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

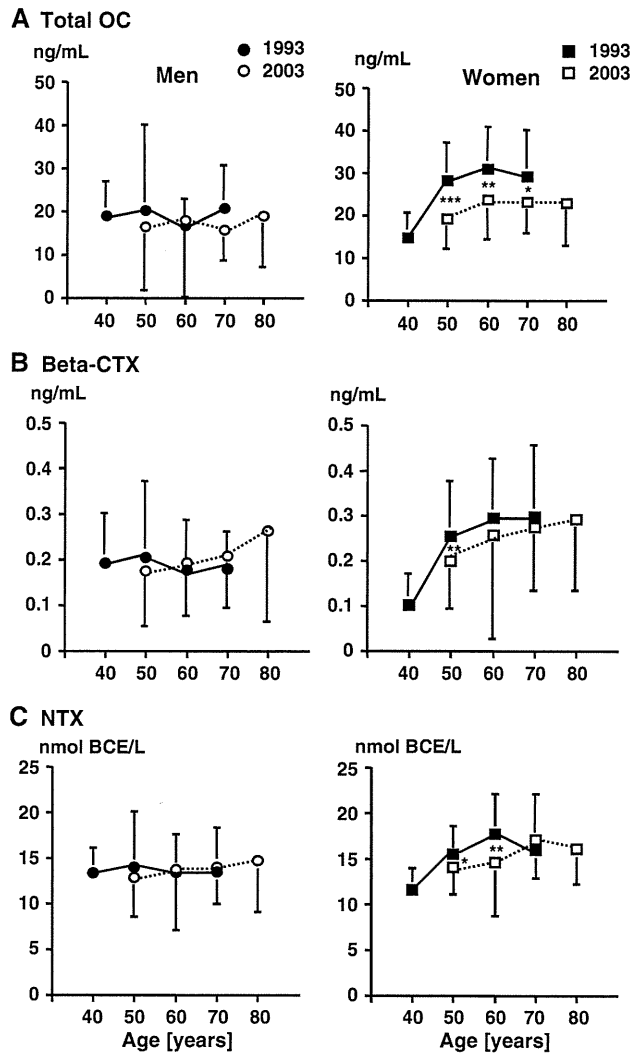

Fig. 2 Changes in serum biochemical markers of bone turnover over 10 years, classified by age strata. **a** Total osteocalcin (*OC*). **b** Beta-isomerized C-terminal cross-linking telopeptide of type I collagen (*beta-CTX*). **c** N-terminal cross-linking telopeptide of type I collagen (*NTX*). *BCE*, 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

Reference values for hand grip strength, muscle mass, walking time, and one-leg standing time as indices for locomotive syndrome and associated disability: the second survey of the ROAD study

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Abstract
Background We established reference values for hand grip strength, muscle mass, walking time, and one-leg standing time as indices reflecting components of locomotive syndrome and associated disability using a large-scale population-based sample from the second survey of the Research on Osteoarthritis/Osteoporosis Against Disability (ROAD) cohort.
Methods We measured the above-mentioned indices in 2,468 individuals ≥40 years old (826 men, 1,642 women; mean age 71.8 years) during the second visit of the ROAD study. Disability was defined as certified disability according to the long-term care insurance system through public health centres of each municipality.
Results Mean values for hand grip strength (weaker side), muscle mass of the thighs, walking time for 6 m at the usual pace, and the fastest pace for men were 32.7 kg, 7.0 kg, 5.6 s, and 3.7 s, respectively, and those for women were 20.8 kg, 5.2 kg, 5.9 s, and 4.1 s, respectively. The median values for one-leg standing time (weaker side) were 14 s for men and 12 s for women. The prevalence of disability in men aged 65–69, 70–74, 75–79, and ≥80 was 0.0, 1.0, 6.3, and 8.8%, respectively, and in women was 3.4, 3.5, 9.2, and 14.7%, respectively. There were significant associations between the presence of disability and walking time for 6 m at the usual pace and at the fastest pace, and between the presence of disability and walking speed.
Conclusions We established reference values for indices reflecting components of locomotive syndrome, and identified significant associations between walking ability and disability.

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Introduction

Musculoskeletal diseases, including osteoarthritis (OA) and osteoporosis (OP), are major public health problems among the elderly that affect activities of daily living (ADL) and quality of life (QOL), leading to increased morbidity and mortality. According to the recent National Livelihood Survey by the Ministry of Health, Labour, and Welfare in Japan, OA is ranked fourth, while falls and osteoporotic fractures are ranked fifth among diseases that cause disabilities and subsequently require support for ADL [1]. Previous studies have reported increased mortality following osteoporotic fractures at the hip and other sites [2], and have estimated that a total of 47,000,000 people (21,000,000 men and 26,000,000 women) aged ≥40 years will eventually be affected by either OA or OP. Considering that the population of Japan is aging very rapidly—more than 22% of the population is aged ≥65 years [3]—a comprehensive and evidence-based prevention strategy for musculoskeletal diseases is urgently needed.

