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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

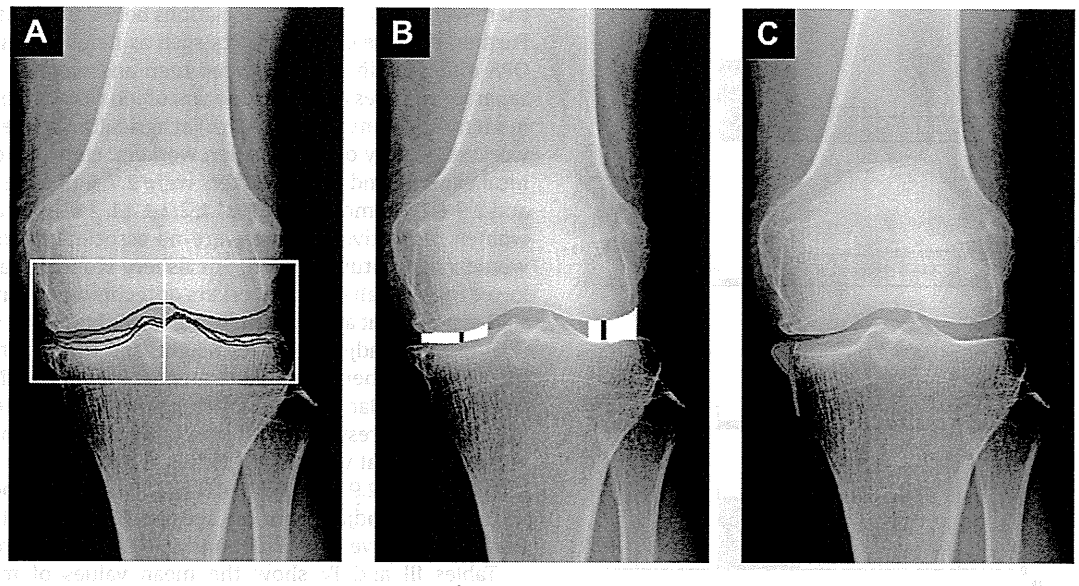

Fig. 1. Schema of image processing by KOACAD (cited from reference number[9]). (A). Outlines of anterior and posterior margins of the tibial plateau. The middle line between the two outlines is defined as the lower rim of the joint space. (B). Medial and lateral mJSWs were defined as the minimum vertical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

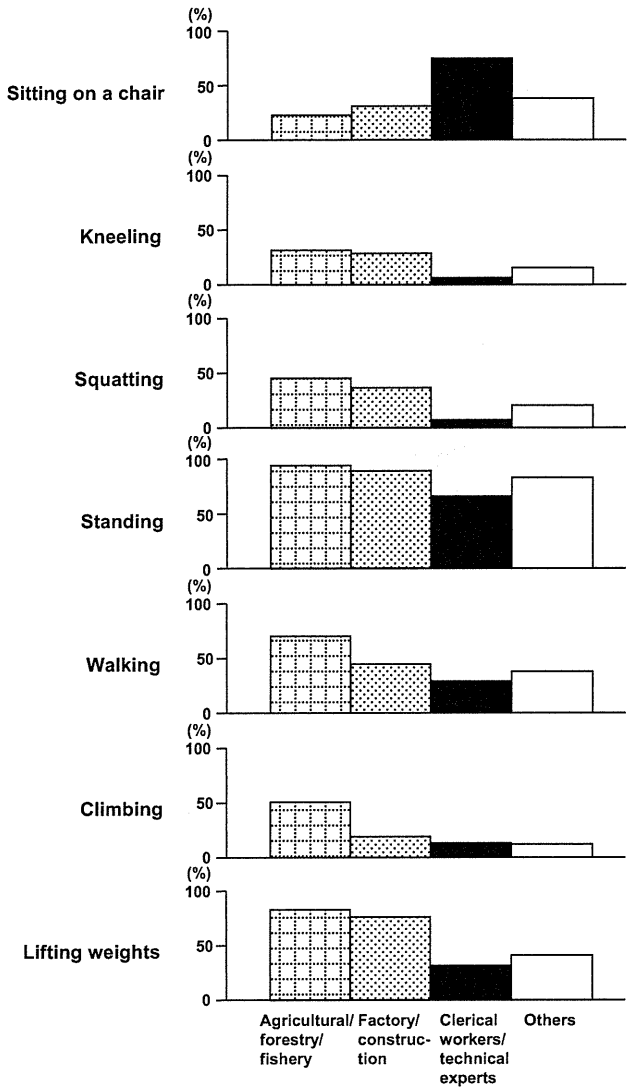

Fig. 2. Percentage of participants engaged in each occupational activity: sitting on a chair ≥2 h/day, kneeling ≥1 h/day, squatting ≥1 h/day, standing ≥2 h/day, walking ≥3 km/day, climbing ≥1 h/day, or lifting weights ≥10 kg ≥1 time/week among agricultural, forestry, and fishery workers; factory and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

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Association of vitamin D status with knee pain and radiographic knee osteoarthritis

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SUMMARY

Objective: The objective of the present study was to explore the association of serum vitamin D concentration and polymorphism in the vitamin D receptor (VDR), with knee pain and radiographic knee osteoarthritis (OA) among men and women in a large population-based UK cohort study.
Methods: Seven hundred and eighty-seven participants in the Hertfordshire Cohort Study (399 men, 388 women; mean age 65.6 ± 2.7 years) underwent a questionnaire on knee pain and radiographic knee examination. This study examined the association of Fok1, Cdx2 and Apa1 polymorphism in the gene for the VDR and serum 25(OH)D concentration with knee pain and radiographic knee OA by a generalized estimating equations population averaged logistic regression analysis in the Hertfordshire Cohort Study.
Results: There were no associations of Fok1, Cdx2 and Apa1 polymorphisms of the VDR with knee OA except for Aa for Apa1 compared with AA [Odds ratio (OR) 0.59, 95% confidence interval (CI) 0.36–0.95, $P = 0.031$]. While, ff for Fok1 (OR 1.60, 95% CI 1.07–2.39, $P = 0.022$) and AA for Cdx2 polymorphism (OR 2.21, 95% CI 1.07–4.56, $P = 0.032$) was significantly associated with higher prevalence of knee pain compared with FF for Fok1 and GG for Cdx2, respectively. None of these are statistically significant after adjusting for the three polymorphisms tested. 25(OH)D level was not significantly associated with radiographic knee OA, while, low tertile of 25(OH)D level tended to be associated with knee pain compared with high tertile of 25(OH)D level.
Conclusion: The present cross-sectional study using a large-scale population from the Hertfordshire Cohort study indicated that vitamin D may be associated with pain rather than radiographic change, but the evidence for an association between vitamin D genetic variation and pain in knee OA is very weak in the present study. Further replication of our results will be required to elucidate the association of vitamin D and knee OA.

