Introduction

Ossification of the posterior longitudinal ligament of the spine (OPLL) is the pathological ectopic ossification of this ligament at the cervical and thoracic spine. It causes myeloradiculopathy as a result of chronic pressure on the spinal cord and nerve roots [1, 2]. Epidemiologic studies have shown a relatively high prevalence of OPLL among the Japanese, a slightly lower prevalence among East Asians and a substantially lower prevalence among whites [3, 4].

In terms of its characteristics, several epidemiological studies have reported that adult-onset obesity and diabetes mellitus (DM) are independent risk factors of OPLL [5, 6]. Further, OPLL often coincides with diffuse idiopathic skeletal hyperostosis (DISH), a systemic disorder of hyperossification. McAfee et al. [7] found that seven (50 %) of 14 patients with OPLL had DISH, and in a Japanese study, DISH was present in 27 (25 %) of 109 patients with OPLL [8].

Besides the coexistence of other disorders such as DM and DISH, little detailed information is available on the profile of OPLL in the general population. These data are important in order to characterise the disease burden. In addition, limited information is available regarding factors associated with OPLL, including biochemical markers of bone turnover, bone mineral density (BMD) values, lifestyle factors, or other coexisting disorders, such as dyslipidaemia, impairment of glucose tolerance, lumbar spondylosis (LS) and knee osteoarthritis (KOA).

Thus, the aims of the present study were to clarify the prevalence of OPLL in the Japanese population and to examine the association of OPLL with biological and environmental factors as well as coexisting disorders. For this, we used a questionnaire survey and the large, population-based cohort Research on Osteoarthritis/osteoporosis Against Disability (ROAD), which included lifestyle factors and nutrition, blood and urinary examinations, BMD measurements and X-ray examinations [9, 10].

Methods

Outline of the ROAD study

We conducted the present study using the cohorts established in 2005 for the ROAD study. The ROAD study is a nationwide, prospective study of OA comprising population-based cohorts from several communities in Japan. The details of the cohort profile have been reported elsewhere [9, 10]. Briefly, in 2005–2007, we created a baseline database that included clinical and genetic information for 3,040 residents of Japan (1,061 men, 1,979 women); the mean age (deviation [SD]) of the participants was 70.3 [11.0]years (71.0 [10.7]years for men and 69.9 [11.2]years for women). The subjects were recruited from resident registration listings in three communities with different characteristics: 1,350 subjects (465 men, 885 women) were from an urban region in Itabashi, Tokyo; 864 subjects (319 men, 545 women) were from a mountainous region in Hidakagawa, Wakayama and 826 subjects (277 men, 549 women) were from a coastal region in Taiji, Wakayama.

The participants completed an interviewer-administered questionnaire of 400 items that included lifestyle information such as occupation, smoking habits and alcohol consumption; family history; medical history; physical activity; reproductive variables and health-related quality of life. A questionnaire was prepared by modifying the one used in the Osteoporotic Fractures in Men Study [11], and some new items were added to the modified questionnaire. The participants were asked whether they took prescription medication daily or nearly every day (0 = no, 1 = yes). If participants did not know the reason for the prescribed medication, they were asked to bring their medications to the medical doctor (NY).

Anthropometric measurements included height (in centimetres), body weight (in kilograms), arm span (in centimetres), bilateral grip strength (in kilograms) and body mass index (BMI; in kilograms per square metre). Experienced orthopaedic surgeons collected medical information on systematic, local and mental status, including information on back, knee and hip pain; swelling and range of motion of the joints and patellar and Achilles tendon reflexes.

In 2008–2010, we attempted to locate and follow up all 3,040 subjects. They were invited for the second survey of the ROAD study, which included a 3-year follow-up of the same examinations as the baseline.

Subjects eligible for the present study

In the present study, we enrolled all 1,690 subjects (men, 596; women, 1,094) from mountainous and coastal areas who had enrolled in the ROAD study. In the ROAD study, X-ray examination of the cervical and thoracic spine had been performed only for these subjects and not for those from the urban region. Further, for all these 1,690 participants, the BMDs for the lumbar spine and the proximal femur had been measured using dual energy X-ray absorptiometry (Hologic Discovery; Hologic, Waltham, MA, USA) during the baseline examination. Additionally, blood and urinary examinations had also been performed for these subjects.

The study participants provided written informed consent, and the study was conducted with the approval of the ethics committees of the University of Tokyo (no. 1264 and no. 1326) and the University of Wakayama Medical University (no. 373).

Radiographic assessment

Plain radiographs were obtained for the cervical, thoracic and lumbar spine in the anteroposterior and lateral views and both knees in the anteroposterior view with weight-bearing and foot-map positioning.

Cervical OPLL was diagnosed using plain radiographs of the cervical spine in the lateral view. OPLL was indicated by the presence of heterotopic ossification in the posterior longitudinal ligament on a lateral cervical radiograph. Radiographic OPLL was diagnosed by a single, experienced orthopaedic surgeon (KN) who was blinded to participants' clinical status. OPLL was classified into the following types: continuous, segmental and mixed. In the original OPLL classification by Tsuyama [3], it was categorised into four modes, namely continuous, segmental, mixed and localised. However, here, because of the small number of subjects in the localised category, these subjects were included in the continuous category. If OPLL was observed, the maximum length (continuous and localised type, upper limit to lower limit; segmental and mixed types, upper limit to lower limit of the longest serial region) and width of ossification were measured using the imaging software OsiriX (http://www.osirix-viewer.com/).

In addition, using radiographs of spine and knees, we determined the grade of OA. The severity of radiographic OA was determined according to the Kellgren–Lawrence (KL) grading [12] as follows: KL0, normal; KL1, slight osteophytes; KL2, definite osteophytes; KL3, joint or intervertebral space narrowing with large osteophytes and KL4, bone sclerosis, joint or intervertebral space narrowing and large osteophytes. Radiographs for each site, i.e. the vertebrae and knees, were examined by a single, experienced orthopaedic surgeon (SM) who was blinded to participants' clinical status. In the present study, the subject's KL grade was considered the maximum grade diagnosed for at least one intervertebral level of the lumbar spine or at least one knee joint.

We also investigated the presence of DISH using whole-spine X-ray films. The criterion for the definite diagnosis of DISH was the presence of four or more vertebral bodies with contiguous ligamentous ossification and calcification, which is known as Resnick and Niwayama's criterion [13]. DISH was diagnosed by a single, experienced orthopaedic surgeon (RK) who was blinded to participants' clinical status.

Blood and urine examinations

Samples were collected from the end of October to the middle of January from both mountainous and coastal areas. All blood and urine samples were extracted between 0900 and 1500 hours. The blood samples were centrifuged, and the sera and urine samples were immediately placed on dry ice and transferred to a deep freezer within 24 h. The samples were stored at −80 °C until assayed.

The blood samples were used to measure haemoglobin A1c (HbA1c, Japan Diabetes Society), serum levels of total cholesterol, uric acid and creatinine levels. The analyses were performed at the same laboratory within 24 h of collection (Osaka Kessei Research Laboratories, Inc., Osaka, Japan).

Serum levels of intact parathyroid hormone (iPTH) were measured using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). As a marker of bone formation, serum levels of N-terminal propeptide of type I procollagen (PINP) were measured using a radioimmunoassay (Orion Diagnostics, Espoo, Finland). The urinary levels of β-isomerised C-terminal cross-linking telopeptide of type I collagen (β-CTX), a bone resorption marker, were determined using an enzyme-linked immunosorbent assay (Fujirebio, Inc., Tokyo, Japan). Urinary β-CTX values were standardised to urinary creatinine concentrations. Plasma pentosidine levels were detected using a competitive ELISA kit (FSK pentosidine ELISA kit; Fushimi Pharmaceutical, Kagawa, Japan) as previously described [14].

Three-year follow-up and definition of OPLL occurrence and progression

In 2008–2010, the 1,690 subjects were invited to enrol in the second survey of the ROAD study, a 3-year follow-up consisting of examinations identical to those conducted at baseline. Spine and knee radiographs were also obtained at follow-up. All cervical radiographs were read by the same orthopaedic surgeon who read them at the baseline (KN), and he was again blinded to participants' clinical status. He simultaneously compared the X-ray films at the baseline and 3-year follow-up and thereby diagnosed OPLL. A new OPLL case was diagnosed if heterotopic ossification in the posterior longitudinal ligament was absent on the lateral cervical radiograph obtained at baseline but present in that obtained during follow-up. OPLL progression was defined as an increase in the maximum length or width of the heterotopic ossification during follow-up compared to that at baseline.

Statistical analysis

All statistical analyses were performed using STATA statistical software (STATA Corp., College Station, TX, USA). Differences in proportions were compared using the chi-square test. Differences in continuous variables were tested for significance using analysis of variance for multiple groups or Scheffe's least significant difference test for pairs of groups. All p values and 95 % confidence intervals (CI) are two sided.

To test the association between OPLL and potential risk factors, we used logistic regression analysis with the presence or absence of OPLL (0 = absence, 1 = presence) as an objective variable and select potential explanatory variables, in addition to basic characteristics such as age (+1 year), gender (0 = men, 1 = women) and regional differences (0 = mountainous area, 1 = coastal area). The selected associated factors were those that showed a significant ($p < 0.05$) association with OPLL status in a simple linear analysis. To test the association between OPLL progression and associated factors, we used multivariate

regression analysis with the change rate (percent per year) of the maximum length or width as an objective variable and the explanatory variables used in the above-mentioned logistic regression analysis. The explanatory variables in the logistic regression analysis and multivariate regression analysis are described in the "Results" section.

Results

Prevalence of radiographic OPLL

The X-ray radiographs of 1,562 of the 1,690 subjects (92.4 %, 520 men, 1,038 women) showed all parts of the lateral cervical spine, from C1 to C7. Among these 1,562 individuals, 30 (17 men, 13 women) were diagnosed with radiographic OPLL; thus, the prevalence of OPLL was estimated at 1.9 % (men, 3.2 %; women, 1.3 %), and it was significantly higher in men than in women ($p=0.007$).

