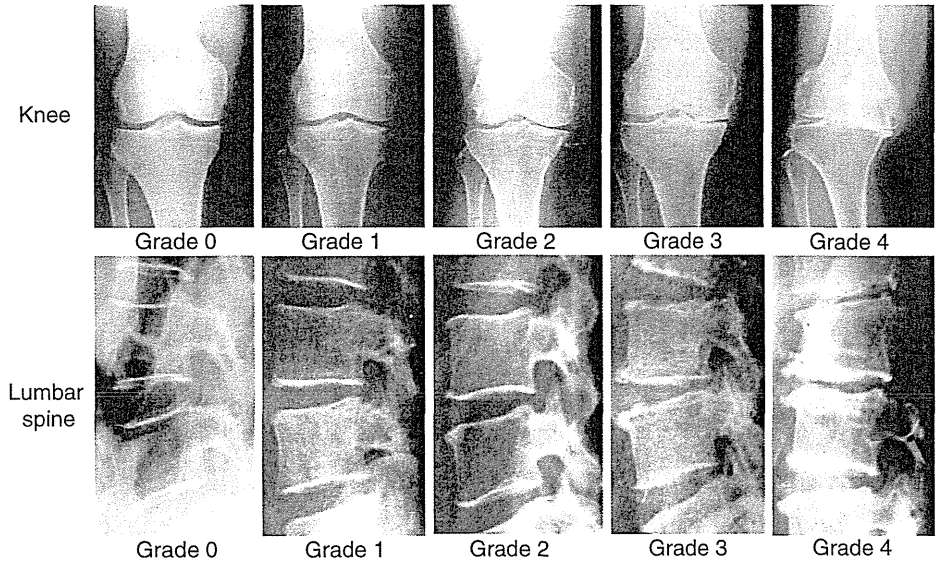

Fig. 1 Kellgren–Lawrence (KL) grade at knee and lumbar spine. *Knee*: *Grade 1* Doubtful narrowing of the joint space and possible osteophytic lipping, *Grade 2* definite osteophytes and possible narrowing of the joint space, *Grade 3* multiple moderate osteophytes, definite narrowing of the joint space, some sclerosis, and possible deformity of bone ends, *Grade 4* large osteophytes, marked narrowing of the joint space, severe sclerosis, and definite deformity of bone ends. *Lumbar spine*: *Grade 1* Minimal osteophytosis only, *Grade 2* definite osteophytosis with some sclerosis of the anterior part of the vertebral plates, *Grade 3* marked osteophytosis and sclerosis of the vertebral plates with slight narrowing of the disk space, *Grade 4* large osteophytes, marked sclerosis of the vertebral plates, and marked narrowing of the disk space

(RP); bodily pain (BP); vitality (VT); social functioning (SF); mental health (MH); role emotional (RE). The SF-8 provides two summary scores for physical and mental health [physical component summary (PCS) and mental component summary (MCS)]. The EQ-5D questionnaire [16] translated into Japanese was also used [22]. This five-dimensional healthcare classification includes questions on the status of morbidity, self-care, usual activities, pain/discomfort, and anxiety/depression. Participants were asked to indicate current health status by checking off the most appropriate of three statements on each of five QOL dimensions. Each statement represents an increasing degree of severity. These results were coded and converted to a score of utility using the tables of values. For disease-specific scales, the WOMAC (version LK 3.0) [17, 18], a 24-item OA-specific index, was utilized. The WOMAC consists of three domains: pain; stiffness; physical function. Domain scores range from 0 to 20 for pain, 0 to 8 for stiffness, and 0 to 68 for physical function. Japanese versions of the WOMAC have been validated [23].

Statistical analysis

We performed a non-paired Student's *t* test to examine differences between subjects with and without knee pain and low back pain. The impact of knee pain and low back pain on QOL was analyzed by multiple regression analysis after adjusting for age and BMI. We also examined the association of KL grade at the knee with the magnitude of QOL loss in subjects with knee pain using the Tukey honestly significant difference (HSD) test. If a subject showed pain in both knees, the more severe KL grade was designated as that of the subject. The Tukey HSD test was also used to examine the association of the presence of VFx and lumbar spondylosis with the magnitude of QOL loss in subjects with low back pain. For the lumbar spine, the most severe KL grade among all intervertebral spaces was designated as that of the subject. Data analyses were performed using SAS ver. 9.0 software (SAS Institute, Cary, NC).

