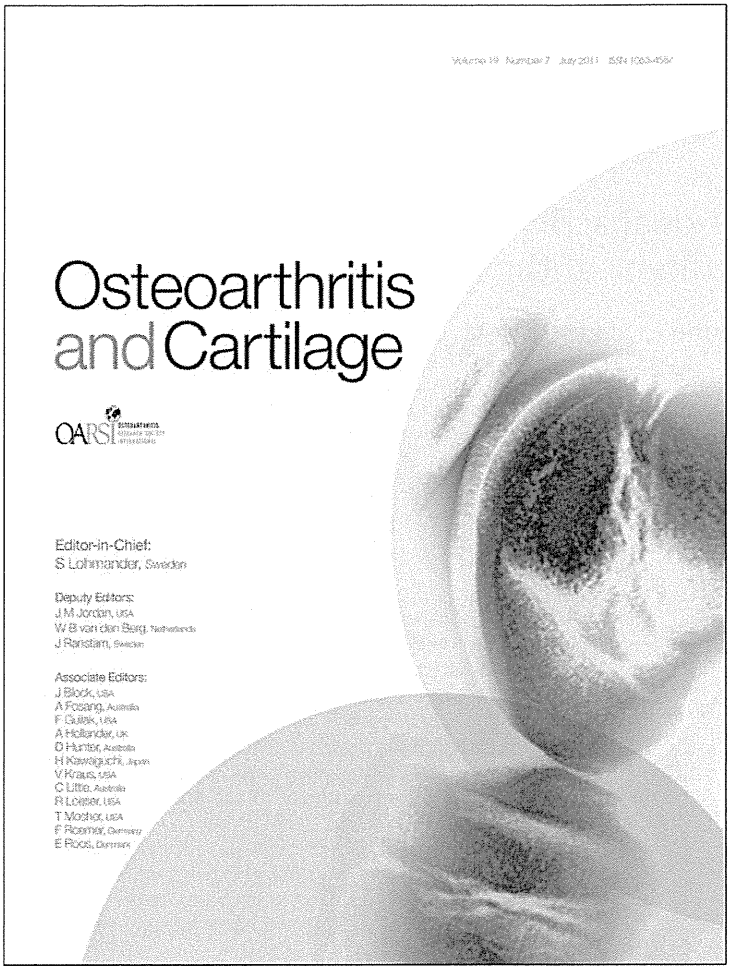
Conflict of interest The authors have no conflicts or disclosures to declare regarding the present manuscript.

References

1. Yoshimura N, Muraki S, Oka H, Mabuchi A, En-yo Y, Yoshida M, Saika A, Suzuki T, Yoshida H, Kawaguchi H, Nakamura K, Akune T (2009) Prevalence of knee osteoarthritis, lumbar spondylosis and osteoporosis in Japanese men and women: the Research on Osteoarthritis/Osteoporosis Against Disability (ROAD). J Bone Miner Metab 27:620–628
2. Ministry of Health, Labour and Welfare (2007) The outline of the results of National Livelihood Survey 2007. http://www.mhlw.go.jp/toukei/list/20-19-1.html. Accessed 7 Jan 2010
3. Sakata T, Yaegashi Y, Hosoi T, Orimo H (2009) The nationwide survey on the incidence of hip fracture in Japan and 20-year trends. Osteoporos Int 20(suppl 1):S63
4. Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J, Committee of Scientific Advisors of the International Osteoporosis Foundation (2000) The use of biochemical markers of bone turnover in osteoporosis. Osteoporos Int 11(suppl 6):S2–S17
5. Szulc P, Delmas PD (2008) Biochemical markers of bone turnover: potential use in the investigation, management of postmenopausal osteoporosis. Osteoporos Int 19:1683–1704
6. Bergmann P, Body JJ, Boonen S, Boutsen Y, Devogelaer JP, Goemaere S, Kaufman JM, Reginster JY, Gangji V, Members of Advisory Board on Bone Markers (2009) Evidence-based guidelines for the use of biochemical markers of bone turnover in the selection and monitoring of bisphosphonate treatment in osteoporosis: a consensus document of the Belgian Bone Club. Int J Clin Pract 63:19–26
7. Garnero P, Sornay-Rendu E, Duboeuf F, Delmas PD (1999) Markers of bone turnover predict postmenopausal forearm bone loss over 4 years: the OFELY study. J Bone Miner Res 14:1614–1621
8. Chapurlat RD, Garnero P, Sprnay-Rendu E, Arlot ME, Claustrat B, Delmas PD (2000) Longitudinal study of bone loss in pre- and perimenopausal women: evidence for bone loss in perimenopausal women. Osteoporos Int 11:493–498
9. Stepan JJ (2000) Prediction of bone loss in postmenopausal women. Osteoporos Int 11(suppl 6):S45–S54
10. Iki M, Morita A, Ikeda Y et al (2006) Biochemical markers of bone turnover predict bone loss in perimenopausal women but not in postmenopausal women—the Japanese Population-based Osteoporosis (JPOS) cohort study. Osteoporosis Int 17:1086–1095
11. Yoshimura N, Hashimoto T, Sakata K, Morioka S, Kasamatsu T, Cooper C (1999) Biochemical markers of bone turnover and bone loss at the lumbar spine and femoral neck. The Taiji study. Calcif Tissue Int 65:198–202
12. Bauer DC, Garnero P, Harrison SL, Cauley JA, Eastell R, Ensrud KE, Orwoll E, Osteoporotic Fractures in Men (MrOS) Research Group (2009) Biochemical markers of bone turnover, hip bone loss, and fracture in older men: the MrOS study. J Bone Miner Res 24:2032–2038
13. Yoshimura N, Kinoshita H, Danjoh S, Takijiri T, Morioka S, Kasamatsu T, Sakata K, Hashimoto T (2002) Bone loss at the lumbar spine and the proximal femur in a rural Japanese community, 1990–2000: the Miyama study. Osteoporos Int 13:803–808
14. Kasamatsu T, Yoshimura N, Morioka S, Sugita K, Hashimoto T (1996) A population survey on bone mineral density in a fishing village in Wakayama Prefecture (Part 1). Distribution of bone mineral density by sex and age on a representative sample of the community. Jpn J Hyg 50:1084–1092 (in Japanese)
15. Yoshimura N, Kasamatsu T, Morioka S, Hashimoto T (1996) A population survey on bone mineral density in a fishing village in Wakayama Prefecture (Part 2). The analysis of the risk factors affecting the bone mineral density. Jpn J Hyg 51:677–684 (in Japanese)
16. Yoshimura N, Hashimoto T, Morioka S, Sakata K, Kasamatsu T, Cooper C (1998) Determinants of bone loss in a rural Japanese community. The Taiji Study. Osteoporos Int 8:604–610
17. Yoshimura N, Kakimoto T, Nishioka M, Kishi T, Iwasaki H, Niwa T, Morioka S, Sakata T, Hashimoto T (1997) Evaluation of reproducibility of bone mineral density measured by dual energy X-ray absorptiometry (DPX-L). J Wakayama Med Soc 48:461–466
18. Schmidt-Gayk H, Spanuth E, Kotting J, Bartl R, Felsenberg D, Pfeilshifter J, Raue F, Roth HJ (2004) Performance evaluation of automated assays for beta-Crosslaps, N-MID Osteocalcin and intact parathyroid hormone (BIROSE Multicenter Study). Clin Chem Lab Med 42:90–95
19. Clemens JD, Herrick MV, Singer FR, Eyre DR (1997) Evidence that serum NTx (collagen-type I N-telopeptides) can act as an immunochemical marker of bone resorption. Clin Chem 43:2058–2063
20. Gertz BJ, Clemens JD, Holland SD, Yuan W, Greenspan S (1998) Application of a new serum assay for type I collagen cross-linked N-telopeptides: assessment of diurnal changes in bone turnover with and without alendronate treatment. Calcif Tissue Int 63:102–106
21. Ebeling PR, Atley LM, Guthrie JR, Burger HG, Dennerstein L, Hopper JL, Wark JD (1996) Bone turnover markers and bone density across the menopausal transition. J Clin Endocrinol Metab 81:3366–3371
22. Sowers MR, Greendale GA, Bondarenko I, Finkelstein JS, Cauley JA, Neer RM, Ettinger B (2003) Endogenous hormones and bone turnover markers in pre- and perimenopausal women: SWAN. Osteoporos Int 14:191–197
23. Yoshimura N, Kasamatsu T, Sakata K, Hashimoto T, Cooper C (2002) The relationship between endogenous estrogen, sex hormone binding globulin and bone loss in female residents of a rural Japanese community: the Taiji study. J Bone Miner Metab 20:303–310
24. Yoshimura N, Takijiri T, Kinoshita H, Danjoh S, Kasamatsu T, Morioka S, Sakata K, Hashimoto T, Takeshita T (2004) Characteristics and course of bone mineral densities among fast bone losers in a rural Japanese community: the Miyama study. Osteoporos Int 15:139–144
25. Fujiwara S, Mizuno S, Ochi Y, Sasaki H, Kodama K, Russell WJ, Hosoda Y (1991) The incidence of thoracic vertebral fractures in a Japanese population, Hiroshima and Nagasaki, 1958–86. J Clin Epidemiol 44:1007–1014
26. Hannon R, Eastell R (2000) Preanalytical variability of biochemical markers of bone turnover. Osteoporos Int 11(suppl 6):S30–S44
27. Seibel MJ (2000) Molecular markers of bone turnover: biochemical, technical and analytical aspects. Osteoporos Int 11(suppl 6):S18–S29