Introduction

Knee osteoarthritis (OA) is a major public health issue that causes chronic pain and disability[1–3], although at present the pathogenesis of this condition remains largely unknown. Several environmental factors have been associated with OA, including obesity[4–6], previous injury[7], knee-bending occupations[8,9], and other metabolic factors[10,11]. A previous population-based UK study of twins has also demonstrated a clear genetic influence on radiologic knee OA in women, with up to 65% of the variance being explained by genetic factors[12].

Vitamin D has been shown to stimulate synthesis of proteoglycan by mature articular cartilage *in vitro*[13], and this suggests that vitamin D may directly affect articular cartilage metabolism. Vitamin D receptor (VDR) is found in many types of tissues, including chondrocytes[14,15]. A previous study showed that VDR gene polymorphism was associated with bone[16], although it is still controversial[17]. The relationship between osteoporosis and OA suggests that VDR gene polymorphisms may be associated with both diseases[18]. However, the association of VDR gene polymorphisms with knee OA is

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controversial[19–23]. This may be partly due to different races, differences in environmental factors related to vitamin D metabolism, or the presence of other genetic factors that influence VDR function.

The association of vitamin D level with knee OA is also controversial[24–28]. In previous studies, McAlindon suggested that low serum levels of vitamin D were associated with progression of knee OA[24]. A recent study has also shown that serum 25(OH)D levels were associated with decreased knee cartilage loss[28], but Hunter *et al.* found that there was no significant association between vitamin D levels and knee osteophytes after adjusting for age, body mass index (BMI) and relatedness[25]. The Framingham study also found no association of vitamin D levels with knee OA worsening[26]. This may be partly explained by VDR gene polymorphism, because vitamin D exerts its endocrine and autocrine/paracrine local effects upon binding to and activating its intracellular receptor VDR. In other words, the association of vitamin D level with knee OA may be different by VDR gene polymorphisms, but, to the best of our knowledge, there were no studies investigating the association of vitamin D level with knee OA by VDR gene polymorphisms.

The principal clinical symptom of knee OA is pain[29], but the correlation between pain and radiographic severity is inconsistent[4,30–32]. Fewer studies have addressed factors which might influence knee pain[32–35]; among these, older age, female gender, and physically demanding work, have all been proposed[30–33]. Previous studies, however, have not addressed the role of vitamin D status or fixed genetic variation in the VDR.

The objective of this study was to clarify the association of VDR gene polymorphism with knee pain and radiographic knee OA among men and women in the general population, as well as to examine the association between circulating vitamin D concentration and these indices of OA.

Subjects and methods

Subjects

The Hertfordshire Cohort Study is a population-based cohort study in the UK. Details of the study design have been published previously[36], thus, a brief summary is provided here. The selection procedure was as follows: using the National Health Service Central Registry at Southport, and Hertfordshire Family Health Service Association, we traced men and women who were born during 1931–1939 in Hertfordshire, and still lived there during the period 1998–2003. After obtaining written permission from each subject's general practitioner (GP), we approached each person by letter, asking him or her if they would be willing to be contacted by one of our research nurses. If subjects agreed, a research nurse performed a home visit and administered a structured questionnaire. This included information on socioeconomic status, medical history, drug history, cigarette smoking, alcohol consumption, and reproductive variables in women.

At a subsequent clinic, height was measured to the nearest 0.1 cm using a Harpenden pocket stadiometer (Chasmors Ltd, London, UK) and weight to the nearest 0.1 kg on a SECAfloor scale (Chasmors Ltd). Fasting venous whole-blood samples were taken at this clinic visit. Eligible subjects were then invited to book a return visit for knee radiography. Weightbearing anteroposterior and lateral semiflexed radiographs of both knees were taken at the same hospital using the same radiographic equipment; a standard tube to film distance of 100 cm was used. Radiographs were performed at a median duration of 6 months [interquartile range (IQR) 4.8–7.2] after the clinic visit. Radiographs were graded at the tibiofemoral joints using the Kellgren Lawrence (KL) grade[37]. One trained reader graded the radiographs; KL grade ≥ 2 was the threshold for a definition of knee OA. Subjects were also asked "Have you had any pain in or around your right knee on most days in the last month?" and "Have you had any pain in or around your left knee on most days in the last month?" Knee pain reported in this way was defined as having knee pain. A total of 498 men and 468 women completed a home questionnaire, attended clinic, and underwent knee radiography.

A fasting morning blood sample was obtained from all subjects at the first clinic visit, and the serum separated and stored at −70°C. 25(OH)vitamin D was assayed using a DiaSorin Liason automated chemiluminescent assay with equal specificity for both D2 and D3 (coefficient of variation for vitamin D across the assays was 10–12% for within batch and 10–15% between batch).

Genomic DNA was extracted from whole-blood samples according to standard procedures. VDR genotype was determined by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis, and three VDR polymorphic sites (Fok1, Cdx2 and Apa1) were analyzed.

Ethical permission for the study was granted by the East and North Hertfordshire Ethical Committees. All participants gave written informed consent.

