Table 5 Standardized partial regression coefficient (β) of changes of BTMs for annual change rate for BMD

BTMs	L2–L4 BMD		Femoral neck BMD		Total hip BMD	
	β	P	β	P	β	P
Men						
Total OC	−0.12	0.139	0.06	0.455	−0.16	0.056
Beta-CTX	0.03	0.747	−0.04	0.632	0.11	0.166
NTX	−0.08	0.323	0.01	0.875	−0.01	0.938
Women						
Total OC	−0.18	0.024	−0.16	0.068	−0.31	<0.001
Beta-CTX	−0.09	0.269	−0.06	0.457	−0.18	0.027
NTX	−0.21	0.006	−0.06	0.495	−0.34	<0.001

Standardized partial regression coefficients were obtained after adjustment for age and body mass index

BMD, bone mineral density; BTMs, biochemical markers of bone turnover; *n*, number of subjects; OC, osteocalcin; beta-CTX, beta-isomerized C-terminal cross-linking telopeptide of type I collagen; NTX, N-terminal cross-linking telopeptide of type I collagen

was performed using change rates of L2–L4 BMD as an objective factor and change rates of each BTM as an explanatory factor after controlling for age, body mass index (BMI), occurrence of clinical vertebral fractures over 10 years in both men and women, and menstrual status over 10 years (0, premenopausal; 1, transition to menopause; 2, menopausal) in women. Furthermore, with regard to the proximal hip, including the femoral neck and total hip, multivariate regression analysis was performed after controlling for age and BMI in both men and women and menstrual status over 10 years in women. Table 5 shows the standardized partial regression coefficient of change rates of BTMs for annual change rates for BMD. For men, there was no significant association of changes of BTMs and changes of BMDs at any of the sites. By contrast, for women, although no significant association was seen between changes of BMD at the femoral neck and changes in BTM, change rates of total OC and NTX were significantly associated with change rates of L2–L4 BMD, and change rates of total OC, beta-CTX, and NTX were significantly associated with change rates of BMD at the total hip (Table 5).

Comparison of mean BTM levels in given age strata classified by birth cohort

The BTM levels of subjects in their fifties, sixties, and seventies in 1993 were compared to those in their fifties, sixties, and seventies in 2003 (Fig. 2). No significant differences in mean values of BTMs were identified in the same age strata or in different birth cohorts in men. By contrast, the BTM levels of female subjects in 1993 tended