Figure 1 shows the prevalence of OPLL classified by age and gender. The prevalence of OPLL was not associated with age in either men or women.

In the 30 subjects with radiographic OPLL, the OPLL was categorised into the continuous type in 13 subjects (six men and seven women, 43.3 %), the segmented type in eight (six men and two women, 26.7 %), the mixed type in seven (four men and three women, 23.3 %) and the localised type in two (one man and one woman, 6.7 %). The largest OPLL region was most commonly observed in C4 (ten individuals; 33.3 %; three men and seven women), followed by C5 (nine individuals; 33.0 %; eight men and one woman), C3 (seven individuals; 23.3 %; four men and three women), C6 (three individuals; 10.0 %; two men and one women) and C2 (one individual; 3.3 %; one woman). The largest OPLL region was not found in C1 or C7 in any subject.

The mean length and width (standard deviation, SD) of the largest region of ossification at the baseline were 27.6 (16.0) and 3.0 (1.5)mm, respectively. The values in men were 26.1 (14.5) and 2.9 (1.4)mm, and those in women were 29.6 (18.1) and 3.2 (1.5)mm, respectively; thus, no significant difference was observed between men and women in this regard.

Factors associated with OPLL

Table 1 shows the baseline characteristics of 1,562 participants with and without OPLL. Overall, subjects with OPLL tended to be taller and heavier than those without OPLL ($p<0.05$). Further, compared to individuals without OPLL, those with OPLL had higher plasma pentosidine levels and higher BMD values for both the lumbar spine (L2–4) and femoral neck ($p<0.05$).

Table 1 also shows the prevalence of LS, KOA and DISH on the basis of OPLL status. The prevalence of LS with ≥grade 2 KL and that of DISH was higher in the group with OPLL than in the one without OPLL ($p<0.05$), although no significant association was observed between the prevalence of KOA and the presence of OPLL.

Logistic regression analysis was performed with the OPLL status as the objective variable (0 = absence, 1 = presence). As explanatory variables, the analysis involved select associated factors that showed a significant ($p<0.05$) association with OPLL status in the simple linear analysis, namely, height (in centimetres), weight (in kilograms), values of plasma pentosidine (+1 μg/mL), BMD of the femoral neck (+1 SD), presence of LS based on KL grade (0 = KL grade 0 or 1, 1 = KL grade≥2) and DISH (0 = absent, 1 = present), after adjustments were made for age (years) and gender (0 = men, 1 = women). As seen from Table 2, plasma pentosidine levels, BMD of the femoral neck and the presence of DISH were found to be significant associated factors for the presence of OPLL (Table 2). Further, when BMD of the lumbar spine (L2–4) was used instead of that of the femoral neck, this factor was also found to be significantly associated with OPLL (+1 SD; odds ratio (OR), 1.52; 95 % CI, 1.05–2.20; $p=0.026$), but the

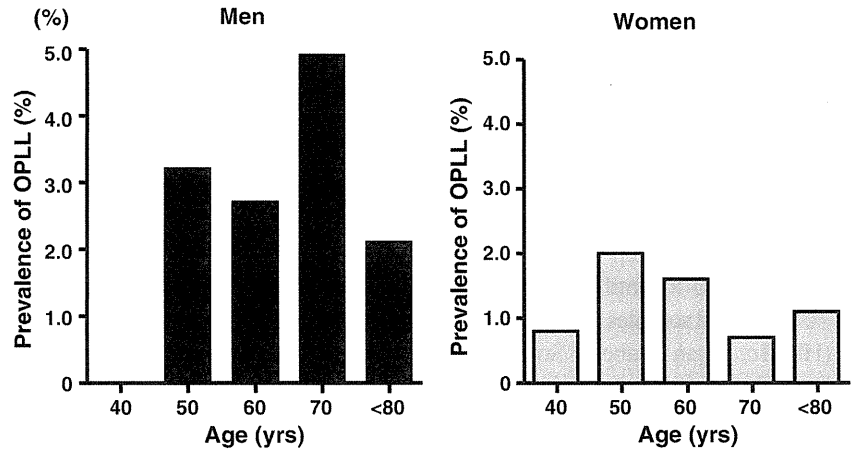

Fig. 1 Prevalence of OPLL classified by age and gender

Table 1 Baseline characteristics of participants classified by the presence or absence of OPLL

	Total (N=1,562)			Men (N=524)			Women (N=1,038)		
	OPLL (−) N=1,532	OPLL (+) N=30	p	OPLL (−) N=507	OPLL (+) N=17	p	OPLL (−) N=1,025	N=1,025 N=13	p
Age distribution (prevalence, %)									
30 years and younger	43	0 (0.0)		12	0 (0.0)		31	0 (0.0)	
40–49 years	141	1 (0.7)		39	0 (0.0)		102	1 (1.0)	
50–59 years	291	7 (2.4)	0.729	92	3 (3.2)	0.604	199	4 (2.0)	0.787
60–69 years	449	9 (2.0)		142	4 (2.7)		307	5 (1.6)	
70–79 years	468	11 (2.3)		175	9 (4.9)		293	2 (0.7)	
80 years and older	140	2 (1.4)		47	1 (2.1)		93	1 (1.1)	
Age (years), mean (SD)	62.9 (12.1)	67.0 (9.3)	0.3495	66.0 (11.7)	70.7 (8.0)	0.0990	64.4 (12.2)	62.2 (9.0)	0.5069
Height (cm), mean (SD)	154.9 (9.1)	159.1 (7.5)	0.0132*	163.3 (7.0)	163.9(5.4)	0.7414	150.8 (6.9)	152.8 (4.6)	0.2945
Weight (kg), mean (SD)	55.0 (10.3)	60.3 (10.1)	0.0053**	61.6 (10.5)	62.7 (8.2)	0.6759	51.7 (8.5)	57.1 (11.7)	0.0219*
BMI (kg/m^2), mean (SD)	22.8 (3.2)	23.8 (3.4)	0.1135	23.0 (3.1)	23.3 (2.1)	0.7434	22.7 (3.3)	24.4 (4.6)	0.0671
Residing in the coastal area (%)	49.4	53.3	0.671	46.4	58.8	0.311	50.9	46.2	0.732
Current smoking habit (regularly, ≥1/month) (%)	12.9	23.3	0.095	31.1	41.2	0.377	3.8	0.0	0.472
Current alcohol consumption (regularly, ≥1/month) (%)	39.1	43.3	0.637	66.1	64.7	0.907	25.8	15.4	0.395
Total cholesterol (mg/dL), mean (SD)	208.8 (34.5)	209.6 (36.2)	0.8954	198.6 (34.1)	204.4 (33.5)	0.4874	213.8 (33.6)	216.4 (39.8)	0.7840
Uric acid (mg/dL), mean (SD)	4.84 (1.30)	5.24 (1.21)	0.0943	5.71 (1.26)	5.71 (1.03)	0.9867	4.42 (1.09)	4.65 (1.21)	0.4528
HbA1c (Japan Diabetes Society) (%), mean (SD)	5.17 (0.70)	5.38 (0.79)	0.1124	5.20 (0.79)	5.44 (0.95)	0.2162	5.16 (0.64)	5.29 (0.56)	0.4595
Serum levels of iPTH (pg/mL), mean (SD)	41.2 (34.4)	41.2 (14.2)	0.9952	42.6 (54.4)	41.1 (13.9)	0.9083	40.5 (17.4)	41.3 (15.1)	0.8748
Serum levels of PINP (μg/L), mean (SD)	57.9 (27.0)	52.6 (29.9)	0.2915	47.5 (22.0)	42.6 (14.9)	0.3619	63.1 (27.8)	65.8 (39.2)	0.7301
Urinary levels of β-CTX (μg/mmol Cr), mean (SD)	187.2 (121.3)	150.4 (79.1)	0.0985	128.4 (78.7)	119.8 (58.3)	0.6529	216.2 (128.0)	190.5 (86.8)	0.4693
Plasma levels of pentosidine (μg/mL), mean (SD)	0.058 (0.037)	0.085 (0.140)	0.0005***	0.061 (0.048)	0.102 (0.184)	0.0042**	0.057 (0.030)	0.062 (0.037)	0.5012
BMD of the lumbar spine L2-4 (g/cm^2), mean (SD)	0.925 (0.205)	1.084 (0.205)	<0.0001***	1.038 (0.203)	1.176 (0.176)	0.0058**	0.868 (0.181)	0.965 (0.181)	0.0575
BMD of the femoral neck (g/cm^2), mean (SD)	0.667 (0.137)	0.747 (0.134)	0.0016**	0.739 (0.132)	0.797 (0.110)	0.0727	0.631 (0.124)	0.681 (0.139)	0.1558
Presence of LS (KL grade≥2) (%)	61.8	83.3	0.016*	76.1	100.0	0.022*	54.7	61.5	0.624
Presence of KOA (KL grade≥2) (%)	49.5	56.7	0.440	41.4	41.2	0.986	53.6	76.9	0.093
Presence of DISH (%)	9.4	33.3	<0.001***	0.7	52.9	0.002**	3.8	7.7	0.469

OPLL ossification of posterior longitudinal ligament, *SD* standard deviation, *BMI* body mass index, *HbA1c* haemoglobin A1c, *iPTH* intact parathyroid hormone, *PINP* N-terminal propeptide of type I procollagen, *β-CTX* β-isomerised C-terminal cross-linking telopeptide of type I collagen, *BMD* bone mineral density, *LS* lumbar spondylosis, *KOA* knee osteoarthritis, *KL grade* Kellgren–Lawrence grade, *DISH* diffuse idiopathic skeletal hyperostosis, *OPLL(−)* absence of OPLL, *OPLL(+)* presence of OPLL

*p<0.05; **p<0.01; ***p<0.001

Table 2 Odds ratios of potential factors associated with the presence of OPLL vs. the absence of OPLL

Explanatory variables	Reference	OR	95 % CI	*p*
Age (years)	+1 year	1.03	0.98–1.07	0.269
Gender	0 = men, 1 = women	1.30	0.39–4.34	0.666
Height (cm)	+1 cm	1.04	0.96–1.12	0.352
Weight (kg)	+1 kg	1.00	0.96–1.05	0.909
Pentosidine (μg/mL)	+0.01 μg/mL	1.05	1.00–1.09	0.038*
BMD (femoral neck) (g/cm2)	+1 SD	1.55	1.04–2.33	0.033*
Presence of LS (KL grade≥2)	0 = no, 1 = yes	1.94	0.67–5.61	0.219
Presence of DISH	0 = no, 1 = yes	2.78	1.11–6.92	0.029*

Logistic regression analysis was performed using the status of OPLL as the objective variable (0 = absence, 1 = presence), and the abovementioned factors were correspondingly adjusted

OPLL ossification of posterior longitudinal ligament, *BMD* bone mineral density, *LS* lumbar spondylosis, *KL grade* Kellgren–Lawrence grade, *DISH* diffuse idiopathic skeletal hyperostosis, *SD* standard deviation, *OR* odds ratios, *95 % CI* 95 % confidence interval

*$p<0.05$; **$p<0.01$; ***$p<0.001$

association of plasma pentosidine levels and DISH weakened (plasma pentosidine +0.01 μg/mL, 1.04, 0.997–1.087, $p=0.069$; presence of DISH 2.37, 0.94–6.00, $p=0.069$).

