Reference Values for Bone Mineral Density and Bone Turnover Markers in the General Elderly Population: A Japanese Cohort Survey Randomly Sampled From A Basic Resident Registry

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Abstract

The aim of this study was to provide definitive reference values for bone mineral density (BMD) and bone turnover markers in the general elderly population.

Registered citizens of 50 to 89 years old were targeted for this survey. After random sampling from the resident registry of Obuse town, we established eight groups based on age (50s, 60s, 70s, and 80s) and gender. A total of 411 people were enrolled. We used a dual-energy x-ray absorptiometry device to measure and evaluate BMD. The bone formation marker bone alkaline phosphatase (BAP) was measured as a bone turnover marker. Bone resorption markers, including pentosidine, urinary total deoxypyridinoline, urinary type I collagen N-telopeptide, tartrate-resistant acid phosphatase 5b (TRACP-5b), 25-hydroxyvitamin D, and whole PTH were also measured as bone turnover markers.

Sixty-three people (15.3%) were diagnosed as OP. In women, BMD decreased with age. On the other hand, there was no characteristic change with age in men. As for bone markers, 25(OH)D, whole parathyroid hormone, and BAP showed no characteristics associated with gender and aging. In terms of the association between low BMD and bone markers, there was a significant association between low BMD and TRACP-5b in females.

In conclusions, BMD decreased with age in women. However, there was no decline with age in men. All bone metabolism markers showed no significant characteristics associated with age or gender, except for a significant association between low BMD and TRACP-5b in females. TRACP-5b was a potentially useful marker for the detection of low BMD.

Introduction

Bone mineral density (BMD) and bone turnover markers have become widely adopted as evaluation tools for osteoporotic disease [1–3]. Although several reports have attempted to determine reference values for BMD and bone turnover markers [4–7], the availability of definitive reference values for BMD and bone turnover markers in the general population are scarce, since earlier studies were based on volunteer cohorts or lumbar disorder patients.

To establish a new population study of the Japanese subjects, we conducted a random sampling from the Obuse Town Registry of Residents to obtain a more representative cohort of the general population with minimal selection bias [8–11]. This epidemiological study is referred to as the "Obuse Study" after the name of the cooperating municipality of Obuse Town. It is the first study of its kind to provide baseline values for age-specific bone turnover markers in a large cohort study.

The present investigation proposes reference values for BMD and bone turnover markers in the Japanese population using the Obuse study cohort.

Materials And Methods
Subjects

The protocols in this study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and STROBE Statement. Informed consent was obtained from participants prior to the initiation of the study. Participants were informed about the purposes of the research both verbally and in writing prior to the study, and written informed consent was obtained from all participants. This study was approved by the Institutional Review Board of Shinshu University (study no: 2792). From October 2014 to June 2017, we conducted an epidemiological study of residents (the Obuse Study) as a joint collaboration with a cooperating town office [8, 9]. Male and female participants between the ages of 50–89 were randomly selected from a pool of 5,352 registrants in the resident registry of a rural town [8, 9]. Those selected from the registry were asked whether they would be able to undergo a bone density examination, and calls for participation were continued until approximately 50 consenting participants were successfully recruited for each age group and sex [8, 9]. Four hundred and thirteen participants were consequently included in the study, excluding 4 participants with incomplete measurements (Fig. 1) [8, 9].

Bone mineral density and young adult mean measurement

We used a dual-energy x-ray absorptiometry device (GE Prodigy, GE healthcare, Chicago, IL, USA) to measure and evaluate bone mineral density (BMD) and T-score. BMD and T-score were measured at the femoral neck, total hip, and lumbar spine (L2-4). Based on the WHO diagnostic criteria, T-score ≥ -1 was classified as healthy, -2.5 < T-score < -1 as osteopenia, and T-score ≤ -2.5 as osteoporosis [12]. Osteoporosis and osteopenia were defined as low BMD.