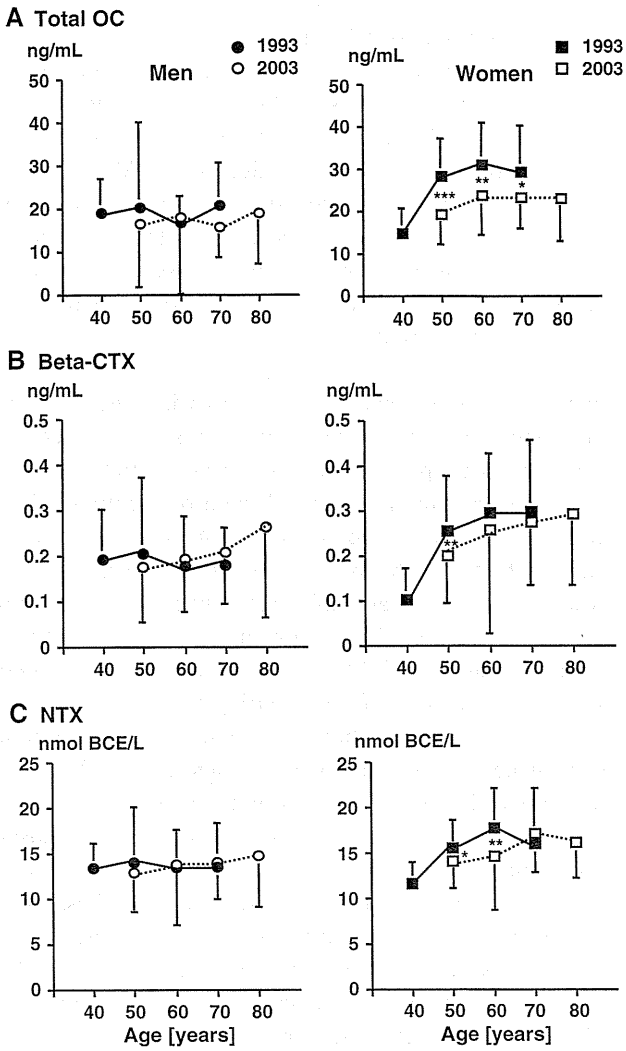

Fig. 2 Changes in serum biochemical markers of bone turnover over 10 years, classified by age strata. **a** Total osteocalcin (*OC*). **b** Beta-isomerized C-terminal cross-linking telopeptide of type I collagen (*beta-CTX*). **c** N-terminal cross-linking telopeptide of type I collagen (*NTX*). *BCE*, bone collagen equivalents. Significantly different from values of participants in the same age strata between different birth-cohorts in 1993 and 2003 (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$)

to be higher than those in 2003 for the same age strata (Fig. 2). This result suggests an effect of birth cohort for serum levels of BTMs in women, particularly those in their fifties, but not in men. That is, BTM levels were significantly lower for women in their fifties in 2003 compared to those in their fifties in 1993.

Discussion

In this 10-year follow-up study, we clarified changes to levels of BTMs in men and women from a rural community

in Japan. Change rates of BTMs over 10 years were influenced by menstrual transition, age, and sex. Increases in both bone formation and bone resorption markers are associated with decreases in BMD at L2–L4 and the total hip in women after controlling for confounding factors. In terms of birth-cohort effect, values of BTMs for participants in 2003 were significantly lower than those in 1993 when compared between the same age strata in women.

We have already reported the age–sex distribution of values of BTMs, such as intact OC, alkaline phosphatase, C-terminal propeptide of type I procollagen, C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase, urinary pyridinoline cross-links of collagen, and deoxypyridinoline cross-links of collagen using the same population as the present study [11]. That report showed that levels of all the aforementioned BTMs were significantly lower in the 40–49 age group than in each of the 50–59, 60–69, and 70–79 age groups in women, whereas no significant differences were apparent among age groups in men [11]. Following the previous study, we clarified changes of BTMs in each age group in the present study, with values of BTMs starting to increase in women in their forties, then stabilizing (beta-CTX, NTX) or mildly decreasing (total OC) among older age groups. The rate of decrease of BTMs was greatest in the menopausal transient group compared to the groups remaining premenopausal or postmenopausal. Although the number of subjects in each category of menstrual status was limited, these results suggest that the onset of menopause in their forties causes dramatic changes in bone metabolism in women. With regard to estrogen and changes of BTMs, Ebeling et al. [21] and Sowers et al. [22] reported that levels of BTMs increased before menopause as a consequence of declining concentrations of serum estradiol (E_2) and increasing concentrations of follicle-stimulating hormone. We have already reported that serum levels of total E_2 were associated with decreased BMD over 3 years among premenopausal women [23].

In terms of the effects of BTM changes on changes in BMD over 10 years, the present study revealed that increases in BTMs over 10 years in women, even for bone formation markers or bone resorption markers, are associated with decreased BMD at L2–L4 and total hip. This association remains after controlling for confounding factors. No previous reports appear to have clarified associations between changes in levels of BTMs and bone loss for one decade. The present study revealed that a higher rise in values of BTMs, particularly total OC and NTX, was associated with faster BMD loss in women. These associations were observed over a reasonably long time period. However, these findings were identified at L2–L4 and total hip, but not at the femoral neck. Although reasons for site-specific differences in the association between BTMs and BMD remain uncertain, we have already reported that bone loss rate differs depending on the site involved in another cohort study [13]. We have also reported that characteristics differ between fast bone loss at the lumbar spine and femoral neck [24]. One reason for these site-specific differences might be that fixing the position for BMD examination using DXA was more difficult for the femoral neck than for L2–L4 or total hip, and as a result, the CV tended to be higher there than at other sites [17]. Changes that increase BMD, such as osteophytosis or sclerotic changes, are also observed most frequently at the lumbar spine, which might be another reason for the site-specific differences. We were unable to perform X-ray examinations of participants in the present study. We thus could not control the influence of degenerative changes and fractures on lumbar L2–L4 BMD. Regarding fractures, we analyzed past clinical vertebral fractures as a confounder, but this was not sufficient. However, these changes seem to increase the BMD, so our results in terms of changes to BMD in the present study may be overestimated. Considering the CV and effect of degenerative changes, measurement for the total hip might be the proper site for observation of BMD change over the long term.