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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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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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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

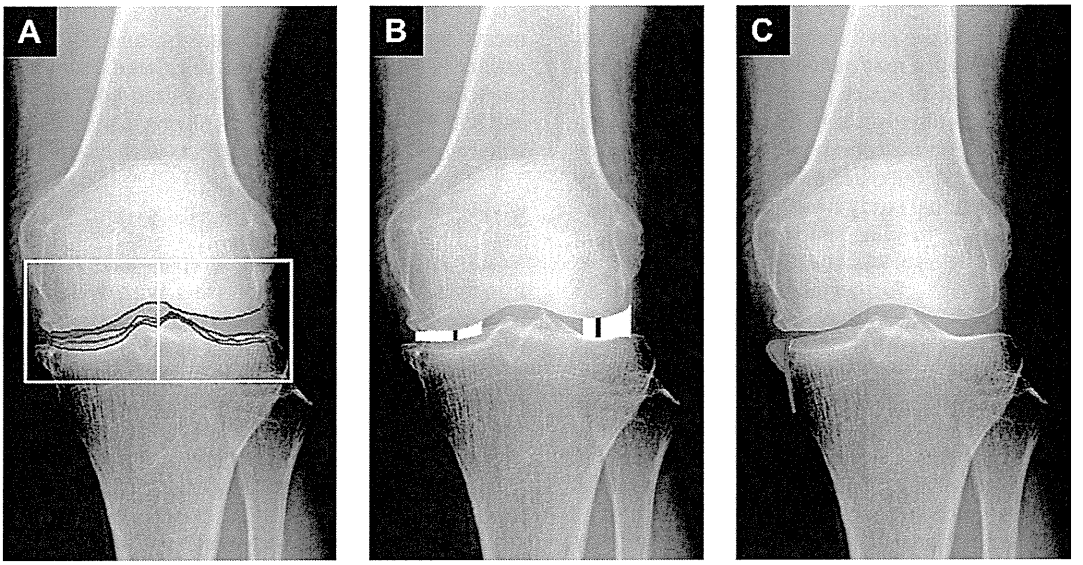

Fig. 1. Schema of image processing by KOACAD (cited from reference number[9]). (A). Outlines of anterior and posterior margins of the tibial plateau. The middle line between the two outlines is defined as the lower rim of the joint space. (B). Medial and lateral mJSWs were defined as the minimum vertical 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.

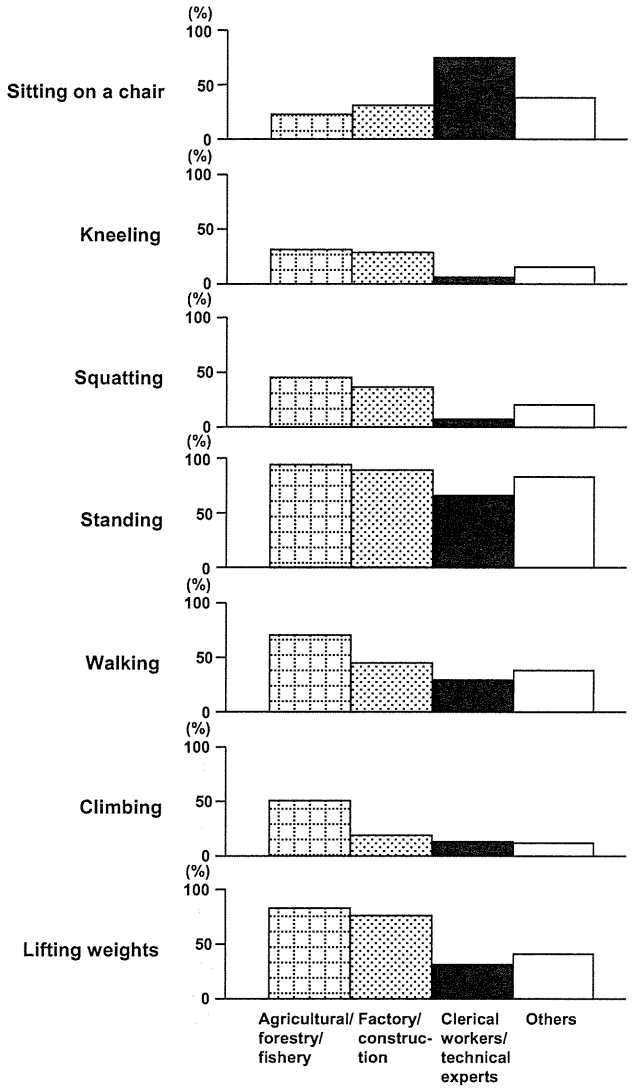

Fig. 2. Percentage of participants engaged in each occupational activity: sitting on a chair ≥2 h/day, kneeling ≥1 h/day, squatting ≥1 h/day, standing ≥2 h/day, walking ≥3 km/day, climbing ≥1 h/day, or lifting weights ≥10 kg ≥1 time/week among agricultural, forestry, and fishery workers; factory and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