Results

Characteristics of the 1,369 women ≥40 years old enrolled in the ROAD study are shown in Table 1. The prevalence of knee pain was higher than that of low back pain, while the prevalence of knee OA and lumbar spondylosis was similar and substantially higher than that of VFx.

Table 2 shows the mean scores for all QOL domains in the SF-8 and EQ-5D utility score according to the presence of knee pain and low back pain. We further examined the independent association of knee pain and low back pain with QOL using multiple regression analysis after

Table 1 Characteristics of the participants

Clinical/demographic/QOL characteristics of study cohort	Values
n	1,369
Age (years)	68.4 ± 11.1
Height (cm)	150.0 ± 6.9
Weight (kg)	51.4 ± 9.0
BMI (kg/m^2)	22.8 ± 3.7
Knee pain (%)	27.9
Low back pain (%)	17.3
VFx (%)	7.7
Knee OA (%)	60.2
Lumbar spondylosis (%)	61.3
SF-8 score	
GH	49.5 ± 5.8
PF	49.5 ± 6.3
RP	49.8 ± 6.5
BP	49.1 ± 9.6
VT	49.3 ± 5.9
SF	51.9 ± 6.2
MH	53.3 ± 6.4
RE	51.4 ± 5.7
PCS	46.8 ± 7.0
MCS	52.5 ± 6.1
EQ-5D score	0.90 ± 0.15
WOMAC index	
Pain (0–20)	1.50 ± 2.57
Stiffness (0–8)	0.77 ± 1.33
Function (0–68)	4.49 ± 8.37

Unless indicated otherwise, values represent the mean ± standard deviation (SD)

QOL Quality of life,*BMI* body mass index, *VFx* vertebral fracture, *OA* osteoarthritis, *SF-8* Medical Outcomes Study Short Form-8 health survey, *GH* general health, *PF* physical function, *RP* role physical, *BP* bodily pain, *VT* vitality, *SF* social function, *MH* mental health, *RE* role emotional, *PCS* physical component summary, *MCS* mental component summary, *EQ-5D* EuroQOL questionnaire, *WOMAC* the Western Ontario and McMaster Universities Osteoarthritis Index

adjustment for age and BMI. Knee pain was significantly associated with lower QOL scores in all domains of the SF-8, with the exception of MH, RE, MCS, and also with lower EQ-5D utility scores. Low back pain was significantly associated with lower QOL scores in almost all domains of the SF-8, except for MCS, and with lower EQ-5D utility scores. The impact of low back pain was greater than that of knee pain in almost all QOL domains.

Scores of the SF-8, EQ-5D, and WOMAC by KL grade of knee in women with knee pain are shown in Table 3. The Tukey HSD test revealed that compared with women with KL = 0/1, PCS in the SF-8 and pain in the WOMAC were significantly lower in women with KL = 3 knee OA, while PF, RP, BP, and PCS in the SF-8 and all domains of the WOMAC were significantly lower in women with KL = 4 knee OA. After adjusting for age and BMI, PCS in the SF-8 and pain and physical function in the WOMAC were also significantly lower in women with KL = 4 knee OA compared with those with KL = 0/1.

Table 4 shows the association of KL grade for the lumbar spine and presence of VFx with QOL in subjects with low back pain. In women with low back pain, no associations were seen between KL grade and any of the domains of the SF-8 or EQ-5D utility scores, while PF, RP, RE, and PCS were significantly lower in subjects with VFx than in those without VFx.

To compare the magnitude of impact on PCS between knee pain graded as KL = 4 knee OA and low back pain with vertebral fracture, we then used multiple regression analysis after adjusting for age and BMI. The impact of knee pain graded as KL = 4 knee OA on PCS was larger than that of low back pain with VFx (beta: −0.11 and −0.09, $p < 0.0001$, respectively).