The present study also found evidence of differences in BTM values for a given age stratum between different birth cohorts in women. Data on levels of BTMs in 1993 and 2003 showed that accelerated bone remodeling seemed to improve for women in their fifties to seventies in younger cohorts. However, those results were affected by potential confounders such as differences in age, anthropometric measurements, and menstrual status. We then compared the aforementioned factors between women in their fifties to seventies in 1993 and in 2003. Mean age (SD) for groups in 1993 and 2003 was 65.0 (8.1) years and 64.6 (8.9) years, and mean BMI (SD) in 1993 and 2003 was 23.2 (3.8) kg/m^2 and 23.5 (3.9) kg/m^2. No significant differences were identified between birth cohorts. The proportion of women in menopause in their fifties to seventies was 94.7% in 1993 and 91.3% in 2003. No significant difference was seen between birth cohorts ($P = 0.26$). Even if analysis was focused on women in their fifties, no significant differences were apparent ($P = 0.25$). Although other confounders resulting from differences in generation might have influenced the cohort effect, we conclude that a birth-cohort effect was seen on bone metabolism in middle-aged and elderly women in the present cohort. Our results are consistent with findings we have reported elsewhere that community-dwelling inhabitants in later birth cohorts show higher BMD in middle age, using another cohort established in a mountainous area [13]. The results are also consistent with the findings of Fujiwara et al. [25], who assessed the effects of birth cohort on the incidence of vertebral fracture in Hiroshima and found that incidence

decreased with successive birth decades. Thus, given all these findings, levels of BTMs appear significantly lower, levels of BMD appear significantly higher, and the incidence of vertebral fractures is lower in women from younger birth cohorts in Japan compared to those from older birth cohorts. These results suggest that the problem of osteoporosis might be less severe than has previously been predicted for the future in Japan.

The present study shows several limitations. The primary weakness involved the methods of sample collection. First, not all samples of participants were extracted at a fixed time (e.g., morning) under fixed conditions (e.g., fasting). Samples in this study were extracted between 0900 and 1500, rather than at a fixed time. Circadian variability is known to affect BTM levels [4]. Hannon and Eastell [26] reviewed the circadian variability of BTMs, noting that serum levels of OC peaked between 0200 and 0400 and reached a nadir between 1200 and 1600, whereas serum CTX levels peaked between 0130 and 0430, reaching a nadir between 1100 and 1400. We could not find any reports on circadian rhythms for serum NTX, but Delmas et al. [4] stated that most BTM levels increased at night, peaked between 0200 and 0800, then decreased rapidly to a nadir between 1300 and 2300. Based on these reports, the timing of sample collection was based on when BTM levels were supposed to be reaching a nadir. The present results might thus have underestimated levels of BTMs compared to collection at a fixed time in the morning. Although adjustment for the time after eating is important, particularly for measurements of serum CTX, we could not collect samples under absolutely controlled conditions. Delmas et al. [4] reported that fasting diminishes the rhythm of serum CTX-I, particularly with regard to the rapid decrease in the morning. Because we could not control the timing for collecting blood samples and fasting, we might not have accurately evaluated interindividual changes in BTMs. However, all participants in examinations in both 1993 and 2003 were allocated randomly to a specific sampling time and the allocated time was associated with eating behaviors. Random noise resulting from variability in sampling time and eating status might thus have occurred with relatively equal probability in both 1993 and 2003. Comparison of BTM levels between cohorts, rather than individuals, in 1993 and 2003 thus appears valid.