Assay of bone turnover markers

The bone resorption marker pentosidine, urinary total deoxypyridinoline (DPD), tartrate-resistant acid phosphatase 5b (TRACP-5b), 25-hydroxyvitamin D (25[OH]D), and whole parathyroid hormone (PTH) were measured as bone turnover markers. Serum pentosidine, TRACP-5b, 25[OH]D, and whole PTH were measured using an enzyme-linked immunosorbent assay kit (SRL), enzyme immunoassay kit (SRL), electro chemiluminescence immunoassay kit (SRL), and chemiluminescent enzyme immunoassay kit (SRL), respectively. Urine DPD was measured using an enzyme immunoassay kit (SRL). The bone formation marker bone alkaline phosphatase (BAP) was also measured as a bone turnover marker. Serum BAP was measured using a chemiluminescent enzyme immunoassay kit (SRL, Tokyo, Japan).

Statistical Analysis

The prevalence of OP was compared for each age group and sex. BMD and bone turnover markers were evaluated for each age and sex using Tukey’s test for comparisons among multiple groups. Multiple logistic regression analysis was applied to determine the association between bone markers and low BMD.

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (version 3.5.2; The R Foundation for Statistical Computing, Vienna, Austria).
Results

Of the 415 participants who were randomly sampled from the resident registry, 4 participants who were unable to measure BMD due to artificial hip joint replacement were excluded (Fig. 1). The physical characteristics and functions of the 411 examinees are shown for each age group and sex in Table 1. The interview results regarding participant comorbidities and menopause were shown in Table 2. Thirty-nine (9.5%) people had been treated osteoporosis. Fifteen (3.6%) people had been treated with hormone therapy. Five (1.2%) people had been treated with steroid.

| Age strata (years) | n   | Height (cm) | Weight (kg) | BMI (kg/m²) |
|-------------------|-----|-------------|-------------|-------------|
| Male 50–59        | 50  | 171.8 (5.9) | 67.1 (9.0) | 22.7 (2.9) |
| 60–69             | 53  | 166.7 (4.7) | 66.9 (7.7) | 24.1 (2.7) |
| 70–79             | 55  | 163.2 (4.9) | 60.0 (10.2)| 22.5 (3.4) |
| 80–89             | 45  | 160.1 (5.6) | 57.5 (8.4) | 22.4 (2.7) |
| total             | 203 | 165.6 (6.8) | 63.0 (0.98)| 22.9 (0.30)|
| Female 50–59      | 47  | 158.1 (4.9) | 55.4 (8.9) | 22.2 (3.8) |
| 60–69             | 61  | 152.8 (5.3) | 52.2 (7.6) | 22.3 (2.7) |
| 70–79             | 55  | 149.3 (5.6) | 50.7 (7.9) | 22.8 (3.5) |
| 80–89             | 45  | 144.7 (6.1) | 48.4 (8.1) | 23.1 (3.4) |
| total             | 208 | 151.3 (7.2) | 51.7 (8.4) | 22.6 (3.3) |

Values represent mean (standard deviation). BMI, Body Mass Index.
Sixty-three people (15.3%) were diagnosed as OP, of which 14 (6.9%) were male and 49 (23.6%) were female. In men and women, BMD decreased with age. The decrease in BMD was particularly pronounced in the femoral neck. On the other hand, there was no characteristic change with age in the lumber spine (Table 3).

| Disease                          | No. of participants | Prevalence |
|----------------------------------|---------------------|------------|
| Hyperthyroidism                  | 5                   | 1.2%       |
| Hyperparathyroidism              | 0                   | 0%         |
| Diabetes mellitus                | 52                  | 12.7%      |
| Paget's disease of bone          | 3                   | 0.7%       |
| Rheumatoid arthritis             | 5                   | 1.2%       |
| Chronic obstructive pulmonary disease | 7               | 1.7%       |
| Fracture                         | 131                 | 31.9%      |
| Menopause (only female)          | 202                 | 96.7%      |
Table 3
Bone mineral density and T-score at the femoral neck, proximal femur, and lumbar 1–4, in addition to prevalence of osteoporosis