Discussion

Few previous studies have examined the associations of knee pain with QOL [4], and there have been no studies published to date on the impact of knee pain and low back pain on QOL in women. The results of our study reveal that among our study cohort of 1,369 Japanese women ≥40 years of age, knee pain and low back pain were significantly associated with lower QOL scores. The multiple regression analysis showed that the impact of knee pain on QOL was weaker than that of low back pain; however, knee pain with severe knee OA had a strong, negative impact on QOL that was greater than that of low back pain with VFx. In fact, the severity of knee OA was significantly associated with the magnitude of QOL loss in subjects with knee pain. In other words, the Tukey HSD test after adjustment for age and BMI showed that in subjects with KL = 4 knee OA, PCS in the SF-8 was significantly lower and pain and physical function in the WOMAC were both significantly higher, while QOL scores of subjects with KL = 2 knee OA were similar to those of subjects with KL = 0/1. These results indicate not only that the prevalence of knee pain is higher but also that the magnitude of knee pain may be more severe in subjects with severe knee OA, whereas the magnitude of knee pain may be similar in subjects with moderate knee OA and in those without knee OA. However, the two features of knee OA, joint space narrowing and osteophytosis, cannot be assessed separately using the KL grade, so we were unable to clarify the independent effects of these two features to the association

Table 2 Scores for QOL in participants with and without knee pain and low back pain and associations with knee and low back pain by multiple regression analysis after adjusting for age, BMI, knee pain, and low back pain

QOL assessment domain	Knee pain			Low back pain		
	No	Yes	Adjusted beta[a]	No	Yes	Adjusted beta[a]
SF-8						
GH	49.9 ± 5.8	48.8 ± 5.8[b]	−0.043[c]	50.1 ± 5.7	47.1 ± 5.5[b]	−0.152[c]
PF	50.1 ± 6.0	47.9 ± 6.8[b]	−0.064[c]	50.2 ± 5.9	46.0 ± 6.9[b]	−0.180[c]
RP	50.4 ± 6.3	48.4 ± 6.9[b]	−0.058[c]	50.6 ± 6.1	47.3 ± 7.5[b]	−0.182[c]
BP	50.4 ± 9.4	45.6 ± 9.2[b]	−0.163[c]	50.3 ± 9.5	43.3 ± 7.7[b]	−0.223[c]
VT	49.7 ± 5.9	48.4 ± 5.8[b]	−0.059[c]	49.7 ± 5.9	47.2 ± 5.0[b]	−0.134[c]
SF	52.4 ± 5.6	50.8 ± 7.3	−0.077[c]	52.4 ± 5.7	49.8 ± 8.0[b]	−0.111[c]
MH	53.6 ± 6.1	52.7 ± 6.8	−0.039	53.7 ± 6.2	51.4 ± 6.9[b]	−0.128[c]
RE	51.8 ± 5.4	50.8 ± 6.4	−0.038	51.9 ± 5.3	49.4 ± 7.1[b]	−0.131[c]
PCS	47.7 ± 6.9	44.5 ± 7.0[b]	−0.113[c]	47.8 ± 6.7	42.4 ± 7.0[b]	−0.218[c]
MCS	52.6 ± 5.9	52.6 ± 6.7	−0.004	52.7 ± 5.9	51.9 ± 7.3	−0.0052
EQ-5D	0.92 ± 0.14	0.85 ± 0.17[b]	−0.127[c]	0.91 ± 0.14	0.82 ± 0.17[b]	−0.150[c]

[a] Adjusted beta values are shown using multiple regression analysis after adjusting for age, BMI, knee pain and low back pain

[b] $p < 0.05$ vs. subjects without the corresponding pain by non-paired t test

[c] $p < 0.05$

Table 3 Scores for SF-8, EQ-5D, and WOMAC by Kellgren–Lawrence (KL) grade in participants with knee pain