Second, long-term storage might have influenced BTM levels. In this study, serum samples were immediately placed in dry ice and transferred within 24 h to a deep freezer kept at −80°C. BTMs in the present study were measured utilizing baseline samples after 7 years, given that methods to identify these BTMs were unavailable in 1993. Storage for 7 years might therefore have influenced BTM levels, even at −80°C. No data are available regarding the influence of such long-term storage, although Seibel [27] stated that BTMs in sera would be stable with a storage temperature of −70°C. Hannon and Eastell [26] reported that long-term CVs for OC, serum NTX-I, and serum CTX-I were 27.3% at 9 months, 24.0% at 3 years, and 13.1% at 1 year. The CV for 7-year storage might well be higher than these results. If so, levels of BTMs collected in 1993 and measured in 2000 would have been systematically greater than those obtained in the present study, underestimating differences between 1993 and 2003. Changes over 10 years would thus have been greater and the effects of birth cohort even more pronounced.

Another limitation involves withdrawal bias. Although we completed the 10-year follow-up with a high participation rate, 80.4%, the dropout rate among men in their seventies was rather high (54.0%). This high dropout rate might have resulted in a withdrawal bias, meaning that healthier survivors would have skewed the results of long-term observation. Increases in femoral neck BMD might have been skewed by any such withdrawal bias. However, the main reasons for dropout among men in their seventies were death (52%) and illness (31%), which seem unavoidable. We think that this represents an inherent limitation of all longitudinal follow-up studies. The possibility of withdrawal bias should be considered when interpreting the data.

In conclusion, the present study found that change rates of BTMs were higher for women in their forties than for women in their fifties to seventies ($P < 0.05$) and were higher in the menstrual transition group than in the pre- and postmenopausal groups ($P < 0.001$). Changes in BTMs during the 10 years showed significant associations between bone loss at L2–L4 and total hip in women, after adjusting for confounders. Levels of all BTMs in women in their fifties were significantly lower than in younger birth cohorts.

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

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ORIGINAL ARTICLE

Biochemical markers of bone turnover as predictors of osteoporosis and osteoporotic fractures in men and women: 10-year follow-up of the Taiji cohort

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Abstract We aimed to assess the capacity of biochemical markers of bone turnover (BTMs) to predict bone loss, osteoporosis (OP), and osteoporotic fractures. We randomly selected 400 individuals (age 40–79 years in 1993; 50 of each gender and age stratum) from a list of registered residents. In the years 1993, 1996, 2000, and 2003, bone mineral density (BMD) of the spine and hip were measured by dual-energy X-ray absorptiometry. The BTMs assessed at baseline were serum intact osteocalcin (OC), total OC, bone-specific alkaline phosphatase, C-terminal propeptide of type I procollagen, N-terminal propeptide of type I procollagen (PINP), C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase, C-terminal cross-linking telopeptide of type I collagen (beta-CTX), N-terminal cross-linking telopeptide of type I collagen (NTX), urinary pyridinoline, and deoxypyridinoline (DPD). For 307 completers, multivariate analysis after adjusting for confounders revealed that serum PINP levels in men [hazard ratio (HR) 2.80, $P < 0.05$] and serum PINP (HR 1.65, $P < 0.05$), beta-CTX (HR 1.80, $P < 0.001$), NTX (HR 1.96, $P < 0.01$), and urinary DPD levels (HR 1.40, $P < 0.05$) in women were significantly related to the occurrence of spinal OP. In addition to adjustment for the baseline status of BMD, i.e., osteopenia or normal range, PINP, beta-CTX, and NTX in women could significantly predict the future occurrence of spinal OP. BTMs were not significant predictors of bone loss, femoral OP, or osteoporotic fractures. In conclusion, various BTMs in women can predict the occurrence of spinal OP.