| Age strata (years) | N   | Femoral neck BMD | Total hip BMD | Lumbar spine BMD | Femoral neck T-score | Total hip T-score | Lumbar spine T-score | Number of OP |
|-------------------|-----|------------------|----------------|------------------|----------------------|-------------------|----------------------|--------------|
| Male              |     |                  |                |                  |                      |                   |                      |              |
| 50–59             | 50  | 0.90 (0.13)      | 0.96 (0.13)    | 1.17 (0.18)      | -0.39 (1.04)         | 0.13 (1.01)       | -0.13 (1.53)         | 2 (4.0%)     |
| 60–69             | 53  | 0.91 (0.11)      | 0.99 (0.13)    | 1.28 (0.22)      | -0.35 (0.88)         | 0.35 (0.99)       | 0.76 (1.84)          | 0 (0.0%)     |
| 70–79             | 55  | 0.88 (0.12)      | 0.97 (0.15)    | 1.35 (0.27)      | -0.51 (0.93)         | 0.21 (1.14)       | 1.31 (2.28)          | 5 (9.1%)     |
| 80–89             | 45  | 0.80 (0.15)abc   | 0.87 (0.15)abc | 1.27 (0.29)      | -1.12 (1.12)         | -0.59 (1.14)      | 0.70 (2.39)          | 7 (15.6%)    |
| total             | 203 | 0.88 (0.13)      | 0.95 (0.15)    | 1.27 (0.25)      | -0.58 (1.03)         | 0.05 (1.12)       | 0.67 (2.08)          | 14 (6.9%)    |
| Female            |     |                  |                |                  |                      |                   |                      |              |
| 50–59             | 47  | 0.80 (0.11)      | 0.87 (0.13)    | 1.09 (0.18)      | -0.85 (0.90)         | -0.52 (1.06)      | -0.24 (1.46)         | 4 (8.5%)     |
| 60–69             | 61  | 0.76 (0.10)      | 0.84 (0.11)    | 1.02 (0.18)      | -1.16 (0.85)         | -0.81 (0.88)      | -0.80 (1.52)         | 12 (19.7%)   |
| 70–79             | 55  | 0.72 (0.10)a     | 0.80 (0.12)a   | 1.03 (0.20)      | -1.47 (0.86)         | -1.08 (0.99)      | -0.80 (1.67)         | 15 (27.3%)   |
| 80–89             | 45  | 0.67 (0.10)ab    | 0.71 (0.10)abc | 1.00 (0.20)      | -1.88 (0.80)         | -1.83 (0.84)      | -0.96 (1.67)         | 18 (40.0%)   |
| total             | 208 | 0.74 (0.11)      | 0.81 (0.13)    | 1.04 (0.19)      | -1.33 (0.92)         | -1.04 (1.05)      | -0.71 (1.60)         | 49 (23.6%)   |

BMD: bone mineral density, OP: Osteoporosis

Values represent mean (standard deviation).

Values of OP represent number (prevalence).

One female patient aged 70's and 3 female patients aged 80's were excluded due to the artificial head replacement surgeries.

a, Significantly different (p < 0.05) values from those aged 50–59 years.

b, Significantly different (p < 0.05) values from those aged 60–69 years.

c, Significantly different (p < 0.05) values from those aged 70–79 years.

In men and women, pentosidine and DPD increased with age. In addition, TRACP-5b increased with age in males. 25(OH)D, whole PTH, and BAP showed no characteristics associated with gender or aging (Table 4).
| Age strata (years) | Pentosidine | DPD | 25(OH)D | TRACP-5b | Whole PTH | BAP |
|-------------------|-------------|-----|---------|----------|-----------|-----|
| Male              |             |     |         |          |           |     |
| 50–59             | 0.05 (0.01) | 3.3 (0.8) | 25.2 (6.0) | 312.2 (88.3) | 21.8 (8.1) | 11.9 (2.4) |
| 60–69             | 0.05 (0.02) | 3.7 (1.2) | 22.9 (5.4) | 380.8 (144.0) | 19.8 (7.7) | 13.5 (3.8) |
| 70–79             | 0.06 (0.02) | 3.9 (1.1) | 29.3 (7.5) | 448.7 (198.4) | 20.7 (7.7) | 13.6 (4.7) |
| 80–89             | 0.07 (0.05) | 5.2 (1.7) | 22.0 (5.6) | 489.7 (194.8) | 22.0 (12.1) | 13.3 (3.7) |
| total             | 0.06 (0.02) | 4.0 (1.4) | 25.0 (6.8) | 406.2 (174.2) | 21.0 (8.9) | 13.1 (3.8) |
| Female            |             |     |         |          |           |     |
| 50–59             | 0.04 (0.01) | 5.9 (1.2) | 22.0 (6.5) | 416.1 (130.9) | 22.6 (11.0) | 15.2 (4.5) |
| 60–69             | 0.05 (0.02) | 5.3 (1.3) | 20.6 (6.2) | 478.5 (141.7) | 20.1 (7.7) | 16.1 (5.1) |
| 70–79             | 0.06 (0.05) | 5.4 (2.0) | 25.0 (9.1) | 490.1 (167.4) | 22.2 (12.3) | 15.5 (5.4) |
| 80–89             | 0.06 (0.02) | 6.2 (2.5) | 19.2 (6.0) | 433.8 (167.9) | 21.0 (10.5) | 14.1 (5.3) |
| total             | 0.06 (0.03) | 5.7 (1.8) | 21.8 (7.4) | 457.6 (154.8) | 21.4 (10.4) | 15.3 (5.1) |