The Japanese Orthopaedic Association has proposed the term 'locomotive syndrome' to designate a condition in high-risk groups with musculoskeletal diseases who are highly likely to require nursing care [4]. Locomotive syndrome is caused by weakening of musculoskeletal organs such as bone, joint, and muscle, which in turn interferes with physical performance, especially self-transportation. Loss of locomotor abilities such as walking causes disabilities requiring support. Therefore, to prevent decline into disability, it is important to maintain a healthy range of bone, joint, muscle, and physical performance.

These four components, bone, joint, muscle, and physical performance, each have objective measurements that can be used as indices to evaluate their present condition. For example, bone mineral density (BMD) is a representative index of the condition of the bone. Joint space width (JSW), joint space area (JSA), and osteophyte area (OPA) are indices reflecting the condition of the joint. Regarding muscle, although the best index remains controversial, hand grip strength can be used to reflect muscle strength [5], and muscle mass is one index of muscle volume [6]. In addition, as objective indices of physical performance, walking speed and/or one-leg standing times are candidates [7, 8]. However, at present, it is difficult to use such indices for evaluating, diagnosing, or predicting the future occurrence and progression of locomotive syndrome in Japan, because there is little information on reference values for such indices to distinguish patients at risk from normal individuals in a large population-based cohort.

In 2005–2007, we began a large-scale population-based cohort study entitled Research on Osteoarthritis/Osteoporosis Against Disability (ROAD), consisting of 3,040 participants in three communities located in urban, mountainous, and coastal areas (baseline study). Following the baseline study, a second survey was performed in the same communities in 2008–2010, in which 2,674 inhabitants participated (second visit).

Through analysis of the baseline data of the ROAD, the age-gender distribution of BMD has been reported as an index for bone mass [3], and the medial and lateral JSW, medial and lateral JSA, OPA, and femorotibial angle of the knee have been reported as indices of the health of joints [9] in these populations. However, there is still scant information regarding the condition of the muscles and physical performance. Therefore, in the present study, we aimed to establish reference values for hand grip strength as an index of muscle power, muscle mass as an index of muscle volume, and walking time and one-leg standing time as indices of physical performance, classified by age and gender, using the data from the second visit of the ROAD study. This information is expected to be valuable for early diagnosis and prevention of locomotive syndrome. In addition, we evaluated the prevalence of disabilities in participants in the ROAD study second visit, and identified associations between hand grip strength, muscle mass, walking time, and one-leg standing time and the presence of disability.

Participants and methods

Participants

Reference values were obtained from the results of cross-sectional measurements of participants enrolled in the second visit of the ROAD study. The ROAD study, which began in 2005, is a nationwide prospective study comprising population-based cohorts established in three communities, such as urban, mountainous, and coastal regions in Japan. Recruitment methods for this study have been described in detail elsewhere [3]. To date, participants in the urban region, aged ≥60 years, were recruited from among those enrolled in a randomly selected cohort study from the previously established Itabashi Ward resident's registration database. The response rate was 75.6%. Participants in the mountainous and coastal regions, aged ≥40 years, were recruited from listings of resident registration. Residents aged ≤60 years in the urban area and ≤40 years in the mountainous and coastal areas who were interested in participating in the study were invited. We have completed development of a baseline database including clinical and genetic information for 3,040 inhabitants aged 23–95 years (1,061 men, 1,979 women).

The second visit of the ROAD study began in 2008 and was completed in 2010. All the participants in the baseline study were invited to participate in the second visit. In addition to the former participants, inhabitants aged ≥60 years in the urban area and those aged ≥40 years in the mountainous and coastal areas who were willing to participate in the ROAD survey performed in 2008–2010 were also included in the second visit. In addition, residents aged ≤60 years in the urban area and ≤40 years in the mountainous and coastal areas who were interested in participating in the study were invited to be examined as well as the baseline.

The inclusion criteria of participants were as follows: (1) ability to walk to the clinic where the survey was performed, (2) ability to provide self-reported data, and (3) ability to understand and sign an informed consent form. No other exclusion criteria were used.

Thus, a total of 2,674 residents (892 men, 1,782 women) aged 21–97 years participated in the second visit. In the present study, we analysed the data for 2,468 individuals (826 men, 1,642 women; mean age 71.8 years); this population comprised 956 individuals (318 men, 638 women) in the urban region, 726 individuals (258 men, 468 women) in the mountainous region, and 786 individuals (250 men, 536 women) in the coastal region who participated in the second visit and were ≥40 years old.

All the participants provided written informed consent, and the study was conducted with the approval of the ethics committees of the participating institutions.

Hand grip strength, muscle mass, walking time, and one-leg standing time

Hand grip strength was measured bilaterally using a Toei Light handgrip dynamometer (Toei Light Co., Ltd., Saitama, Japan). Both hands were tested, and the better value was used to characterise the maximum muscle strength of the subject.

Among the 2,468 participants who participated in the second visit of the ROAD study, 778 residents (248 men, 530 women) in the coastal town of Taiji were examined to determine their segmental muscle mass using the bioelectrical impedance method (BIP; Physion MD; Physion Inc., Kyoto, Japan). We obtained values for the muscle masses of the right and left forearms, upper arms, upper limbs, quadriceps, thighs, lower legs, and lower limbs. This method had previously been validated as having a close correlation to muscle volume as measured by magnetic resonance imaging [10].