Keywords Biochemical markers of bone turnover · Bone resorption · Bone formation · Bone mineral density · Osteoporotic fracture

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Introduction

Osteoporosis (OP) impairs the activities of daily living (ADL) and quality of life (QOL), leading to increased morbidity and mortality in the elderly [1, 2]. With the rapid aging of the population, an urgent need has been identified for the development of methods to prevent OP. The estimated number of patients with OP in Japan is about 11 million [3], and osteoporotic fractures are ranked fifth among the diseases responsible for disabilities requiring support in Japan [4].

As the restoration of diminished bone volume seems quite difficult to achieve, the early diagnosis of OP is the most valuable strategy for preventing osteoporotic fractures. However, the prediction of rapid bone loss, incidence of OP, and osteoporotic fractures remains difficult.

Biochemical markers of bone turnover (BTMs) reflect the status of bone metabolism in various processes coupled with bone resorption and formation, and are widely used in clinical situations to evaluate the efficacy of treatments for OP [5–8]. Several population-based epidemiological

studies have shown that BTMs can predict bone loss and the incidence of osteoporotic fractures in women [9–15], but the effectiveness of BTMs for predicting such epidemiological indices over the long-term, such as 10 years, is unclear. In addition, few reports, besides our own previous reports [16, 17], have evaluated BTM values and bone loss or osteoporotic fractures in men.

We established a cohort comprising men and women in a rural area in Japan, and followed this cohort for 10 years. The present study was performed for the purpose of evaluating the capacity of baseline urinary and serum concentrations of various BTMs to predict future bone loss and the occurrence of OP and osteoporotic fractures in men and women randomly selected from a rural population in Japan.

Subjects, materials, and methods

Establishment of the baseline cohort

Details of the cohort survey at the Japanese town of Taiji have already been reported [18–20], and the Taiji cohort is therefore described here only in brief. The town of Taiji is located in the southern coastal area of Wakayama Prefecture in the south-western area of the main island of Japan. A list of all inhabitants born between 1913 and 1952, and therefore aged between 40 and 79 years in 1993, was compiled based on resident registrations as of the end of 1992. A cohort of 2,261 inhabitants (1,028 men, 1,233 women) was identified, and all members of the cohort completed a self-administered, 125-item questionnaire addressing lifestyle factors such as dietary habits, smoking habits, alcohol consumption, and physical exercise (whole cohort).

From this whole cohort, 50 men and 50 women in each of four age groups between 40 and 79 years by decade of birth year (1913–1922, 1923–1932, 1933–1942, and 1943–1952), for a total of 400 participants, were selected randomly, using a table of random numbers, and underwent measurement of bone mineral density (BMD) in 1993. At this time, blood and urine samples were taken from all participants. An interviewer administered a second questionnaire to these 400 participants, covering items of past medical history, including questions related to OP, osteoporotic fractures and falls, family history, calcium intake, dietary habits, physical exercise, occupational activities, and sun exposure, and, for women, additional questions about reproductive variables such as menstrual status (premenopause, perimenopause, or menopause), age at menopause, age at menarche, number of childbirths, lactation, use of estrogen for treatment, history of ovariectomy, and history of uterectomy (BMD cohort, baseline study).

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

BMD measurements at baseline and follow-up surveys

Baseline BMD was measured in 1993, using dual-energy X-ray absorptiometry (DXA) (QDR 1000; Hologic, Bedford, MA, USA), providing antero-posterior images of lumbar vertebrae L2–4 and the proximal femur (femoral neck, Ward's triangle, trochanter). These measurements were repeated on the same participants after 3 (2nd visit, 1996), 7 (3rd visit, 2000) and 10 years (4th visit, 2003).

At each follow-up survey, an interviewer-administered questionnaire survey was performed regarding changes in lifestyle factors during the observation period, and covering items of medical history, including questions related to OP, osteoporotic fractures and falls, anti-OP treatment, calcium intake, dietary habits, physical exercise, occupational activities, and sun exposure, and, for women, additional questions about reproductive variables such as menstrual status (premenopause, perimenopause, or menopause), age at menopause, use of estrogen for treatment, history of ovariectomy, and history of uterectomy.