Variables	KL 0/1	KL 2	KL 3	KL 4
Prevalence (%)	26.8	37.5	22.8	12.9
SF-8				
GH	49.3 ± 5.9	49.1 ± 5.7	48.5 ± 6.3	47.2 ± 5.3
PF	49.3 ± 6.8	48.3 ± 6.1	47.2 ± 7.6	45.0 ± 6.3[a]
RP	49.8 ± 6.4	48.4 ± 6.4	48.1 ± 7.8	46.1 ± 7.3[a]
BP	46.7 ± 8.9	46.9 ± 9.2	44.2 ± 9.2	42.0 ± 8.7[a]
VT	49.2 ± 6.0	49.0 ± 5.5	47.2 ± 6.2	46.8 ± 4.9
SF	51.6 ± 6.8	50.4 ± 7.2	50.5 ± 8.0	50.8 ± 7.3
MH	52.6 ± 7.6	52.5 ± 6.5	52.8 ± 6.8	53.6 ± 6.2
RE	51.4 ± 6.5	50.6 ± 5.9	50.6 ± 7.0	50.3 ± 6.7
PCS	46.1 ± 6.5	45.4 ± 6.4	43.5 ± 7.9[a]	40.6 ± 6.1[a,b]
MCS	52.5 ± 7.2	52.0 ± 6.1	52.7 ± 7.2	54.2 ± 6.3
EQ-5D	0.89 ± 0.15	0.84 ± 0.19	0.84 ± 0.16	0.81 ± 0.18[a]
WOMAC				
Pain	1.67 ± 2.72	2.33 ± 2.99	2.80 ± 2.76[a]	4.38 ± 3.29[a,b]
Stiffness	0.96 ± 1.59	1.14 ± 1.61	1.34 ± 1.50	1.88 ± 2.20[a]
Function	4.58 ± 9.38	6.95 ± 9.80	8.05 ± 9.56	14.94 ± 12.46[a,b]

Except where indicated otherwise, values represent the mean ± SD

[a] $p < 0.05$ vs. KL 0/1 in the corresponding group by the Tukey HSD test

[b] $p < 0.05$ vs. KL 0/1 in the corresponding group by the Tukey HSD test after adjustment for age and BMI

of knee pain with QOL. Furthermore, radiographic joint space narrowing represents not only joint cartilage destruction but also meniscal loss or extrusion. In addition, knee pain may arise from a variety of structures other than joint cartilage, including menisci, synovium, ligaments, bursae, bone, and bone marrow [24–28]. Comprehensive mechanistic studies of knee pain taking various tissues in and around the knee joint into consideration are thus needed to elucidate the relationships between radiographic OA and QOL.

The results of our previous study showed that lumbar spondylosis is weakly associated with low back pain. In the

Table 4 Scores for SF-8 and EQ-5D by KL grade and VFx in subjects with low back pain

Variables	Lumbar spondylosis				VFx	
	KL 0/1	KL 2	KL 3	KL 4	No	Yes
Prevalence (%)	28.3	12.9	26.6	32.2	10.7	89.3
SF-8						
GH	48.1 ± 5.6	47.1 ± 5.7	46.4 ± 5.7	46.9 ± 5.1	47.2 ± 5.5	46.1 ± 5.4
PF	46.8 ± 7.4	45.9 ± 6.7	44.7 ± 6.7	46.3 ± 6.6	46.2 ± 6.9	43.9 ± 6.3[a]
RP	47.2 ± 7.4	47.1 ± 6.9	44.7 ± 8.2	46.7 ± 7.2	46.7 ± 7.4	43.4 ± 7.6[a]
BP	43.8 ± 8.0	44.1 ± 8.3	43.4 ± 7.9	42.6 ± 7.2	43.6 ± 7.7	41.1 ± 7.4
VT	48.3 ± 5.3	45.6 ± 6.7	47.3 ± 5.5	46.9 ± 5.0	47.3 ± 5.6	46.3 ± 3.9
SF	51.4 ± 6.6	50.8 ± 6.5	47.8 ± 9.8	49.7 ± 7.9	50.0 ± 7.9	48.3 ± 8.7
MH	52.8 ± 6.0	52.0 ± 7.4	50.0 ± 7.5	51.2 ± 6.8	51.5 ± 6.9	49.8 ± 7.0
RE	50.7 ± 5.9	51.2 ± 5.2	47.8 ± 8.8	49.0 ± 6.7	49.7 ± 7.0	46.9 ± 7.1[a]
PCS	42.9 ± 7.7	42.3 ± 7.2	41.8 ± 7.0	42.4 ± 6.3	42.6 ± 7.0	40.2 ± 6.2[a]
MCS	53.5 ± 6.0	52.8 ± 6.7	50.3 ± 8.6	51.5 ± 7.1	52.0 ± 7.3	50.6 ± 6.8
EQ-5D	0.86 ± 0.15	0.87 ± 0.18	0.77 ± 0.18[a]	0.81 ± 0.17	0.83 ± 0.17	0.80 ± 0.21