New occurrence or progression of OPLL

During the three study years, 1,380 individuals (88.3 %; 466 men, 914 women) among the 1,562 subjects at baseline returned for follow-up, and their radiographs were available for observation. Among the 30 individuals with radiographic cervical OPLL at baseline, 25 (83.3 %; 14 men and 11 women) participated in the second survey.

The remaining 1,355 individuals who did not have cervical OPLL at baseline and who participated in the initial and second surveys were regarded as members of the population at risk for the occurrence of OPLL. Among them, only one woman was diagnosed with newly developed radiographic OPLL (incidence 2.46/10,000 per year).

At follow-up, the mean length (in millimetres, SD) and width (in millimetres, SD) of the maximum region of ossification among the 25 individuals with OPLL was 28.7 (16.1) and 3.5 (1.5)mm, respectively. Since the mean values of length and width of the maximum region of ossification of these 25 subjects were 27.0 (16.2) and 3.0 (1.5)mm at the baseline, respectively, both the length and width of the maximum region of ossification increased, although a significant difference was not observed.

To clarify the risk factors associated with this increase in the length and width of the ossification, we performed multivariate regression analysis using the rate of change in these parameters as objective variables and the explanatory variables as those used in the logistic regression analysis, namely height (in centimetres), weight (in kilograms), plasma pentosidine levels (+1 μg/mL), BMD of the femoral neck (+1 SD), presence of LS based on the KL grade (0 = KL grade 0 or 1, 1 = KL grade≥2) and DISH (0 = absence, 1 = presence). Adjustments for age (years) and gender (0 = men, 1 = women) were made. However, none of the abovementioned variables was found to be significantly associated with the rate of changes in the length or width.

Discussion

In the present population-based study, we clarified the prevalence of radiographic OPLL in the general Japanese population, and we found that it is significantly associated with high plasma pentosidine levels, high BMD and the presence of DISH. The 3-year follow-up study also showed that new cases were very rare, and the length and width of the maximum region of ossification among the subjects with OPLL tended to increase.

The prevalence of OPLL in Japan has been reported to be 1.9 to 4.3 % among individuals aged 30 years and older [1, 15–17]. In other Asian countries, such as in Korea [18, 19] and Taiwan [20], a similar prevalence was reported, but it was lower in Western countries [21], suggesting that ethnic and/or genetic factor(s) could be associated with the onset of OPLL. In the present study, the prevalence of OPLL was found to be 1.9 %. This is consistent with the value found in previous reports. However, it is difficult to clearly distinguish localised-type OPLL from osteophytic changes, and we included two individuals with localised-type OPLL in the OPLL group. Thus, we may have overestimated the presence of radiographic OPLL. If we exclude individuals with localised-type OPLL from the OPLL group, the prevalence of the OPLL in the present study is 1.8 %.

With regard to the gender difference in OPLL prevalence, the prevalence was previously reported to be three times higher in men than in women [22]. We found that men are 2.5 times more likely to have OPLL than women (men 3.2 %, women

1.3 %), which is consistent with results reported previously among Japanese subjects. In contrast, symptomatic OPLL was reported to be usually observed in the sixth decade of life [22], although we were unable to find a significant association between age and the presence of OPLL. This might be explained by the fact that previous studies on the characteristics of OPLL were performed on the subjects with symptomatic OPLL, i.e. they had been clinically diagnosed with OPLL, while our subjects had radiographic OPLL that had not been clinically diagnosed. If the OPLL in our subjects progresses in the future, the peak age at which the symptoms could be expressed may be their 60s.

With regard to the comorbidities of OPLL, several reports have indicated that obesity and DM might be associated with OPLL [5, 6]. In the present study, the values of BMI tended to be higher in the group with OPLL than in that without OPLL, although this difference was not significant. A similar pattern was found in the values of HbA1c, and this finding could be explained by previous findings that the extent of ossification was significantly associated with the fasting serum insulin level but not with the fasting glucose level or the HbA1c level [23]. However, in the ROAD study, since all subjects could not be requested to fast, we could not confirm the association between fasting serum insulin levels and OPLL.

With regard to the association between biochemical markers of bone turnover and OPLL, Matsui et al. showed that the levels of the bone markers serum procollagen type I carboxyl-terminal peptide and intact osteocalcin were higher in patients with OPLL than in normal subjects [24]. This suggested that OPLL was associated with biochemical markers of bone turnover. In the present study, to evaluate the role of bone metabolism in OPLL, we compared the serum levels of iPTH and PINP as bone formation markers and the urinary levels of β-CTX between the groups with and without OPLL. However, we could not find significant differences between the groups.

Instead, the plasma pentosidine levels of the OPLL group were found to be significantly higher than those of the group without OPLL. This tendency remained after potential associated factors were adjusted for. Pentosidine is an advanced glycation end product, products generated by the sequential nonenzymatic glycosylation of protein amino groups [25] that accumulate in various tissues including kidney and coronary arteries, resulting in the development of diabetic vascular complications [26]. The concentrations of pentosidine in cortical and trabecular bone are reported to be adversely associated with bone strength [27–29]. Yamamoto et al. [30] found that serum pentosidine levels were positively associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes. Renal insufficiency was reported to be a dominant determinant of serum pentosidine levels [31] because of which serum pentosidine levels are increased in patients with chronic renal failure [32, 33]. However, no report has shown the association between pentosidine levels and the presence of OPLL. On the basis of the abovementioned reports, we performed multivariate logistic regression analysis using the same explanatory factors we had used in the analysis shown in Table 2, along with the estimated glomerular filtration rate. We found that the plasma pentosidine levels were still significantly related to the presence of OPLL (OR, 1.05; 95 % CI, 1.00–1.09; $p=0.042$). We speculate that the levels of pentosidine might be associated with ectopic ossification, such as vascular calcification in patients with renal dysfunction, or the presence of OPLL, directly or indirectly, although the currently available information is inadequate to prove this hypothesis. One reason for the inadequacy of the information obtained in this study could be that we did not evaluate genetic factors in the present study. Further investigations are needed to clarify whether the observed relationship between pentosidine levels and OPLL remains after analysis of other possible confounders, including genetic factors.

In addition to the biochemical markers, high BMDs have been observed in patients with OPLL [24, 34, 35]. However, Morio et al. reported that the BMD was lower in patients with advanced OPLL [36], suggesting that the disuse atrophy may result during advanced-stage OPLL. Our results also showed that subjects with OPLL had higher BMDs. However, the subjects in the present study all had radiographically determined OPLL but few clinical symptoms, so their condition may not have been in the advanced stage. Therefore, based solely on the results of the present study, we were unable to discuss the association between BMD and advanced-stage OPLL.

Several reports have shown that the coexistence of OPLL and DISH is quite common [4, 7, 8]. The pathogenesis of DISH and OPLL has been speculated to be similar, although the details remain unclear. For example, Havelka et al. analysed intron 6 (−4) polymorphisms in the COL 11 A2 gene in Czech patients with DISH and Japanese patients with OPLL, but they found no agreement between the data of subjects with DISH and OPLL [37]. Additional studies with a broader spectrum of genotyping and a larger cohort of patients may clarify the presence or absence of genetic relations between DISH and OPLL.

Few studies have been reported regarding the incidence of OPLL in the general population because OPLL is relatively rare and based on ethnicity, as noted. Using data collected in a pilot study in the corporation of 360 Japanese hospitals [3], Tsuyama described the incidence of OPLL and found that 2,142 patients were treated in these hospitals and the estimated incidence of OPLL was 19 patients per million persons of the total population [3]. In the present study, only one new case of OPLL was detected, so we could not accurately estimate the incidence of OPLL and compare our results to those of previous reports. In order to confirm the incidence of OPLL, we need to follow this cohort for a longer time.

Several studies have investigated the course of OPLL. Chiba et al. use computer-assisted measurement to examine OPLL

progression, and they found that the rate of OPLL progression was 56.5 % over 2 years, and this rate was most common in younger patients with continuous- and mixed-type OPLL [38]. Murakami et al. followed the case of a 67-year-old man who had had cervical OPLL for more than 26 years, and they found that the rate of OPLL progression was 2.2, 8.8 and 2.0 mm/year from 1–4, 4–8 and 8–10 years after the first visit, respectively [39]. However, to our knowledge, no study has reported the progression of radiographically defined OPLL in the general population. In the present study, we found that both the length and width of the maximum region of ossification increased during the 3 years of the study, although it was not a significant change. A previous report [39] found no evidence of OPLL progression after 10 years. We must carefully examine whether or not radiographically defined OPLL progresses to clinical OPLL.