Statistical analysis

To assess gene polymorphism effects on radiographic knee OA and knee pain, indicator variables were created for Fok1 (FF and ff), Cdx2 (GA and AA) and Apa1 (AA and aa) polymorphism. As both knees have a pain score and a radiographic grade, a generalized estimating equations (GEE) population averaged logistic regression model was used to adjust for clustering of knees within patients. To examine 25(OH)D levels and their association with knee OA and knee pain, we classified subjects into three categories; high tertile (>51.5 nmol/l), middle tertile (35.5–51.5 nmol/l) and low tertile (<35.5 nmol/l). A GEE population averaged logistic regression analysis was used to determine the association of vitamin D level with knee OA and knee pain with and without adjustment for age, gender, BMI, season of the clinic visit and KL grade. To decide whether statically significant associations between VDR polymorphisms and knee outcomes are noteworthy, we used Wacholder's method to calculate the False Positive Report Probability (FPRP) [Wacholder in JNCI 2004]. Data analyses were performed using SAS version 9.0 (SAS Institute, Cary, NC, USA) and Stata version 11.2 (Stata, College Station, TX, USA).

Results

Of 984 subjects, 170 (17.3%) provided incomplete pain questionnaires. A further 19 (1.9%) lacked genotypic information. We also excluded eight subjects with total knee arthroplasty, leaving 787 (399 males and 388 females) participants in this analysis. Comparison between the 787 subjects with complete information and those without complete information revealed no statistically significant differences in mean age (responders 65.6 years, nonresponders 65.7 years; $P = 0.66$), sex (responders 80.3% women, nonresponders 79.8% women; $P = 0.87$), BMI (responders 27.0, nonresponders 27.4; $P = 0.19$) or prevalence of knee OA (responders 15.2% women, nonresponders 16.3% women; $P = 0.74$). The characteristics of these participants are shown in Table I. The men were slightly younger than the women, and they had a lower mean BMI; serum vitamin D concentration was also significantly higher among men than women. There were no significant differences in mean values [IQR] of 25(OH)D concentration (nmol/l) among VDR gene polymorphisms of Fok1 [FF 45.5 (30.0–56.0), Ff 47.1 (31.5–56.8), ff 51.3 (31.0–69.0)], Cdx2 [GG 45.9 (30.6–56.0), AG 47.5 (31.2–60.2), AA 54.1 (36.7–68.0)] and Apa1 [AA 48.4 (32.9–61.3), Aa 47.5 (30.0–59.1), aa 42.7 (31.0–50.9)]. There were no significant

Table I
Characteristics of participants

	Overall	Men	Women	*P*-value
Number of subjects	787	399	388	
Age, years	65.6 (2.7)	64.8 (2.6)	66.4 (2.6)	<0.001
BMI, kg/m^2	27.0 (4.3)	26.8 (3.6)	27.2 (4.9)	0.22
25(OH)D level, nmol/l* means, (IQR)	42.5 (30.8, 57.3)	44.4 (34.7, 64.2)	41.0 (28.3, 54.1)	<0.001
Radiographic knee OA, *n*, (%)	120 (15.3)	70 (17.5)	50 (12.9)	0.069
Knee pain, *n*, (%)	309 (39.3)	147 (36.8)	162 (41.8)	0.16

Except where indicated otherwise, values represent means (standard deviation). The differences in age, BMI and 25(OH)D level between men and women were examined by the non-paired Student's *t*-test. The differences in prevalence of radiographic knee OA and knee pain between men and women were examined by chi square test.

* Of 787 subjects, 25(OH)D was measured in 683 subjects.

differences in the prevalence of radiographic knee OA and knee pain between genders. Of 120 subjects with radiographic knee OA, 79 (65.8%) had knee pain, while, of 667 subjects without radiographic knee OA, 230 (34.5%) had knee pain. Knee pain was significantly associated with radiographic knee OA after adjustment for age, gender and BMI [Odds ratio (OR); 3.03, 95% confidence interval (CI); 1.98–4.68].

We examined the association of VDR gene polymorphisms and radiographic knee OA (Table II). There were no associations of Fok1, Cdx2 and Apa1 polymorphisms of the VDR with knee OA except for Aa for Apa1 compared with AA after adjustment for age, gender and BMI, and FPRP values were low for association of Apa1 (Aa) on radiographic knee OA suggesting this association may be noteworthy. We also examined the associations of the alleles with knee OA. f for Fok1 tended to associate with higher prevalence of knee OA than F ($P = 0.06$). The alleles for Cdx2 and Apa1 were not significantly associated with knee OA ($P = 0.94$ and 0.64).

We also examined the association of VDR gene polymorphisms and knee pain (Table III). Unlike radiographic knee OA, Fok1 and Cdx2 polymorphism was significantly associated with prevalence of knee pain after adjustment for age, gender BMI and KL grade, and FPRP values were low for association of Fok1 (ff) for knee pain, suggesting this association may be noteworthy. There were no associations of Apa1 polymorphisms with knee pain. When analyzed in men and women separately, Fok1 polymorphism was significantly associated with knee pain after adjustment for age, BMI and KL grade in women (Ff: OR; 1.17, 95% CI; 0.75–1.81, $P = 0.486$, ff: OR; 2.46, 95% CI; 1.38–4.39, $P = 0.002$, compared with FF), while, not in men (Ff: OR; 1.10, 95% CI; 0.71–1.73, $P = 0.649$, ff: OR; 1.01, 95% CI; 0.58–1.76, $P = 0.98$, compared with FF). We also examined the associations of the alleles with knee pain. f for Fok1 had significantly associated with higher prevalence than F ($P = 0.01$). The alleles for Cdx2 and Apa1 were not significantly associated with knee pain ($P = 0.49$ and 0.64, respectively).

We next examined the association of 25(OH)D level and knee OA (Table IV). GEE logistic regression analysis showed that 25(OH)D level was not significantly associated with radiographic knee OA. For knee pain effect of vitamin D level was non-linear, so we classified subjects into three groups; high tertile (>51.5 nmol/l, $n = 225$), middle tertile (35.5–51.5 nmol/l, $n = 229$) and low tertile (<35.5 nmol/l, $n = 229$); low tertile of 25(OH)D level tended to be associated with knee pain compared with high tertile of 25(OH)D level after adjustment for age, gender, BMI, season of the clinic visit and KL grade (Table IV).