Except where indicated otherwise, values represent the mean score ± SD

[a] $p < 0.05$ vs. KL 0/1 in the corresponding group by the Tukey HSD test

present study, we found that low back pain was strongly associated with lower QOL scores, while the severity of lumbar spondylosis was not significantly associated with the magnitude of QOL loss in women with low back pain. These results may be partly explained by the weak association between lumbar spondylosis and low back pain, as reported by us and other researchers [1, 29, 30]. KL grade encompasses assessments of both osteophytosis and disk space narrowing, but not of narrowing of the spinal canal, spondylolisthesis, or scoliosis, all of which are associated with low back pain. In addition, low back pain arises from a number of disorders other than disc space narrowing, such as nociceptive stimuli, inflammation, muscle weakness, and abnormal load on muscles, ligaments, or capsular tissues [31]. Indeed, disc degeneration was detected by magnetic resonance imaging (MRI) at at least one lumbar level in all but one asymptomatic volunteer in a 60- to 80-year-old age group [32]. Pain is also influenced by psychological status, such as depression, since significant associations between low back pain and depression have been confirmed in many longitudinal studies [33, 34]. In terms of VFx, previous studies have shown strong effects of clinical VFx on QOL in clinical studies [35, 36], and associations of subclinical vertebral deformity with QOL were found in women in a population-based study [37]. The results of our also show that VFx was significantly associated with the magnitude of QOL loss as measured by the PF, RP, RE, and PCS of the SF-8 in subjects with low back pain, indicating that low back pain with VFx has a strong impact on QOL in women.

Knee pain and low back pain were not significantly associated with lower scores for the MCS of the SF-8 in this study. MCS questions within the SF-8 include generic questions on energy levels, feelings of being "downhearted and blue", and interference with daily activities as a result of emotional problems. As such, this summary score is less sensitive to the presence of mental health issues than disease-specific scales such as the Kessler psychological distress scale [38]. In fact, although in one study psychological distress was significantly more frequent in individuals with pain than in those without [39], the MCS score did not differ significantly between these two groups [40]. Whether the MCS is not associated with knee pain and low back pain is thus unclear. A further complication is that previous research has shown that chronic pain patients who accept their diagnosis display lower levels of pain and affective distress than those who are uncertain [41, 42], which may be one reason why in our study MCS was not associated with pain. The ROAD study is a longitudinal survey, and analysis of its data over time may elucidate the association of QOL measured by MCS and pain.

This study has several limitations. First, it was a large-scaled population-based study, but the baseline data were cross-sectional, so causal relationships could not be determined. The ROAD study is a longitudinal survey that will eventually shed light on the causal relationships. Second, we only used a semi-quantitative method to assess VFx. In addition, the KL system was used for knee OA and lumbar spondylosis. The KL system is the most conventional grading system to detect the radiographic severity of knee OA, but joint space narrowing and osteophyte formation cannot be assessed separately in this categorical system. In addition, since the KL system emphasizes osteophytosis, the handling of data on lumbar spondylosis

