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Original article

Normal and threshold values of radiographic parameters for knee osteoarthritis using a computer-assisted measuring system (KOACAD): the ROAD study

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Abstract

Background. Although radiographic severity of the knee is commonly determined by the Kellgren-Lawrence (KL) grading scale, it does not separately assess joint space narrowing or osteophyte formation. The present study aimed to establish normal and threshold values of radiographic parameters for knee osteoarthritis (OA) using the knee osteoarthritis computer-aided diagnosis (KOACAD) measuring system on a large-scale population-based cohort of the Research on Osteoarthritis/Osteoporosis Against Disability (ROAD) population.

Methods. From a total of 3040 participants in the ROAD study, standing anteroposterior radiographs of the knee were obtained from 2975 subjects (1041 men, 1934 women) in the ROAD cohort, and 5950 knees were evaluated using the KOACAD system to obtain the medial and lateral minimum joint space width (mJSW), medial and lateral joint space area (JSA), osteophyte area (OPA), and femorotibial angle (FTA). These indices were compared with the KL scores, and cutoff values for radiographic knee OA were determined by receiver operating characteristic (ROC) curve analysis.

Results. The mean KOACAD parameters for KL = 0 were as follows: medial mJSW 3.70 mm; lateral mJSW 4.77 mm, medial JSA 125.0 mm^2, lateral JSA 140.0 mm^2, OPA 0, and FTA 176.1° in men; for women they were medial mJSW 3.26 mm, lateral mJSW 4.22 mm, medial JSA 100.9 mm^2, lateral JSA 111.0 mm^2, OPA 0, and FTA 174.9°. Threshold values for KL ≥ 2 provided by ROC curve analysis with area under the curve (AUC) > 0.7 were medial mJSW 2.8 mm and medial JSA 107.3 mm^2 in men and medial mJSW 2.7 mm in women. Those for KL ≥ 3 were medial mJSW 2.1 mm, medial JSA 81.1 mm^2, OPA 2.4 mm^2, and FTA 179.6° in men; and medial mJSW 2.1 mm, medial JSA 66.6 mm^2, OPA 2.5 mm^2, and FTA 178.1° in women. We then determined the cutoff values for medial knee OA and lateral knee OA.

Conclusions. The present study established normal and threshold values of parameters for knee OA using an automated computer-assisted program on plain radiographs.

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Introduction

Osteoarthritis (OA) is a major public health problem among the elderly that affects activities of daily living (ADL) and quality of life (QOL), leading to increased morbidity and mortality. According to the recent National Livelihood Survey by the Ministry of Health, Labor, and Welfare in Japan, OA is ranked first and fourth among the diseases that cause disabilities requiring support and long-term care, respectively.[1]

Given the increasing proportion of the elderly among the Japanese population, a comprehensive, evidence-based prevention strategy for OA is urgently needed. However, few indices have been identified that can predict the future occurrence and progression of OA. Risk factors for knee OA are known to include age, heavy weight, previous knee injury, and history of work involving overloading the knees.[2–4]

One of the reasons for the scarcity of epidemiological studies on OA might be the diagnostic criteria. Most cases of OA are radiographically determined based on a rating of grade 2 or more on the Kellgren-Lawrence (KL) grading scale.[5] The KL scale is the most conventional grading system for determining the radiographic severity of knee OA, but this categorical scale does not assess joint space narrowing or osteophyte formation separately. Accumulating evidence has shown that osteophytosis and joint space narrowing display distinct etiological mechanisms, and their progression is neither constant nor proportional.[6–8] When examining factors associated with knee OA, these two features should thus be assessed separately. In addition, other indices

of knee OA on plain radiographs, such as the femorotibial angle (FTA) and joint space area, should be determined for evaluation of the severity of knee OA. However, reference values of these indices have yet to be established for a general population.

In the present study, we obtained values for medial and lateral minimum joint space width (mJSW), medial and lateral joint space area (JSA), osteophyte area (OPA), and FTA using the knee osteoarthritis computer-aided diagnosis (KOACAD) measuring system, which we developed and reported elsewhere.[9] We used it in a large-scale population-based cohort study called the Research on Osteoarthritis/Osteoporosis Against Disability (ROAD)[10,11] to establish normal reference and threshold values of radiographic parameters for knee OA.

Participants and methods

Participants

Reference values were obtained based on the results of measurements from the participants of the ROAD study, a nationwide prospective study comprising population-based cohorts established in several communities in Japan. Recruitment methods for this study have been described in detail elsewhere.[9,10] To date, we have completed creation of a baseline database that includes clinical and genetic information of 3040 inhabitants (1061 men, 1979 women) in the age range of 23–95 years (mean 70.6 years), recruited from resident registrations in three communities. All participants provided written informed consent, and the study was conducted with the approval of the ethics committees of the participating institutions.

In the present study, from among a total cohort of 3040 participants, 2975 individuals (1041 men, 1934 women) with knee radiographs that could be evaluated by the KOACAD system were selected as subjects for analysis. Among 65 dropouts in the present analysis, 29 underwent total knee arthroplasty (TKA), and 1 had unilateral knee arthroplasty (UKA). The radiographic conditions for the remaining 35 subjects were insufficient for automated analysis due to severe flexion contracture, so we excluded them from the overall analysis.

A summary of the characteristics of subjects are shown in Table 1. No significant differences in baseline characteristics were seen between the 3040 participants in the whole cohort and the 2975 subjects in the present analysis.