Discussion

This is the first study to examine the association of radiographic knee OA and knee pain with vitamin D level and VDR gene polymorphism at the same time. A Fok1 polymorphism of the VDR was significantly associated with radiographic knee OA and knee pain. There were no associations between radiographic knee OA and 25(OH) D level, while 25(OH)D level tended to be associated with knee pain.

The association of VDR gene polymorphism with OA is controversial[19–23]. In previous studies, a nested case-control study in Britain showed the 'T' allele was associated with knee OA in women[19]. The Rotterdam Study showed that the 'bAT' haplotype was associated with reduced prevalence of OA[20]. While, The Framingham study found no evidence for an association of the VDR gene with knee OA[23]. In a case-control study in Japan, there was also no significant association between VDR gene polymorphism and knee OA[21], although cases were sampled from hospital attenders in the study and controls did not undergo X-rays, causing the inevitable selection bias to occur. This inconsistency may also be due to differences in the relative importance of this gene in different races, differences in environmental factors related to vitamin D metabolism, or the presence of other genetic factors that influence VDR function. Further, the association of genetic factors with knee OA is diminishing later in life due to the effects of lifestyle factors, thus it may be difficult to find out their association in the elderly. In the present study, a Fok1 polymorphism of the VDR was significantly associated with radiographic knee OA. Vitamin D has been shown to stimulate synthesis of proteoglycan by mature

Table II
Association of VDR gene polymorphisms and radiographic knee OA

	Total	Number (%) with knee OA	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)*	*P*-value	Power†	FPRP prior probability	
								0.1	0.01
Fok1									
FF	328	39 (11.9)	1.00		1.00				
Ff	333	60 (18.0)	1.50 (0.96, 2.34)	0.072	1.54 (0.96, 2.46)	0.071	0.47	0.58	0.94
ff	108	18 (16.7)	1.38 (0.75, 2.57)	0.301	1.56 (0.82, 2.94)	0.173	0.27	0.85	0.98
Cdx2									
GG	491	75 (15.3)	1.00		1.00				
AG	248	37 (14.9)	1.03 (0.66, 1.59)	0.903	0.94 (0.60, 1.48)	0.781	0.49	0.93	0.99
AA	29	5 (17.2)	1.30 (0.48, 3.57)	0.605	1.09 (0.43, 2.74)	0.858	0.11	0.99	1.00
Apa1									
AA	213	36 (16.9)	1.00		1.00				
Aa	388	51 (13.1)	0.64 (0.40, 1.03)	0.068	0.59 (0.36, 0.95)	0.031	0.39	0.42	0.89
aa	166	31 (18.7)	1.12 (0.65, 1.91)	0.687	1.04 (0.60, 1.81)	0.884	0.28	0.97	1.00

Of 787 subjects, genotyping was completed for 769, 768 and 767 with Fok1, Csk2 and Apa1 polymorphism of the VDR, respectively.

* As both knees have a radiographic grade, GEE population averaged logistic regression analysis after adjustment for age, gender and BMI was used to calculate adjusted OR.

† To detect an OR of 1.5, we are looking for a difference in proportions of 15.3% vs 21.3% for radiographic knee OA.

Table III
Association of VDR gene polymorphisms and knee pain

	Total	Number (%) with knee pain	Crude OR (95% CI)	*P*-value	Adjusted OR* (95% CI)	*P*-value	Power†	FPRP prior probability	
								0.1	0.01
Fok1									
FF	328	115 (35.1)	1.00		1.00				
Ff	333	139 (41.7)	1.19 (0.89, 1.61)	0.244	1.14 (0.84, 1.56)	0.398	0.71	0.83	0.98
ff	108	51 (47.2)	1.50 (1.00, 2.24)	0.052	1.60 (1.07, 2.39)	0.022	0.40	0.33	0.84
Cdx2									
GG	491	189 (38.5)	1.00		1.00				
AG	248	94 (37.9)	1.05 (0.78, 1.42)	0.733	0.99 (0.73, 1.34)	0.936	0.71	0.92	0.99
AA	29	15 (51.7)	2.20 (1.08, 4.47)	0.03	2.21 (1.07, 4.56)	0.032	0.14	0.67	0.96
Apa1									
AA	213	87 (40.8)	1.00		1.00				
Aa	388	151 (38.9)	0.90 (0.65, 1.23)	0.5	0.93 (0.67, 1.30)	0.678	0.62	0.91	0.99
aa	166	64 (38.6)	0.97 (0.65, 1.43)	0.864	0.92 (0.61, 1.40)	0.71	0.45	0.93	0.99

Of 787 subjects, genotyping was completed for 769, 768 and 767 with Fok1, Csk2 and Apa1 polymorphism of the VDR, respectively.
* As both knees have a pain score, GEE population averaged logistic regression analysis after adjustment for age, gender, BMI and KL grade was used to calculate adjusted OR.
† To detect an OR of 1.5, we are looking for a difference in proportions of 39.3% vs 49.3% for knee pain.

articular cartilage *in vitro*[13], and this suggests that vitamin D may directly affect articular cartilage metabolism. Further, *in vitro* experiments confirmed that loss of VDR in chondrocytes reduced osteoclastogenesis by inducing receptor activator of NF-κB ligand (RANKL) expression[38], indicating that polymorphism of the VDR may affect osteophyte formation. In addition, the VDR gene has a thymine to cytosine single nucleotide polymorphism (SNP) at the Fok1 restriction site in the first of two potential start (ATG) codons located in the 50 region, resulting in a VDR protein that is shorter by three amino acids[39]. The F allele lacks the first ATG; thus, translation starts at the second ATG, instead of the first ATG, where translation of the f allele starts[40]. Most data indicate that the F allele is more effective than the f allele in transactivation of the 1,25-dihydroxyvitamin D signal[41]. However, a meta-analysis studying the association between VDR polymorphisms and OA[42] found no associations between VDR variation and OA. The ongoing GWAS studies on OA did not also find the foci polymorphism[43,44]. In the present study, the best *P*-value is only 0.022 which would be at least 0.066 when adjusted. Given the lack of a replication cohort, the evidence for an association between vitamin D genetic variation and pain in knee OA is very weak. In addition, considering that the sample size is modest for association studies in general, and more specifically for genetic association studies, the significant association of VDR gene polymorphism with radiographic knee OA in the present study may be due to random error. Additional and larger studies will be required, and, longitudinal studies may also determine whether this locus has any influence on the progression of joint damage at the knee.

