

# One-year Changes in Bone Mineral Density and Bone Turnover Markers in Premenopausal Amateur Runners: A Prospective Study

Sachiko Sumida,<sup>1,2</sup> Jun Iwamoto,<sup>3</sup> Kazuhiro Uenishi<sup>4</sup> and Toshiro Otani<sup>1,3</sup>

<sup>1</sup>Graduate School of Health Management, Keio University, Kanagawa, Japan

<sup>2</sup>Sports Medicine Research Center, Keio University, Kanagawa, Japan

<sup>3</sup>Institute for Integrated Sports Medicine, School of Medicine, Keio University, Tokyo, Japan

<sup>4</sup>Laboratory of Physiological Nutrition, Kagawa Nutrition University, Saitama, Japan

(Received for publication on November 15, 2013)

(Revised for publication on January 8, 2014)

(Accepted for publication on January 16, 2014)

(Published online in advance on June 10, 2014)

A prospective study was conducted to clarify the 1-year changes in lumbar spine and hip bone mineral density (BMD) and bone turnover markers in premenopausal amateur runners and to determine whether jumping and muscle-strengthening exercises have additive effects on the bone parameters in these runners. Thirty-six premenopausal amateur runners were recruited and were divided into the following two groups: a jumping plus muscle-strengthening exercise group (n =21) and a control group (n =15). All participants continued their running practice for 1 year, and the lumbar spine and total hip BMD and bone turnover markers were monitored. For all participants, the lumbar spine and total hip BMD increased modestly after 1 year (1.31% and 1.54%, respectively) in addition to increases in the bone-specific alkaline phosphatase, osteocalcin, and tartrate-resistant acid phosphatase 5b levels (13.2%–27.8%), indicating mild effects of running activity on bone turnover and BMD at clinically relevant skeletal sites. Jumping plus muscle-strengthening exercises did not significantly influence any bone parameters; however, it was difficult to draw definite conclusions because compliance was poor. These results suggest that long-distance running at the recreational level may be useful in maintaining bone health in premenopausal women. (doi: 10.2302/kjm.2013-0010-OA; Keio J Med 63 (3) : 43–51, September 2014)

**Keywords:** premenopausal women, long-distance runner, bone mineral density (BMD), jumping exercise, muscle-strengthening exercise

## Introduction

Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture.<sup>1</sup> Because estrogen deficiency after menopause causes rapid bone loss in women, osteoporosis commonly affects postmenopausal women and places them at an increased risk of fractures. Osteoporotic fractures, particularly vertebral and hip fractures,

are known to increase morbidity and mortality.<sup>2</sup> Thus, the spine and hip are clinically important skeletal sites; appropriate management to prevent vertebral and hip fractures is considered to be crucial.

Peak bone mass is reached by age 30 years, with 90% of bone development completed by age 18.<sup>3</sup> For most women, bone mass remains stable until menopause, when the loss of estrogen in conjunction with aging is associated with a decline in bone mineral density (BMD).<sup>4</sup> Strate-

gies for preventing osteoporotic fractures in women include maximizing peak bone mass, increasing bone mass before menopause, counteracting menopause-related bone loss, and preventing falls.<sup>5</sup> Primarily, sports and exercise in the presence of sufficient calcium and vitamin D intake play an important role in the prevention of osteoporotic fractures throughout a woman's life. Available evidence suggests that exercise stimulates bone growth in children (leading to an increase in peak bone mass), attenuates loss of BMD in postmenopausal women, and prevents falls in elderly women.<sup>5</sup> Cross-sectional studies among young female athletes have revealed that runners and swimmers have a lower BMD than athletes performing weight-bearing activities.<sup>6</sup> A systematic review study demonstrated that in young women, high-impact exercises such as jumping increase the femoral neck BMD, and muscle-strengthening exercises are useful for increasing the lumbar spine BMD.<sup>7,8</sup>

Recently, the number of young adult women who attempt to maintain their general health through running activities has increased. However, whether long-distance running enhances bone health in young adult women remains uncertain, because very few prospective longitudinal studies have demonstrated the effect of long-distance running at the recreational level on the lumbar spine and hip BMD in premenopausal women. In female athletes, Nikander *et al.*<sup>9</sup> showed that long-term sport-specific exercises involving ground impacts (e.g., endurance running, ball games, jumping) were associated with thicker cortex at the distal and diaphyseal sites of the tibia, whereas Wilks *et al.*<sup>10</sup> reported that the cortical BMD of the tibia in runners decreased systematically with running distance or training volume. Thus, the influence of endurance running on bone mass remains controversial. The purposes of the present study were (1) to clarify the 1-year changes in the lumbar spine and total hip BMD as well as bone turnover markers in premenopausal amateur runners and (2) to determine whether jumping and muscle-strengthening exercises have additive effects on the bone parameters in those runners.