Among the 2,468 participants who participated in the second visit of the ROAD study, 1,637 residents (559 men, 1,078 women) of the mountainous town of Hidakagawa and the coastal town of Taiji were examined to determine their walking time. Walking time was measured as the time required to complete a 6-m course. All participants walked the 6-m course twice; they first walked at their usual walking speed and then repeated the course at their fastest pace.

Among the 2,468 participants who participated in the second visit of the ROAD study, one-leg standing time with eyes open was measured on both sides for 2,433 individuals (816 men, 1,617 women). The time until the raised leg was set down on the floor was measured, with a maximum time of 60 s recorded for those who could stand on one leg for at least that length of time. The shorter value of the two measurements was used as the worse side and the longer measurement as the better side for the one-leg standing time of the subject.

Mean values and standard deviations (SDs) of hand grip strength, muscle mass, and walking time were classified by gender and age group (40, 50, 60, 70, and ≥80 s) to establish age-gender reference values for the general population. However, reference values classified by gender and age group for one-leg standing time were established using median (50th percentile) values and 25th–75th percentile ranges. These values were recorded using a maximum time of 60 s for anyone who could exceed that time; thus, the data do not fit a normal distribution, and use of means and SDs is unsuitable for one-leg standing time reference values.

Presence of disability

Disability in the present study was defined as 'cases requiring long-term care' as determined by the long-term care insurance system based on the Long-Term Care Insurance Act of 1997 in Japan. The procedure for identifying cases requiring long-term care is as follows: (1) each municipality establishes a long-term care approval board consisting of clinical experts, physicians, and specialists at the Division of Health and Welfare in each municipal office; (2) the long-term care approval board investigates the insured person using an interviewer-administered questionnaire consisting of 82 items regarding mental and physical condition and makes a screening judgement based on the opinion of a regular doctor; and (3) 'cases requiring long-term care' are determined according to standards for long-term care certification uniformly and objectively applied nationwide [11].

During the 3 years between the baseline and the second visit of the ROAD study, we annually obtained information on the participating residents regarding deaths, changes of residence, and presence or absence of certified disability according to the long-term care insurance system from the public health centres of the participating municipalities.

Statistical analysis

All statistical analyses were performed using Stata statistical software (Stata, College Station, TX, USA). Differences in the values of the indices were tested for significance using analysis of variance for comparisons among multiple groups. Scheffé's least significant difference test was then used for pairs of age groups.

To ascertain associations between the presence of disability and hand grip strength, muscle mass, walking time, and one-leg standing time, logistic regression analyses were performed using the presence of disability (yes, 1; no, 0) as an objective factor, and values for hand grip strength, muscle mass, walking time, and one-leg standing time as the explanatory factor after adjusting for age, gender, and body mass index (BMI, kg/m^2).

Results

Characteristics of participants

Summary characteristics including age, height, weight, and BMI of the participants in the present study are shown in Table 1. Two-thirds of the 2,468 subjects were women, and the mean age of the female participants was 1 year younger than that of the male participants. Height and weight were significantly lower for women than for men, but no significant difference in BMI was noted between the genders. All anthropometric measurements other than BMI of females tended to decrease with age. BMI of women in their 80s and older was significantly lower than that in younger age groups, while there were no significant differences among age groups 40–70 years old.

Reference values for hand grip strength, muscle mass, walking time, and one-leg standing time

Table 1 also shows the age-gender distribution of hand grip strength for both the better and worse sides. Mean hand grip strength in men was significantly higher than that in women ($p < 0.001$) and decreased with age in both men and women ($p < 0.001$).

Mean muscle mass for both forearms, both upper arms, both upper limbs, both quadriceps, both thighs, both lower legs, and both lower limbs are shown in Table 2. Muscle masses for all parts of the body were significantly higher in men than in women ($p < 0.001$). Mean muscle mass in men decreased with age for all areas except the lower leg. Particularly in the quadriceps and thighs, muscle masses in men aged ≥70 were significantly lower than those in their 40s–50s ($p < 0.05$). By contrast, although muscle mass for women aged ≥80 and older tended to be lower than those of younger age groups (other than the lower legs), there

Table 1 Mean values (standard deviation) of anthropometric measurements and hand grip strength of the participants classified by sex and gender