IOF Working Group suggests that 75 nmol/L is the appropriate target level of serum 25(OH)D for individuals[45]. Vitamin D insufficiency, defined as 25(OH)D levels <75 nmol/L is prevalent worldwide[46], and the present study also showed that 604/683 (88.4%) had vitamin D insufficiency defined as <75 nmol/L. While, the association of serum vitamin D level and radiographic knee OA is controversial[24–27], McAlindon suggested that subjects with low serum levels of vitamin D are approximately three times more likely to have progression of established knee OA than subjects with high serum levels[24], but the number of subjects with progressive knee OA were comparably small in the study. Hunter *et al.* found that there was evidence of decreased vitamin D levels in subjects with knee osteophytes compared to those without osteophyte, but after adjusting for age, BMI and relatedness, the significant differences disappeared[25]. While, the Framingham study also found no association of vitamin D levels with knee OA worsening, defined as joint space loss on radiography or as worsening cartilage score on magnetic resonance imaging (MRI)[26]. In the present study, contrary to VDR gene polymorphisms, there were no significant association between vitamin D level and radiographic knee OA. Further, there were no differences in association of vitamin D level with radiographic knee OA among VDR gene polymorphisms.

Like radiographic knee OA, a Fok1 polymorphism of the VDR was significantly associated with knee pain in the present study. Further, knee pain also tended to be associated with vitamin D level, although it was not associated with radiographic knee OA. The correlation with the radiographic severity of knee OA is controversial[4,30–32]. In our previous study, 10% of men and 20% of women without radiographic knee OA had knee pain, and approximately 50% of men and 40% of women with severe radiographic knee OA had no knee pain in the elderly[4]. This indicates

Table IV
Association of 25(OH)D level with radiographic knee OA and knee pain

	Radiographic knee OA					Knee pain				
	n (%)	Crude OR (95% CI)	*P*-value	Adjusted OR* (95% CI)	*P*-value	*n* (%)	Crude OR (95% CI)	*P*-value	Adjusted OR† (95% CI)	*P*-value
25(OH)D level		0.99 (0.90, 1.10)	0.889	1.03 (0.92, 1.16)	0.627		–	–	–	–
Tertile 3 (51.2–147)	30/225 (13.3)	–	–	–	–	79/225 (35.1)	1.00		1.00	
Tertile 2 (35.9–51)	41/229 (17.9)	–	–	–	–	89/229 (38.9)	1.10 (0.77, 1.58)	0.598	1.04 (0.70, 1.56)	0.832
Tertile 1 (17–35.8)	36/229 (15.7)	–	–	–	–	105/229 (45.9)	1.48 (1.04, 2.10)	0.031	1.47 (0.95, 2.25)	0.08

OR of continuous vitamin D is for a 10-unit increase. For knee pain effect of vitamin D level was non-linear, so stratified into tertiles. Of 787 subjects, 25(OH)D was measured in 683 subjects.
* As both knees have and a radiographic grade, GEE population averaged logistic regression analysis after adjustment for age, gender, BMI and season of the clinic visit was used to calculate adjusted OR.
† As both knees have a pain score, GEE population averaged logistic regression analysis after adjustment for age, gender, BMI, season of the clinic visit and KL grade was used to calculate adjusted OR.

that there may be other factors associated with knee pain rather than radiographic knee OA, but there were few studies regarding factors associated with knee pain. Previous studies have shown that age, female sex and physical demanding work were associated with knee pain[32–35], but these factors were also reported as those associated with radiographic knee OA[4,9]. In the present study, vitamin D level tended to be associated with knee pain without association with radiographic knee OA, indicating that the association of vitamin D level with knee pain may be independent of radiographic knee OA. In fact, the result was almost similar after adjustment for radiographic knee OA, although it did not reach significance. Previous study has shown that vitamin D deficiency was related to quadriceps weakness[47], which is strongly associated with knee pain and disability in the community, even when activation and psychological factors are taken into account[48]. This may partly explain the association of vitamin D level and knee pain.

There are several limitations in the present study. First, the sample size was modest for association studies in general, and more specifically for genetic association studies. Further, we did not make multiple testing adjustments in the present study. In addition, studies reporting biomarker associations and, even more so, genetic associations have suffered from the report of false positives and the best way of addressing this is by testing these associations in independent cohorts and replicating the results. Thus the association of VDR gene polymorphisms with knee pain may be due to random error. However, FPRP values were low for association of Apa1 (Aa) on radiographic knee OA, and Fok1 (ff) for knee pain, suggesting these associations may be noteworthy, thus, these may merit replication in further studies. Second, we did not analyze Bsm and Taq, although these SNP are near Apa1. Third, 25(OH)D should have different association with different feature of ROA such as joint space narrowing or osteophytosis, but we did not analyze the association of joint space narrowing or osteophytosis with 25(OH)D or VDR polymorphisms.

In conclusion, the present cross-sectional study using a large-scale population from the Hertfordshire Cohort study revealed that a Fok1 and Cdx2 polymorphism of the VDR were significantly associated with knee pain, but not with radiographic knee OA. There were no associations between radiographic knee OA and vitamin D level, but it tended to be associated with knee pain. Further replication of our results will be required to elucidate the association of vitamin D and knee OA.

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.

Conflict of interest

There are no conflicts of interest.

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ORIGINAL ARTICLE

Capacity of endogenous sex steroids to predict bone loss in Japanese men: 10-year follow-up of the Taiji Cohort Study

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Abstract This prospective cohort study aimed to evaluate the capacity of endogenous sex steroids to predict male osteoporosis (OP) among community-dwelling inhabitants. Among 1,028 male residents aged 40–79 years, 50 men belonging to each age stratum (200 in total) were randomly selected from a resident registration list. In the years 1993, 1996, 2000, and 2003, bone mineral density (BMD) of the lumbar spine and proximal femur was measured by dual-energy X-ray absorptiometry. Serum total estradiol (E_2) and free testosterone (FT) were measured using samples extracted in 1993. Among the 200 participants at baseline, 153 subjects completed 10-year follow-ups. Mean values of serum E_2 and FT were 22.4 and 9.4 pg/ml, respectively. Rates of change for BMD at the lumbar spine and femoral neck were 0.8% and 0.5% during the first 3 years, 0.0% and 0.5% during 7 years, and 0.8% and –0.3% over 10 years, respectively. According to multivariate regression analysis after adjusting for age and body mass index, mean values of FT were significantly related to the rate of change of BMD at the femoral neck at 3 years (beta = 0.21; $r^2 = 0.05$; $P < 0.01$), but not at 7 or 10 years. Serum FT level could offer a useful predictor of bone loss within 3 years.