## Materials and Methods

### Study design

This study was a 1-year prospective longitudinal study. The study was approved by the research ethics board of the Graduate School of Health Management, Keio University (Kanagawa, Japan). Written informed consent was obtained from all participants.

### Participants

Forty-three premenopausal women were recruited from three running clubs and at two running events in

Kanagawa and Tokyo, Japan. The inclusion criteria included premenopausal women, amateur runners who participated in running clubs and/or running events regularly, and running activity more than twice per week. The exclusion criteria included pregnancy, lactation, suffering from diseases such as rheumatoid arthritis, kidney disease, hyperthyroidism, fractures within 1 year, and medication related to bone metabolism (e.g., glucocorticoid, vitamin D<sub>3</sub>, bisphosphonates). The participants were assigned randomly to a running plus jumping and muscle-strengthening exercise group (exercise group) or a running only group (control group). The random assignment procedure was performed using random numbers generated by a computer program. The duration of the study was 1 year, and running activities were continued throughout the year. In the exercise group, the subjects were instructed to perform a maximum jumping exercise ten times a day, 3 days per week, and trunk muscle (four core muscles) strengthening exercises ten times a day, 3 days per week. The lumbar spine and total hip BMD, biochemical markers including bone turnover markers, physical function parameters (muscle strength and muscle power), and nutritional intake were evaluated over 1 year. The primary endpoint was the BMD. The secondary endpoints included biochemical markers and physical function parameters.

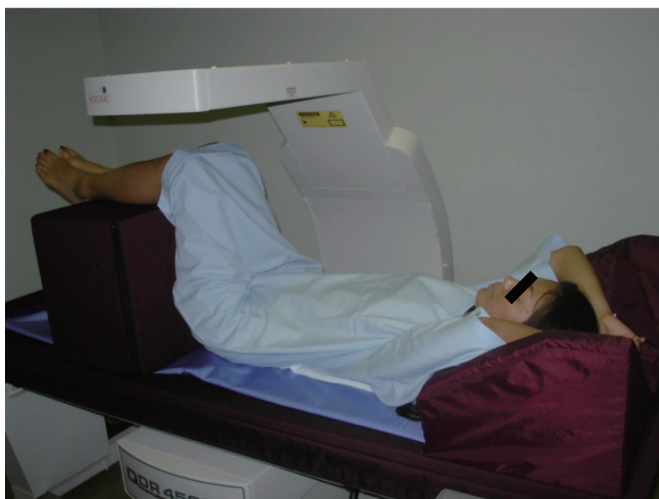
### Measurement of BMD

The BMD of the lumbar spine (L2–L4) and the total hip (the sum of the neck, trochanter, and intertrochanteric values) was measured using dual-energy X-ray absorptiometry (DXA: QDR 4500A; Hologic, Bedford, MA, USA) (**Fig. 1**).<sup>11</sup> Measurements of the lumbar spine and hip BMD have a precision error of 1%–1.5% based on adult scans.<sup>12</sup> The BMD was measured at baseline and at 1 year after the start of the trial.

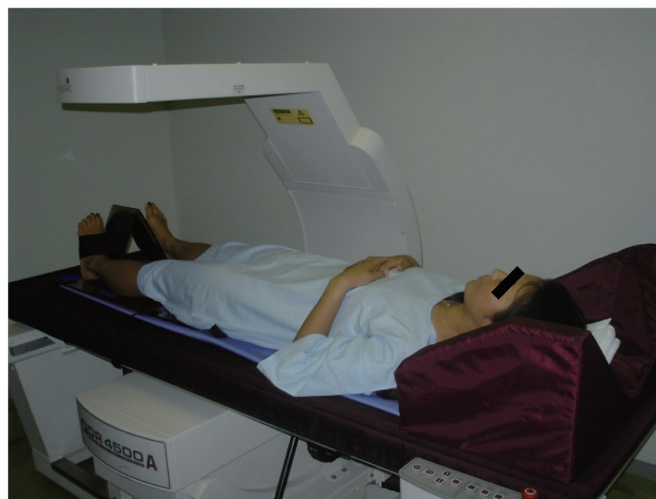
### Measurement of biochemical markers

Blood and urine samples were obtained from each participant after the participant had fasted in the morning. The serum level of bone-specific alkaline phosphatase (BAP) as a bone formation marker was measured using an enzyme immunoassay (EIA) or a chemiluminescent enzyme immunoassay (CLEIA). EIA was available at the beginning of the trial, but CLEIA only became available thereafter. BAP levels measured using EIA were converted into BAP levels measured using CLEIA with the following formula: BAP measured using CLEIA = 0.778 × (BAP measured using EIA) – 7.059.<sup>13</sup> The serum level of osteocalcin as a bone formation marker was measured using EIA. The serum level of tartrate-resistant acid phosphatase 5b (TRAP5b) and the urinary level of cross-linked N-terminal telopeptides of type I collagen