DPD: deoxypyridinoline, 25(OH)D: 25-hydroxyvitamin D, TRACP-5b: tartrate-resistant acid phosphatase 5b, PTH: parathyroid hormone, BAP: bone alkaline phosphatase

Values represent mean (standard deviation).

a, Significantly different (p < 0.05) values from those aged 50–59 years.

b, Significantly different (p < 0.05) values from those aged 60–69 years.

c, Significantly different (p < 0.05) values from those aged 70–79 years.

Relevant factors selected by the unifactorial analysis were subjected to multiple logistic regression analysis with gender. The results showed that when considering low BMD as a dependent variable, bone markers were not associated with significantly low BMD in males (Table 5). However, there was a significant association between low BMD and TRACP-5b in females (Table 6).
Table 5
Independent association between low BMD and bone markers in males

| Factor       | Univariate |               | Multivariate |               |
|--------------|------------|---------------|--------------|---------------|
|              | Odds ratio (95%CI) | p value | Odds ratio (95%CI) | p value |
| Age          | 1.02 (0.99–1.04) | 0.20 |               |               |
| Pentosidine  | 1.11 (0.84–1.47) | 0.47 |               |               |
| DPD          | 1.27 (1.03–1.57) | 0.026 | 1.13 (0.88–1.44) | 0.38 |
| 25(OH)D      | 0.98 (0.94–1.02) | 0.30 | 1.00 (1.00–1.00) | 0.072 |
| TRACP-5b     | 1.00 (1.00–1.00) | 0.0079 |               |               |
| Who1e PTH    | 1.03 (0.99–1.07) | 0.064 |               |               |
| BAP          | 1.03 (0.95–1.11) | 0.50 |               |               |

CI: confidence interval, DPD: deoxypyridinoline, 25(OH)D: 25-hydroxyvitamin D, TRACP-5b: tartrate-resistant acid phosphatase 5b, PTH: parathyroid hormone, BAP: bone alkaline phosphatase

Table 6
Independent association between low BMD and bone markers in females

| Factor       | Univariate |               | Multivariate |               |
|--------------|------------|---------------|--------------|---------------|
|              | Odds ratio (95%CI) | p value | Odds ratio (95%CI) | p value |
| Age          | 1.08 (0.0023-0.20) | 0.00074 | 1.06 (1.02–1.10) | 0.0015 |
| Pentosidine  | 1.94 (1.01–3.71) | 0.045 | 1.36 (0.70–2.66) | 0.37 |
| DPD          | 1.18 (0.96–1.45) | 0.11 | 1.36 (0.70–2.66) | 0.075 |
| 25(OH)D      | 0.96 (0.92-1.00) | 0.048 | 1.00 (1.02–1.10) | 0.0015 |
| TRACP-5b     | 1.00 (1.00–1.00) | 0.011 |               |               |
| Who1e PTH    | 1.02 (0.99–1.06) | 0.25 |               |               |
| BAP          | 1.04 (0.98–1.12) | 0.19 |               |               |

CI: confidence interval, DPD: deoxypyridinoline, 25(OH)D: 25-hydroxyvitamin D, TRACP-5b: tartrate-resistant acid phosphatase 5b, PTH: parathyroid hormone, BAP: bone alkaline phosphatase

Discussion

In this present cohort study, we were able to calculate mean BMD and bone turnover markers by age and sex for the elderly aged 50 years and older according to the Japanese population ratio in more than 400 subjects randomly selected from a rural town registry in Japan. We were able to create a cohort that more accurately reflects the general population in comparison to traditional population studies that recruit active volunteers. Another feature of this study was the uniform distribution of age and gender ratios between 50
and 89 years old, as a result of collecting about 50 physical examination participants by age and gender. This uniform distribution is advantageous for making accurate statistical comparisons between men and women and between age groups.