Keywords Testosterone · Estrogen · Bone loss · Male osteoporosis · Population-based cohort study

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Introduction

Osteoporosis (OP) is associated with impairment of activities of daily living (ADL) and quality of life (QOL), leading to increased morbidity and mortality in the elderly [1, 2]. As the proportion of the elderly population is rapidly increasing, an urgent need exists for the development of methods to prevent OP. The estimated number of patients with OP in Japan is about 10 million [3], and cases of hip fracture, as the most severe complication of OP and a key cause of bedridden status, are increasing annually, according to the results of a national survey [4].

Although OP is widely considered as a disorder that mainly affects women, 13% of cases of lumbar spine OP and 24% of cases of femoral neck OP involve men [3]. Up to 20% of hip fractures occur in men, and the number of men with fractures has been rising in Japan [3, 4]. In addition, several studies have shown higher mortality rates after hip fracture in men than in women [5–8], suggesting that male OP warrants urgent attention.

Estrogen is a well-known determinant of low bone mass, bone loss, and osteoporotic fracture in women [9–12]. Reports from the study of osteoporotic fracture suggest that in elderly women, undetectable levels of estradiol, which occur in about one-third of the population, are strongly associated with low bone mineral density (BMD), rapid

bone loss, and increased fracture risk [13–15]. In addition, lower androgen concentrations are reportedly weakly associated with lower BMD and rapid bone loss at some skeletal sites [13].

By contrast, less epidemiological evidence has been gathered regarding the influence of serum sex hormone levels on bone loss, OP, and osteoporotic fracture in men. Some studies of BMD in men have reported positive associations with endogenous androgen levels [16–19], but others have found no significant association [20, 21]. The influence of endogenous sex hormone concentrations on bone loss in men thus remains controversial.

In the present study, to clarify the age distribution of serum levels of endogenous sex steroids and to explore the predictive capacity of these levels for bone loss in men for the early detection of male OP, we measured baseline concentrations of endogenous sex steroids in male subjects randomly selected from a rural population in Japan and conducted follow-up for 10 years.

Materials and methods

Establishment of baseline cohort

This survey was performed in the Japanese town of Taiji. The Taiji cohort has been profiled in detail elsewhere [22–24] and so is summarized here only briefly. Taiji is located in the southern coastal area of Wakayama Prefecture. A list of all inhabitants born in 1913–1952, and therefore aged between 40 and 79 years old 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 topics such as dietary habits, smoking habits, alcohol consumption, and physical exercise (whole cohort).

From the whole cohort, 50 men 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 200 participants, were randomly selected. BMD was measured for these 200 participants in 1993. At this time, blood samples of all participants were taken. An interviewer administered a second questionnaire to these 200 participants covering items of past medical history, including questions related to osteoporotic fractures and falls, family history, calcium intake, dietary habits, physical exercise, occupational activities, sun exposure, and, for women, additional questions about reproductive variables (baseline study).

Measurements of endogenous sex steroids

At the baseline study in 1993, blood samples were taken from all participants. After centrifugation of blood samples, sera were immediately placed in dry ice, transferred to a freezer within 24 h, and kept at –80°C until assayed. Serum levels of total estradiol (E_2) and free testosterone (FT) were measured using an immunoradiometric assay (DPC-free estradiol kit and DPC-free testosterone kit, respectively; Mitsubishi Kagaku, Tokyo, Japan). The lowest measurable levels of E_2 and FT were 10 and 0.4 pg/ml, respectively, and percent of coefficient of variation (CV%) for E2 and FT were both less than 15% (unpublished data).

BMD measurements

Baseline BMD was measured in 1993 using dual-energy X-ray absorptiometry (DXA) (QDR 1000; Hologic, Bedford, MA, USA), providing anteroposterior images of lumbar vertebrae L2–L4 and the proximal femur (femoral neck, Ward's triangle, trochanter). These measurements were repeated on the same participants after 3, 7, and 10 years (1996, 2000, and 2003).

To control for the precision of DXA, the equipment was checked at every examination in 1993, 1996, 2000, and 2003 using the same phantom, and BMD of the phantom was 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.). Intraobserver variability for DXA scans by this investigator was 0.35% using the phantom, as reported previously [25].

Annual rates of change for BMD during 3-, 7-, and 10-year observations were calculated as follows:

$$\text{Annual rate (\%/year)} = [(\text{BMD follow-up} - \text{BMD baseline})/\text{BMD baseline/follow-up years}] \times 100$$

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.

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 (LSD) test for pairs of groups. Significant items were selected, and multiple regression analysis was performed with adjustment of suitable variables.

Results

Eligible participants and baseline characteristics

Background data including physical characteristics for all male participants at baseline are shown in Table 1. Mean weight and height in their fifties, sixties, and seventies, and mean body mass index (BMI) in their seventies were significantly lower than those in their forties ($P < 0.05$).

Among the 200 male participants at baseline, 1 man in his sixties declined to undergo blood and urinary examinations for endogenous hormones. Examinations at baseline were thus performed on 199 men. The second visit, aimed at evaluating changes in BMDs over 3 years, obtained measurements for 181 of the 200 initially recruited participants (90.5%). The following reasons were given for the loss of 19 participants at the 3-year follow-up: 8 men had died, 1 man had moved, 1 man was ill, 4 men declined to participate, and 2 men were away from the area at the time of follow-up. The third visit, aimed at evaluating changes in BMDs over 7 years, evaluated 170 of the 200 initially recruited participants (85%). Loss of 30 participants at the 7-year follow-up was explained as follows: 14 men had died, 3 men had moved, 6 men were ill, 5 men declined to participate, and 2 men were away from the area at the time of follow-up. Among the 200 male participants initially recruited, 153 men participated in the fourth visit held in 2003 (76.5%). Loss of 47 participants at the 10-year follow-up was explained as follows: 33 men had died, 6 men had moved, 4 men were ill, 2 men declined to participate, and 2 men were away from the area at the time of follow-up.