Table 1. Summary characteristics of participants

Characteristic	Men	Women
No. of participants	1041	1934
Age (yrs)	71.0 (10.7)	69.8 (11.3)
Height (cm)	162.5 (6.7)	149.8 (6.5)
Weight (kg)	61.3 (10.0)	51.5 (8.6)
Body mass index (kg/m^2)	23.2 (3.0)	22.9 (3.5)
Current smokers	25.7%	3.5%
Current drinkers	64.2%	25.9%

Results are the mean (SD) unless otherwise specified

Radiographic assessment

All participants underwent radiographic examination of both knees using an anteroposterior (AP) view with weight-bearing and foot-map positioning. Fluoroscopic guidance with a horizontal AP X-ray beam was used to visualize the joint space properly, and images were downloaded into digital imaging and communication in medicine (DICOM) format files. The KOACAD system has been described in detail elsewhere[9] and is only briefly summarized here. The KOACAD was programmed to measure mJSW and JSA in the medial and lateral compartments, OPA at the medial tibia, and FTA 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) including the tibiofemoral joint space, a vertical neighborhood difference filter was applied to identify points with high absolute values for difference of scales. The center of all points was then calculated, and the ROI was chosen. Within the ROI, the outline of the femoral condyle was designated as the upper rim of the joint space. The two ends were determined, 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. 1A). A straight regression line for the lower rim outline was then drawn, and the intersections of the lower rim outline and the regression line were designated as the inside rims.

Medial and lateral JSAs 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 JSA (Fig. 1B).

To measure the osteophyte area and FTA, medial and lateral outlines of the femur and tibia were drawn. Inflection points for these outlines were then calculated. The medial outline of the tibia from the inflection point was drawn upward to the joint level, and the area that was medially prominent over the smoothly extended outline was designated the osteophyte area (Fig. 1C).

For FTA, a middle line between the medial and lateral outlines of the femur from the top of the image

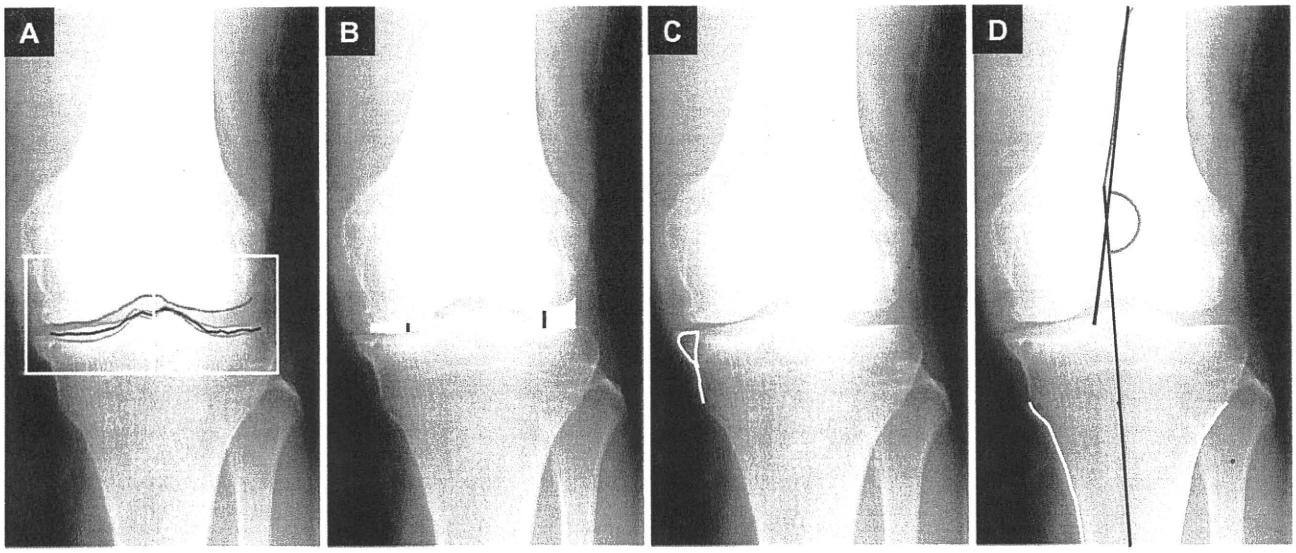

Fig. 1. Schema of image processing by the knee osteoarthritis computer-aided diagnosis (KOACAD) measuring system. **A** Outlines of anterior and posterior margins of the tibial plateau. The *middle line* between these two outlines represents the lower rim of the joint space. **B** Medial and lateral minimum joint space widths (mJSWs) as minimum vertical distances in joint space areas (JSAs). **C** Osteophyte area (area surrounded by *white lines*) determined as the medial prominence over the smoothly extended outline of the tibia. **D** Tibiofemoral angle as the lateral angle between straight regression lines (*black lines*) of the *middle lines* above in the femur and tibia. (From Oka et al.[9], with permission)

to the inflection points was drawn, and the straight regression line was determined as the axis of the femur. Similarly, the straight regression line of the middle line of the tibia from the bottom to the inflection points was designated as the axis of the tibia. The lateral angle between the two axis lines was calculated as the FTA (Fig. 1D).

This system can thus quantify the major features of knee OA on standard radiographs. Moreover, it allows objective, accurate, simple, easy assessment of the structural severity of knee OA without any manual operation in general clinical practice.

The severity of OA was radiographically determined according to the KL grading scale as follows[5]: KL0, normal joint; KL1, slight osteophytes; KL2, definite osteophytes; KL3, disc-space narrowing and large osteophytes; and KL4, bone sclerosis, joint space narrowing, and large osteophytes. In the ROAD study, joints that exhibited only joint space narrowing and no large osteophytes were graded as KL3. All radiographs were examined by a single, experienced orthopedic surgeon (S.M.) who was blinded to the clinical status of the participants.

Establishment of normal values and threshold values for parameters for knee OA

Each index determined using the KOACAD system — medial mJSW, lateral mJSW, medial JSA, lateral JSA, OPA, FTA — was compared with KL scores. First, we established normal values of these parameters using mean values for knees with KL grade 0. Cutoff values for radiographic knee OA for KL ≥ 2 and KL ≥ 3 were then determined using receiver operating characteristic (ROC) curve analysis. The present study adopted cutoff points that maximized the area under the ROC curve (AUC) as threshold values. Threshold values were determined for total OA including medial and lateral OA, and then separate threshold values were determined for medial and lateral OA. Although parameters for medial OA could be obtained separately for each sex, those for lateral OA were evaluated for the combined number of men and women, given the small number of cases with lateral OA.