## Lumbar spine (L2-L4)



## Total hip



**Fig. 1** Bone mineral density (BMD) measurement by dual-energy X-ray absorptiometry (DXA).

The BMD of the lumbar spine (L2–L4) and the total hip was measured using QDR 4500A DXA. First, the participants reclined on the DXA table with a large square cushion under their lower legs with the thighs as close to a 90° angle to the body as possible (left panel). Then, the lumbar spine was scanned and the L2–L4 BMD was measured. Second, the participants reclined on the DXA table placing the hip scan positioning device at the far end of the table near the participants' feet. The leg to be examined was rotated inward so that the foot could be placed against the positioning device and secured with a strap (right panel). The abduction of the leg was adjusted so that the shaft of the femur was parallel with the center of the table. The hip to be examined was then scanned and the BMD of the total hip (the sum of the neck, trochanter, and intertrochanteric values) was measured.

(NTX) as bone resorption markers were both measured using EIA. The serum levels of 25-hydroxyvitamin D (25-OHD), undercarboxylated osteocalcin (ucOC), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) were measured using a competitive protein binding assay, an electro chemiluminescence immunoassay, a chemiluminescent immune assay (CLIA), and CLIA, respectively.

The standard values of BAP (CLEIA), osteocalcin, TRAP5b, and urinary NTX in premenopausal healthy women are 2.9–14.5 µg/L, <7.0 ng/mL, 120–420 mU/dL, and 9.3–54.3 nmol BCE/mmol Cr, respectively.<sup>14</sup> The cut-off values for vitamin D and vitamin K insufficiency are 25-OHD <28 ng/mL and ucOC >4.5 ng/mL, respectively.<sup>14,15</sup> The normal range of FSH is 3.2–14.4 µIU/mL for the follicular phase, 3.4–17.1 µIU/mL for the ovulatory phase, and 1.4–8.4 µIU/mL for the luteal phase. The normal range of TSH is 0.436 – 3.78 µIU/mL. Biochemical markers were measured at baseline and at 1 year after the start of the trial.

### *Evaluation of physical function*

Muscle strength was evaluated by measuring the isometric maximum extension and flexion strength of the right knee joint using a hand-held dynamometer (µTas

F-100; Anima, Tokyo, Japan). The measurement was performed while the participants assumed a sitting position with the knee and hip joints flexed at 90° (knee extension strength) and a prone position with the knee joint flexed at 90° (knee flexion strength). The measurement was performed twice, and the higher value of the two measurements was adopted.

Muscle power was evaluated by measuring the leg extension power using a dynamometer (Aneropress 3500; Combi Wellness, Tokyo, Japan). Participants sat on the seat of the dynamometer with bilateral feet fixed on the footplate and the knee joints flexed at 90°. Then, they were instructed to push the footplate as strongly and quickly as possible. The parameter was measured five times, and the highest value of five measurements was adopted. These physical function parameters were evaluated at baseline and at 1 year after the start of the trial.

### *Assessment of nutritional intake*

A food frequency questionnaire for the prevention and management of osteoporosis, developed by Uenishi et al.,<sup>16</sup> was used to evaluate nutritional status. The daily intakes of energy, protein, calcium, vitamin D, and vitamin K were calculated using this questionnaire. Nutritional intakes were evaluated at baseline and at 1 year after the

**Table 1** Baseline anthropometry, BMD, and bone turnover markers

	All participants (n=36)	Control group (n=15)	Exercise group (n=21)
Age (years)	37.9 ± 6.6	37.5 ± 7.4	38.2 ± 6.2
Height (m)	1.58 ± 0.05	1.59 ± 0.05	1.57 ± 0.05
Body weight (kg)	51.8 ± 5.6	51.9 ± 5.1	51.8 ± 6.1
Body mass index (kg/m <sup>2</sup> )	20.8 ± 1.9	20.5 ± 1.9	21.0 ± 2.0
Duration of running activity (years)	5.24 ± 4.27	4.85 ± 3.83	5.52 ± 4.64
Lumbar spine BMD (g/cm <sup>2</sup> )	0.992 ± 0.110	1.002 ± 0.118	0.985 ± 0.106
Total hip BMD (g/cm <sup>2</sup> )	0.843 ± 0.105	0.819 ± 0.087	0.859 ± 0.116
Serum BAP (µg/L)	8.69 ± 3.50	7.91 ± 2.71	9.24 ± 3.94
Serum osteocalcin (ng/mL)	5.11 ± 1.33	5.26 ± 1.65	5.02 ± 1.11
Serum TRAP5b (mU/dL)	173 ± 67	155 ± 49	186 ± 76
Urinary NTX (nmol BCE/mmol Cr)	30.3 ± 11.9	32.6 ± 9.5	28.6 ± 13.3

Data are expressed as the means ±SD. An unpaired *t* test was used to compare parameters between the two groups. There were no significant differences in any parameters between the two groups. BMD, bone mineral density; BAP, bone-specific alkaline phosphatase; TRAP5b, tartrate-resistant acid phosphatase 5b; NTX, cross-linked N-terminal telopeptides of type I collagen.

start of the trial.