Age strata (years)	Number of subjects	Weight (kg)	Height (cm)	Body mass index (g/cm^2)	Grip strength (better side) (kg)	Grip strength (worse side) (kg)
Men						
40–49	32	73.5 (10.2)	170.3 (7.3)	25.4 (3.6)	49.5 (8.2)	49.3 (8.4)
50–59	100	68.8 (10.6)	168.0 (5.2)	24.3 (3.3)	47.3 (7.0)	42.6 (6.9)
60–69	137	65.4 (11.1)[a]	165.2 (6.2)[a,b]	23.9 (3.5)	41.4 (6.6)[a,b]	36.9 (7.9)[a,b]
70–79	308	60.0 (8.1)[a,b,c]	161.1 (5.7)[a,b,c]	23.1 (2.7)[a,b]	35.4 (6.8)[a,b,c]	31.5 (7.1)[a,b,c]
80 and older	249	57.2 (8.9)[a,b,c,d]	159.7 (6.0)[a,b,c]	22.4 (2.9)[a,b,c]	29.7 (6.2)[a,b,c,d]	26.3 (6.3)[a,b,c,d]
Total	826	61.6 (10.3)	162.5 (6.7)	23.3 (3.1)	36.6 (9.1)	32.7 (9.1)
Women						
40–49	93	55.9 (9.5)	157.0 (4.4)	22.6 (3.5)	31.2 (4.3)	28.2 (4.4)
50–59	191	55.3 (8.9)	154.4 (5.8)[a]	23.2 (3.7)	28.7 (4.9)[a]	25.4 (4.9)[a]
60–69	316	54.2 (8.0)	152.0 (5.5)[a,b]	23.4 (3.2)	26.6 (4.3)[a,b]	23.77 (4.5)[a,b]
70–79	599	51.3 (8.5)[a,b,c]	148.4 (5.9)[a,b,c]	23.3 (3.5)	22.6 (4.6)[a,b,c]	19.7 (4.7)[a,b,c]
80 and older	443	47.4 (8.3)[a,b,c,d]	145.5 (5.9)[a,b,c,d]	22.4 (3.6)[c,d]	19.4 (4.4)[a,b,c,d]	16.6 (4.6)[a,b,c,d]
Total	1,642	51.6 (8.9)	149.5 (6.7)	23.0 (3.5)	23.7 (5.8)	20.8 (5.8)

[a] Significantly different ($p < 0.05$) from values of the age group in their 40s

[b] Significantly different ($p < 0.05$) from values of the age group in their 50s

[c] Significantly different ($p < 0.05$) from values of the age group in their 60s

[d] Significantly different ($p < 0.05$) from values of the age group in their 70s

Table 2 Mean values (standard deviation) of segmental muscle mass (kg) in total right and left sides classified by age and gender

Age strata (years)	Number of subjects	Forearm	Upper arm	Upper limb	Quadriceps	Thigh	Lower leg	Lower limb
Men								
40–49	25	1.20 (0.19)	1.59 (0.36)	2.79 (0.54)	3.91 (0.64)	7.76 (1.19)	3.41 (0.66)	11.16 (1.69)
50–59	60	1.18 (0.16)	1.53 (0.28)	2.71 (0.41)	3.73 (0.64)	7.45 (1.22)	3.41 (0.66)	10.86 (1.60)
60–69	67	1.15 (0.17)	1.50 (0.28)	2.65 (0.42)	3.50 (0.68)	7.02 (1.28)	3.52 (0.86)	10.54 (1.89)
70–79	66	1.17 (0.20)	1.43 (0.28)	2.60 (0.46)	3.37 (0.66)[a]	6.78 (1.26)[a]	3.51 (0.68)	10.29 (1.70)
80 and older	30	1.11 (0.17)	1.37 (0.26)	2.48 (0.38)	3.10 (0.62)[a,b]	6.27 (1.18)[a,b]	3.92 (1.11)	10.18 (2.05)
Total	248	1.16 (0.18)	1.48 (0.29)	2.65 (0.44)	3.52 (0.69)	7.04 (1.30)	3.53 (0.80)	10.57 (1.79)
Women								
40–49	67	0.77 (0.12)	0.86 (0.19)	1.63 (0.30)	2.65 (0.60)	5.37 (1.12)	2.65 (0.47)	8.02 (1.45)
50–59	124	0.76 (0.10)	0.82 (0.16)	1.58 (0.24)	2.56 (0.44)	5.20 (0.82)	2.58 (0.51)	7.78 (1.20)
60–69	161	0.78 (0.11)	0.84 (0.16)	1.62 (0.25)	2.55 (0.45)	5.18 (0.84)	2.57 (0.42)	7.74 (1.10)
70–79	130	0.80 (0.12)[b]	0.85 (0.16)	1.66 (0.27)	2.54 (0.46)	5.17 (0.85)	2.66 (0.53)	7.83 (1.24)
80 and older	48	0.79 (0.43)	0.82 (0.16)	1.61 (0.28)	2.39 (0.45)	4.90 (0.84)	2.91 (0.69)[b,c]	7.81 (1.38)
Total	530	0.78 (0.11)	0.84 (0.16)	1.62 (0.26)	2.55 (0.47)	5.18 (0.88)	2.63 (0.51)	7.81 (1.23)

[a] Significantly different ($p < 0.05$) from values of the age group in their 40s

[b] Significantly different ($p < 0.05$) from values of the age group in their 50s

[c] Significantly different ($p < 0.05$) from values of the age group in their 60s

were no specific trends in muscle mass among age groups ≤79 years old. However, as for men, the muscle mass of the quadriceps in women tended to decline with age, although the difference was not statistically significant.