Statistical analysis

All statistical analyses were performed using STATA statistical software (STATA, College Station, TX, USA). First, the Kolmogrov-Smirnov Lilliefors test was used to evaluate the normality of distribution of each variable. All parameters were confirmed to fit normal distributions (*P* values after Kolmogrov-Smirnov Lilliefors test: medial mJSW 0.40; lateral mJSW 0.6; medial JSA 0.37; lateral JSA 0.76; OPA 0.10; FTA 0.49). Differences in values of the parameters were tested for significance using analysis of variance (ANOVA) for comparisons among multiple groups. Trends of values

Table 2. Various parameters classified by age and sex

Age strata (years)	No. of knees	Medial mJSW (mm)	Lateral mJSW (mm)	Medial JSA (mm^2)	Lateral JSA (mm^2)	OPA (mm^2)	FTA (°)
Men							
<40	28	4.12 (0.92)	4.91 (1.22)	141.6 (27.3)	153.2 (30.1)	0	176.5 (2.2)
40–49	82	3.67 (0.75)	5.06 (1.10)	132.9 (27.9)	156.2 (34.4)	0.40 (1.98)	177.5 (2.4)
50–59	214	3.63 (0.75)	4.88 (1.06)	124.1 (26.9)	148.5 (33.1)	0.30 (1.51)	176.5 (3.2)
60–69	334	3.37 (0.93)[a]	4.59 (0.96)[bc]	113.9 (28.4)[abc]	138.9 (28.8)[bc]	0.96 (3.60)	177.0 (3.0)
70–79	1052	3.13 (0.96)[abcd]	4.40 (1.02)[bc]	102.5 (26.7)[abcd]	125.7 (30.3)[abcd]	1.35 (4.68)[c]	177.1 (3.3)
80+	372	2.94 (0.98)[abcd]	4.22 (0.87)[abcd]	97.2 (27.4)[abcd]	121.3 (28.0)[abcd]	1.31 (4.06)	177.0 (4.0)
Total	2082	3.22 (0.96)	4.48 (1.02)	107.3 (29.0)	130.6 (31.7)	1.12 (4.07)	177.0 (3.3)
Women							
<40	62	3.37 (0.61)	4.31 (1.23)	106.3 (24.0)	116.8 (32.6)	0.18 (1.25)	175.6 (3.0)
40–49	210	3.22 (0.64)	4.36 (1.01)	104.0 (22.2)	116.9 (25.8)	0.46 (2.09)	175.5 (2.7)
50–59	418	3.03 (0.78)	4.23 (1.15)	97.5 (26.2)	112.3 (28.3)	0.96 (2.87)	175.8 (4.0)
60–69	762	2.80 (0.98)[abc]	4.03 (1.09)[b]	89.8 (28.7)[abc]	106.6 (28.7)[bc]	2.33 (6.39)	176.4 (3.8)
70–79	1764	2.52 (0.92)[abcd]	3.87 (1.00)[bcd]	79.5 (26.3)[abcd]	99.4 (26.8)[abcd]	4.60 (11.2)[abcd]	176.9 (4.2)[bc]
80+	652	2.32 (0.95)[abcde]	3.80 (1.08)[abcd]	77.4 (26.9)[abcd]	97.9 (28.0)[abcd]	6.39 (12.70)[abcde]	177.1 (4.6)[bc]
Total	3868	2.65 (0.95)[#]	3.96 (1.07)[#]	84.9 (28.0)[#]	103.2 (28.2)[#]	3.76 (9.87)[#]	176.6 (4.1)[#]

Results are the mean (SD)
mJSW, minimal joint space width; JSA, joint space area; OPA, osteophyte area; FTA, femoro-tibial angle; KL grade: Kellgren-Lawrence grade
[a] Significantly different from those of the group <40 years ($P < 0.05$)
[b] Significantly different from those of the group in their 40s ($P < 0.05$)
[c] Significantly different from those of the group in their 50s ($P < 0.05$)
[d] Significantly different from those of the group in their 60s ($P < 0.05$)
[e] Significantly different from those of the group in their 70s ($P < 0.05$)
[#] Significantly different from those of men ($P < 0.001$)

according to the KL grade were tested using the Jonckheere-Terpstra trend test. Scheffe's least significant difference test was then used for pairs of groups.

Results

The mean values for mJSW, JSA, OPA, and FTA, classified by sex, are shown in Table 2. The values for medial and lateral mJSW, medial and lateral JSA, OPA, and FTA all differed significantly between the sexes ($P < 0.001$). The mean values for medial mJSW, JSA, and FTA were significantly greater in men than in women ($P < 0.001$). By contrast, the OPA values in both knees were significantly lower in men than in women ($P < 0.001$).

The mean values for mJSW, JSA, OPA, and FTA classified by age are also shown in Table 2. The medial mJSW for men in their sixties was significantly smaller than that for men <40 years; and that for men ≥70 years was significantly smaller than that of men <70 years ($P < 0.05$). Lateral mJSW for subjects in their sixties and seventies was significantly smaller than that for subjects in their forties and fifties ($P < 0.05$). Medial JSA for subjects in their sixties was significantly smaller than that for subjects <60 years old ($P < 0.05$); and that for subjects ≥70 years was significantly smaller than that for subjects <70 years ($P < 0.05$). Lateral JSA for subjects in their sixties was significantly smaller than that for subjects in their forties and fifties ($P < 0.05$); and that for subjects ≥70 years was significantly smaller than that for subjects <70 years ($P < 0.05$). No significant differences in OPA or FTA were seen according to age in men except for OPA in subjects between their fifties and seventies. In women, the medial mJSW for subjects in their sixties was significantly smaller than that for subjects <60 years ($P < 0.05$); that for women in their seventies was significantly smaller than that for subjects <70 years ($P < 0.05$); and that for women ≥80 years was significantly smaller than that of subjects <80 years ($P < 0.05$). The lateral mJSW for subjects in their sixties was significantly smaller than that for subjects in their forties ($P < 0.05$); that for subjects in their seventies was significantly smaller than that for subjects in their forties, fifties, and sixties ($P < 0.05$); and that for subjects ≥80 years was significantly smaller than that for subjects <70 years ($P < 0.05$). The medial JSA and lateral JSA showed trends with age similar to those of men. However, the mean OPA was significantly larger in women ≥70 years than in younger women ($P < 0.05$), and the FTA was significantly larger for subjects ≥70 years than for women in their forties and fifties ($P < 0.05$).