To control for the precision of DXA, the equipment was checked at all examinations using the same phantom, and the BMD of the phantom was regulated to 1.030 ± 0.016 g/cm^2 (1.5%) during all examinations. The same physician (N.Y.) obtained all BMD measurements. Intra-observer variability for DXA scans done by this investigator was 0.35%, using the phantom as described [21].

Detection of the occurrence of OP

OP was defined based on World Health Organization criteria, according to which OP is diagnosed based on T scores of BMD $\leq$2.5 standard deviations (SDs) lower than peak bone mass [22]. The mean L2–4 BMD for young adult men and women measured using the Hologic QDR 1000 in Japan is reported as 1.011 g/cm^2, and the SD is 0.119 g/cm^2 [23]. The mean femoral neck BMD (SD) in Japan is reported as 0.863 (0.127) for young men and 0.787 (0.109) for young women [23]. This study therefore defined OP, using these indices, as lumbar spine BMD $<$0.714 g/cm^2 for both men and women, and as femoral neck BMD $<$0.546 g/cm^2 for men and $<$0.515 g/cm^2 for women.

To define the incidence of OP among the 400 participants at the initial survey, individuals with spinal or femoral neck OP were excluded. Among the remaining participants without OP at the lumbar spine and/or femoral neck at baseline, the number of new cases of OP was

counted at the 3, 7, and 10-year follow-up surveys. Incidences of OP were estimated using the number of new cases divided by the person-years, consisting of years of individuals diagnosed with OP and years of drop-outs. The annual incidence of lumbar and femoral neck OP was then estimated.

Measurements of BTMs

All blood and urine samples were collected between 09:00 and 15:00. After centrifugation of the blood samples, sera were immediately placed in dry ice and transferred to a deep freezer within 24 h. Spot urine samples were frozen using the same procedure. These samples were kept at −80°C until needed for assays.

From the samples of participants in the baseline study, the following BTMs were measured to establish values. As markers of bone formation, serum intact osteocalcin (OC), serum total OC, serum bone-specific alkaline phosphatase (BAP), serum C-terminal propeptide of type I procollagen (PICP), and serum N-terminal propeptide of type I procollagen (PINP) were utilized. To monitor bone resorption, products of collagen breakdown, i.e., serum C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase (ICTP), serum beta-isomerized C-terminal cross-linking telopeptide of type I collagen (beta-CTX), serum N-terminal cross-linking telopeptide of type I collagen (NTX), urinary pyridinoline cross-links of collagen (PYR), and urinary deoxypyridinoline cross-links of collagen (DPD) were used.

Reference values classified by age and gender for serum intact OC, PICP, and ICTP and urinary PYR and DPD have already been described [17]. Measurement methods for these compounds are therefore only described in brief. Serum intact OC was measured using an immunoradiometric assay (Osteocalcin IRMA kit; Mitsubishi Kagaku BCL, Tokyo, Japan) [24]. Serum PICP and ICTP were measured using a radioimmunoassay (RIA) (Orion Diagnostics, Espoo, Finland) [25]. Urinary PYR and DPD in hydrolyzed urine specimens were analyzed by high-performance liquid chromatography followed by fluorescent detection using essentially the same methods [26]. The values of these urinary markers were standardized to urinary creatinine concentrations.

Total OC was measured using an electrochemiluminescence immunoassay (ECLIA) (Elecsys N-MID Osteocalcin; Roche Diagnostics, Mannheim, Germany) [27] with an intraassay coefficient of variation (CV) of 0.5%, and sensitivity of 0.5 ng/mL. We measured BAP using an enzyme immunoassay (Metra BAP; Quidel, San Diego, CA, USA) [28] with an intraassay CV of 3.9–5.2% and sensitivity of 0.7 U/L. Serum PINP was measured using an RIA (Orion Diagnostics) with an intraassay CV of 3.1–9.3% and sensitivity of 2 ng/mL [29, 30].