Mean 6-m walking time and the calculated walking speed (m/s) using the walking time, classified by age and gender, are shown in Table 3. Six-meter walking time was significantly lower in men than in women ($p < 0.05$), indicating that men tended to walk faster than women in this study population. Mean 6-m walking time for both men and women increased with age. In particular, 6-m walking times for men and women ≥70 years old were significantly higher than those in younger age groups ($p < 0.05$).

Table 4 shows median one-leg standing time classified by age and gender with 25th–75th percentile ranges. For both men and women in their 40s–50s, all median, 25th percentile, and 75th percentile values were 60 s, with no gender difference. One-leg standing times for men ≥60 years old tended to be higher than those for women, and median values declined with age in both men and women.

Prevalence of disability among subjects ≥65 years old

Among the 2,468 participants in the second visit of the ROAD study, we surveyed 1,845 subjects (625 men, 1,220 women) ≥65 years old and obtained information on the presence or absence of disability certified for long-term care insurance. We found a total of 149 individuals (8.1%; 36 men, 5.8%; 113 women, 9.3%) that were certified as requiring support. Figure 1 shows the prevalence of disability classified by gender and age. The prevalence of disability in men 65–69, 70–74, 75–79, and ≥80 years old was 0.0, 1.0, 6.3, and 8.8%, respectively, and that in women in the same age groups was 3.4, 3.5, 9.2, and 14.7%, respectively (Fig. 1). The prevalence of disability in women was significantly higher than that in men ($p < 0.05$) and increased with age in both genders ($p < 0.01$).

Associations between disability and hand grip strength, muscle mass, walking speed, and one-leg standing time

Logistic regression analysis was performed using the presence of disability (1, yes; 0, no) as an objective factor, and hand grip strength on the better side and the worse side; muscle mass of the forearms, upper arms, upper limbs, quadriceps, thighs, lower legs, and lower limbs; walking time for 6 m at the usual pace and at the fastest pace; and quartile of one-leg standing time [0: 0–25% (highest quartile), 1: 25–50% (higher quartile), 2: 50–75% (lower quartile), 3: 75–100% (the lowest quartile] on the better and worse sides as explanatory factors, after adjusting for age, gender, and BMI. No significant associations were found between the presence of disability and hand grip strength, muscle mass, or one-leg standing time. However, there were significant associations between the presence of disability and 6-m walking time at the usual

Table 3 Mean values (standard deviation) of 6-m walking time (s) and walking speed (m/s) with usual pace and the fastest pace classified by age and gender

Age strata (years)	Number of subjects	Usual pace		Fastest pace	
		Time for 6 m (s)	Walking speed (m/s)	Time for 6 m (s)	Walking speed (m/s)
Men					
40–49	32	4.4 (0.6)	1.38 (0.19)	3.0 (0.5)	2.09 (0.43)
50–59	100	4.8 (0.9)	1.29 (0.20)	3.2 (0.6)	1.97 (0.36)
60–69	134	5.1 (0.9)	1.21 (0.20)[a]	3.4 (0.7)	1.82 (0.33)[a]
70–79	196	5.9 (1.8)[a,b,c]	1.09 (0.25)[a,b,c]	4.0 (1.4)[a,b,c]	1.62 (0.39)[a,b,c]
80 and older	97	6.8 (3.0)[a,b,c,d]	0.99 (0.33)[a,b,c,d]	4.5 (1.8)[a,b,c,d]	1.48 (0.44)[a,b,c]
Total	559	5.6 (1.9)	1.15 (0.27)	3.7 (1.3)	1.73 (0.42)
Women					
40–49	92	4.7 (1.0)	1.32 (0.24)	3.2 (0.6)	1.95 (0.31)
50–59	190	4.9 (0.9)	1.27 (0.23)	3.3 (0.7)	1.87 (0.33)
60–69	299	5.1 (1.1)	1.22 (0.23)	3.7 (0.8)	1.71 (0.32)[a,b]
70–79	345	6.3 (2.4)[a,b,c]	1.03 (0.25)[a,b,c]	4.4 (1.5)[a,b,c]	1.46 (0.36)[a,b,c]
80 and older	152	8.4 (3.9)[a,b,c,d]	0.82 (0.27)[a,b,c,d]	5.8 (2.7)[a,b,c,d]	1.17 (0.36)[a,b,c,d]
Total	1,078	5.9 (2.4)	1.12 (0.29)	4.1 (1.6)	1.60 (0.42)

[a] Significantly different ($p < 0.05$) from values of the age group in their 40s

[b] Significantly different ($p < 0.05$) from values of the age group in their 50s

[c] Significantly different ($p < 0.05$) from values of the age group in their 60s

[d] Significantly different ($p < 0.05$) from values of the age group in their 70s

Table 4 Values of median (25–75 percentile) of one-leg standing time (s, maximum = 60 s) in a better side and a worse side classified by age and gender