This study has several limitations. First, although the ROAD study includes a large number of participants, these participants may not truly be representative of the general population. To address this, we compared the anthropometric measurements and the frequencies of smoking and alcohol consumption between the study participants and the general Japanese population. No significant differences were found, with the exception that male ROAD study participants aged 70–74 years were significantly smaller in terms of body structure than men from the overall Japanese population ($p < 0.05$) [10]. This difference should be considered when evaluating potential risk factors for men aged 70–74 years; factors such as body build, particularly weight, are known to be associated with metabolic risk factors and KOA. Therefore, our results may have underestimated the prevalence of these conditions. Second, the total number of subjects with confirmed OPLL was very small, which might make the results somewhat less credible. In the present study, we used logistic regression analysis to adjust for gender differences. When the total number of the objective variable, namely OPLL cases, is small, using the multivariate model to adjust for gender differences may be more useful than using a gender-specific analysis. This is because the total number of cases in a gender-specific analysis will be even smaller, which reduces the statistical power. Although the significant associations between OPLL and the plasma levels of pentosidine and between OPLL and DISH were observed only in men in the simple comparative analysis, the pentosidine levels and DISH remained significant factors associated with the presence of OPLL even in the logistic regression analysis with adjustments for gender. We interpreted this result to mean that the female sex might dilute the strength of the association between OPLL and DISH, but the tendency in both genders remained significant.

To clarify the effect of sex differences in the interaction among OPLL, pentosidine levels and DISH, the logistic regression analysis was performed in men and women separately (Supplementary Table 1). In this logistic regression analysis, the presence of OPLL was significantly associated with the pentosidine levels and femoral neck BMD in men, but the association of OPLL with the presence of DISH was diluted to a marginal association ($p = 0.080$). Further, since all male patients with DISH had radiographic LS, we could not evaluate the association between OPLL and LS. In women, the associations among OPLL, pentosidine levels and DISH were not significant. Although these results may indicate that the significant associated factors were observed only in men, they may even be skewed by the small number of female cases. Under these circumstances, it is difficult to distinguish which model should be used, i.e. logistic regression analysis or the multivariate model. It may be necessary to first include an adequate number of OPLL cases before this can be decided. To compensate for these limitations, we decided to include the urban cohort of the ROAD study in the OPLL survey. Thus, more participants will be included in the third ROAD survey planned from 2012 to 2013, and further detailed investigation regarding the risk factors for the presence, occurrence or exacerbation of OPLL may be possible.

In summary, the present study clarified that the prevalence of radiographic cervical OPLL in 1,562 individuals was 1.9 %, which was significantly higher in men than in women ($p = 0.007$), but no association with age was observed. In logistic regression analysis, OPLL showed a significant association with the femoral neck BMD, presence of DISH and plasma pentosidine levels. Only one new case of radiographic OPLL was detected, but OPLL progressed in all affected subjects.

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Conflicts of interest None.

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ORIGINAL ARTICLE

Profiles of vitamin D insufficiency and deficiency in Japanese men and women: association with biological, environmental, and nutritional factors and coexisting disorders: the ROAD study

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Abstract
Summary Assessments of serum 25-hydroxyvitamin D levels in 1,683 Japanese from a population-based cohort revealed prevalences of vitamin D insufficiency and deficiency were 81.3 and 1.2 %, respectively. Vitamin D deficiency was significantly associated with female sex, examined month, current smoking, lack of regular walking, higher intact parathyroid hormone (iPTH), and poor daily vitamin D intake.
Introduction To clarify the characteristics of subjects with vitamin D insufficiency and deficiency among men and women in the general Japanese population.
Methods We initiated research on osteoarthritis/osteoporosis against disability (ROAD), a large-scale population-based cohort study, in 2005–2007. Blood examination was performed to measure serum 25-hydroxyvitamin D (25D) and iPTH levels and biochemical markers of bone turnover in 1,683 participants (595 men, 1,088 women). Participants completed an interviewer-administered questionnaire, measurements of bone mineral density, and x-ray examination. Vitamin D deficiency and insufficiency were defined by serum 25D levels <10 and ≥10 but <30 ng/mL, respectively.
Results The prevalence of vitamin D insufficiency and deficiency was 81.3 and 1.2 %, respectively. Multinominal logistic regression analyses using potentially confounding variables revealed vitamin D insufficiency was significantly associated with age (+1 year, relative risk ratio, 0.98; 95 % confidence interval, 0.96–0.99), gender (women vs. men, 2.28; 1.59–3.30), residing areas (coastal area vs. mountainous area, 0.58; 0.41–0.81), examined month (October, November, December vs. January, 0.51; 0.34–0.76), and serum levels of iPTH (+1 pg/mL, 1.02; 1.01–1.03). Vitamin D deficiency was significantly characterised by female sex (20.5; 3.1–136.7), examined month (0.28; 0.09–0.95), current smoking habit (6.39; 1.78–23.0), lack of regular outside walking (3.96; 1.34–11.7), higher iPTH (1.02; 1.01–1.03) and poor daily vitamin D intake (+10 μg/day, 0.48; 0.24–0.93).
Conclusions A high prevalence of vitamin D insufficiency and a low prevalence of vitamin D deficiency were found in

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Japanese men and women, and the characteristics of vitamin D status were clarified.

Keywords Epidemiology · Population-based study · Prevalence · Risk factor · Vitamin D deficiency · Vitamin D insufficiency

Introduction

Vitamin D influences bone quality and is important, in particular, for maintaining bone density [1, 2]. Vitamin D deficiency results in decreased bone mineralisation, secondary hyperparathyroidism, and increased cortical bone loss, and has been linked to the pathogenesis of osteoporosis (OP) and hip fractures [1, 3]. Furthermore, vitamin D supplementation may help decrease fractures and falls [4, 5].

However, vitamin D status in populations varies widely. Mithal et al. reviewed the population-based reports regarding vitamin D status in six different regions in the world (i.e., Asia, Europe, the Middle East and Africa, Latin America, North America, and Oceania) and observed that serum 25-hydroxyvitamin D (25D) levels below 75 nmol/L (<30 ng/mL) were prevalent in every region studied, and that levels below 25 nmol/L (<10 ng/mL) were most common in regions such as South Asia and the Middle East [6]. In addition, the International Osteoporosis Foundation (IOF) has reported vitamin D deficiency in postmenopausal women (defined as 25D levels <30 ng/mL), to be approximately, 50 % in Thailand and Malaysia, 75 % in the United States, and 90 % in eastern Asia [7].

The prevalence of vitamin D inadequacy in postmenopausal women in Japan is well known to be very high [8, 9]. Regarding reports of vitamin D concentrations of community-dwelling inhabitants in Japan, Nakamura et al. measured serum levels of 25D of 600 postmenopausal women, and found that higher serum 25D concentrations are associated with higher bone mineral density (BMD) of the femoral neck, and at least 20 ng/mL is needed to achieve normal PTH levels and prevent low BMD [10]. In terms of the Japanese elderly, Suzuki et al. screened 2,957 elderly men and women with an age range of 65–92 years and reported a low 25D level was significantly associated with a high prevalence of falls in Japanese elderly women because of their inferior physical performance [11]. However, little detailed information is still available on the profiles of vitamin D insufficiency and deficiency in the general population, especially in premenopausal women and men. In addition, there is yet little information regarding associated factors for vitamin D insufficiency and deficiency, such as biochemical markers of bone turnover, lifestyle factors (e.g., dietary habits), and other coexisting disorders.

In the present study, we performed a survey using the population-based cohort known as the research on osteoarthritis/osteoporosis against disability (ROAD), which consists of a large number of participants and various outcomes. The ROAD study uses a questionnaire survey consisting of lifestyle factors and nutrition, blood and urinary examinations, measurements of BMD, and x-ray examinations [12, 13]. The aim of our study was to clarify the prevalence of vitamin D insufficiency and deficiency in the general population, including among men and premenopausal and postmenopausal women, and to examine the association of biological, environmental, and nutritional factors and coexisting disorders with vitamin D insufficiency and deficiency.

Subjects and methods

Outlines of the ROAD study

We conducted the present study using the cohorts established in 2005 for the ROAD study. The ROAD study is a nationwide, prospective study of osteoarthritis (OA) comprised of population-based cohorts in several communities in Japan. Details of the cohort profile have been reported elsewhere [12, 13]. Briefly, in 2005–2007, we created a baseline database that included clinical and genetic information for 3,040 residents of Japan (1,061 men and 1,979 women); the mean age (standard deviation (SD)) of the participants was 70.3 (11.0) years (71.0 (10.7) years for men and 69.9 (11.2) years for women). The subjects were recruited from resident registration listings in three communities with different characteristics: 1,350 subjects (465 men, 885 women) in an urban region in Itabashi, Tokyo; 864 subjects (319 men, 545 women) in a mountainous region in Hidakagawa, Wakayama; and 826 subjects (277 men, 549 women) in a coastal region in Taiji, Wakayama.

Participants completed an interviewer-administered questionnaire of 400 items that included lifestyle information such as occupation, smoking habits, alcohol consumption, family history, medical history, physical activity, reproductive variables, and health-related QOL. A questionnaire was prepared by modifying the questionnaire used in the Osteoporotic Fractures in Men Study [14], and some new items were added to the modified questionnaire. The participants were asked whether they took prescription medication daily or nearly every day (0: no, 1: yes). If participants did not know the reason for the prescribed medication, they were asked to bring their medications to the medical doctor (NY).

Anthropometric measurements included height, weight, and body mass index (BMI; weight/height^2, kg/m^2). Systolic and diastolic blood pressure (BP) was measured by an experienced public health nurse using a mercury sphygmomanometer. Medical information, including information on knee joints, was

collected by experienced orthopaedic surgeons (SM and HO). All participants underwent radiographic examination of both knees and the lumbar spine.