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Supplementary material

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

References

1. Sharma L, Kapoor D. Epidemiology of osteoarthritis. In: Moskowitz RW, Altman RD, Hochberg MC, Buckwalter JA, Goldberg VM, Eds. Osteoarthritis: Diagnosis and Medical/Surgical Management. 4th edn. 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. 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.
5. Yoshimura N, Muraki S, Oka H, Mabuch 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 (ROAD). J Bone Miner Metab 2009;27:620–8.
6. Ministry of Health, Labour and Welfare. The outline of the results of National Livelihood Survey 2007. Available at: http://www.mhlw.go.jp/toukei/list/20-19-1.html.
7. Jackson JP. Degenerative changes in the knee after meniscectomy. Br Med J 1968;2:525–7.
8. Fairbank TJ. Knee joint changes after meniscectomy. J Bone Joint Surg Br 1948;30:664–70.
9. Jacobsen K. Osteoarthrosis following insufficiency of the cruciate ligaments in man. A clinical study. Acta Orthop Scand 1977;48:520–6.
10. Sommerlath K, Gillquist J. The long-term course of various meniscal treatments in anterior cruciate ligament deficient knees. Clin Orthop Relat Res 1992;283:207–14.
11. Neyret P, Donell ST, Dejour H. Results of partial meniscectomy related to the state of the anterior cruciate ligament. Review at 20–35 years. J Bone Joint Surg Br 1993;75:36–40.
12. Felson DT. Epidemiology of hip and knee osteoarthritis. Epidemiol Rev 1988;10:1–28.
13. Kellgren JH, Lawrence JS. Rheumatism in miners. II. X-ray study. Br J Ind Med 1952;9:197–207.
14. Partridge RE, Duthie JJ. Rheumatism in dockers and civil servants. A comparison of heavy manual and sedentary workers. Ann Rheum Dis 1968;27:559–68.
15. Lindberg H, Montgomery F. Heavy labor and the occurrence of gonarthrosis. Clin Orthop Relat Res 1987;214:235–6.
16. Kohatsu ND, Schurman DJ. Risk factors for the development of osteoarthrosis of the knee. Clin Orthop Relat Res 1990;261:242–6.
17. Vingård E, Alfredsson L, Goldie I, Hogstedt C. Occupation and osteoarthrosis of the hip and knee: a register-based cohort study. Int J Epidemiol 1991;20:1025–31.
18. Vingård E, Alfredsson L, Fellenius E, Hogstedt C. Disability pensions due to musculo-skeletal disorders among men in heavy occupations. Scand J Soc Med 1992;20:31–6.
19. 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.
20. Felson DT, Hannan MT, Naimark A, Berkeley J, Gordon G, Wilson PW, *et al.* Occupational physical demands, knee bending, and knee osteoarthritis: results from the Framingham study. J Rheumatol 1991;18:1587–92.
21. Cooper C, McAlindon T, Coggon D, Egger P, Dieppe P. Occupational activity and osteoarthritis of the knee. Ann Rheum Dis 1994;53:90–3.
22. Coggon D, Croft P, Kellingray S, Barrett D, McLaren M, Cooper C. Occupational physical activities and osteoarthritis of the knee. Arthritis Rheum 2000;43:1443–9.
23. Sandmark H, Hogstedt C, Vingard E. Primary osteoarthrosis of the knee in men and women as a result of lifelong physical load from work. Scand J Work Environ Health 2000;26:20–5.
24. Manninen P, Heliovaara M, Riihimaki H, Suoma-Iainen O. Physical workload and the risk of severe knee osteoarthritis. Scand J Work Environ Health 2002;28:25–32.
25. 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.
26. Kellgren JH, Lawrence JS, Eds. The Epidemiology of Chronic Rheumatism: Atlas of Standard Radiographs of Arthritis. Oxford: Blackwell Scientific; 1963
27. 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.
28. 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.
29. 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.
30. Muraki S, Oka H, Mabuchi A, Akune T, En-yo Y, Yoshida M, *et al.* Prevalence of radiographic lumbar spondylosis and its association with low back pain in the elderly of population-based cohorts: the ROAD study. Ann Rheum Dis 2009;68:1401–6.
31. 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.
32. Oka H, Muraki S, Akune T, Mabuchi A, Suzuki T, Yoshida H, *et al.* Fully automatic quantification of knee osteoarthritis severity on plain radiographs. Osteoarthritis Cartilage 2008;16:1300–6.
33. Oka H, Muraki S, Akune T, Nakamura K, Kawaguchi H, Yoshimura N. Normal and threshold values of joint space width, joint space area, osteophyte area and fibro-tibial angle using a computer-assisted measuring system (KOACAD) to evaluate knee osteoarthritis: the ROAD study. J Orthop Sci 2010;15:781–9.
34. Rossignol M, Leclerc A, Allaert FA, Rozenberg S, Valat JP, Avouac B, *et al.* Primary osteoarthritis of hip, knee, and hand in relation to occupational exposure. Occup Environ Med 2005;62:772–7.
35. Walker-Bone K, Palmer KT. Musculoskeletal disorders in farmers and farm workers. Occup Med (Lond) 2002;52:441–50.
36. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, *et al.* Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000;133:635–46.
37. 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.
38. McAlindon TE, Cooper C, Kirwan JR, Dieppe PA. Determinants of disability in osteoarthritis of the knee. Ann Rheum Dis 1993;52:258–62.
39. O'Reilly SC, Jones A, Muir KR, Doherty M. Quadriceps weakness in knee osteoarthritis: the effect on pain and disability. Ann Rheum Dis 1998;57:588–94.
40. 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.
41. Jordan JM, Helmick CG, Renner JB, Luta G, Dragomir AD, Woodard J, *et al.* Prevalence of knee symptoms and radiographic and symptomatic knee osteoarthritis in African Americans and Caucasians: the Johnston County osteoarthritis project. J Rheumatol 2007;34:172–80.
42. Piperno M, Hellio Le Graverand MP, Conrozier T, Bochu M, Mathieu P, Vignon E, *et al.* Quantitative evaluation of joint space width in femorotibial osteoarthritis: comparison of three radiographic views. Osteoarthritis Cartilage 1998;6:252–9.
43. Schmidt JE, Amrami KK, Manduca A, Kaufman KR. Semiautomated digital image analysis of joint space width in knee radiographs. Skeletal Radiol 2005;34:639–43.