Table 3 shows mean values for mJSW, JSA, OPA, and FTA classified by KL grade. All values increased significantly according to the severity of the KL grade (P for trends < 0.0001). Regarding differences in the above-mentioned indices, values for medial mJSW and

Table 3. Various parameters classified by Kellgren-Lawrence grade

KL grade	Proportion of knees (%)	Medial mJSW (mm)	Lateral mJSW (mm)	Medial JSA (mm^2)	Lateral JSA (mm^2)	OPA (mm^2)	FTA (°)
Men							
KL0	24.4	3.70 (0.77)	4.77 (1.01)	125.0 (27.1)	140.0 (33.6)	0	176.1 (2.6)
KL1	38.4	3.40 (0.76)[a]	4.50 (0.93)[a]	109.8 (23.5)[a]	128.9 (29.0)[a]	0.48 (2.24)	176.6 (2.7)
KL2	28.5	3.02 (0.78)[ab]	4.38 (1.02)[a]	99.3 (22.5)[ab]	125.1 (29.8)[a]	1.08 (3.25)[ab]	177.5 (3.1)[ab]
KL3	6.3	2.10 (1.00)[abc]	4.06 (1.40)[abc]	84.1 (31.3)[abc]	129.5 (38.2)[a]	5.37 (8.70)[abc]	178.1 (4.5)[ab]
KL4	2.4	0.79 (0.84)[abcd]	4.04 (1.12)[ab]	44.7 (32.7)[abcd]	137.3 (39.5)	12.05 (10.36)[abcd]	184.2 (6.2)[abcd]
Total	100.0	3.22 (0.96)	4.48 (1.02)	107.3 (29.1)	130.8 (31.8)	1.12 (4.08)	177.0 (3.3)
Women							
KL0	13.9	3.26 (0.65)	4.22 (1.08)	100.9 (23.7)	111.0 (29.4)	0	174.9 (2.9)
KL1	30.6	2.95 (0.73)[a]	3.95 (0.99)[a]	89.7 (24.3)[a]	101.3 (26.0)[a]	0.68 (2.26)	175.6 (3.0)[a]
KL2	38.3	2.66 (0.73)[ab]	3.93 (0.96)[a]	84.5 (23.5)[ab]	100.3 (25.5)[a]	3.39 (6.67)[ab]	176.6 (3.3)[ab]
KL3	13.1	1.85 (0.92)[abc]	3.91 (1.20)[a]	73.3 (27.4)[abc]	106.5 (30.2)[bc]	11.15 (17.54)[abc]	178.7 (4.8)[abc]
KL4	4.1	0.67 (1.02)[abcd]	3.83 (1.68)[a]	34.6 (34.8)[abcd]	112.1 (43.7)[bc]	19.70 (20.65)[abcd]	183.8 (7.1)[abcd]
Total	100.0	2.65 (0.94)[#]	3.97 (1.06)[#]	84.9 (27.9)[#]	103.4 (28.1)[#]	3.76 (9.90)[#]	176.6 (4.1)[#]

Results are the mean (SD)
[a] Significantly different from those of KL0 ($P < 0.05$)
[b] Significantly different from those of KL1 ($P < 0.05$)
[c] Significantly different from those of KL2 ($P < 0.05$)
[d] Significantly different from those of KL3 ($P < 0.05$)
[#] Significantly different from those of men ($P < 0.05$)

medial JSA in both sexes tended to be smaller with increasing KL grade ($p < 0.05$). Values for OPA and FTA in both sexes were significantly larger in the KL 2–4 group than in the KL 0–1 group ($P < 0.05$). Age differences in values of lateral mJSW and JSA were smaller than those for medial mJSW and JSA.

We performed ROC curve analysis to identify threshold values of these indices to determine the knee OA defined by KL ≥ 2 and KL ≥ 3. ROC curve analysis provided threshold values of KL ≥ 2 and KL ≥ 3 in OA parameters for the two knees (Table 4). Threshold values of KOACAD parameters for KL ≥ 2 with AUC > 0.7 were medial mJSW 2.8 mm and medial JSA 107.3 mm^2 in men and medial mJSW 2.7 mm in women. Those for KL ≥ 3 were medial mJSW 2.1 mm, medial JSA 81.1 mm^2, OPA 2.4 mm^2, and FTA 179.6° in men; and they were medial mJSW 2.1 mm, medial JSA 66.6 mm^2, OPA 2.5 mm^2, and FTA 178.1° in women. In contrast, the AUC of the lateral mJSW and lateral JSA for KL ≥ 2 and KL ≥ 3 in OA parameters was near 0.5, meaning that the capacity of these parameters to distinguish diseased knees from normal knees was low.

In addition, we provided threshold values for parameters for both the medial and lateral knee OA using ROC curve analysis (Table 4). Medial OA comprised 97.8% of total OA cases, with the lateral type making up the remaining 2.2%. Although most threshold values for medial OA were similar to those for total OA, the AUC values for parameters of medial OA (e.g., medial mJSW, medial JSA) were higher than for total OA. In contrast, for lateral OA, the AUC values for lateral mJSW and lateral JSA for KL ≥ 2 and KL ≥ 3 in OA parameters were higher than those for total OA, which were near 0.99, meaning that the capacity of these parameters to distinguish disease states from the normal population was high.

Discussion

We have reported elsewhere the automated computer-assisted program KOACAD, which can accurately measure values of mJSW, JSA, OPA, and FTA.[9] In the previous report,[9] we clarified that KOACAD allows accurate, easy assessment of the structural severity of knee OA without any manual operation. The present study applied this system to baseline data from the ROAD study, obtaining normal and threshold values of the above-mentioned indices for objective diagnosis of knee OA.