Mean levels of serum concentration of sex steroids at baseline

Age distributions of mean E_2 and FT levels at the initial survey are also shown in Table 1. Because data below the measurable range were excluded from analysis, E_2 and FT data could be obtained for 178 and 198 participants, respectively. Mean serum levels of E_2 and FT were 22.4 and 9.4 pg/ml, respectively. Although no significant age-related trends were seen for E_2, a significant trend toward low values of FT was noted according to age ($P < 0.001$). In addition, mean serum FT was significantly higher for men in their forties than for men in their sixties and seventies ($P < 0.05$).

Predictive capacity of endogenous sex steroids for bone change

Initial mean values and rates of change in L2–L4 BMD over the 3-, 7-, and 10-year periods, classified by age stratum, are shown in Table 2. BMD values at L2–L4 for men had increased slightly by the 10-year follow-up in their fifties and sixties but had decreased a little in the forties and seventies. BMD values at the femoral neck over 10 years had decreased for men in their forties and fifties and had increased considerably in their seventies.

According to multivariate regression analysis using each rate of change for BMD at the lumbar spine over 3, 7, and 10 years as an objective factor and serum levels of E_2 as an explanatory factor after adjusting for age and BMI, beta values for the rate of change for BMD for the first 3, 7, and 10 years were 0.02, 0.04, and –0.02, respectively. Similarly, on multivariate regression analysis using each rate of change for BMD at the femoral neck over 3, 7, and 10 years as an objective factor and serum levels of E_2 as an explanatory factor after adjusting for age and BMI, beta values for the rate of change for BMD for the first 3, 7, and 10 years were –0.07, 0.09, and –0.01, respectively. Total E_2 values could not predict bone change at the lumbar spine or femoral neck at 3, 7, or 10 years.

Again, using the results of multivariate regression analysis to clarify associations between serum FT and

Table 1 Summary characteristics for male participants at baseline classified by age

Birth cohort	Age-group (years)	*n*	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	E2 (pg/mL)		FT (pg/mL)	
			Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
1943–1952	40–49	50	44.2 (2.6)	168.8 (5.2)	69.0 (10.4)	24.2 (3.2)	46	22.1 (7.4)	50	10.9 (2.8)
1933–1942	50–59	50	54.8 (2.7)	165.6 (5.0)[a]	63.5 (9.4)[a]	23.1 (2.9)	43	22.2 (7.0)	50	9.8 (2.6)
1923–1932	60–69	50	64.6 (2.5)	163.0 (4.8)[a]	62.9 (9.6)[a]	23.6 (3.2)	46	23.1 (8.5)	49	8.8 (2.6)[a]
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)[a]	43	22.3 (7.7)	49	8.2 (3.1)[a]
1913–1952	40–79	200	59.4 (11.4)	164.5 (5.9)	63.2 (10.2)	23.3 (3.1)	178	22.4 (7.6)	198	9.4 (2.9)

BMI body mass index, *E2* total estradiol, *FT* free testosterone, *n* number of participants, *SD* standard deviation

[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

Table 2 Mean values (SD) of bone mineral density (g/cm^2) and change rate (%) at lumbar spine L2–L4 and femoral neck over 3, 7, and 10 years, classified by age and gender

Birth cohort	Age-group (years)	L2–L4								Femoral neck			
		Baseline		2nd visit (3-year follow-up)		3rd visit (7-year follow-up)		4th visit (10-year follow-up)		Basesline	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)
1943–1952	40–49	50	1.05 (0.15)	48	0.6 (3.8)	46	−0.6 (5.1)	43	−0.2 (5.8)	0.86 (0.09)	0.3 (4.6)	−1.8 (4.8)	−1.5 (10.9)
1933–1942	50–59	50	0.98 (0.17)	47	1.0 (3.3)	46	−0.0 (6.3)	46	1.6 (8.0)	0.80 (0.13)[a]	−0.2 (4.9)	0.7 (10.0)	−3.0 (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)	23	−1.5 (9.2)	0.71 (0.08)[a,b,c]	0.9 (6.3)	4.6 (10.2)[a]	6.6 (16.2)[b]
1913–1952	40–79	200	1.01 (0.18)	181	0.8 (4.0)	170	0.0 (6.6)	153	0.8 (8.1)	0.79 (0.12)	0.5 (5.7)	0.5 (8.9)	−0.3 (11.7)

SD standard deviation, *BMD* bone mineral density, *n* number of participants

[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

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

BMD changes at the lumbar spine and femoral neck, beta values of FT for the rate of change for BMD at the lumbar spine at the first 3, 7, and 10 years were 0.08, 0.08, and 0.03, respectively, and those at the femoral neck were 0.21, 0.14, and 0.06, respectively. Mean FT levels were significantly related to the rate of change for BMD at the femoral neck during the first 3 years ($R^2 = 0.05$, $P < 0.01$), but could not predict bone change at any site at 7 or 10 years.

Discussion

The present study examined endogenous hormone levels among men in Japan, measuring changes in BMD over spans of 3, 7, and 10 years. The present study clarified the age distribution of endogenous sex steroids, and a significant trend was seen toward low FT levels with age. FT tended to be significantly lower in the sixties and older when compared with levels in the forties in the present study. Our results support the findings of other reports. Orwoll et al. [26] showed that testosterone levels, particularly FT levels, for 2,623 men 65 years or older were associated with increasing age. Similar findings have been described in other cross-sectional and longitudinal studies [27–29]. Based on these results, we concluded that older men tended to show lower testosterone levels than younger men, similar to the situation with E_2 in women. Some men might display testosterone insufficiency, as seen in women with E_2 insufficiency. However, we do not yet have enough evidence regarding normal ranges in young men and thresholds for testosterone insufficiency. In addition, levels of testosterone may vary among individuals and be influenced by body composition such as adipose tissue, muscle, and bone.