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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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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

had been prescribed calcitonin or alfacalcidol, and confirmed that all participants without OP in our examinations had not been given these treatments. For this reason, we did not include the presence or absence of anti-OP treatment into the analysis as an adjustment factor. Moreover, we did not exclude from our analysis subjects with experience of using calcium supplements, because we decided that such supplements should be regarded as a kind of food intake and that the pharmacological effects on BTMs and BMD were small.

Results

Eligible participants and baseline characteristics

Table 1 shows the background data, including physical characteristics, for all 400 participants at baseline. The mean body weight of men in their 40s was significantly higher than that in men in their 50s, 60s, and 70s ($P < 0.05$), whereas that of women was significantly lower in their 40s, 60s, and 70s than during their 50s ($P < 0.05$).

Among the 400 participants at baseline, one man in his 60s refused to undergo blood and urinary examinations for BTMs. As a result, BTMs at baseline were examined in 399 participants (199 men, 200 women). At the 2nd examination, to evaluate changes in BMDs over 3 years, 369 (92.3%; 181 men, 188 women) of the 400 initially recruited individuals participated. At the 3rd examination, to evaluate changes in BMDs and BTMs over 7 years, 338 (84.5%; 170 men, 168 women) of the 400 initial recruits participated. At the 4th (10-year) follow-up in 2003, 322 (80.5%; 153 men, 169 women) of the original 400 recruits participated. Among the 322 participants at the 4th follow-up, 6 men and 9 women who had missed the 3rd visit were excluded from analysis. Detailed reasons for drop-outs at each visit are summarized in Fig. 1. We also specifically searched for participants who had been treated for primary hyperparathyroidism, or who had undergone estrogen or steroid therapy for more than 3 months between 1993 and 2003, but no such individuals were identified. In addition, we confirmed that the main reason for illness or death was not attributable to osteoporotic fracture in any cases. The remaining 307 participants (76.8%; 147 men, 160 women) completed all examinations over the 10-year study period.

Changes in BMD over 10 years

Table 2 shows the initial mean values and rates of change in L2–4 BMD classified by sex and age stratum over the 10-year study period. BMD values at L2–4 for men in their 50s and 60s had slightly increased by the 10-year follow-up, but had decreased slightly for those in their 40s and 70s. By contrast, BMD at L2–4 had decreased in all age strata for women over the 10 years, at a mean rate of −7.5%/10 years. BMD at the femoral neck had increased for men in their 40s and 50s, and had considerably increased for those in their 70s, while BMD at the same site

Table 1 Summary of participants' characteristics at baseline, classified by age and gender

Birth cohort	Age group (years)	*n*	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Men						
1943–1952	40–49	50	44.2 (2.6)	168.8 (5.2)	69.0 (10.4)	24.2 (3.2)
1933–1942	50–59	50	54.8 (2.7)	165.6 (5.0)[a]	63.5 (9.4)[a]	23.1 (2.9)
1923–1932	60–69	50	64.6 (2.5)	163.0 (4.8)[a]	62.9 (9.6)[a]	23.6 (3.2)
1913–1922	70–79	50	74.0 (2.7)	160.7 (5.4)[a,b]	57.5 (8.3)[a,b,c]	22.2 (2.8)
1913–1952	40–79	200	59.4 (11.4)	164.5 (5.9)	63.2 (10.2)	23.3 (3.1)
Women						
1943–1952	40–49	50	44.0 (2.8)	154.4 (5.0)	54.1 (8.3)	22.7 (3.1)
1933–1942	50–59	50	55.8 (2.8)	154.9 (5.3)	59.4 (10.0)[a]	24.8 (4.0)[a]
1923–1932	60–69	50	64.8 (2.6)	151.1 (4.6)[a,b]	52.1 (9.1)[b]	22.8 (3.5)[b]
1913–1922	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]
1913–1952	40–79	200	59.8 (11.6)	152.0 (5.8)	53.5 (9.7)	23.1 (3.6)

Values are given as means with standard deviations in parentheses

BMI body mass index, *n* number of participants

[a] Significantly different ($P < 0.05$) from values of participants in their 40s

[b] Significantly different ($P < 0.05$) from values of participants in their 50s

[c] Significantly different ($P < 0.05$) from values of participants in their 60s

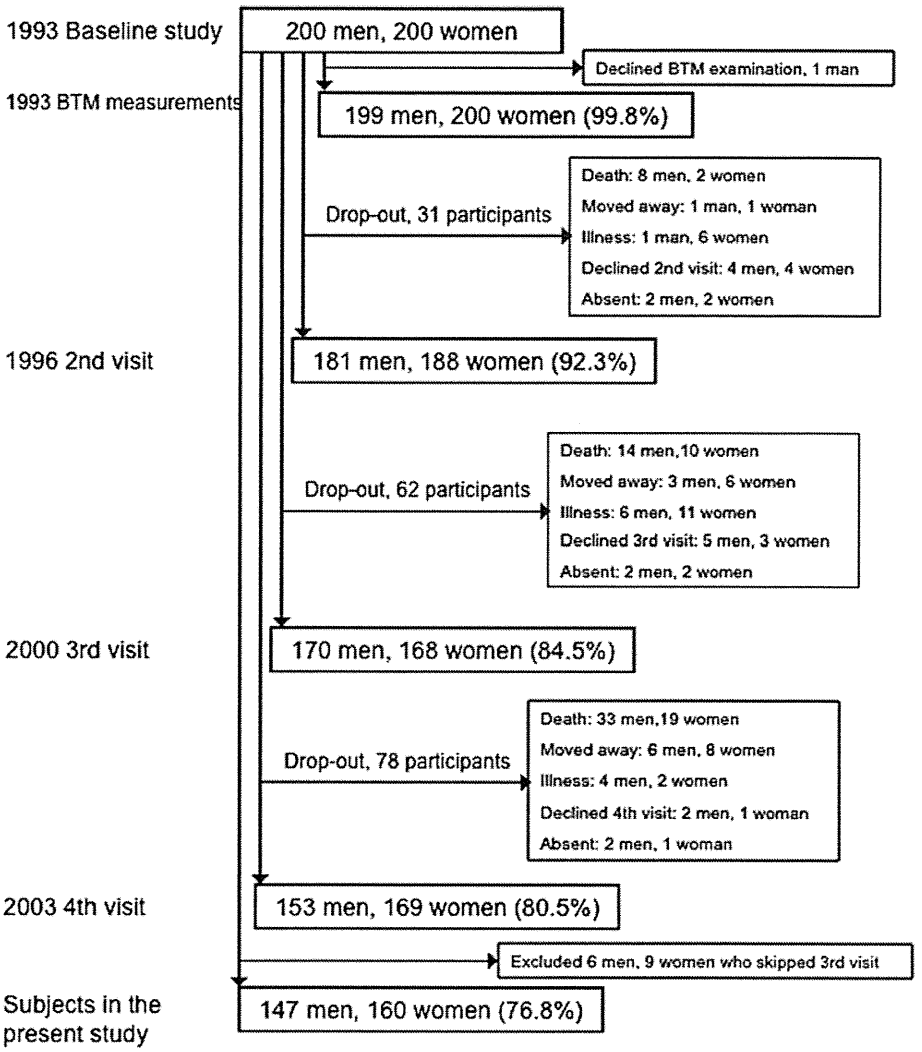

Fig. 1 Flow chart of participants in the cohort. *BTM* Biochemical marker of bone turnover

in women had decreased in all age strata. Rates of change did not differ significantly among age strata.