Age strata (years)	Number of subjects	One-leg standing time (better side) (s)	One-leg standing time (worse side) (s)
Men			
40–49	32	60 (60–60)	60 (60–60)
50–59	99	60 (60–60)	60 (60–60)
60–69	136	60 (34.5–60)	45 (14.25–60)
70–79	303	27 (9–60)	9 (4–35)
80 and older	246	8 (4–32)	4 (2–12)
Total	816	39.5 (8–30)	14 (4–60)
Women			
40–49	92	60 (60–60)	60 (60–60)
50–59	191	60 (60–60)	60 (43–60)
60–69	317	60 (41.5–60)	43 (13–60)
70–79	593	21 (8–57.5)	8 (3–25)
80 and older	424	7 (3–18.8)	3 (2–7)
Total	1,617	31 (8–60)	12 (4–60)

pace [+1 s, odds ratio (OR) 1.15, 95% confidential interval (CI) 1.07–1.24, $p < 0.001$] and at the fastest pace (+1 s, OR 1.22, 95% CI 1.08–1.38, $p < 0.01$). In addition, there were significant associations between the presence of disability and walking speed at the usual pace (+1 m/s, OR 0.07, 95% CI 0.02–0.27, $p < 0.001$) and at the fastest pace (+1 m/s, OR 0.16, 95% CI 0.06–0.41, $p < 0.001$).

Discussion

In this study, we established age-gender-classified mean values for hand grip strength as an index of muscle strength, muscle mass as an index of muscle volume, and walking time and median one-leg standing time as indices of physical performance, using data for a large-scale

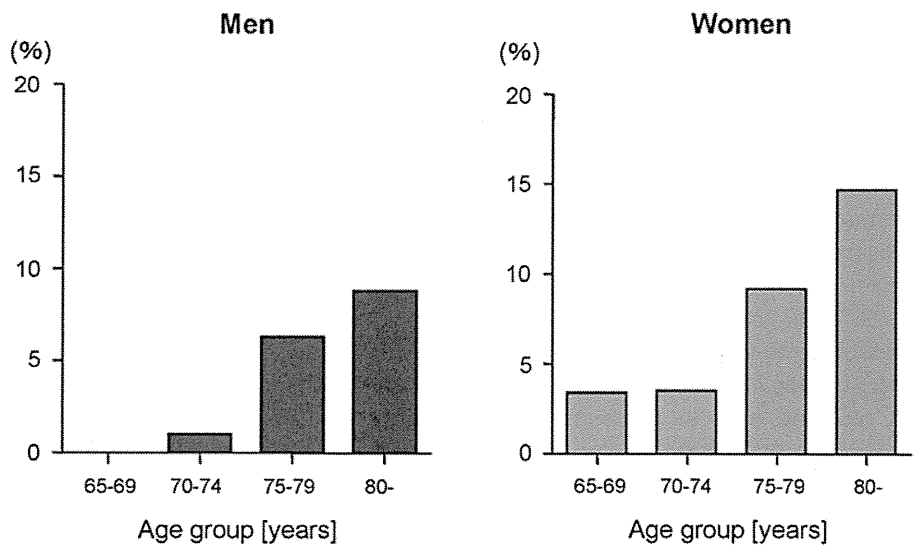

Fig. 1 Prevalence of disability among subjects ≥65 years old classified by gender and age

population-based cohort. We found that mean hand grip strength, muscle mass, walking time, and median one-leg standing time were higher in men than in women, and decreased with age (with the exception of the muscle mass of the lower legs).

The Japanese Ministry of Education, Culture, Sports, Science, and Technology has reported ranges for physical strength and sporting ability in 69,745 Japanese men and women 6–79 years old. Mean hand grip strength in both men and women reaches peak values between the ages of 20–40, and decreases with age after 40 [12]. In the present study of a population aged ≥40 years, hand grip strength declined significantly with age, consistent with the previous report of the Japanese government.

Although computed tomography or MRI scans are the most reliable methods of measuring segmental muscle mass, these methods are not suitable for a large-scale population-based study. The BIA method is rapid, inexpensive, portable, and importantly, a noninvasive measuring method. Previous studies have shown that there is a strong correlation between BIA resistance and measurements of skeletal muscle mass in the arms [13], legs [13], and whole body [14]. For the BIA method used in the present study, Miyatani et al. [10] had previously compared values for muscle mass using a series of cross-sectional images of the forearm, upper arm, lower leg, and thigh on the right side of 22 male subjects as determined by the BIA and MRI methods. The BIA impedance index (L^2/Z) for every segment, calculated as the ratio of the segment length squared to the impedance, was significantly correlated with the muscle volume measured by MRI, with $r = 0.902$–0.976 ($p < 0.05$). These previous results demonstrate that the BIA method used in the present study is sufficiently reliable as an index of muscle volume.

