significantly related to the occurrence of spinal OP. In addition to adjustment for the baseline status of BMD, i.e., osteopenia or normal range, PINP, beta-CTX, and NTX in women could significantly predict the future occurrence of spinal OP. BTMs were not significant predictors of bone loss, femoral OP, or osteoporotic fractures. In conclusion, various BTMs in women can predict the occurrence of spinal OP.

Keywords Biochemical markers of bone turnover · Bone resorption · Bone formation · Bone mineral density · Osteoporotic fracture

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Introduction

Osteoporosis (OP) impairs the activities of daily living (ADL) and quality of life (QOL), leading to increased morbidity and mortality in the elderly [1, 2]. With the rapid aging of the population, an urgent need has been identified for the development of methods to prevent OP. The estimated number of patients with OP in Japan is about 11 million [3], and osteoporotic fractures are ranked fifth among the diseases responsible for disabilities requiring support in Japan [4].

As the restoration of diminished bone volume seems quite difficult to achieve, the early diagnosis of OP is the most valuable strategy for preventing osteoporotic fractures. However, the prediction of rapid bone loss, incidence of OP, and osteoporotic fractures remains difficult.

Biochemical markers of bone turnover (BTMs) reflect the status of bone metabolism in various processes coupled with bone resorption and formation, and are widely used in clinical situations to evaluate the efficacy of treatments for OP [5–8]. Several population-based epidemiological

studies have shown that BTMs can predict bone loss and the incidence of osteoporotic fractures in women [9–15], but the effectiveness of BTMs for predicting such epidemiological indices over the long-term, such as 10 years, is unclear. In addition, few reports, besides our own previous reports [16, 17], have evaluated BTM values and bone loss or osteoporotic fractures in men.

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 evaluating the capacity of baseline urinary and serum concentrations of various BTMs to predict future bone loss and the occurrence of OP and osteoporotic fractures in men and women randomly selected from a rural population in Japan.

Subjects, materials, and methods

Establishment of the baseline cohort

Details of the cohort survey at the Japanese town of Taiji have already been reported [18–20], and the Taiji cohort is therefore described here only in brief. The town of Taiji is located in the southern coastal area of Wakayama Prefecture in the south-western area of the main island of Japan. A list of all inhabitants born between 1913 and 1952, and therefore aged between 40 and 79 years in 1993, was compiled based on 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 whole cohort, 50 men and 50 women in each of four age groups between 40 and 79 years by decade of birth year (1913–1922, 1923–1932, 1933–1942, and 1943–1952), for a total of 400 participants, were selected randomly, using a table of random numbers, and underwent measurement of bone mineral density (BMD) in 1993. At this time, blood and urine samples were taken from all participants. An interviewer administered a second questionnaire to these 400 participants, covering items of past medical history, including questions related to OP, osteoporotic fractures and falls, family history, calcium intake, dietary habits, physical exercise, occupational activities, and sun exposure, and, for women, additional questions about reproductive variables such as menstrual status (premenopause, perimenopause, or menopause), age at menopause, age at menarche, number of childbirths, lactation, use of estrogen for treatment, history of ovariectomy, and history of uterectomy (BMD cohort, baseline study).

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

BMD measurements at baseline and follow-up surveys

Baseline BMD was measured in 1993, using dual-energy X-ray absorptiometry (DXA) (QDR 1000; Hologic, Bedford, MA, USA), providing antero-posterior images of lumbar vertebrae L2–4 and the proximal femur (femoral neck, Ward's triangle, trochanter). These measurements were repeated on the same participants after 3 (2nd visit, 1996), 7 (3rd visit, 2000) and 10 years (4th visit, 2003).

At each follow-up survey, an interviewer-administered questionnaire survey was performed regarding changes in lifestyle factors during the observation period, and covering items of medical history, including questions related to OP, osteoporotic fractures and falls, anti-OP treatment, calcium intake, dietary habits, physical exercise, occupational activities, and sun exposure, and, for women, additional questions about reproductive variables such as menstrual status (premenopause, perimenopause, or menopause), age at menopause, use of estrogen for treatment, history of ovariectomy, and history of uterectomy.

To control for the precision of DXA, the equipment was checked at all examinations using the same phantom, and the BMD of the phantom was regulated to 1.030 ± 0.016 g/cm^2 (1.5%) during all examinations. The same physician (N.Y.) obtained all BMD measurements. Intra-observer variability for DXA scans done by this investigator was 0.35%, using the phantom as described [21].

Detection of the occurrence of OP

OP was defined based on World Health Organization criteria, according to which OP is diagnosed based on T scores of BMD $\leq$2.5 standard deviations (SDs) lower than peak bone mass [22]. The mean L2–4 BMD for young adult men and women measured using the Hologic QDR 1000 in Japan is reported as 1.011 g/cm^2, and the SD is 0.119 g/cm^2 [23]. The mean femoral neck BMD (SD) in Japan is reported as 0.863 (0.127) for young men and 0.787 (0.109) for young women [23]. This study therefore defined OP, using these indices, as lumbar spine BMD <0.714 g/cm^2 for both men and women, and as femoral neck BMD <0.546 g/cm^2 for men and <0.515 g/cm^2 for women.