Incidence of OP and osteoporotic fractures over 10 years

Among the 400 participants at the initial survey, 47 (9 men, 38 women) with spinal OP were excluded from estimation of the incidence of OP. Among the remaining 353 participants (191 men, 162 women), 29 (4 men, 25 women) developed OP at the lumbar spine over the 10-year period. Incidences of OP at the lumbar spine over the 10-year period in men and women aged 40–79 years were 23.8 and 176.0 per 10,000 person-years, respectively. Similarly, 22 (3 men, 19 women) of 383 participants (200 men, 183 women) developed OP at the femoral neck over the 10-year period. Incidences of OP at the femoral neck in men and women aged 40–79 years were 17.1 and 114.5 per 10,000 person-years, respectively. The annual incidence of lumbar and femoral neck OP was thus approximately sevenfold higher among women than among men.

Incidence of OP classified by age was then examined in detail. Incidences of lumbar OP in men aged in their 40s, 50s, 60s, and 70s were 0, 22.1, 0, and 96.2 per 10,000 person-years, respectively, with a peak in the oldest stratum. By contrast, these values for women were 63.8, 205.5, 380.2, and 120.5 per 10,000 person-years, respectively, with peaks in the 50s and 60s. Incidences of OP at the femoral neck in men in their 40s, 50s, 60s, and 70s were 0, 0, 42.9, and 28.4 per 10,000 person-years, respectively, with peaks in the 60s and 70s. These values in women were 0, 20.4, 224.4, and 301.0 per 10,000 person-years, respectively, with the highest peak in the oldest stratum, followed by the 60s.

During the 10-year observation period, we detected 32 osteoporotic fractures (10 in men, 22 in women) after the exclusion of traumatic fractures (results of traffic accidents and falls from more than the subject's height). These 32

Table 2 Mean values (standard deviation) of bone mineral density (g/cm^2) and change rate (%) at lumbar spine L2–4 and femoral neck over 3, 7, and 10 years, classified by age and gender

Birth cohort	Age group (years)	L2–4								Femoral neck			
		Baseline		2nd visit (3-year follow-up)		3rd visit (7-year follow-up)		4th visit (10-year follow-up)		Baseline	2nd visit	3rd visit	4th visit
		n	BMD (g/cm^2)	*n*	Change rate (%/3 years)	*n*	Change rate (%/7 years)	*n*	Change rate (%/10 years)	BMD (g/cm^2)	Change rate (%/3 years)	Change rate (%/7 years)	Change rate (%/10 years)
Men													
1943–1952	40–49	50	1.05 (0.15)	48	0.6 (3.8)	46	−0.6 (5.1)	42	−0.2 (5.8)	0.86 (0.09)	0.3 (4.6)	−1.8 (4.8)	−1.3 (10.9)
1933–1942	50–59	50	0.98 (0.17)	47	1.0 (3.3)	46	−0.0 (6.3)	43	2.1 (8.0)	0.80 (0.13)[a]	−0.2 (4.9)	0.7 (10.0)	−2.6 (6.8)
1923–1932	60–69	50	1.04 (0.21)	49	1.3 (3.6)	47	1.4 (7.1)	41	2.3 (9.4)	0.77 (0.11)[a]	1.0 (7.0)	−0.1 (9.3)	0.3 (12.5)
1913–1922	70–79	50	0.97 (0.19)	37	0.1 (5.3)	31	−1.2 (7.9)	21	−1.1 (9.2)	0.71 (0.08)[a,b,c]	0.9 (6.3)	4.6 (10.2)	7.1 (16.7)[b]
1913–1952	40–79	200	1.01 (0.18)	181	0.8 (4.0)	170	0.0 (6.6)	147	1.1 (8.1)	0.79 (0.12)	0.5 (5.7)	0.5 (8.9)	−0.4 (11.7)
Women													
1943–1952	40–49	50	1.07 (0.14)	48	−1.1 (4.2)	47	−8.2 (9.3)	45	−11.6 (10.0)	0.79 (0.10)	−1.7 (5.0)	−3.0 (9.5)	−8.8 (9.3)
1933–1942	50–59	50	0.92 (0.16)[a]	50	−3.1 (5.7)	47	−8.5 (9.4)	47	−7.9 (11.8)	0.70 (0.11)[a]	0.1 (4.9)	−4.5 (8.1)	−6.4 (9.4)
1923–1932	60–69	50	0.78 (0.17)[ab]	47	−0.3 (3.9)[b]	42	−3.9 (5.3)	39	−3.5 (7.1)[a]	0.62 (0.09)[a,b]	1.5 (5.8)	−3.3 (8.3)	−5.0 (7.9)
1913–1922	70–79	50	0.77 (0.12)[ab]	43	−0.6 (4.9)	32	−2.8 (7.1)[ab]	29	−6.0 (9.4)	0.59 (0.10)[a,b]	−0.7 (6.7)	−3.8 (9.1)	−6.8 (10.7)
1913–1952	40–79	200	0.89 (0.19)	188	−1.3 (4.8)	168	−6.2 (8.4)	160	−7.5 (10.2)	0.68 (0.13)	−0.2 (5.7)	−3.6 (8.7)	−6.8 (9.3)

BMD bone mineral density, *n* number of subjects

[a] Significantly different ($P < 0.05$) from values of subjects in their 40s

[b] Significantly different ($P < 0.05$) from values of subjects in their 50s

[c] Significantly different ($P < 0.05$) from values of subjects in their 60s

osteoporotic fractures included 6 hip fractures (1 man, 5 women), 5 clinical vertebral fractures (1 man, 4 women), 2 wrist fractures (2 men), and 9 costal fractures (4 men, 5 women). Incidences of osteoporotic fractures in men in their 40s, 50s, 60s, and 70s were 20.8, 90.1, 44.4, and 82.6 per 10,000 person-years, respectively, and incidences in women were 20.2, 157.0, 158.2, and 170.3 per 10,000 person-years, respectively. Osteoporotic fractures tended to increase with age in women, whereas an age-related but non-significant tendency was seen in men.

Capacity of BTMs at baseline to predict rates of change of BMD and the occurrence of OP and osteoporotic fractures

Age-gender distributions of mean BTM levels at the initial survey are shown in Table 3. No significant difference was seen among age groups for BTM levels in men, while significant differences were seen for each marker between the 40s and 50–70s in women ($P < 0.05$ each).