In addition, the brief diet history questionnaire (BDHQ) was used for the dietary assessment. Each participant was given the questionnaire and provided with a detailed explanation on how to fill it out at home; the unclear parts were addressed by well-trained interviewers. BDHQ is a 4-page structured questionnaire that inquires about the consumption frequency of 80 principal foods, with specified serving sizes described in terms of a natural portion or the standard weight and volume measurement of servings commonly consumed in general Japanese populations. It was modified from a comprehensive (16-page) version of a validated self-administered diet history questionnaire [15]. A total of 141 components, including energy and dietary nutrient intakes, were calculated using the ad hoc computer algorithm for BDHQ. Dietary intake levels of total energy and 27 nutrients (animal protein, vegetable protein, animal fat, vegetable fat, carbohydrate, sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, copper, manganese, vitamins B1, 2, 6, and 12, niacin, folate, vitamins C, D, E, and K, cholesterol, dietary fibre, and salt) were analysed. We used the values obtained for these nutrients to estimate the total amount of calcium, phosphorus, and vitamin D intake during a day.

Eligible subjects of the present study

Among the above-mentioned regions, the measurements of BMD were performed on subjects in the mountainous and coastal regions. For all 1,690 participants (596 men, 1094 women) in the mountainous and coastal regions, BMD was measured for the lumbar spine and the proximal femur using dual energy x-ray absorptiometry (DXA) (Hologic Discovery; Hologic, Waltham, MA, USA) during the baseline examination.

Blood and urinary examinations had also been performed in subjects in mountainous and coastal regions. Among the participants, we were able to measure the serum levels of 25D in 1,683 individuals (99.6 %; 595 men, 1,088 women). Hence, the data from these 1,683 subjects were used for the analysis in the present study. The study participants provided written informed consent, and the study was conducted with the approval of the ethics committees of the University of Tokyo (No 1264).

Blood and urine examinations

Samples were collected from the end of October to the middle of January in both mountainous and coastal areas. All blood and urine samples were extracted between 0900 and 1500 hours. After centrifugation of the blood samples, the sera and urine samples were immediately placed on dry ice and transferred to a deep freezer within 24 h. These samples were stored at −80 °C until assayed.

The blood samples were used to measure haemoglobin A1c (HbA1c, Japan Diabetes Society), blood sugar, high-density lipoprotein cholesterol (HDL-cho), total cholesterol, triglyceride, and creatinine levels. The analyses were performed at the same laboratory within 24 h of extraction (Osaka Kessei Research Laboratories, Inc., Osaka, Japan).

Serum levels of 25D were measured by radioimmunoassay with a ^{125}I-labelled tracer (DiaSorin, Stillwater, MN, USA) [16], and intact parathyroid hormone (iPTH) was measured by an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). As a marker of bone formation, serum N-terminal propeptide of type I procollagen (PINP) was measured using a radioimmunoassay (Orion Diagnostics, Espoo, Finland). The urinary levels of β-isomerized C-terminal cross-linking telopeptide of type I collagen (β-CTX), a bone resorption marker, were determined using an enzyme-linked immunosorbent assay (Fujirebio, Inc. Tokyo, Japan). Urinary β-CTX values were standardised to urinary creatinine concentrations.

Definitions of vitamin D insufficiency and deficiency

For revealing the severity of 25D status worldwide, the IOF reported vitamin D status on a global map using four categories defined according to the mean (or median) 25D levels as follows: 30, 20–30, 10–20, and <10 ng/mL [17]. In the present study, based on the IOF report, vitamin D deficiency was defined as 25D serum levels <10 ng/mL, whereas vitamin D insufficiency was defined as 25D serum levels ≥10 and <30 ng/mL.

Radiographic assessment

Plain radiographs of the lumbar spine in the anteroposterior and lateral views and bilateral knees in the anteroposterior view with weight-bearing and foot map positioning were obtained. The severity of radiographic OA was determined according to Kellgren–Lawrence (KL) grading as follows [18]: KL0, normal; KL1, slight osteophytes; KL2, definite osteophytes; KL3, joint or intervertebral space narrowing with large osteophytes; KL4, bone sclerosis, joint or intervertebral space narrowing and large osteophytes. In the ROAD study, participants were classified into KL3 if they had joint or intervertebral space narrowing without large osteophytes. Radiographs at each site, i.e., the knees, hips and vertebrae, were examined by a single, experienced orthopaedic surgeon (SM), who was masked regarding participants' clinical status. If at least one knee joint was graded as KL2 or higher, the participant was diagnosed with

radiographic knee osteoarthritis. Similarly, if at least one intervertebral level of the lumbar spine was graded as KL2 or higher, the participant was diagnosed with radiographic lumbar spondylosis.

Definition of coexisting disorders, such as hyperparathyroidism, OP, hypertension, dyslipidaemia, impaired glucose tolerance, and renal dysfunction

For clarifying the association between vitamin D status and comorbidities, the prevalence of the following disorders was investigated in relation to vitamin D status: hyperparathyroidism; OP at the lumbar spine (L2–4), the femoral neck, or both sites; hypertension; dyslipidaemia; impaired glucose tolerance; and renal dysfunction. Hyperparathyroidism was defined by serum iPTH >65 pg/mL. OP was defined based on World Health Organization criteria, in which OP was mainly diagnosed when the BMD T-scores were lower than peak bone mass by −2.5 SDs [19]; the mean (SD) for the L2–4 BMD in young adult men and women, as measured by the Hologic DXA in Japan, was 1.011 (0.119) g/cm^2 [20]. The present study therefore defined OP at the lumbar spine as an L2–4 BMD <0.714 g/cm^2. Furthermore, the mean (SD) for the femoral neck BMD in young adult men and women was 0.863 (0.127) g/cm^2 and 0.787 (0.109) g/cm^2, respectively [20]. Therefore, OP at the femoral neck in men and women was defined as a femoral neck BMD <0.546 g/cm^2 and <0.515 g/cm^2, respectively.

In the present study, hypertension was defined as a systolic BP ≥130 mm Hg and/or a diastolic BP ≥85 mm Hg [21]. Dyslipidaemia was defined by a serum HDL-cho level <40 mg/dL [21, 22]; and impaired glucose intolerance, by a serum HbA1c level ≥5.5 % [22]. Renal dysfunction was defined as chronic kidney disease at stage 3 or higher, which was determined by an estimated glomerular filtration rate <60 mL/min/1.73 m^2 [23].

Furthermore, subjects being treated with medication for OP, hypertension, dyslipidaemia, impaired glucose intolerance and/or diabetes mellitus, or renal disease were regarded as having these respective conditions.

Statistical analysis

All statistical analyses were performed using STATA statistical software (STATA Corp., College Station, Texas, USA). Differences in proportions were compared using the Chi-square test. Differences in continuous variables were tested for significance using analysis of variance for comparisons among multiple groups or Scheffe's least significant difference test for pairs of groups.

To test the association between the presence of vitamin D insufficiency and deficiency and various biological, environmental, behavioural, and nutritional factors, we used multinominal logistic regression analysis. In the analysis, we used the presence of vitamin D insufficiency and vitamin D deficiency as the objective variable (0: group with normal vitamin D levels, 1: vitamin D insufficiency group, 2: vitamin D deficiency group) and selected explanatory variables in addition to the basic characteristics, such as age (+1 year), gender (0: men, 1: women), BMI (+1 kg/m^2), regional differences (0: mountainous area, 1: coastal area), month of examination (October, November, December vs. January). The following potential risk factors were included in the multinominal logistic regression analysis and showed a significant or marginal (p <0.1) association with vitamin D status in the simple linear analysis: smoking (0: never, ever, 1: current), alcohol consumption (0: never, ever, 1: current), lack of regular walking outside (0: ≥5 times/week, 1: <5 times/week), regular exercise outdoors (0: yes, 1: no), serum levels of iPTH (+1 pg/mL), serum levels of PINP (+1 SD), urinary levels of β-CTX (+1 SD), daily total energy from amount of intake (+100 Kcal/day), calcium (+100 mg/day), phosphorus (+100 mg/day), vitamin D (+10 μg/day) calculated based on the BDHQ questionnaire, and the values of the baseline BMD at the lumbar spine (+1 SD) or femoral neck (+1 SD). Selected explanatory variables for each analysis are described in the Results section. After the multinominal logistic regression analysis, the relative risk ratios (RRRs) were evaluated.

Results

Figure 1 shows the prevalence of vitamin D insufficiency and deficiency according to gender and age groups. The overall prevalence of vitamin D insufficiency and deficiency was 81.3 and 1.2 %, respectively, and was higher in women than in men (vitamin D insufficiency, men, 72.1 %; women, 86.3 %; vitamin D deficiency, men, 0.3 %; women, 1.7 %) (p <0.001). In terms of menstrual status, 926 women (85.1 %) had postmenopausal status. The prevalence of vitamin D insufficiency and deficiency classified by menstrual status was 89.4 and 2.5 % in premenopausal women and 85.8 and 1.5 % in postmenopausal women, respectively. No significant differences were observed in the prevalence of vitamin D insufficiency and deficiency between pre- and postmenopausal women (p=0.181). Figure 1 also shows the prevalence of vitamin D insufficiency and deficiency according to age groups. The prevalence of vitamin D insufficiency (%) for individuals in their 30s and younger and those in their 40s, 50s, 60s, 70s, and 80s or older was 86.4, 91.2, 81.0, 80.8, 78.4, and 82.4, respectively. Similarly, the prevalence of vitamin D deficiency (%) for individuals in these same age groups was 2.3, 1.4, 1.0, 0.8, 1.5, and 1.3, respectively. The prevalence of vitamin D insufficiency and deficiency in men and women according to these different age groups is shown in Figure 1.