In the present study, we first established normal values for mJSW, JSA, OPA, and FTA using mean values of these parameters for knees with KL grade 0. The mean values were medial mJSW 3.70 mm, lateral mJSW 4.77 mm, medial JSA 125.0 mm^2, lateral JSA 140.0 mm^2, OPA 0, and FTA 176.1° in men; and medial mJSW 3.26 mm, lateral mJSW 4.22 mm, medial JSA 100.9 mm^2, lateral JSA 111.0 mm^2, OPA 0, and FTA 174.9° in women. All these indices except OPA were significantly lower in women than in men, suggesting that the values are influenced by differences in stature. We concluded that normal and threshold values for knee OA should be established for each sex.

The JSW has been recommended as a candidate index for progression of knee OA,[12] but few data

Table 4. Threshold values of various parameters, by Kellgren-Lawrence grades 2 and 3

Parameter	Threshold value	AUC	Sensitivity	Specificity (%)
Total				
KL ≥ 2				
Men				
Medial mJSW (mm)	2.8	0.726	58.4	76.8
Lateral mJSW(mm)	4.3	0.566	52.3	59.0
Medial JSA (mm^2)	107.3	0.715	71.0	60.3
Lateral JSA (mm^2)	115.5	0.551	39.5	68.2
OPA (mm^2)	1.0	0.599	23.9	95.5
FTA (°)	178.5	0.633	42.7	79.3
Women				
Medial mJSW (mm)	2.7	0.730	63.7	72.5
Lateral mJSW(mm)	4.3	0.521	66.4	38.5
Medial JSA (mm^2)	85.9	0.654	64.5	59.9
Lateral JSA (mm^2)	79.2	0.509	19.8	83.4
OPA (mm^2)	1.0	0.691	44.3	92.4
FTA (°)	177.4	0.664	48.6	77.0
KL ≥ 3				
Men				
Medial mJSW (mm)	2.1	0.875	73.6	92.1
Lateral mJSW(mm)	4.3	0.608	65.2	54.3
Medial JSA (mm^2)	81.1	0.800	58.4	88.9
Lateral JSA (mm^2)	135.7	0.522	50.0	60.1
OPA (mm^2)	2.4	0.739	52.8	93.5
FTA (°)	179.6	0.702	52.5	85.5
Women				
Medial mJSW (mm)	2.1	0.842	65.3	92.0
Lateral mJSW(mm)	2.5	0.507	15.7	93.0
Medial JSA (mm^2)	66.6	0.717	48.7	83.2
Lateral JSA (mm^2)	116.4	0.562	38.8	73.0
OPA (mm^2)	2.5	0.768	66.1	82.2
FTA (°)	178.1	0.744	64.6	76.3
Medial OA				
KL ≥ 2				
Men				
Medial mJSW (mm)	2.8	0.728	58.5	76.8
Lateral mJSW(mm)	4.3	0.560	51.7	59.0
Medial JSA (mm^2)	107.3	0.717	71.3	60.3
Lateral JSA (mm^2)	115.5	0.545	38.8	68.2
OPA (mm^2)	1.2	0.599	23.9	95.5
FTA (°)	178.5	0.639	43.2	79.3
Women				
Medial mJSW (mm)	2.7	0.732	63.9	72.5
Lateral mJSW(mm)	5.4	0.505	92.9	10.9
Medial JSA (mm^2)	85.9	0.655	64.7	59.9
Lateral JSA (mm^2)	97.9	0.505	56.1	46.3
OPA (mm^2)	1.0	0.693	44.7	92.4
FTA (°)	177.4	0.677	49.9	77.0
KL ≥ 3				
Men				
Medial mJSW (mm)	2.1	0.888	76.3	90.4
Lateral mJSW(mm)	4.3	0.598	64.2	54.4
Medial JSA (mm^2)	81.1	0.809	59.0	89.0
Lateral JSA (mm^2)	135.3	0.536	52.6	59.7
OPA (mm^2)	2.4	0.741	53.2	93.4
FTA (°)	179.6	0.719	54.0	85.5
Women				
Medial mJSW (mm)	2.1	0.854	66.6	92.2
Lateral mJSW(mm)	4.6	0.512	29.7	75.8
Medial JSA (mm^2)	66.6	0.727	49.4	83.4
Lateral JSA (mm^2)	116.5	0.587	40.8	72.8
OPA (mm^2)	2.5	0.774	67.3	82.1
FTA (°)	178.1	0.771	67.9	76.0

Table 4. *Continued*

Parameter	Threshold value	AUC	Sensitivity	Specificity (%)
Lateral OA				
KL ≥ 2				
Men and women				
Medial mJSW (mm)	2.1	0.683	43.1	92.4
Lateral mJSW(mm)	2.2	0.995	100.0	98.1
Medial JSA (mm^2)	75.7	0.664	50.0	84.2
Lateral JSA (mm^2)	69.7	0.990	100.0	95.4
OPA (mm^2)	1.2	0.626	30.6	93.8
FTA (°)	173.3	0.795	65.3	81.5
KL ≥ 3				
Men and women				
Medial mJSW (mm)	2.1	0.680	46.0	92.0
Lateral mJSW(mm)	2.2	0.992	100.0	97.0
Medial JSA (mm^2)	75.1	0.638	48.7	84.5
Lateral JSA (mm^2)	69.1	0.987	100.0	95.6
OPA (mm^2)	4.8	0.706	43.2	96.5
FTA (°)	173.3	0.805	64.9	80.8

AUC, area under the curve

regarding normal values have been accumulated.[13] Gensburger et al. showed that the mean medial and lateral JSW in women were 5.1 mm and 6.0 mm, respectively,[13] suggesting that those values in Caucasian populations may be larger than our results in women; no normal values for men were available. In addition, although evaluations of knee alignment are known to be useful for diagnosing arthritic conditions affecting the knee joint and also serve as a guide for conservative management and surgical planning,[14,15] few reports have shown normal values of FTA along with JSA and OPA.