Table 4 shows mean BTM levels in women at the initial survey classified by menstrual status, categorized into the following three groups: premenopausal group with regular period; perimenopausal group with irregular period; and postmenopausal group with no period within at least 1 year. No significant differences in BTM levels were seen between the pre- and perimenopausal groups, with the exception of serum PINP. Although a tendency toward increased levels in the perimenopausal group was seen compared to the premenopausal group, this was slight and non-significant. Conversely, all BTM levels measured in the present study were significantly higher in the postmenopausal group than in the premenopausal group ($P < 0.001$). In addition, serum total OC, PICP, PINP, beta-CTX, NTX, and urinary DPD were significantly lower in the perimenopausal group than in the postmenopausal group ($P < 0.05$). These results suggest that BTM levels were significantly accelerated after menopause.

We clarified whether values of BTMs at baseline could predict rates of change of BMD over 10 years, using multiple regression analysis with rate of change of BMD (%/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. No associations were identified between any BTMs and rate of change in BMDs at L2–4 or the femoral neck over 10 years.

Table 5 shows the hazard ratios (HRs) of BTMs (/SD) for the incidence of OP at L2–4 and the femoral neck. Cox proportional hazards modeling using the occurrence of OP (yes 1; no 0) as an objective factor and BTM level (/SD) at baseline as an explanatory factor after adjusting for age and weight, and menstrual status in women, at baseline identified only the PINP level as being significantly related to the occurence of OP at L2–4 in men ($P < 0.05$). By contrast, serum PINP, beta-CTX, and NTX and urinary DPD levels were significantly related to the occurence of OP at L2–4 in women (PINP, $P < 0.05$; beta-CTX, $P < 0.001$; NTX, $P < 0.01$; DPD, $P < 0.05$). In addition to the above-mentioned analysis, we then added the baseline BMD status, i.e., osteopenia or normal range, as an adjusted factor. Using the baseline L2–4 BMD, 36 men and 71 women were categorized into the group of spinal osteopenia. After adjusting for the baseline status of L2–4 BMD (1 osteopenia; 0 normal) in addition to age, weight, and menstrual status in women, the association between PINP and the occurrence of spinal OP was diluted in men (HR 3.88, 95% confidence interval [CI] 0.92–16.4, $P = 0.066$), but the association of the BTMs, with the exception of DPD, remained significant in women (PINP, HR 1.57, 95% CI 1.02–2.43, $P < 0.05$; beta-CTX, HR 1.99, 95% CI 1.30–3.05, $P < 0.01$; NTX, HR 1.68, 95% CI 1.69–2.65, $P < 0.05$; DPD, HR 1.42, 95% CI 0.99–2.04, $P = 0.056$). However, no BTMs were identified as significant predictors of the incidence of OP at the femoral neck in either men or women.

We estimated the HRs of BTMs (/SD) for the incidence of osteoporotic fracture by Cox proportional hazards modeling, using the occurrence of osteoporotic fractures (yes 1; no 0) as an objective factor, and BTM levels (/SD) at baseline as explanatory factors after adjusting for age and weight, and menstrual status in women, at baseline. No BTMs were identified as significant predictors of the incidence of osteoporotic fractures in men or women.

Discussion

The present study first clarified rates of bone loss at the lumbar spine and femoral neck over 10 years in the general population. BMD values for men had changed slightly by the 10-year follow-up, with the exception of femoral neck BMD for men in their 70s. Although the reason of the considerable increase in femoral neck BMD among men in their 70s is uncertain, it might be partially attributable to bone proliferative degeneration, such as hip osteoarthritis. Conversely, BMD values had decreased in all age strata for women over the 10 years, at an approximate mean rate of −7%/10 years, at both L2–4 and the femoral neck. BMD values decreased most rapidly among women in their 40s, suggesting a menopausal effect, whereas rates of change did not differ significantly among age strata.

We then clarified the incidence of OP and osteoporotic fractures over the 10 years among the general population. We have previously reported the incidence of OP in individuals aged 40–79 years living in a mountain village [33].

Table 3 Mean values of biochemical markers of bone turnover for participants at baseline, classified by age and gender

Age group (years)	*n*	Sera								Urine	
		Intact OC (ng/mL)	Total OC (ng/mL)	BAP (U/L)	PICP (μg/L)	PINP (ng/mL)	ICTP (μg/L)	β-CTX (ng/mL)	NTX (nmol BCE/L)	PYR (pmol/μmol Cr)	DPD (pmol/μmol Cr)
Men											
40–49	50	3.63 (1.70)	18.8 (7.5)	26.1 (9.3)	126.8 (36.1)	41.4 (17.3)	2.85 (1.71)	0.190 (0.107)	13.3 (2.8)	18.0 (6.5)	3.02 (1.28)
50–59	50	3.80 (4.28)	19.7 (18.3)	26.7 (16.6)	115.5 (33.0)	39.4 (28.1)	2.61 (0.83)	0.197 (0.162)	14.0 (6.0)	18.9 (7.7)	3.24 (2.41)
60–69	49	3.58 (1.55)	16.4 (6.1)	26.4 (7.1)	113.1 (28.5)	34.5 (13.3)	3.00 (1.07)	0.174 (0.107)	13.6 (4.1)	19.0 (7.5)	2.81 (0.97)
70–79	50	3.41 (1.77)	18.9 (8.1)	26.9 (10.7)	121.8 (34.3)	37.4 (16.0)	3.39 (1.06)[b]	0.187 (0.099)	13.5 (3.4)	21.5 (4.9)	3.17 (0.94)
40–79	199	3.60 (2.54)	18.5 (11.1)	26.5 (10.7)	119.3 (33.3)	38.2 (19.5)	2.96 (1.24)	0.187 (0.121)	13.6 (4.2)	19.3 (6.8)	3.06 (1.53)
Women											
40–49	50	3.58 (1.72)	14.9 (5.7)	19.7 (5.2)	96.1 (25.6)	30.6 (13.3)	2.51 (0.60)	0.103 (0.066)	11.6 (2.3)	20.7 (5.9)	3.20 (1.18)
50–59	50	5.68 (2.42)[a]	28.1 (8.8)[a]	30.2 (7.5)[a]	130.2 (41.1)[a]	57.8 (20.9)[a]	3.07 (0.62)[a]	0.255 (0.121)[a]	15.4 (3.3)[a]	27.5 (5.3)[a]	5.05 (1.34)[a]
60–69	50	6.61 (3.10)[a]	32.6 (12.4)[a]	32.3 (11.2)[a]	136.2 (35.7)[a]	59.2 (20.0)[a]	3.51 (1.25)[a]	0.301 (0.136)[a]	17.8 (4.4)[a,b]	32.0 (10.8)[a]	5.79 (2.14)[a]
70–79	50	6.09 (3.51)[a]	28.3 (10.3)[a]	32.2 (10.8)[a]	143.3 (39.2)[a]	52.8(20.0)[a]	3.56 (1.22)[a]	0.275 (0.153)[a]	16.0 (3.2)[a]	29.7 (8.9)[a]	4.95 (1.84)[a]
40–79	200	5.60 (3.01)	26.0 (11.6)	28.6 (10.4)	126.4 (40.0)	50.1 (21.9)	3.16 (1.06)	0.234 (0.145)	15.2 (4.0)	27.5 (9.0)	4.76 (1.91)