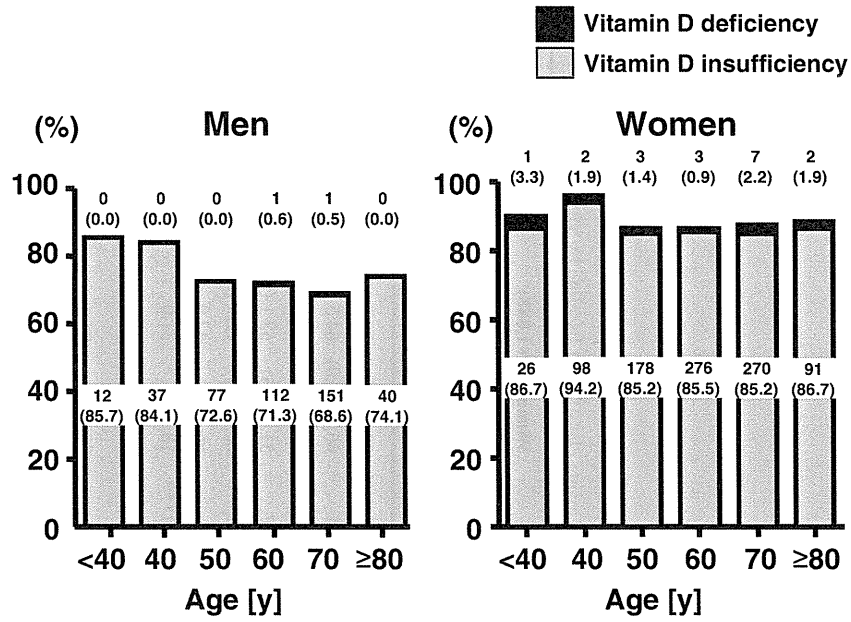

Fig. 1 The number of individuals (prevalence, %) with vitamin D insufficiency and deficiency as classified by gender. The numbers along the middle line indicate the number of individuals (prevalence, %) with vitamin D insufficiency for each bar, while those along the upper line indicate the number of individuals (prevalence, %) with vitamin D deficiency

The mean level (standard deviation, SD) of serum 25D in the total participants was 23.3 (6.6) ng/mL. The blood examinations were performed from October to January; consequently, we compared vitamin D levels classified by the month of examination. Mean values of serum 25D were the highest for the subjects in October (26.2 (6.3) ng/mL), followed by November (23.9 (7.1) ng/mL), December (23.1 (6.3) ng/mL), and January (22.0 (5.7) ng/mL). These results suggested the analysis to clarify the factors associated with 25D status should be adjusted for the month of the examinations.

Table 1 shows the results for the serum 25D levels, the anthropometric measurements, and the prevalence of lifestyle factors of individuals with vitamin D insufficiency and deficiency as compared to those with normal vitamin D levels. The mean levels (SD) of serum 25D were 33.2 (3.0) in the normal group; the individuals in this group tended to reside in the coastal area. Meanwhile, drinking alcohol, the habit of walking outside, and regular exercise were most common in individuals with normal vitamin D levels, less common in individuals with vitamin D insufficiency, and least common in those with vitamin D deficiency. Smokers were most common in the vitamin D-deficiency group, followed respectively by the normal vitamin D and vitamin D-insufficiency groups.

The mean levels of serum iPTH, PINP, and urinary β-CTX were compared across groups with normal vitamin D, vitamin D insufficiency, and vitamin D deficiency (Table 2). The serum iPTH and urinary β-CTX levels significantly increased across groups from the normal vitamin D group to the vitamin D-deficiency group (p=0.0076 and p=0.0003, respectively). No significant trends were observed in the serum levels of PINP and the vitamin D status. By contrast, the BMD values at both the lumbar spine (L2–4) and the femoral neck significantly decreased across groups from the normal vitamin D group to the vitamin D-deficiency group (L2–4, p=0.0094; femoral neck, p=0.0179). These various trends were observed across groups within both men and women; however, with the exception of iPTH values in women, none of these trends were significant.

Table 3 shows the mean values of the total nutrient intake per day in relation to vitamin D status. The daily amount of total energy and calcium, phosphorus, and vitamin D intake all significantly decreased across groups from the normal vitamin D group to the vitamin D insufficiency group. These trends were mainly due to the observations in women, since no significant association was observed between nutrient intake and vitamin D status in men.

Table 4 shows the relative risk ratios obtained from the multinominal logistic regression analysis using the presence of vitamin D insufficiency and vitamin D deficiency as the objective variable (0: normal vitamin D levels, 1: vitamin D insufficiency, 2: vitamin D deficiency), and the factors listed in the 'Subjects and methods' section, such as age (+1 year), gender (0: men, 1: women), regional differences (0: mountainous area, 1: coastal area), BMI (+1 kg/m^2), month of examination (0: October, November, December, 1: January), smoking (0: never, ever, 1: current), alcohol consumption (0: never, ever, 1: current), lack of regular walking outside (0: ≥5 times/week, 1: <5 times/week), regular exercise outdoors (0: yes, 1: no), serum levels of iPTH (+1 pg/mL), urinary levels of β-CTX (+1 SD), daily total energy from amount of intake (+100 Kcal/day), vitamin D (+10 μg/day) calculated based on the BDHQ questionnaire, and the values of the baseline BMD at the lumbar spine (+1 SD) or femoral neck (+1 SD). Daily intake of calcium (+100 mg/day) and

Table 1 Mean values (standard deviations) of the anthropometric measurements and the prevalence (%) of lifestyle factors for the participants classified at baseline by vitamin D status

	Total (N=1,683)				Men (N=595)				Women (N=1,088)			
	Normal (N=295)	Vitamin D insufficiency (N=1,368)	Vitamin D deficiency (N=20)	p for trend	Normal (N=164)	Vitamin D insufficiency (N=429)	Vitamin D deficiency (N=2)	p for trend	Normal (N=131)	Vitamin D insufficiency (N=939)	Vitamin D deficiency (N=18)	p for trend
Serum levels of 25 (OH) D (ng/mL)	33.2 (3.0)	21.4 (4.8)[a]	8.2 (1.2)[a,b]	<0.0001***	33.6 (3.3)	22.8 (4.6)[a]	9.0 (0.0)[a, b]	<0.0001***	32.8 (2.5)	20.7 (4.7)[a]	8.1 (1.2)[a,b]	<0.0001***
Age (years)	67.1 (11.1)	64.9 (12.1)[a]	66.0 (14.4)	0.0143*	67.6 (11.2)	65.8 (11.9)	72.5 (7.8)	0.1851	66.5 (11.0)	64.5 (12.2)	65.2 (14.9)	0.1865
Height (cm)	157.2 (9.7)	154.8 (9.2)[a]	154.8 (5.7)	0.0003***	162.8 (7.4)	163.6 (7.1)	161.6 (2.2)	0.4378	150.1 (7.4)	150.7 (6.9)	154.0 (5.5)	0.0755
Weight (kg)	56.8 (10.8)	55.4 (10.8)	53.5 (9.0)	0.0811	61.2 (10.5)	62.7 (11.0)	53.2 (8.2)	0.1557	51.4 (8.6)	52.1 (8.9)	53.5 (9.3)	0.5434
BMI (kg/m^2)	22.9 (3.3)	23.0 (3.4)	22.4 (4.0)	0.5983	23.0 (3.1)	23.3 (3.2)	20.3 (2.6)	0.2295	22.8 (3.5)	22.9 (3.5)	22.6 (4.1)	0.8983
Residing in the coastal area	59.0	46.7	45.0	0.001**	53.1	44.1	50.0	0.145	66.4	47.9	44.4	<0.001***
Blood examination performed in January	17.0	31.2	40.0	<0.001***	15.9	31.7	50.0	<0.001***	18.3	31.0	38.9	0.003**
Current smoking habit (regularly, ≥1/month)	16.2	12.3	25.0	0.056	27.6	30.6	50.0	0.643	1.6	3.7	22.2	<0.001***
Current alcohol consumption (regularly, ≥1/month)	50.2	37.7	25.0	<0.001***	71.8	64.6	100.0	0.152	23.1	25.4	16.7	0.602
Regularly walking outside (≥5 times/week, including job)	61.7	56.6	25.0	0.004**	68.0	60.7	50.0	0.261	53.7	54.7	22.2	0.023*
Regularly exercising outdoors (football, tennis, baseball, golf, etc.) after graduation from the last school	19.7	13.2	10.0	0.013*	33.5	32.4	0.0	0.594	2.3	4.4	11.1	0.186

25D 25-hydroxyvitamin D, *BMI* body mass index

*p <0.05; **p <0.01; ***p <0.001

[a] significantly different (p <0.05) from values of the normal group

[b] significantly different (p <0.05) from values of the VD-insufficient group

Table 2 Mean values (standard deviations) of serum iPTH, serum and urinary biochemical markers of bone turnover, and bone mineral densities for the participants classified at baseline by vitamin D status

	Total (N=1,683)				Men (N=595)				Women (N=1,088)			
	Normal (N=295)	Vitamin D insufficiency (N=1,368)	Vitamin D deficiency (N=20)	p for trend	Normal (N=164)	Vitamin D insufficiency (N=429)	Vitamin D deficiency (N=2)	p for trend	Normal (N=131)	Vitamin D insufficiency (N=939)	Vitamin D deficiency (N=18)	p for trend
Serum levels of iPTH (pg/mL)	36.7 (16.3)	42.2 (36.7)[a]	55.4 (24.9)	0.0076**	37.8 (17.0)	44.6 (60.2)	55.0 (28.3)	0.3415	35.4 (15.4)	41.1 (17.3)[a]	55.4 (25.5)[a,b]	<0.0001***
Serum levels of PINP (μg/L)	56.0 (26.9)	57.6 (26.9)	65.0 (28.8)	0.3078	46.4 (18.9)	47.5 (22.8)	48.1 (15.8)	0.8705	68.0 (30.4)	62.2 (27.3)	67.0 (29.7)	0.0716
Urinary levels of β-CTX (μg/mmol Cr)	166.6 (105.8)	188.3 (125.8)[a]	266.6 (171.1)[a,b]	0.0003***	121.8 (64.0)	132.6 (85.2)	157.0 (131.5)	0.3157	221.7 (120.3)	213.8 (133.0)	278.8 (173.5)	0.1032
BMD (L2-4) (g/cm^2)	0.965 (0.227)	0.926 (0.203)[a]	0.894 (0.183)	0.0094**	1.061 (0.214)	1.040 (0.200)	1.055 (0.234)	0.5360	0.844 (0.181)	0.873 (0.181)	0.876 (0.176)	0.2253
BMD (femoral neck) (g/cm^2)	0.692 (0.146)	0.669 (0.136)[a]	0.637 (0.133)	0.0179*	0.744 (0.146)	0.746 (0.126)	0.769 (0.113)	0.9595	0.625 (0.116)	0.633 (0.126)	0.622 (0.130)	0.7415

iPTH intact parathyroid hormone, *PINP* N-terminal propeptide of type I procollagen, *β-CTX* β-isomerized C-terminal cross-linking telopeptide of type I collagen, *BMD* bone mineral density, *L2–4* lumbar spine L2–L4