Koshino measured the FTA of 85 knees in men and 97 knees in women aged 25–35 years and reported normal FTA values of 178° in men and 176° in women.[16] These results seem broadly consistent with our results, although no sex differences were apparent in our study, with values of 176° for both men and women. In any case, this represents the first report of reference values for the above-mentioned parameters using a population-based cohort. The results may thus be useful for diagnosing knee OA. Furthermore, by a longitudinal follow-up of the present cohort, these parameters would be expected to predict the progress of knee OA.

We then determined the threshold values for knee OA using ROC curve analysis. In this analysis, we regarded parameters with AUC > 0.7 as good indices for features of knee OA according to a previous report.[17] For KL ≥ 2, threshold values of KOACAD parameters with AUC > 0.7 were only the mJSW in men and women and the medial JSA in men. AUCs > 0.7 on ROC curve analysis means that the threshold of parameters might show good capacity for accurate diagnosis of the disorder in question. In contrast, AUCs of threshold values of parameters regarding the lateral region (i.e., KL ≥ 2; 0.566 for lateral mJSW 4.3 mm, 0.551 for lateral JSA 115.5 mm^2 in men; 0.521 for lateral mJSW 4.3 mm, 0.509 for lateral JSA 79.2 mm^2 in women) seem insufficient as indicators for knee OA. In contrast, for KL ≥ 3, OPA and FTA seem to represent good predictors with satisfactory AUCs. These results suggest that such parameters are more useful in severe knee OA than in mild knee OA.

We also tried to determine threshold values for medial knee OA and lateral knee OA. Because most cases of knee OA were medial knee OA (97.8%), the above-mentioned threshold values were considered applicable for medial OA. Conversely, in the diagnosis for lateral OA, for both KL ≥ 2 and KL ≥ 3, threshold values for medial mJSW and medial JSA were no longer parameters with good predictive capacity. By contrast, AUCs of threshold values for parameters of the lateral region (KL ≥ 2: 0.995 for lateral mJSW 2.2 mm, 0.990 for lateral JSA 69.7 mm^2; KL ≥ 3: 0.992 for lateral mJSW 2.2 mm, 0.987 for lateral JSA 69.1 mm^2) were preferable as good predictors. Similar to medial knee OA, for KL ≥ 3 the OPA and FTA seem to represent good predictors with satisfactory AUC. These results suggest that parameters at the medial side are useful in medial knee OA, and parameters at the lateral side are useful in lateral knee OA. However, evaluation of lateral OA was performed in only 65 participants (2.2%), so we could not analyze data for men and women separately. Regarding lateral OA and threshold KOACAD parameters, further investigation is warranted.

On the other hand, discrepancies between continuous values obtained from the KOACAD system and categorical scales such as the KL scale might add to the limitations of the KL grading scale. Most previous

studies have been performed in patients with knee OA defined by a KL score; but utilizing this categorical scale at the diagnosis of OA seems to result in the loss of a considerable amount of information, as the contribution of joint space narrowing and osteophytes is relatively small. Even though these indices are linear and constant in number, joint space narrowing is simply categorized as mild or severe and osteophytes as slight, definite, or large. In addition, the optimal method for handling joints with severe joint space narrowing but no osteophyte formation is unclear.

One solution to such problems might be found in a radiographic atlas of individual features published by the Osteoarthritis Research Society International (OARSI).[18] OARSI proposed a new grading scale in which joint space narrowing and osteophyte formation at the medial and lateral tibiofemoral compartments on radiographs should be evaluated separately. Several studies have evaluated the severity of joint space narrowing and osteophytes in the osteoarthritic knee utilizing the OARSI scale,[19] although these studies did not assess distinct features of knee OA such as joint space narrowing, osteophyte formation, or joint angulation in one sitting. To the best of our knowledge, no quantitative assessment systems for osteophytes have been described other than in the KOACAD,[9] so the present study is the first to assess threshold values for knee OA in a population-based cohort.

Unlike categorical methods for grading the severity of knee OA (e.g., KL or OARSI scales), KOACAD enables measurement of independent parameters for knee OA. We have already confirmed that low medial mJSW and high FTA are associated with the presence of knee pain, unlike lateral mJSW or an osteophyte area.[9] These accurate and continuous parameters obtained by KOACAD might be candidates for predictors of rapid progress from mild knee OA. These parameters might also be helpful for assessing risk factors for the occurrence of OA. We assumed that 25.3 million people (8.6 million men, 16.7 million women) ≥40 years would be affected by radiographic knee OA, and 7.8 million people (2.2 million men, 5.6 million women) ≥40 years would be affected by knee OA with knee pain.[10] Preventive strategies for OA are certainly in urgent demand. At the planning stage for the strategies against knee OA, the provision of accurate, objective, quantitative indices to measure outcomes seems highly important.

However, some limitation might apply to automated systems for all knee OA. First, as we stated, the number of cases with lateral knee OA was small for accurate determination of thresholds. Second, with radiographs of cases showing severe flexion contracture of the knee (>20°), the KOACAD system failed to measure parameters automatically. However, the system includes a manual mode, and in such cases orthopedic specialists can obtain values by manual measurement.

We believe this system may not only be useful for objective evaluation of knee OA in daily clinical practice or population-based epidemiological studies, it also acts as a proper surrogate measure for the development of disease-modifying drugs for OA. We hope in the future that this system will be applied worldwide to develop international criteria and for the diagnosis and treatment of knee OA.

Conclusion

We have established normal and threshold values of parameters for knee OA using an automated computer-assisted program, KOACAD, on plain radiographs.

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No conflict of interest has been declared by the authors.