In the present study, age-related differences were observed in the thighs and quadriceps. In addition, the age-related decreases in muscle mass were greater in the lower limbs than in the upper limbs, and in proximal sites than in distal sites. Yamada et al. [15] estimated the bioelectrical impedance (BI) index, calculated as the ratio of the square of segmental length to impedance in a Japanese population consisting of 1,006 individuals (374 men, 632 women) and reported that the BI index decreased most with age in the thighs, whereas there were no significant age-related changes in the forearms or lower limbs. Miyatani et al. [16] studied muscle thickness at nine sites, the forearm, anterior and posterior upper arm, abdomen, subscapular, anterior and posterior thigh, and anterior and posterior lower leg, using brightness-mode ultrasonography in 348 Japanese men aged 20–79 years. They found a greater decrease in muscle thickness in the trunk and anterior thigh than at other sites, consistent with our results. They speculated that site-related differences in muscle loss with aging may be attributed to age-related changes in the patterns of loading to and/or activation of individual muscles in daily life. However, because these results were obtained from a cross-sectional study, longitudinal data would be required to determine the mechanism of these differences. We have begun the third visit of the ROAD study, 6 years after the baseline and 3 years after the second visit, to measure losses of skeletal muscle mass at various sites. Losses of muscle mass in the quadriceps and/or thighs may result in a decrease in walking ability, including walking speed. Therefore, establishment of reference values for muscle mass is useful for prediction of future disability.

Walking ability is regarded as the most important activity for the elderly to maintain an independent life in the community, and walking speed is an important index of

walking ability. Reference values have been published for populations in western countries [17, 18]; however, there has been little information available for the Japanese population. Takahashi et al. [19] surveyed walking speed at 130 crosswalks and reported that at least 1.0 m/s was required to safely cross the street. In the present study, we determined that the mean 6-m walking time at the usual pace at an age of ≥40 years was 5.6 s for men and 5.9 s for women, and the mean 6-m walking time at the fastest pace was 3.7 s in men and 4.1 s in women. These walking speeds for both the usual pace and the fastest pace provide a baseline for clinical judgments of patient performance and could be used to determine which subjects would benefit from therapeutic intervention to improve locomotive function.

The Japanese Ministry of Education, Culture, Sports, Science and Technology published mean values for one-leg standing time with a maximum time of 120 s using 5,500 individuals (2,741 men, 2,759 women) with an age range of 65–79 years in each prefecture who participated in an examination of sporting ability, including walking ability [12]. They reported that mean one-leg standing times for men 65–69, 70–74, and 75–79 years old were 79.9, 66.5, and 50.5 s, respectively, and those for women were 80.8, 62.1, and 45.0 s, respectively. These values were measured up to 120 s, and ours were measured up to 60 s. Because the measuring method was different and their outcomes are means while our results are medians, the results cannot be compared directly. However, one-leg standing time was significantly lower with age in both studies. Again, establishment of reference values for physical performance, including walking and standing ability, would be useful for prediction of future disability.

We then evaluated associations between hand grip strength, muscle mass, walking time, and one-leg standing time, as indices reflecting components of locomotive syndrome and the presence of disability. We found that the 6-m walking time may be a useful index for detection of disability. To evaluate the independence of elderly persons in daily life, physical performance has been measured using various outcomes. Walking speed has been reported to be one important index that can predict future disability, hospitalisation, and mortality in the general geriatric population [20, 21]. In a Japanese population, Shinkai et al. [22] demonstrated that lower scores on baseline performance measures, particularly maximum walking speed, predicted an increased risk of onset of functional dependence, based on their 6-year follow-up of a cohort in a rural community consisting of 736 participants. In the present study, a 1-s slower normal walking time for 6 m was associated with a 15% increase in the presence of disability, and a 1-s slower fastest walking time for 6-m was associated with a 22% increase in the presence of disability. Our study evaluated only walking ability and the presence of disability, not the occurrence of disability; however, we expect to follow these populations and clarify the predictive ability of walking speed for the occurrence of disability over the next few years.

On the other hand, no associations were found between indices such as hand grip strength, muscle mass, and one-leg standing time and the presence of disability. There is growing evidence that reduced hand grip strength is associated with adverse outcomes in older years, including morbidity, lower quality of life, higher fracture rates, increased length of hospital stay, and mortality [23–25]. Progressive decline in muscle mass has been defined as sarcopenia, which represents an impaired state of health associated with morbidity disorders, increased risk of falls and fractures, impaired ADL, loss of independence, and increased risk of death [6, 26–29]. Lang et al. [29] stated that loss of muscle mass and power increases the difficulties associated with procuring adequate nutrition and the effort required to undertake exercise; the combination of nutritional loss and reduced physical activity levels results in further loss of muscle mass and power. The resulting decrements in power, endurance, and physical performance lead to a loss of independence. In addition to muscle strength and mass, balance appears to be an important index of disability. Shinkai et al. [22] measured the one-leg standing time of 736 participants in a cohort established in a rural community, and the individuals in the lowest performance quartile had a significantly higher occurrence of disability.