To define the incidence of OP among the 400 participants at the initial survey, individuals with spinal or femoral neck OP were excluded. Among the remaining participants without OP at the lumbar spine and/or femoral neck at baseline, the number of new cases of OP was

counted at the 3, 7, and 10-year follow-up surveys. Incidences of OP were estimated using the number of new cases divided by the person-years, consisting of years of individuals diagnosed with OP and years of drop-outs. The annual incidence of lumbar and femoral neck OP was then estimated.

Measurements of BTMs

All blood and urine samples were collected between 09:00 and 15:00. After centrifugation of the blood samples, sera were immediately placed in dry ice and transferred to a deep freezer within 24 h. Spot urine samples were frozen using the same procedure. These samples were kept at −80°C until needed for assays.

From the samples of participants in the baseline study, the following BTMs were measured to establish values. As markers of bone formation, serum intact osteocalcin (OC), serum total OC, serum bone-specific alkaline phosphatase (BAP), serum C-terminal propeptide of type I procollagen (PICP), and serum N-terminal propeptide of type I procollagen (PINP) were utilized. To monitor bone resorption, products of collagen breakdown, i.e., serum C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase (ICTP), serum beta-isomerized C-terminal cross-linking telopeptide of type I collagen (beta-CTX), serum N-terminal cross-linking telopeptide of type I collagen (NTX), urinary pyridinoline cross-links of collagen (PYR), and urinary deoxypyridinoline cross-links of collagen (DPD) were used.

Reference values classified by age and gender for serum intact OC, PICP, and ICTP and urinary PYR and DPD have already been described [17]. Measurement methods for these compounds are therefore only described in brief. Serum intact OC was measured using an immunoradiometric assay (Osteocalcin IRMA kit; Mitsubishi Kagaku BCL, Tokyo, Japan) [24]. Serum PICP and ICTP were measured using a radioimmunoassay (RIA) (Orion Diagnostics, Espoo, Finland) [25]. Urinary PYR and DPD in hydrolyzed urine specimens were analyzed by high-performance liquid chromatography followed by fluorescent detection using essentially the same methods [26]. The values of these urinary markers were standardized to urinary creatinine concentrations.

Total OC was measured using an electrochemiluminescence immunoassay (ECLIA) (Elecsys N-MID Osteocalcin; Roche Diagnostics, Mannheim, Germany) [27] with an intraassay coefficient of variation (CV) of 0.5%, and sensitivity of 0.5 ng/mL. We measured BAP using an enzyme immunoassay (Metra BAP; Quidel, San Diego, CA, USA) [28] with an intraassay CV of 3.9–5.2% and sensitivity of 0.7 U/L. Serum PINP was measured using an RIA (Orion Diagnostics) with an intraassay CV of 3.1–9.3% and sensitivity of 2 ng/mL [29, 30].

As markers of bone resorption, serum beta-CTX was measured using an ECLIA (Elecsys beta-CrossLaps; Roche Diagnostics) with an intraassay CV of 2.0% and sensitivity of 0.01 ng/mL [27]. Serum NTX was measured using an enzyme-linked immunosorbent assay (Osteomark NTX serum; Ostex International, Seattle, WA, USA) [31, 32] with an intraassay CV of 4.6% and sensitivity of 3.2 nM BCE/L.

Fracture assessment

All participants completed a detailed questionnaire at baseline, including a history of fragility fractures (that is, fractures resulting from low-impact trauma) that had occurred since the age of 40 years. Thereafter, at each subsequent examination, information about the occurrence of fractures since the previous visit was extracted from interviewer-registered questionnaires and registered. Information about fractures considered to be osteoporotic was analyzed. Osteoporotic fractures comprised those of the spine, pelvis, ribs, distal radius, forearm, humerus, and hip that occurred in the absence of high-impact trauma.

Statistical analysis

All statistical analyses were performed using STATA statistical software (STATA, College Station, TX, USA). Differences were tested for significance using analysis of variance for comparisons among multiple groups and Scheffe's least significant difference test for pairs of groups. Causal relationships between bone changes and serum and urinary concentrations of BTMs at baseline were clarified using multiple regression analysis with the rate of change of BMD (% per year) as an objective factor, and values of BTMs (/SD) at baseline after adjusting for age, weight, and menstrual status (0 pre- and perimenopause; 1 menopause) in women at baseline. Causal relationships between the incidence of OP and osteoporotic fractures, and serum and urinary concentrations of BTMs at baseline were clarified using Cox proportional hazards modeling using the occurrence of OP (yes 1; no 0) and occurrence of osteoporotic fractures (yes 1; no 0) as objective factors, and BTM level (/SD) at baseline as an explanatory factor after adjusting for age and weight, and menstrual status in women at baseline. Regarding anti-OP drugs, during the observation period from 1993 to 2003, bisphosphonates such an alendronate and risedronate and selective estrogen receptor modulator (SERM) agents had not been approved for use in Japan for the treatment of OP. In addition, we asked participants, in the questionnaires at each follow-up at the 2nd, 3rd, and 4th visits, whether they