*p <0.05; **p <0.01; ***p <0.001

[a] significantly different (p <0.05) from values of the normal group

[b] significantly different (p <0.05) from values of the VD-insufficient group

Table 3 Mean values (standard deviations) of total amount of energy/(day) and nutrient intake of the participants classified at the baseline by vitamin D status

	Total (N=1,683)				Men (N=595)				Women (N=1,088)			
	Normal (N=295)	Vitamin D insufficiency (N=1,368)	Vitamin D deficiency (N=20)	p for trend	Normal (N=164)	Vitamin D insufficiency (N=429)	Vitamin D deficiency (N=2)	p for trend	Normal (N=131)	Vitamin D insufficiency (N=939)	Vitamin D deficiency (N=18)	p for trend
Total energy (Kcal)	2,079.6 (589.1)	1,911.3 (585.6)a	1,623.3 (435.0)[a]	<0.0001***	2,299.2 (595.2)	2,306.8 (679.5)	1,966.1 (283.2)	0.7615	1,806.3 (452.8)	1,730.7 (430.0)	1585.2 (437.5)	0.0562
Calcium (mg)	605.2 (237.9)	537.3 (229.1)[a]	503.4 (212.1)	<0.0001***	606.6 (245.3)	565.7 (263.8)	691.3 (275.2)	0.1896	603.5 (229.2)	524.3 (210.3)[a]	482.5 (203.0)	0.0002***
Phosphorus (mg)	1,208.2 (379.5)	1,084.9 (373.2)[a]	918.2 (320.7)[a]	<0.0001***	1,259.8 (394.6)	1,220.3 (428.7)	1,257.3 (141.3)	0.5903	1,144.1 (350.6)	1,023.1 (327.0)[a]	880.5 (314.2)[a]	0.0001***
Vitamin D (μg)	23.7 (12.9)	19.7 (12.4)[a]	13.3 (9.5)[a]	<0.0001***	24.3 (13.9)	22.2 (14.5)	28.8 (11.4)	0.2183	22.9 (11.6)	18.6 (11.1)[a]	11.6 (7.9)[a, b]	<0.0001***

*p <0.05; **p <0.01; ***p <0.001

[a] significantly different (p <0.05) from values of the normal group

[b] significantly different (p <0.05) from values of the VD insufficient group

phosphorus (+100 mg/day) were excluded from the model because of the high correlation coefficient (r) between daily intake of vitamin D (intake of calcium vs. vitamin D, r=0.73, p <0.001; intake of phosphorus and vitamin D, r=0.83, p <0.001).

Compared to normal vitamin D subjects, individuals with vitamin D insufficiency were significantly younger and significantly more likely to be female, to reside in a mountainous area, and undergone measurements in January. The serum iPTH levels of individuals with vitamin D insufficiency were higher than those of the normal vitamin D group. Meanwhile, individuals with vitamin D deficiency, when compared to those with normal vitamin D levels, were more likely to be female, to have undergone measurements in January, to have a smoking habit, and to perform less outside walking. In addition, serum levels of iPTH were significantly higher and mean intake of vitamin D significantly lower than those of the normal vitamin D group. This tendency remained after changing the variable of the baseline BMD at the lumbar spine to the baseline BMD at the femoral neck.

The prevalence of the coexisting disorders listed in the 'Subjects and methods' classified by vitamin D status are shown in Table 5. The prevalence of hyperparathyroidism diagnosed by the serum levels of iPTH was the highest in the group with vitamin D deficiency, followed respectively by the vitamin D insufficiency and normal groups (p <0.001). This trend was observed separately in both men and women (men, p=0.057, women, p <0.001). The prevalence of OP at L2–4 or femoral neck at the baseline tended to be the highest in the vitamin D-deficiency group, followed by the vitamin D insufficiency and normal groups, but was not significantly different across groups. The prevalence of lumbar spondylosis tended to be the highest in the normal group, followed by the vitamin D insufficiency and deficiency groups, but was not significantly different across groups, either. No significant differences in the prevalences of knee osteoarthritis, hypertension, dyslipidaemia, impaired glucose tolerance, and chronic kidney disease were observed.

Finally, to clarify the association of hyperparathyroidism and vitamin D status, we performed a multinominal logistic regression analysis using the presence of vitamin D insufficiency and vitamin D deficiency as the objective variable (0: normal vitamin D levels, 1: vitamin D insufficiency, 2: vitamin D deficiency), and presence of hyperparathyroidism (0: no, 1: yes) as an explanatory variable, after adjustment for age, gender, regional differences, BMI, and month of examination (0: October, November, December, 1: January). The results revealed that vitamin D deficiency was still significantly associated with hyperparathyroidism (RRR, 95 % confidence intervals, vitamin D insufficiency vs. normal group: 1.25, 0.75–2.09, p=0.385, vitamin D deficiency vs. normal group: 12.7, 4.52–35.7, p <0.001).

Discussion

In the present study, using the baseline samples from 1,683 individuals in the population-based cohort ROAD, we found a very high prevalence of vitamin D insufficiency and a low prevalence of vitamin D deficiency in Japanese men and women. These prevalences were not significantly different between pre- and postmenopausal women. It was clarified individuals with vitamin D insufficiency were characterised by a younger age, the female sex, residing in a mountainous area, measurements performed in January, and higher serum iPTH values. Meanwhile, vitamin D deficiency was characterised by the female sex, measurements performed in January, smoking habit, less outside walking, higher serum iPTH, and a low intake of vitamin D.

Several reports stated that vitamin D insufficiency in postmenopausal women in eastern Asia is very high [9, 10]. The high prevalence detected in the present study was consistent with the previous reports. By contrast, few studies have reported gender and age differences in terms of vitamin D insufficiency and deficiency using the data of a general population. In the present study, we found the prevalence of vitamin D insufficiency and deficiency were more common in women than in men and that the frequency of vitamin D inadequacy in women was not associated with menstrual status. In terms of gender difference in the prevalence of vitamin D inadequacy in the Japanese population, Suzuki et al. found the mean serum levels of 25D in elderly men were higher than those in women [11]. Greene-Finestone et al. [24] investigated the vitamin D status in a Canadian population, including men and premenopausal women, and reported that the proportion of individuals with serum vitamin D levels <30 ng/mL was higher in women (60.7 %) than that in men (57.5 %). These results show that vitamin D inadequacy is more prevalent in women than men in both western and eastern populations.

Regarding age differences in vitamin D inadequacy, we observed that in the vitamin D insufficiency group, an age of 1 year older decreased the risk of vitamin D insufficiency; however, this tendency was not found in the vitamin D deficiency group. Moreover, as shown in Table 1, the mean age of the normal vitamin D group, the vitamin D insufficiency group, and the vitamin D deficiency group was 67.1, 64.9, and 66.0 years, respectively, with the vitamin D insufficiency group showing the lowest mean age. We were unable to provide an adequate hypothesis for the U-shaped phenomenon in the vitamin D status in regards to age, in particular the association between age and the risk of vitamin D insufficiency. Nonetheless, one possible explanation is that this phenomenon might be due to the birth-cohort effect. However, because the design of the present study was cross-sectional, we were unable to confirm the cohort effect. Hence, we should follow our cohort longer and confirm whether the vitamin D insufficiency in the younger age group will develop into vitamin D deficiency.

Residents of the seaside town showed a significantly lower prevalence of vitamin D insufficiency and deficiency compared to those in the mountainous area. The total amount of sun exposure might be one of the factors affecting the vitamin D status of the residents. The Japan Meteorological Agency reported that the mean sunlight time in Ryujin town (the area neighbouring Hidakagawa, the mountainous area in the present study) was 1541.7 hours/year and in Shionomisaki (the neighbouring area of Taiji town, the seaside area in the present study) was 2201.2 hours/year [25], which suggests the total sun exposure in the seaside area is much higher than that in the mountainous area. Nutritional differences could also account for these geographical discrepancies. The mean intake of vitamin D estimated in the group residing in the seaside area was 21.1 μg/day and was significantly higher than that in the group residing in the mountainous region (19.6 μg/day, p <0.05). This difference in vitamin D intake might be due to the frequency of fatty fish intake. Indeed, we found that a low intake of vitamin D was one of the emphasised characteristics for vitamin D deficiency in the present study (Table 4). Moreover, in the multinominal logistic regression analysis to assess potentially associated factors, including regional differences and vitamin D intake evaluated in the same model, regional difference was no longer significantly associated with vitamin D deficiency, but vitamin D intake remained a significant explanatory variable.

In terms of iPTH levels and the vitamin D status, a number of reports have shown an inverse association between serum 25D and iPTH levels [1, 26, 27]. Moreover, a low-serum 25D concentration leads to a decrease in calcium absorption. The lower serum calcium concentration causes an increase in iPTH secretion and a relatively higher serum iPTH concentration. Regarding the Japanese population, hypovitaminosis D was reported to adversely affect serum iPTH levels, especially in the very elderly [28]. In the present study, this tendency was observed not only in the very elderly, but also in the middle-aged and aged Japanese population, while the mean values of serum iPTH in vitamin D deficiency remained within the upper limits of the normal range. However, since the current study was cross-sectional in nature, we unable to detect a causal relationship between serum iPTH and 25D status. Thus, we were unable to clarify whether lower 25D caused hyperparathyroidism or whether hyperparathyroidism lowered 25D. In a future follow-up study, we would like to clarify the causal relationship between 25D and iPTH.