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Editorial

Human genetic studies on osteoarthritis from clinicians' viewpoints

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Despite high prevalence and social impact, osteoarthritis (OA) is far behind other skeletal diseases like osteoporosis in the development of disease-modifying treatments. This is mainly because little is known about the underlying molecular mechanism that could be the therapeutic target. Since OA is a multifactorial disease caused by complex interplay between environmental and genetic factors with estimates of around 50% heritability depending on the site[1], numerous efforts and great expense have been spent on human genetic studies on OA worldwide. Although linkage studies have shown large areas of chromosomes associated with the disease, they have failed to detect the susceptible genes. Candidate gene studies have proposed over 100 genes as being responsible; however, most of them have not later been reproduced in larger meta-analysis studies. Recently, while genome-wide association studies (GWAS) have led to the discovery of over 600 gene loci in over 50 common multifactorial diseases, most of the gene variants are of only minimal individual effect. Even though the identified genes with such small effect sizes could possibly be therapeutic targets or at least prognostic markers, it is questionable whether or not these conventional OA genetic studies are worthy of such enormous investment. Aiming at a well-powered approach for this highly polygenic disease with multiple risk loci conferring small effects, consortium studies have been developed to enlarge the sample size. Considering the disease characteristics and prevalence, however, it is our opinion that not only the quantity but also the quality of studies is critical for identification of the genetic architecture. In this sense, the conventional OA genetic studies do not seem to us who are clinicians, although not genetic experts, to have been performed with sufficient scientific strictness, even as compared to those on other common diseases.

In this issue of *Osteoarthritis and Cartilage*, Kerkhof *et al.* clearly indicate that inconsistent and ambiguous definition of OA is a critical limitation of conventional genetic studies[2]. In addition to the stringency of disease definition raised by them, here we propose two other capital issues in the conventional studies: selection of appropriate controls and adjustment for environmental/clinical factors, from a clinician's point of view.

Stringency of disease definition

Kerkhof *et al.* show that there are five different definitions of knee OA in 28 studies involved in the Treat-OA consortium, and the definitions significantly influence the prevalence and association results[2]. Although most conventional genetic studies determine OA on radiographs as Kellgren–Lawrence (KL) score = 2 or higher (Table I)[3–7], the KL grading is limited in reproducibility and sensitivity due to the subjective judgment of observers and the categorical classification into only a five-grade scale[8]. In the ROAD (Research on Osteoarthritis Against Disability) study with a high-quality population-based cohort database of detailed environmental and genetic information of more than 3,000 participants[9], we delete the middle and ambiguous KL = 2 subgroup for the case-control analysis to increase the detection power. For example, our recent association analysis of the *EPAS1* gene which was identified to be crucial for OA development in mice was able to detect a significant difference of the minor allelic frequency (mAF) of a single nucleotide polymorphism (SNP) in the gene between KL = 3 & 4 (case; mAF = 11.1%) and KL = 0 & 1 (control; mAF = 15.2%)[10]. The mAF of the omitted KL = 2 subgroup was 12.3%, confirming an inverse relationship between mAF of the SNP and KL scores. This clearly indicates that inclusion of the KL = 2 subjects in the case group had caused a decrease in the detection power. In fact, this association was not reproduced by conventional Japanese and Chinese studies that include KL = 2 in the case group[11]. Considering that prevalence of the KL = 2 subgroup is shown to be fairly high in representative epidemiologic studies (17.3–41.3%; difference between KL ≥ 2 and KL ≥ 3 in Table II), removal of this subgroup may inevitably cause a decrease in the total sample size. However, we agree with the Kerkhof's opinion that improvement of the definition stringency may compensate a moderate decrease of the sample size to achieve a high detection power[2].

Generally, a lack of objective and quantitative measure for the disease definition remains a fatal limitation of clinical OA studies. The ROAD study has recently established the fully automatic program KOACAD (knee OA computer-aided diagnosis) to quantify the major OA parameters (joint space, osteophyte, *etc.*) on plain radiographs[8]. We believe that the KOACAD system as well as magnetic resonance image systems[12] will serve as optimal measures for the definition of OA in the near future, just as bone mineral density does in osteoporosis.

Selection of appropriate controls

In genetic studies on common diseases with a high prevalence, selection of disease-free controls is essential to avoid the potential bias due to contamination of affected subjects in the control. In representative epidemiologic studies worldwide, the prevalence of radiographic knee OA (KL ≥ 2) in the elderly was ≥ 30% in all

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Table I
Source of subjects, definition, and adjustment for confounders in representative knee OA genetic studies

Gene	GDF5[3]	PTGS2[4]	DVWA[5]	Chromosome 7q22[6]	HLA Class II/III[7]
	Candidate	GWAS	GWAS	GWAS	GWAS
Discovery population	**Case**	**Case**	**Case**	**Case**	**Case**
Source (N)	PBC+HP (718)	HP (243); PBC (114)	HP (740)	PBC (698)	HP (899)
Definition	KL≥2 or CL	CL; KL≥2	CL	KL≥2	CL
Mean age, %female	72y, 83%	NA, 100%; NA, 100%	72y, 90%; 72y, 82%	NA, NA	72y, 84%
	Control	**Control**	**Control**	**Control**	**Control**
	PBC+HP (861)	PBC (196); HS (89)	HP (1,289)	PBC (1,893)	HP+HS (3,396)
	KL≤1 or OR	KL≤1	OR	KL≤1	OD or NA
	49y, 54%	NA, 100%; NA, 87%	49y, 44%; 54y, 46%	NA, NA	53y, 44%
Replication population	**Case**	**Case**	**Case**	**Case**	**Case**
Source (N)	HP (313)	PBC (647); HP (530)	HP (417); PBC (242)	HP (3,142); PBC (741)	HP (813); PBC(167)
Definition	CL	KL≥2; CL	CL; KL≥2	CL, TKR; KL≥2	TKR; KL≥2
Mean age, %female	59y, 66%	NA, 100%; NA, 100%	71y, 75%; 60y, 70%	NA, NA; NA, NA	74y, 74%; 68y, 81%; 66y, 82%
	Control	**Control**	**Control**	**Control**	**Control**
	HS (485)	PBC (1,712); HS (660)	PBC (485); HS (413)	HS (33,825); PBC (2,718); HP (294)	HS (1,071); PBC (347)
	NS	KL≤1; ND	KL≤1; NS	NS; KL≤1 or KL=0; NS	NS; KL≤1 or KL=0
	57y, 65%	NA, 100%; NA, 100%	68y, 63%; 56y, 74%	NA, NA; NA, NA; NA, NA	66y, 64%; 68y, 39%; 60y, 65%
Adjustment	Population	Gender, population	Population	Gender, population	Population