with disc space narrowing but no osteophytosis is unclear. In addition, in terms of the lumbar spine, we did not include lumbar spinal canal stenosis (LSCS), scoliosis, spondylolisthesis, or narrowing of the nerve canal in our analysis, although these changes are also associated with QOL. To determine the associations of these changes of the lumbar spine and knee with QOL, we are currently developing a computer-aided diagnostic program to enable automatic measurement of the major features of VFx, disc space narrowing, osteophytosis, LSCS, scoliosis, spondylosis, and narrowing of the nerve canal in the lumbar spine, and of joint space narrowing and osteophytosis at the knee on plain radiographs [43]. Third, we did not include the onset of VFx in the analysis, although the severity of low back pain often appears to be associated with the interval from the onset of VFx. With respect to clinical fractures, we examined the history of fracture, including vertebral fracture, in the ROAD study by self-report, and no clinical vertebral fractures occurred within 1 month prior to baseline examination. However, we could not compare radiographs of the lumbar spine at baseline examination with those before the examination as the subjects had not undergone radiography of the lumbar spine prior to that examination. We were therefore unable to assess the incidence of subclinical fracture within the month prior to the baseline examination. Both clinical and subclinical vertebral fractures are associated with lower QOL in women [44], but the association between the severity of low back pain and the interval from onset of subclinical VFx may be weaker than that for clinical VFx; consequently, the absence of data on the incidence of subclinical VFx may not strongly affect the present results.

In conclusion, the results of our cross-sectional study using a large-scale population (1,369 Japanese women $\geq$40 years of age) from the ROAD study reveal that knee pain and low back pain were significantly associated with the QOL of these women. In women with knee pain, KL = 4 knee OA was strongly associated with QOL loss. In women with low back pain, no significant associations were seen between KL grade and QOL, while VFx may have some associations with QOL loss. The impact of knee pain with KL = 4 knee OA for PCS was larger than that of low back pain with VFx. Future studies, along with the continued longitudinal survey in the ROAD study, will elucidate the environmental and genetic backgrounds of knee pain and low back pain.

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Conflict of interest statement None.

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ORIGINAL ARTICLE

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

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

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

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Introduction

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

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

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

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

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

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

Materials and methods

Establishment of baseline cohort

This survey was performed in the Japanese town of Taiji. The Taiji cohort has been profiled in detail elsewhere [22–24] and so is summarized here only briefly. Taiji is located in the southern coastal area of Wakayama Prefecture. A list of all inhabitants born in 1913–1952, and therefore aged between 40 and 79 years old in 1993, was compiled based on resident registrations as of the end of 1992. A cohort of 2,261 inhabitants (1,028 men, 1,233 women) was identified, and all members of the cohort completed a self-administered, 125-item questionnaire addressing topics such as dietary habits, smoking habits, alcohol consumption, and physical exercise (whole cohort).

From the whole cohort, 50 men in each of four age groups between 40 and 79 years by decade of birth year (1913–1922, 1923–1932, 1933–1942, and 1943–1952), for a total of 200 participants, were randomly selected. BMD was measured for these 200 participants in 1993. At this time, blood samples of all participants were taken. An interviewer administered a second questionnaire to these 200 participants covering items of past medical history, including questions related to osteoporotic fractures and falls, family history, calcium intake, dietary habits, physical exercise, occupational activities, sun exposure, and, for women, additional questions about reproductive variables (baseline study).

Measurements of endogenous sex steroids

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

BMD measurements

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

To control for the precision of DXA, the equipment was checked at every examination in 1993, 1996, 2000, and 2003 using the same phantom, and BMD of the phantom was regulated to 1.030 ± 0.016 g/cm^2 (1.5%) during all examinations. All BMD measurements were performed by the same medical doctor (N.Y.). Intraobserver variability for DXA scans by this investigator was 0.35% using the phantom, as reported previously [25].

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

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

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

Statistical analysis

All statistical analyses were performed using STATA statistical software (STATA, College Station, TX, USA). Differences were tested for significance using analysis of variance for comparisons among multiple groups, and Scheffe's least significant difference (LSD) test for pairs of groups. Significant items were selected, and multiple regression analysis was performed with adjustment of suitable variables.