Values are given as means with standard deviations in parentheses

OC osteocalcin, *BAP* bone-specific alkaline phosphatase, *PICP* C-terminal propeptide of type I procollagen, *PINP* N-terminal propeptide of type I procollagen, *ICTP* C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase, *β-CTX* β-isomerized C-terminal cross-linking telopeptide of type I collagen, *NTX* N-terminal cross-linking telopeptide of type I collagen, *PYR* pyridinoline cross-links of collagen, *DPD* deoxypyridinoline cross-links of collagen, *Cr* creatinine

[a] Significantly different ($P < 0.05$) from values of participants in their 40s

[b] Significantly different ($P < 0.05$) from values of participants in their 50s

Table 4 Mean values of biochemical markers of bone turnover at baseline classified by menstrual status in women

Menstrual status	*n*	Age (years)	Sera							Urine	
			Intact OC (ng/mL)	Total OC (ng/mL)	BAP (U/L)	PICP (μg/L)	PINP (ng/mL)	ICTP (μg/L)	β-CTX (ng/mL)	NTX (nmol BCE/L)	PYR (pmol/μmol Cr)	DPD (pmol/μmol Cr)
Premenopause	41	44.2 (3.0)	3.35 (1.28)	14.5 (4.4)	19.0 (5.1)	96.5 (26.6)	29.5 (11.1)	2.49 (0.56)	0.099 (0.060)	11.3 (2.0)	20.7 (4.9)	3.12 (0.89)
Perimenopause	14	47.4 (4.9)	4.83 (2.75)	20.4 (10.2)	25.7 (7.0)	94.4 (24.8)	45.2 (25.9)[a]	2.88 (0.82)	0.165 (0.120)	13.0 (2.8)	24.1 (7.8)	4.09 (1.84)
Menopause	145	65.3 (8.1)[a,b]	6.20 (3.07)[a]	29.8 (10.8)[a,b]	31.6 (10.1)[a]	138.0 (38.3)[a,b]	56.4 (20.2)[a,b]	3.38 (1.10)[a]	0.278 (0.138)[a,b]	16.5 (3.7)[a,b]	29.7 (9.0)[a]	5.28 (1.86)[a,b]
Total	200	59.7 (11.6)	5.60 (3.01)	26.0 (11.6)	28.6 (10.4)	126.4 (40.0)	50.1 (21.9)	3.16 (1.06)	0.234 (0.145)	15.2 (4.0)	27.5 (9.0)	4.76 (1.91)

Values are given as means with standard deviations in parentheses

n number of subjects, *OC* osteocalcin, *BAP* bone-specific alkaline phosphatase, *PICP* C-terminal propeptide of type I procollagen, *PINP* N-terminal propeptide of type I procollagen, *ICTP* C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase, *β-CTX* β-isomerized C-terminal cross-linking telopeptide of type I collagen, *NTX* N-terminal cross-linking telopeptide of type I collagen, *PYR* pyridinoline cross-links of collagen, *DPD* deoxypyridinoline cross-links of collagen, *Cr* creatinine

[a] Significantly different ($P < 0.05$) from values of participants in the premenopausal group

[b] Significantly different ($P < 0.05$) from values of participants in the perimenopausal group

Estimated incidences of lumbar spine OP in men and women in that village over 10 years were 55.6 and 231.7 per 10,000 person-years, respectively. The incidences of spinal OP in the present study were approximately half of those found in the mountain village, indicating regional differences in the incidence of OP among Japanese populations. We have already reported on regional differences between cohorts in mountainous and seaside (the present cohort) areas [34–36]. Residents of the mountainous area tended to show lower BMDs [34], and proportions of fast bone losers were higher [35] than those in residents of the seaside area. In addition, we found that levels of BTMs, including serum intact OC and urinary PYR and DPD, in the residents of the mountainous area were significantly higher than those in residents of the seaside area [36]. These differences in the incidence of OP between regions support the concept of regional differences in bone metabolism in Japan, and might be due to environmental differences. From meteorological data in 1990, when the cohort from the mountainous area was started, the total annual duration of sunlight exposure was 1340.9 h in the mountainous area, lower than the 2224.4 h in the seaside area. In addition, based on the results of a nutrition survey of Wakayama Prefecture in 1993, total calcium intake for inhabitants of the mountainous area was 542 mg/day, compared to 563 mg/day for the seaside area [37]. However, this finding was only a result of an ecological study, not a direct survey of participants from our two cohorts. In addition, cohorts in both studies comprised only 400 individuals each, so these differences need to be confirmed in larger population-based cohorts. We have therefore established larger-scale cohorts based on the cohort in the present study, entitled the ROAD study [3, 38], in which BMD and X-ray examinations were performed in all 1,690 participants, and serum and urinary samples were collected. This enlarged population-based cohort study may confirm regional differences in bone metabolism and in OP and osteoporotic fractures.

The present study found no significant differences in baseline levels of the various BTMs among men in any age groups, with the exception of serum ICTP. By contrast, each marker showed significant differences between women in their 40s and those in their 50–70s ($P < 0.05$). In addition, the values of the BTMs in women started to increase in the perimenopausal period, with rapidly accelerating elevations after menopause, according to estrogen deficiency. We had already measured levels of endogenous estrogen and sex hormone-binding globulin in the present cohort, and reported serum estradiol (E2) and BMD levels among postmenopausal women at the 3-year follow-up [39], but cannot confirm any association between BTMs and endogenous sex steroids yet. Further studies to clarify levels of E2 and BTMs in women are warranted. In

Table 5 Hazard ratios of biochemical markers of bone turnover for the occurence of osteoporosis over the 10-year study period

	BTMs		Occurence of osteoporosis (L2–4)			Occurence of osteoporosis (femoral neck)		
	At baseline	Reference	HR	95% CI	Significance	HR	95% CI	Significance
Men								
Serum	Intact OC	+1SD	1.23	0.35–4.27		1.50	0.61–3.71	
	Total OC	+1SD	1.86	0.73–4.75		1.06	0.28–4.07	
	BAP	+1SD	0.95	0.23–3.93		1.61	0.73–3.59	
	PICP	+1SD	0.95	0.33–2.70		0.85	0.24–3.03	
	PINP	+1SD	2.80	1.18–6.63	*	1.09	0.32–3.69	
	ICTP	+1SD	0.74	0.17–3.25		1.10	0.26–4.58	
	β-CTX	+1SD	2.02	0.76–5.34		1.12	0.31–4.02	
	NTX	+1SD	0.95	0.29–3.08		0.64	0.09–4.54	
Urine	PYR	+1SD	1.79	0.79–4.06		2.11	0.98–4.53	+
	DPD	+1SD	2.86	0.78–10.50		1.53	0.63–3.73	
Women								
Serum	Intact OC	+1SD	0.78	0.47–1.29		0.99	0.64–1.53	
	Total OC	+1SD	1.52	0.92–2.52		1.32	0.90–1.93	
	BAP	+1SD	1.46	0.94–2.25	+	1.03	0.65–1.63	
	PICP	+1SD	1.13	0.69–1.84		1.00	0.62–1.64	
	PINP	+1SD	1.65	1.11–2.47	*	1.26	0.73–2.18	
	ICTP	+1SD	1.44	0.90–2.30		1.01	0.66–1.55	
	β-CTX	+1SD	1.80	1.27–2.56	***	1.21	0.76–1.91	
	NTX	+1SD	1.96	1.23–3.13	**	1.13	0.73–1.75	
Urine	PYR	+1SD	1.28	0.97–1.69	+	1.06	0.772–1.56	
	DPD	+1SD	1.40	1.06–1.84	*	1.23	0.84–1.80	