In this study, the BMD decreased with age in the femoral neck and total hip in males and females. The BMD of the femoral neck, total hip, and lumbar spine were comparable with previous studies in our country and elsewhere after accounting for gender and age (Table 7). Previous studies have demonstrated that BMD decreased with aging [13, 14], and this study obtained the same results as have been described in the literature.

| Study    | Country          | Sex   | Number | Age(years) | Femoral neck | Total hip | Lumbar spine |
|----------|------------------|-------|--------|------------|--------------|-----------|--------------|
| Lee, 2019| Korea            | Male  | 244    | age > 65   | 0.78 (0.007) | 0.87 (0.008) | 0.94 (0.005) |
|          |                  | Female| 319    |            | 0.56 (0.005) | 0.70 (0.005) | 0.73 (0.008) |
| Schacht, 2019| Denmark      | Male  | 98     | 69.0 (6.0) | 0.95 (0.18)  | 1.10 (0.24) | 1.31 (0.26)  |
|          |                  | Female| 86     | 70.0 (5.8) | 0.83 (0.19)  | 0.88 (0.24) | 1.13 (0.25)  |
| Fuggle, 2018| England and Wales| Male  | 194    | 64.4 (2.5) | 1.03 (0.14)  |           | 1.06 (0.15)  |
|          |                  | Female| 171    | 66.5 (2.7) | 0.89 (0.13)  |           | 0.95 (0.17)  |
| Fujiwara, 2003| Japan        | Male  | 763    | 62.9 (9.8) | 0.73 (0.11)  |           | 0.98 (0.16)  |
|          |                  | Female| 1593   | 65.4 (9.8) | 0.62 (0.11)  |           | 0.82 (0.11)  |
| Our study| Japan           | Male  | 203    | 69.5 (11.2)| 0.88 (0.13)  | 0.95 (0.15) | 1.27 (0.25)  |
|          |                  | Female| 208    | 70.0 (11.0)| 0.74 (0.11)  | 0.81 (0.13) | 1.04 (0.19)  |

Values represent mean (standard deviation).

All bone markers in this study were within the standard value for males and females in each generation [5, 7]. In men and women, pentosidine and DPD increased with age. TRACP-5b increased with age in males. However, other markers showed no association with age. In this study, subjects were randomly selected from a rural Japanese town; thus, subjects may be healthy. Aging may exert little influence on bone markers, while the prevalence of osteoporosis and osteopenia increased with aging in both males and females.
In terms of the association between low BMD and bone markers, there was a significant association between low BMD and TRACP-5b in females. TRACP-5b is a bone resorption marker that is not affected by renal dysfunction and has a low diurnal variability [15, 16]. Thus, TRACP-5b has been considered a useful marker. TRACP-5b was inversely correlated with BMD in females [17]. Furthermore, TRACP-5b has been described to be associated with increased fracture risk in elderly females [18, 19]. TRACP-5b could be a potential marker to predict fractures. In this study, TRACP-5b was related to low BMD in randomly selected female residents in the area. High bone resorption may be a factor for low BMD in female residents. TRACP-5b may be marker which was useful for the detection of low BMD.

There is a limitation in this study. Although the research design reduces the sampling bias by adopting random sampling from the resident register, we may not have been able to control for all potential biases as a result of the low participation rate.

**Conclusion**

A characteristic feature of this study was the collection of participants with age ranging from 50 to 89 years by randomly sampling from the resident register.

Therefore, this research was designed to create a cohort that more accurately reflects common residents. BMD decreased with age in the femoral neck, total hip, and lumbar spine in women. Furthermore, all bone metabolism markers showed no significant characteristics associated with age or gender. However, a significant association between low BMD and TRACP-5b in females was observed; therefore, high bone resorption may be a factor for low BMD in female residents.

**Declarations**

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