Results

Eligible participants and baseline characteristics

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

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

Mean levels of serum concentration of sex steroids at baseline

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

Predictive capacity of endogenous sex steroids for bone change

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

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

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

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

Birth cohort	Age-group (years)	*n*	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	E2 (pg/mL)		FT (pg/mL)	
			Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
1943–1952	40–49	50	44.2 (2.6)	168.8 (5.2)	69.0 (10.4)	24.2 (3.2)	46	22.1 (7.4)	50	10.9 (2.8)
1933–1942	50–59	50	54.8 (2.7)	165.6 (5.0)[a]	63.5 (9.4)[a]	23.1 (2.9)	43	22.2 (7.0)	50	9.8 (2.6)
1923–1932	60–69	50	64.6 (2.5)	163.0 (4.8)[a]	62.9 (9.6)[a]	23.6 (3.2)	46	23.1 (8.5)	49	8.8 (2.6)[a]
1913–1922	70–79	50	74.0 (2.7)	160.7 (5.4)[a,b]	57.5 (8.3)[a,b,c]	22.2 (2.8)[a]	43	22.3 (7.7)	49	8.2 (3.1)[a]
1913–1952	40–79	200	59.4 (11.4)	164.5 (5.9)	63.2 (10.2)	23.3 (3.1)	178	22.4 (7.6)	198	9.4 (2.9)

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

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

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

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

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

Birth cohort	Age-group (years)	L2–L4								Femoral neck			
		Baseline		2nd visit (3-year follow-up)		3rd visit (7-year follow-up)		4th visit (10-year follow-up)		Basesline	2nd visit	3rd visit	4th visit
		n	BMD (g/cm^2)	*n*	Change rate (%/3 years)	*n*	Change rate (%/7 years)	*n*	Change rate (%/10 years)	BMD (g/cm^2)	Change rate (%/3 years)	Change rate (%/7 years)	Change rate (%/10 years)
1943–1952	40–49	50	1.05 (0.15)	48	0.6 (3.8)	46	−0.6 (5.1)	43	−0.2 (5.8)	0.86 (0.09)	0.3 (4.6)	−1.8 (4.8)	−1.5 (10.9)
1933–1942	50–59	50	0.98 (0.17)	47	1.0 (3.3)	46	−0.0 (6.3)	46	1.6 (8.0)	0.80 (0.13)[a]	−0.2 (4.9)	0.7 (10.0)	−3.0 (6.8)
1923–1932	60–69	50	1.04 (0.21)	49	1.3 (3.6)	47	1.4 (7.1)	41	2.3 (9.4)	0.77 (0.11)[a]	1.0 (7.0)	−0.1 (9.3)	0.3 (12.5)
1913–1922	70–79	50	0.97 (0.19)	37	0.1 (5.3)	31	−1.2 (7.9)	23	−1.5 (9.2)	0.71 (0.08)[a,b,c]	0.9 (6.3)	4.6 (10.2)[a]	6.6 (16.2)[b]
1913–1952	40–79	200	1.01 (0.18)	181	0.8 (4.0)	170	0.0 (6.6)	153	0.8 (8.1)	0.79 (0.12)	0.5 (5.7)	0.5 (8.9)	−0.3 (11.7)

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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ORIGINAL ARTICLE

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

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

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

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Introduction

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

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

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

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

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

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

Materials and methods

Establishment of baseline cohort

This survey was performed in the Japanese town of Taiji. The Taiji cohort has been profiled in detail elsewhere [22–24] and so is summarized here only briefly. Taiji is located in the southern coastal area of Wakayama Prefecture. A list of all inhabitants born in 1913–1952, and therefore aged between 40 and 79 years old in 1993, was compiled based on resident registrations as of the end of 1992. A cohort of 2,261 inhabitants (1,028 men, 1,233 women) was identified, and all members of the cohort completed a self-administered, 125-item questionnaire addressing topics such as dietary habits, smoking habits, alcohol consumption, and physical exercise (whole cohort).

From the whole cohort, 50 men in each of four age groups between 40 and 79 years by decade of birth year (1913–1922, 1923–1932, 1933–1942, and 1943–1952), for a total of 200 participants, were randomly selected. BMD was measured for these 200 participants in 1993. At this time, blood samples of all participants were taken. An interviewer administered a second questionnaire to these 200 participants covering items of past medical history, including questions related to osteoporotic fractures and falls, family history, calcium intake, dietary habits, physical exercise, occupational activities, sun exposure, and, for women, additional questions about reproductive variables (baseline study).