The hazard ratio was estimated using Cox proportional hazards modeling after adjustment for age and weight, and menstrual status of women, at the baseline

BTMs biochemical markers of bone turnover, *HR* hazard ratio, *CI* confidence interval, *OC* osteocalcin, *BAP* bone-specific alkaline phosphatase, *PICP* C-terminal propeptide of type I procollagen, *PINP* N-terminal propeptide of type I procollagen, *ICTP* C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase, *β-CTX* β-isomerized C-terminal cross-linking telopeptide of type I collagen, *NTX* N-terminal cross-linking telopeptide of type I collagen, *PYR* pyridinoline cross-links of collagen, *DPD* deoxypyridinoline cross-links of collagen

$^{+}$ $P < 0.1$; * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$

addition, we had already measured serum free testosterone (FT) levels in male subjects from the present cohort, and found that the serum FT level could offer a useful predictor of bone loss within 3 years [40]. The use of these data might clarify relationships among endogenous sex steroids and BTMs in men and women, and provide some clues to the distinct gender differences in BTM levels.

Regarding the capacity of BTMs to predict bone loss, Garnero et al. [9] reported that BTMs could be useful for forecasting BMD changes in the forearm over 4 years. Others have found that BTMs can only poorly predict bone loss at the spine and hip [10, 11]. Iki et al. [12] found an association between CTX and bone loss at the hip during the first 3 years of follow-up in a female population-based cohort followed for 6 years. In a previous report, we clarified that urinary PYR in men and serum intact OC in women were significantly related to BMD changes at the spine over 3 years [17]. Nevertheless, the present study could not identify any significant associations between BTMs and rates of change in BMDs over 10 years. The influence of BTMs measured at one specific point during BMD change thus appears to be limited to within a relatively short period, such as up to 4 years.

As few reports have examined associations between BTMs and the incidence of OP, evaluating the usefulness of BTMs as predictors of future OP is difficult. However, the present study found that high PINP levels in both men and women and high levels of serum beta-CTX, serum NTX, and urinary DPD in women were significant predictors of future OP at the lumbar spine. This association with PINP, beta-CTX, and NTX in women remained significant after adding baseline BMD status as an adjustment factor. This means that these BTMs could predict the future occurrence of spinal OP in women independent of baseline BMD status showing either osteopenia or normal range BMD. This shows that high bone turnover becomes an important determinant of the occurrence of spinal OP, particularly in women.

We could not establish any BTMs as useful predictors of OP at the femoral neck. Although the reasons for site differences in the predictive capacity of BTMs are obscure, we have previously reported that the characteristics of the lumbar spine and femoral neck differ among individuals who rapidly lose bone [41]. These results suggest that the predictive capacity of BTMs might differ according to the site involved. Different strategies are therefore required to prevent OP of the lumbar spine and that of the femoral neck.

Several reports have found that the risk of osteoporotic fractures could be predicted by BTM levels independently of BMD. Prospective studies of postmenopausal French women have clarified that higher levels of bone resorption markers are associated with an increased risk of osteoporotic fractures [13–15]. In contrast, the present study could not identify any associations between osteoporotic fractures and BTMs. The sample size and characteristics of the present cohort might explain this difference. Our cohort comprised 400 participants aged 40–79 years, and the mean age was approximately 60 years, which might be too young to collect a sufficient number of individuals with new osteoporotic fractures. In fact, only 32 fractures (10 in men, 22 in women) were accumulated during 10 years in the present cohort. Further observation in larger cohorts, such as that in the above-mentioned ROAD study, might be required to confirm the absence of an association between BTMs and osteoporotic fractures.

Besides the small sample size, the present study shows several limitations. First, samples were not all taken at a fixed time. Circadian variability is known to affect BTM levels, with levels of most BTMs increasing at night and peaking between 02:00 and 08:00, then rapidly decreasing to a nadir between 13:00 and 23:00 [42]. Because we collected samples at the point when BTM levels would have been decreasing towards the nadir, our results might represent underestimations. Second, long-term storage might have influenced the BTM levels. In this study, serum and urine samples were immediately frozen in dry ice and then stored in a deep freezer at −80°C within 24 h. However, serum total OC, BAP, PINP, beta-CTX, and NTX were measured in baseline samples after 7 years, as technical methods for identifying these BTMs were unavailable in 1993. Storage for 7 years at −80°C might thus have influenced BTM values, although Seibel et al. [43] stated that BTMs should remain stable in serum and urine samples if stored at −70°C or below and at −20°C or below, respectively.

On the other hand, one advantage of the present survey was that various BTMs were measured in men and women who were randomly selected from the general population and followed for a decade, with a high degree of compliance. Another advantage was that the effects of various BTMs on changes in BMD, the presence of individuals who rapidly lose bone, and the occurrence of OP and osteoporotic fractures could be estimated directly.

In conclusion, we clarified that various BTMs, including markers of both bone resorption and bone formation, such as PINP, beta-CTX, NTX, and DPD in women, and PINP in men, could predict the occurrence of spinal OP. Among these, PINP, beta-CTX, and NTX in women could predict the occurrence of spinal OP, independent of baseline BMD status. We therefore speculate that BTM levels could help to predict OP at the lumbar spine, especially in women, but not OP at the femoral neck, the rate of change in BMD, or osteoporotic fractures.

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Conflict of interest None.

References

1. Jornell O, Kanis JA, Oden A, Sembo I, Redlund-Johnell I, Petterson C, et al. Mortality after osteoporotic fractures. Osteoporos Int. 2004;15:38–42.
2. Muraki S, Yamamoto S, Ishibashi H, Nakamura K. Factors associated with mortality following hip fracture in Japan. J Bone Miner Metab. 2006;24:100–4.
3. 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 (ROAD). J Bone Miner Metab. 2009;27:620–8.
4. Ministry of Health, Labour and Welfare. The outline of the results of National Livelihood Survey 2007. Available at: http://www.mhlw.go.jp/toukei/list/20-19-1.html Accessed 21st December 2010.
5. Rosen CJ, Hochberg MC, Bonnick SL, McClung M, Miller P, Broy S, et al. Fosamax Actonel Comparison Trial Investigators. Treatment with once-weekly alendronate 70 mg compared with once-weekly risedronate 35 mg in women with postmenopausal osteoporosis. A randomized double-blind study. J Bone Miner Res. 2005;20:141–51.