Self-selection bias is suggested as a possible reason for the lack of associations between hand grip strength, muscle mass, and one-leg standing time and disability observed here, compared with previous reports. Self-selection bias is one type of sampling bias exhibited by subjects who voluntarily enrol in an epidemiological study. In this second visit of the ROAD study, volunteers who could walk to the clinic where the survey was performed, and could understand and sign an informed consent form, and who wanted to learn about their bone and joint conditions were welcomed. Therefore, the participants in the second survey may have been healthier than the general Japanese population. In fact, the estimated number of persons with disability in Japan using the age-gender prevalence of the second visit and the age-gender distribution of the Japanese population based on the national census in 2007 would be estimated at 1,510,000 (350,000 men, 1,160,000 women), considerably lower than the 4,940,000 reported by the government in 2010. Thus, self-selection bias likely affected the reference values; the reference values for hand grip strength, muscle mass, and one-leg standing time obtained from the present study may be higher, and walking speed faster, than the actual values. However, self-selection bias is somewhat unavoidable in such an

examination, because it is impossible to obtain measurements for individuals who cannot grasp a handgrip dynamometer or walk 6 m. This bias should be taken into consideration when reference values are used, including not only those obtained from the present study, but also from the national survey of physical strength and sporting ability published by the government.

In addition to self-selection bias, this study has several limitations. First, our results were obtained from a cross-sectional study of the second visit of the ROAD study; thus, we can not conclude causal relationships between such indices and disability, since some of the indices, such as muscle mass and one-leg standing time, were first introduced and performed during the second visit. We have begun the third visit of the ROAD study to clarify the relationships between physical performance and the occurrence of disability. Once the significance of indices reflecting components of locomotive syndrome can be determined as predictors for the occurrence of disability, appropriate thresholds can be developed as predictors of future disability. In addition, because of the lack of sufficient information, we could not determine the disorders that caused the disability. Thus, the disabled status of the participants in the present study might have been affected by various diseases such as cardiovascular diseases, dementia, or other diseases. However, regardless of the cause of disability, we found that walking ability was significantly associated with the presence of disability.

Conclusions

We have established reference values for hand grip strength, muscle mass, walking time, and one-leg standing time using data for a large-scale population-based cohort, and identified gender and age differences in the reference values. In addition, we determined that walking ability, including walking time and walking speed at the usual and maximum pace, was significantly associated with the presence of disability.

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Conflict of interest No conflict of interest has been declared by the authors.

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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

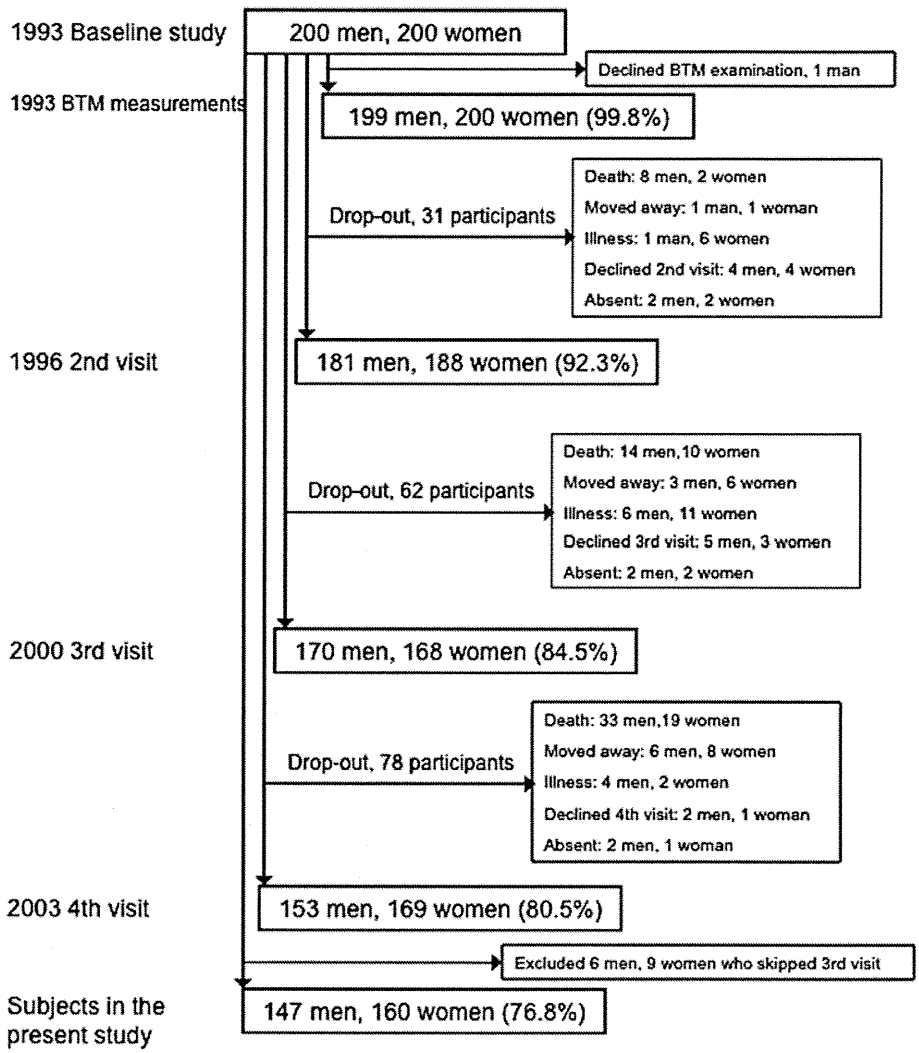

Fig. 1 Flow chart of participants in the cohort. *BTM* Biochemical marker of bone turnover

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