By contrast, current smoking habit and less outside walking were emphasised characteristics for vitamin D deficiency in the present study. In terms of the smoking habit and vitamin D status, Lange et al. reported that vitamin D

Table 4 Relative risk ratios (RRR) of potentially associated factors for the presence of VD insufficiency and deficiency vs. normal VD

		Vitamin D insufficiency			Vitamin D deficiency		
Explanatory variables	Reference	RRR	95 % CI	*p*	RRR	95 % CI	*p*
Age (years)	+1 year	0.98	0.96–0.99	0.001**	1.01	0.96–1.06	0.726
Gender	0: men, 1: women	2.28	1.58–3.30	<0.001***	20.5	3.08–136.7	0.002**
Region	0: mountainous area, 1: coastal area	0.58	0.41–0.81	0.002**	0.65	0.19–2.25	0.492
BMI (kg/m^2)	+1 kg/m2	1.00	0.96–1.05	0.970	0.92	0.79–1.08	0.318
Month of examination (October, November, December)	vs. January	0.51	0.34–0.76	0.001**	0.28	0.09–0.95	0.040*
Smoking	0: ex or never smoker, 1: current smoker	1.05	0.69–1.59	0.822	6.39	1.78–23.0	0.005**
Alcohol consumption	0: ex or never drinker, 1: current drinker	0.78	0.56–1.07	0.120	0.57	0.18–1.80	0.339
Regularly walking outside	0: yes, 1: no	1.27	0.94–1.70	0.121	3.96	1.34–11.7	0.013*
Regularly exercising outdoors	0: yes, 1: no	1.09	0.73–1.63	0.657	0.95	0.17–5.18	0.950
Serum levels of iPTH (pg/mL)	+1 pg/mL	1.02	1.01–1.03	0.001**	1.02	1.01–1.03	<0.001***
Urinary levels of β-CTX (μg/mmol Cr)	+1 SD	0.95	0.81–1.10	0.471	1.41	0.94–2.12	0.099
BMD (L2–4) (g/cm^2)	+1 SD	0.98	0.84–1.15	0.828	1.42	0.82–2.46	0.208
Total energy (Kcal/day)	+100 Kcal	1.00	0.97–1.03	0.918	0.98	0.87–1.11	0.776
Vitamin D (μg/day)	+10 μg	0.91	0.81–1.03	0.138	0.48	0.24–0.93	0.031*

RRR, relative risk ratios; 95 % CI, 95 % confidence interval

BMI body mass index, *iPTH* intact parathyroid hormone, β-*CTX* β-isomerized C-terminal cross-linking telopeptide of type I collagen, *BMD* bone mineral density, *L2–4* lumbar spine L2–L4

*p <0.05; **p <0.01; ***p <0.001

deficiency was associated with lower lung function and a more rapid decline in lung function in smokers over 20 years in their longitudinal cohort, which consisted of 626 elderly men [29]. In the present study, smoking remained a strongly associated factor for vitamin D deficiency after adjustment for gender and other potentially confounding factors (Table 4). In addition, after an identical multinominal logistic analysis performed only in women, smoking was still associated with vitamin D deficiency (vs. non- or ex- smoking: 9.82, 1.35–71.5, p=0.024), which suggests that vitamin D deficiency in both men and women may be influenced by smoking. In the present study, we could not confirm the effect of smoking on lung function because of the lack of information. Hence, future studies are required to confirm the relationship between vitamin D status, smoking, and lung function. In regards to the walking habit and 25D status, the lower sun exposure caused by less outside walking might be associated with lower levels of 25D. Our results showed outside walking and the above-mentioned dietary intake of vitamin D might prevent vitamin D deficiency, which are consistent with statements or recommendations [10].

Besides bone and mineral diseases such as rickets [30], osteomalacia [30], OP [1, 3], osteoporotic fractures [4] and falls [5], a number of studies have reported an association between inadequate vitamin D and other chronic diseases, such as cardiovascular disease [31], diabetes [32], cancer [33–35], and autoimmune diseases such as multiple sclerosis [36]. In the present study, we clarified the association between 25D status and various coexisting disorders including hyperparathyroidism, OP, osteoarthritis, hypertension, dyslipidaemia, impaired glucose tolerance, and chronic kidney disease. We found that vitamin D deficiency was significantly associated with hyperparathyroidism, although no significant relationship was observed for the presence of other diseases after the adjustment for confounding factors. However, to clarify whether vitamin D inadequacy might cause the occurrence or the progression of the above-mentioned diseases, we have already been prepared to perform a follow-up of our cohorts, so that we can report the effect of vitamin D inadequacy on health dysfunction in the general population as a next step.

This study has several limitations. First, although the ROAD study includes a large number of participants, these participants may not truly be representative of the general population. To address this, we compared the anthropometric measurements and the frequencies of smoking and alcohol consumption between the entire group of study participants and the general Japanese population. No significant differences were found, with the exception that the male ROAD study participants aged 70–74 years were

Table 5 Prevalence (%) of coexisting disorders, such as hyperparathyroidism, osteoporosis, osteoarthritis, metabolic risk factors, and renal dysfunction, among the participants classified at the baseline by vitamin D status

	Total (N=1,683)				Men (N=595)				Women (N=1,088)			
	normal (N=295)	Vitamin D insufficiency (N=1,368)	Vitamin D deficiency (N=20)	p for trend	normal (N=164)	Vitamin D insufficiency (N=429)	Vitamin D deficiency (N=2)	p for trend	normal (N=131)	Vitamin D insufficiency (N=939)	Vitamin D deficiency (N=18)	p for trend
Hyperparathyroidism (iPTH >65 pg/mL)	6.8	8.0	45.0	<0.001*	6.1	8.2	50.0	0.057	7.6	7.9	44.4	<0.001*
Osteoporosis (L2–4)	11.5	14.1	10.0	0.442	2.4	3.7	0.0	0.712	22.9	18.9	11.1	0.376
Osteoporosis (femoral neck)	10.2	13.0	25.0	0.109	4.9	3.3	0.0	0.623	17.1	17.4	27.8	0.514
Osteoporosis (L2–4 or femoral neck)	14.6	20.5	25.0	0.057	4.9	6.3	0.0	0.758	26.9	27.1	27.8	0.997
Knee osteoarthritis (KL >=3)	19.4	20.0	35.0	0.238	15.3	14.5	0.0	0.813	24.4	22.5	38.9	0.243
Lumbar spondylosis (KL >=3)	42.7	35.9	30.0	0.072	45.1	34.5	50.0	0.054	38.7	36.5	27.8	0.568
Hypertension	67.9	70.2	60.0	0.473	71.2	76,0	100.0	0.344	63.9	67.6	55.6	0.411
Dyslipidaemia	11.5	12.5	10.0	0.855	9.8	15.6	0.0	0.156	13.7	11.1	11.1	0.667
Impaired glucose tolerance	21.3	21.5	30.0	0.653	23.2	24.7	50.0	0.648	19.1	20.0	27.8	0.688
Chronic kidney disease	43.7	42.9	40.0	0.932	46.3	42.7	0.0	0.333	40.5	43.2	44.4	0.847

iPTH intact parathyroid hormone, *L2–4* lumbar spine L2–L4, *KL* Kellgren–Lawerence grade, *BMI* body mass index

*p <0.001

significantly smaller in terms of body structure than the overall Japanese population (p <0.05) [13]. This difference should be considered when evaluating potential risk factors for men aged 70–74 years; factors such as body build, particularly greater weight, are known to be associated with metabolic risk factors and knee osteoarthritis. Therefore, our results may represent an underestimate of the prevalence of these conditions. Second, in the present study, this study was only the data of the baseline study. Thus, we were not able to confirm the causal relationship between vitamin D status and other associated factors. However, we have already completed a 3-year after follow-up study, so that we can clarify the causal relationship between vitamin D status and the above-mentioned factors in the following reports after the baseline profile of vitamin D status described in the present study. Third, the total number of individuals in the vitamin D deficiency group was very small (n=20), which could make the results pertaining to vitamin D deficiency somewhat less credible than those relating to vitamin D insufficiency. To address this limitation, we performed a multiple regression analysis using the serum levels of vitamin D as the objective variable and the identical explanatory variables as used in the multinominal regression analysis shown in Table 4. The explanatory variables were as follows: age (+1 year), gender (0: men, 1: women), regional differences (0: mountainous area, 1: coastal area), BMI (+1 kg/m^2), month of examination (0: October, November, December, 1: January), smoking (0: never, ever, 1: current), alcohol consumption (0: never, ever, 1: current), lack of regular walking outside (0: ≥5 times/week, 1: <5 times/week), regular exercise outdoors (0: yes, 1: no), serum levels of iPTH (+1 pg/mL), urinary levels of β-CTX (+1 SD), daily total energy from amount of intake (+100 Kcal/day), vitamin D (+10 μg/day), and the values of the baseline BMD at the lumbar spine (+1 SD). Supplementary Table 1 shows the lower serum levels of vitamin D were characterised by younger age, female sex, residing in a mountainous area, measurements performed in January, smoking habit, non- or ex-drinking, higher serum iPTH, and a low intake of vitamin D. This tendency was almost similar to the characteristics of vitamin D insufficiency and deficiency, with the exception that alcohol drinking was also significantly associated with lower serum levels of vitamin D. However, as shown in Table 1, the frequency of alcohol drinking was confirmed to be significantly different according to the 25D status. Thus, the results of the multinominal logistic regression did not differ substantially from those of the multiple regression analysis using continuous values for vitamin D. Rather, these results may support the characteristics of each 25D status as clarified in the present study. Finally, the nutrition survey in the present study was performed using a questionnaire with a multiple-choice style for each meal. Although this questionnaire is widely used in Japanese studies, it might raise the