Measurements of endogenous sex steroids

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

BMD measurements

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

To control for the precision of DXA, the equipment was checked at every examination in 1993, 1996, 2000, and 2003 using the same phantom, and BMD of the phantom was regulated to 1.030 ± 0.016 g/cm^2 (1.5%) during all examinations. All BMD measurements were performed by the same medical doctor (N.Y.). Intraobserver variability for DXA scans by this investigator was 0.35% using the phantom, as reported previously [25].

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

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

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

Statistical analysis

All statistical analyses were performed using STATA statistical software (STATA, College Station, TX, USA). Differences were tested for significance using analysis of variance for comparisons among multiple groups, and Scheffe's least significant difference (LSD) test for pairs of groups. Significant items were selected, and multiple regression analysis was performed with adjustment of suitable variables.

Results

Eligible participants and baseline characteristics

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

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

Mean levels of serum concentration of sex steroids at baseline

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

Predictive capacity of endogenous sex steroids for bone change

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

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

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

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

Birth cohort	Age-group (years)	*n*	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	E2 (pg/mL)		FT (pg/mL)	
			Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
1943–1952	40–49	50	44.2 (2.6)	168.8 (5.2)	69.0 (10.4)	24.2 (3.2)	46	22.1 (7.4)	50	10.9 (2.8)
1933–1942	50–59	50	54.8 (2.7)	165.6 (5.0)[a]	63.5 (9.4)[a]	23.1 (2.9)	43	22.2 (7.0)	50	9.8 (2.6)
1923–1932	60–69	50	64.6 (2.5)	163.0 (4.8)[a]	62.9 (9.6)[a]	23.6 (3.2)	46	23.1 (8.5)	49	8.8 (2.6)[a]
1913–1922	70–79	50	74.0 (2.7)	160.7 (5.4)[a,b]	57.5 (8.3)[a,b,c]	22.2 (2.8)[a]	43	22.3 (7.7)	49	8.2 (3.1)[a]
1913–1952	40–79	200	59.4 (11.4)	164.5 (5.9)	63.2 (10.2)	23.3 (3.1)	178	22.4 (7.6)	198	9.4 (2.9)

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

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

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

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

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

Birth cohort	Age-group (years)	L2–L4								Femoral neck			
		Baseline		2nd visit (3-year follow-up)		3rd visit (7-year follow-up)		4th visit (10-year follow-up)		Basesline	2nd visit	3rd visit	4th visit
		n	BMD (g/cm^2)	*n*	Change rate (%/3 years)	*n*	Change rate (%/7 years)	*n*	Change rate (%/10 years)	BMD (g/cm^2)	Change rate (%/3 years)	Change rate (%/7 years)	Change rate (%/10 years)
1943–1952	40–49	50	1.05 (0.15)	48	0.6 (3.8)	46	−0.6 (5.1)	43	−0.2 (5.8)	0.86 (0.09)	0.3 (4.6)	−1.8 (4.8)	−1.5 (10.9)
1933–1942	50–59	50	0.98 (0.17)	47	1.0 (3.3)	46	−0.0 (6.3)	46	1.6 (8.0)	0.80 (0.13)[a]	−0.2 (4.9)	0.7 (10.0)	−3.0 (6.8)
1923–1932	60–69	50	1.04 (0.21)	49	1.3 (3.6)	47	1.4 (7.1)	41	2.3 (9.4)	0.77 (0.11)[a]	1.0 (7.0)	−0.1 (9.3)	0.3 (12.5)
1913–1922	70–79	50	0.97 (0.19)	37	0.1 (5.3)	31	−1.2 (7.9)	23	−1.5 (9.2)	0.71 (0.08)[a,b,c]	0.9 (6.3)	4.6 (10.2)[a]	6.6 (16.2)[b]
1913–1952	40–79	200	1.01 (0.18)	181	0.8 (4.0)	170	0.0 (6.6)	153	0.8 (8.1)	0.79 (0.12)	0.5 (5.7)	0.5 (8.9)	−0.3 (11.7)

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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