As markers of bone resorption, serum beta-CTX was measured using an ECLIA (Elecsys beta-CrossLaps; Roche Diagnostics) with an intraassay CV of 2.0% and sensitivity of 0.01 ng/mL [27]. Serum NTX was measured using an enzyme-linked immunosorbent assay (Osteomark NTX serum; Ostex International, Seattle, WA, USA) [31, 32] with an intraassay CV of 4.6% and sensitivity of 3.2 nM BCE/L.

Fracture assessment

All participants completed a detailed questionnaire at baseline, including a history of fragility fractures (that is, fractures resulting from low-impact trauma) that had occurred since the age of 40 years. Thereafter, at each subsequent examination, information about the occurrence of fractures since the previous visit was extracted from interviewer-registered questionnaires and registered. Information about fractures considered to be osteoporotic was analyzed. Osteoporotic fractures comprised those of the spine, pelvis, ribs, distal radius, forearm, humerus, and hip that occurred in the absence of high-impact trauma.

Statistical analysis

All statistical analyses were performed using STATA statistical software (STATA, College Station, TX, USA). Differences were tested for significance using analysis of variance for comparisons among multiple groups and Scheffe's least significant difference test for pairs of groups. Causal relationships between bone changes and serum and urinary concentrations of BTMs at baseline were clarified using multiple regression analysis with the rate of change of BMD (% per year) as an objective factor, and values of BTMs (/SD) at baseline after adjusting for age, weight, and menstrual status (0 pre- and perimenopause; 1 menopause) in women at baseline. Causal relationships between the incidence of OP and osteoporotic fractures, and serum and urinary concentrations of BTMs at baseline were clarified using Cox proportional hazards modeling using the occurrence of OP (yes 1; no 0) and occurrence of osteoporotic fractures (yes 1; no 0) as objective factors, and BTM level (/SD) at baseline as an explanatory factor after adjusting for age and weight, and menstrual status in women at baseline. Regarding anti-OP drugs, during the observation period from 1993 to 2003, bisphosphonates such an alendronate and risedronate and selective estrogen receptor modulator (SERM) agents had not been approved for use in Japan for the treatment of OP. In addition, we asked participants, in the questionnaires at each follow-up at the 2nd, 3rd, and 4th visits, whether they

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

Results

Eligible participants and baseline characteristics

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

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

Changes in BMD over 10 years

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

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

Birth cohort	Age group (years)	*n*	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Men						
1943–1952	40–49	50	44.2 (2.6)	168.8 (5.2)	69.0 (10.4)	24.2 (3.2)
1933–1942	50–59	50	54.8 (2.7)	165.6 (5.0)[a]	63.5 (9.4)[a]	23.1 (2.9)
1923–1932	60–69	50	64.6 (2.5)	163.0 (4.8)[a]	62.9 (9.6)[a]	23.6 (3.2)
1913–1922	70–79	50	74.0 (2.7)	160.7 (5.4)[a,b]	57.5 (8.3)[a,b,c]	22.2 (2.8)
1913–1952	40–79	200	59.4 (11.4)	164.5 (5.9)	63.2 (10.2)	23.3 (3.1)
Women						
1943–1952	40–49	50	44.0 (2.8)	154.4 (5.0)	54.1 (8.3)	22.7 (3.1)
1933–1942	50–59	50	55.8 (2.8)	154.9 (5.3)	59.4 (10.0)[a]	24.8 (4.0)[a]
1923–1932	60–69	50	64.8 (2.6)	151.1 (4.6)[a,b]	52.1 (9.1)[b]	22.8 (3.5)[b]
1913–1922	70–79	50	74.4 (2.8)	147.7 (5.4)[a,b,c]	48.4 (8.2)[a,b]	22.2 (3.4)[b]
1913–1952	40–79	200	59.8 (11.6)	152.0 (5.8)	53.5 (9.7)	23.1 (3.6)