In contrast to testosterone, no significant age-related trend in E_2 was found in the present study. Little information is available regarding E_2 levels in older men. Orwoll et al. [26] reported that E_2 concentrations decreased as age increased, and similar findings have been described in various reports [30–33]. However, other studies have noted stable [34–36] or rising [37] E_2 levels with increasing age. Although the reasons for these discrepancies are unclear, E_2 levels may vary among individuals and may be influenced by body composition such as adipose tissue, muscle, and bone, as well as testosterone.

Regarding the ethnic variations in serum sex steroid levels, as most previous reports have been based on studies of Caucasian men, ethnic variations in FT levels among men remain unclear. To the best of our knowledge, the Osteoporotic Fractures in Men Study (MrOS) is the only study in which a sufficient number of Asian men have participated [26]. For reasons of differences in measurement methods, direct comparison of the present results and

those from the MrOS study is inappropriate, but FT levels among Japanese men tended to be lower than those in MrOS participants, although no significant difference in E_2 levels was apparent. Orwoll et al. [26] analyzed ethnic differences in the MrOS study and stated that FT levels were lower in Asian men than in other races such as Caucasian, African-American, and Hispanic subjects, but no such differences were seen for E_2. The present results support these findings.

The present study found that serum levels of FT could offer a useful predictor of bone loss at the femoral neck within 3 years, but this effect was diluted with longer observation. Regarding the effects of testosterone on bone loss at the hip, Cauley et al. [38] reported, in an epidemiological study of 1,327 men ≥65 years old, that men in the lowest FT category experienced greater hip bone loss over 1.8 years. In addition, Ensrud et al. [39] reported that among men with weight loss, the rate of decline in total hip BMD showed a stepwise increase in magnitude with greater decreases in bioavailable testosterone from baseline. In the present study, the effect of FT levels on bone loss within the relatively short term up to 3 years was observed at the femoral neck, independent of age and BMI, supporting previous reports. Although reasons for site-specific differences in the predictive capacity of FT remain uncertain, we have already reported that bone loss rate differs depending on the site involved in another cohort study [40]. We have also reported that characteristics differ between fast bone losers at the lumbar spine and femoral neck [41]. One reason for site-specific differences might be because degenerative changes that increase BMD, such as osteophytosis or sclerotic change, are observed more frequently at the lumbar spine than at the femoral neck. These results suggest that the predictive capacity of FT might differ according to the sites involved.

A recent study showed that older men with total testosterone or E_2 deficiency were more likely to be osteoporotic [19], but no report evaluated the capacity of serum sex steroids to predict occurrence of OP. Regarding the relationship between testosterone and fracture risk, Mellstrom et al. [42] reported that FT within the normal range was independently associated with the presence, but not occurrence, of osteoporotic fracture in elderly men. In contrast, an analysis from the Rotterdam Study failed to confirm any association between testosterone and fracture risk [43]. Data from the Framingham study indicated that men with low serum testosterone and E_2 levels were at increased risk for incident hip fractures [44]. A recent report from the Dubbo osteoporosis epidemiology study revealed that in men older than 60 years, serum testosterone is independently associated with the risk of osteoporotic fracture [45]. We also tried to evaluate the predictive capacity of serum levels of sex steroids and occurrence of OP based on WHO criteria [46] and osteoporotic fractures, but only identified 7 cases of OP and 10 cases of osteoporotic fractures including 1 vertebral fracture, 1 hip fracture, 2 wrist fractures, 3 costal fractures, 2 ankle fractures, and 1 finger fracture. After analysis using Cox proportional hazards models adjusted for age and BMI, serum levels of FT were significantly related to incidence of OP (hazard ratio, 0.42; 95% confidence interval, 0.19–0.90), but not to incidence of osteoporotic fractures. This analysis suggests the possibility of serum FT as a predictor for OP occurrence over 10 years. However, the number of occurrences of OP seems to be too small to reach any conclusion regarding the presence or absence of associations between sex steroids and OP or osteoporotic fractures.

There are several limitations in the present study. First, the small sample size seemed to be the most severe weakness. In fact, as already noted, only 7 cases of OP and 10 cases of osteoporotic fractures were accumulated during the 10 years of the study. Longer observation in the present cohort might be required to confirm the association between sex steroids and OP or osteoporotic fracture. Second, the dropout rate over 10 years for patients in their seventies was considerably high (54.0%). This high dropout rate might cause bias. In fact, the tendency toward an increase in BMD at the femoral neck for patients in their seventies was skewed by withdrawal bias. On the basis of this hypothesis, we reanalyzed the multivariate regression analysis to assess the change rate of BMD at the femoral neck and serum FT with exclusion of subjects in their seventies. However, the results were similar, with serum levels of FT predicting bone loss at the femoral neck within 3 years (beta = 0.17, $P = 0.05$), but diluted effects with longer observation (7 years: beta = 0.8, $P = 0.38$; 10 years: beta = 0.03, $P = 0.77$). Third, all serum samples were extracted between 0900 and 1500, not at a fixed time in the morning, although samples for measurement of FT are recommended to be collected in the morning. Serum levels of testosterone tend to increase toward night, peaking in the early morning, then decreasing rapidly and reaching a nadir between 1300 and 2300. We collected samples when FT levels would probably have been decreasing toward the nadir. The present study might thus have underestimated FT values compared to collection at a fixed time in the morning.

Conversely, the study design shows several notable strengths. In this population-based cohort study, subjects were selected randomly from the resident registration list. BMD was carefully measured by a single observer (N.Y.), and measurements were repeated 3, 7, and 10 years later with high participation rate by the same device and same observer. Another strength was that the effect of serum levels of sex steroids on changes in BMD could be estimated directly.

In conclusion, we clarified that serum levels of FT could predict bone loss within 3 years, but not longer. Further observations are required to confirm the relationship between FT, E_2, and spinal OP and osteoporotic fractures. Other environmental and genetic factors should also be evaluated to develop strategies for the early prevention of OP.

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

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