GDF5: growth differentiation factor 5, PTGS2: prostaglandin-endoperoxide synthase 2, DVWA: double von Willebrand factor A domains, HLA: human leukocyte antigen. PBC: population-based cohort, HP: hospital patients, HS: healthy subjects, CL: clinical diagnosis, TKR: total knee replacement, OR: orthopaedic disease or injury, OD: other disease than OA, ND: not diagnosed for OA, NS: no sign of OA, NA: not available.

populations and >60% in Asian populations like Japan (ROAD study) and China (Shanghai) (Table II)[13]. Furthermore, the prevalence of asymptomatic knee OA was 24–36% in all populations. Hence, if so-called healthy subjects without knee symptoms were collected as controls, a considerable number of OA subjects would be included in the control group. Even in a series of genetic studies in Japan with a high OA prevalence[13], the control subjects are miscellaneous mixtures of various populations including considerable numbers of so-called healthy subjects and other disease patients without radiographic diagnosis (Table I)[3,5,7], indicating that a substantial percentage in the control groups are affected subjects. A recent analysis of the effect of controls selected with different levels of stringency on the association of known knee OA susceptibility genes demonstrates that a control with poor selection or without selection cannot be compensated by increase of the sample size[14]. Hence, selection of appropriate controls confirmed to be disease-free may be crucial to achieve a high detection power.

Adjustment for confounding environmental/clinical factors

Lastly, we should again note that OA is a multifactorial disease with environmental and genetic backgrounds and that the genetic contribution is less than half in knee OA[1]. A recent report by Takahashi *et al.* constructed knee OA prediction models based on genotype (combination of three risk alleles of asporin, GDF5 and DVWA) and environmental/clinical information (age, gender and body mass index), and evaluated the predictive power by area under the curve (AUC; range, 0.5 [worst] to 1 [best]) on a receiver operating characteristic (ROC) curve in a case-control association study[15]. The result was that the power by the genotype information was very small (AUC = 0.554), implicating uselessness of the three famous genotypes as a prognostic marker. Contrarily, the environmental/clinical information was a much better predictor (AUC = 0.678), but was little improved by the combination with the genotype information (AUC = 0.685), again confirming its uselessness. Hence, to achieve a high detection power for the susceptibility gene, all efforts should be made to exclude the influence of environmental/clinical factors. Surprisingly, however, there are big differences in age and gender between case and control groups in previous representative studies (Table I). Even a sole difference in age of about 20 years between case and control groups that is seen in the Japanese studies[3,5,7] is calculated to cause an increase of odds ratio for OA to 2.65 ($=1.05^{20}$), according to the authors' own estimation (1.05/year)[15]. Indeed, we are not opposed to recent activities of OA consortiums to pool subjects worldwide; however, we should note that the pooled subjects are miscellaneous mixtures of various populations with different backgrounds. Selection of case and control subjects with similar backgrounds is essential to minimize selection bias which strongly influences the results in genetic studies with small effect sizes of the risk alleles. Hence, at least for the initial screening, case and control groups should be selected from a single population-based

Table II
Prevalence of radiographic knee OA in representative population-based cohorts

Cohort	ROAD	Framingham	Zoetermeer	Johnston county	Beijing	Shanghai	NHANES III
Ethnicity	Japan	White in USA	Netherlands	Black & whites in USA	China	China	Black & whites in USA
Age	≥60	≥63	≥60	≥65	≥60	60–69	≥60
Total number	2,282	1,420	1,123	1,175	1,781	700	2,415
Radiographic knee OA (%)							
KL ≥ 2	61.9	33.0	30.0	40.6	38.8	64.1	37.4
KL ≥ 2 (symptomatic)	26.1	9.5		13.6	12.0		12.1
KL ≥ 2 (asymptomatic)	35.8	23.5		27.0	26.8		25.3
KL ≥ 3	20.6	15.7	10.2	13.6			10.2

KL ≥ 2 (asymptomatic) was defined as KL ≥ 2 (radiographic) but KL ≥ 2 (symptomatic). NHANES: National Health and Nutrition Examination Survey.
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cohort to adjust the living environment and stratified by confounding environmental/clinical factors which have been identified in preparatory epidemiologic analysis in the cohort. The reproducibility may then be examined in other replication cohorts of the worldwide consortiums, after adjustment for the specific confounding factors in the respective cohorts.

Taken together, conventional OA genetic studies appear to compare a case group containing a substantial number of subjects with ambiguous definition vs a control group containing a substantial number of affected subjects, plus without adjustment for confounding environmental/clinical factors. Contrary to the genetic studies, studies of clinical trial and observational epidemiology are performed under a sound scientific rigidity in compliance with very strict rules to examine the accurate effect sizes of interventions and environmental/clinical factors, respectively. Although genetic studies also examine the effect sizes of genes, they seem to have their fling in the lawless zone. Introduction of strict regulation in the genetic field, just like Consolidated Standards of Reporting Trials (CONSORT) guidelines in the clinical trial field[16], might improve the scientific rigidity. Otherwise, genetic studies seem to be unable to reach a genuine therapeutic target or even a prognostic marker of OA despite numerous efforts and great expense.

Author contributions

All authors contributed to writing and editing of the manuscript, and approved the final submitted manuscript.

Conflicts of interest

One author (HK) is an associate editor for Osteoarthritis and Cartilage. We declare no conflict 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)		Baseline	2nd visit	3rd visit	4th visit
		n	BMD (g/cm^2)	*n*	Change rate (%/3 years)	*n*	Change rate (%/7 years)	*n*	Change rate (%/10 years)	BMD (g/cm^2)	Change rate (%/3 years)	Change rate (%/7 years)	Change rate (%/10 years)
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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