Values are given as means with standard deviations in parentheses

BMI body mass index, *n* number of participants

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

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

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

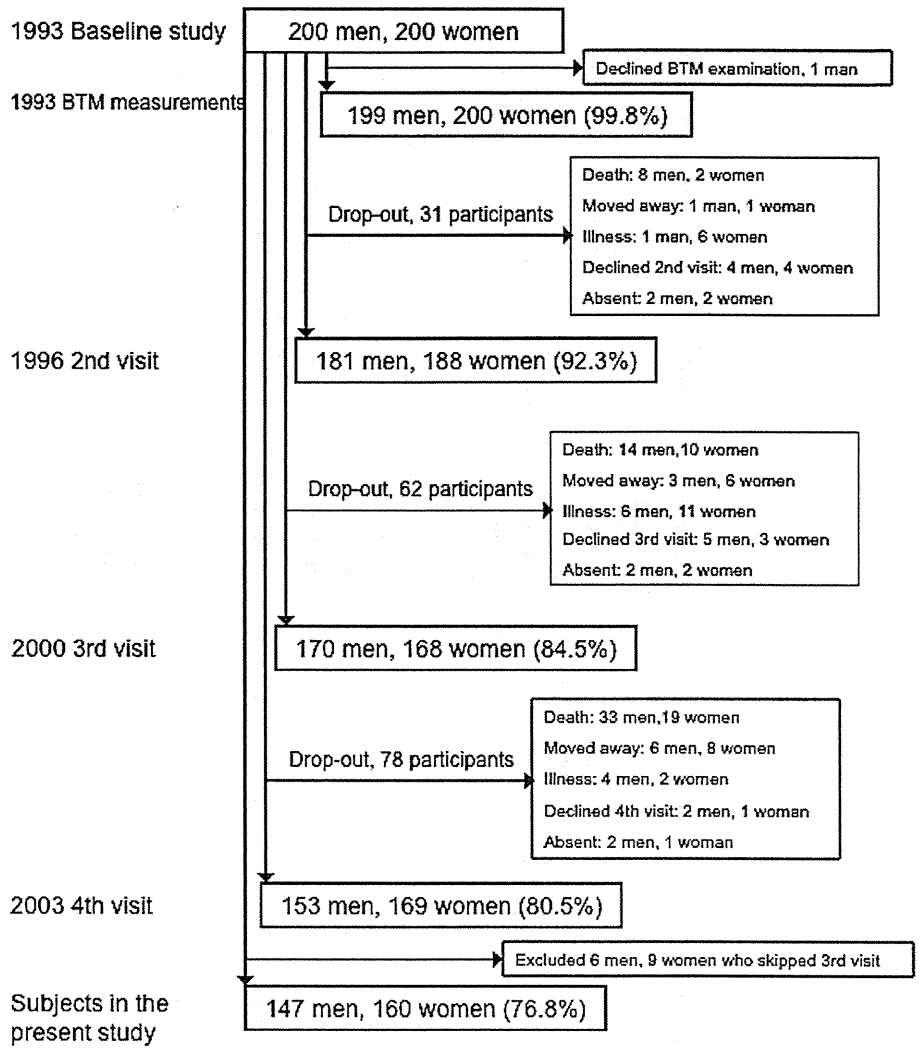

Fig. 1 Flow chart of participants in the cohort. *BTM* Biochemical marker of bone turnover

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

Incidence of OP and osteoporotic fractures over 10 years

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

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

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

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

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

BMD bone mineral density, *n* number of subjects

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

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

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

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

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

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

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

We clarified whether values of BTMs at baseline could predict rates of change of BMD over 10 years, using multiple regression analysis with rate of change of BMD (%/year) as an objective factor, and values of BTMs (/SD) at baseline after adjusting for age, weight, and menstrual status (0 pre- and perimenopause; 1 menopause) in women at baseline. No associations were identified between any BTMs and rate of change in BMDs at L2–4 or the femoral neck over 10 years.

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

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

Discussion

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

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

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

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

Values are given as means with standard deviations in parentheses

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

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

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

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

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

Values are given as means with standard deviations in parentheses

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Conflict of interest None.

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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