### Monitoring of adherence

The frequency and distance of the daily running practice and compliance with the jumping and muscle-strengthening exercises were monitored using a self-report sheet. The sheets were collected every 6 months.

### Statistical analyses

Data are expressed as means ±standard deviations (SD) in the tables and means ±standard errors (SE) in the figures. An unpaired *t* test was used to compare parameters between the two groups. One-way analysis of variance (ANOVA) with repeated measurements was used to examine the longitudinal changes in parameters in all participants. Two-way ANOVA with repeated measurements was used to examine differences in the changes in parameters between the two groups. A significance level of  $p < 0.05$  was used for all the comparisons. All statistical analyses were performed using the IBM SPSS Statistics 20 (International Business Machines, Armonk, NY, USA) and the Stat View J-5.0 programs (SAS Institute, Cary, NC, USA).

## Results

### Breakdown of study participants

Five women (25.0%) in the control group and two women (8.7%) in the exercise group dropped out from the trial. The reasons for the dropouts were non-compliance (n=3), health problems (n=2), trauma (n=1), and house moving (n=1). None of the reasons were related to running

practice or the jumping plus muscle-strengthening exercises. Fifteen women (75.0%) in the control group and 21 women (91.3%) in the exercise group completed the trial and were included in the analyses.

### Baseline characteristics of the study participants who were included in the analyses

**Table 1** shows the baseline anthropometry, BMD, and bone turnover markers of the study participants who completed the trial. For all participants, the mean duration of running activity was 5.2 years. None of the study participants had any menstruation disorders. The mean age at baseline was 37.9 years and the mean body mass index was 20.8 kg/m<sup>2</sup>. The mean lumbar spine and total hip BMD at baseline were 0.992 g/cm<sup>2</sup> and 0.843 g/cm<sup>2</sup>, respectively. These values corresponded to Z-scores (compared with age-matched controls) of -0.14 and -0.20. The mean serum BAP, osteocalcin, TRAP5b, and urinary NTX levels were within the normal ranges. There were no significant differences in the baseline anthropometry, BMD, or bone turnover markers between the two groups.

**Table 2** includes baseline biochemical markers other than the bone turnover markers, physical functions and nutritional intakes. Although the mean serum uOC level (2.85 ng/mL) was within the normal range in all participants,<sup>14</sup> the mean serum 25-OHD level (21.3 ng/mL) was lower than the cut-off value for vitamin D insufficiency (28 ng/mL).<sup>15</sup> There were no significant differences in biochemical markers, physical functions, or nutritional intakes between the two groups.

**Table 2** Changes in biochemical markers, physical functions, and nutritional intakes

	All participants (n=36)		Control group (n=15)		Exercise group (n=21)	
	Baseline	1 year	Baseline	1 year	Baseline	1 year
<b>Biochemical markers</b>						
25-OHD (ng/mL)	21.3 ± 7.1	21.0 ± 5.8	21.4 ± 5.2	20.1 ± 5.4	21.1 ± 8.3	21.6 ± 6.2
ucOC (ng/mL)	2.85 ± 0.99	3.07 ± 1.19	2.83 ± 1.22	3.14 ± 1.35	2.86 ± 0.84	3.03 ± 1.10
FSH (mIU/mL)	7.05 ± 9.97	9.18 ± 12.90	4.29 ± 1.66	8.52 ± 11.38	8.89 ± 12.58	9.67 ± 14.21
TSH (μIU/mL)	1.90 ± 1.15	1.89 ± 1.01	2.08 ± 1.42	2.17 ± 1.05	1.78 ± 0.94	1.68 ± 0.95
<b>Physical functions</b>						
Knee extension strength (kg)	31.1 ± 6.6	31.4 ± 6.8	33.7 ± 8.4	31.8 ± 7.0	29.4 ± 4.5	31.1 ± 6.9
Knee flexion strength (kg)	9.5 ± 2.2	9.5 ± 2.2	9.95 ± 1.51	9.45 ± 2.08	9.14 ± 2.61	9.59 ± 2.26
Leg press power (W)	721 ± 200	844 ± 179	792 ± 234	843 ± 211	669 ± 159	844 ± 157
<b>Nutritional intakes</b>						
Energy (kcal/day)	1877 ± 426	1829 ± 355	1724 ± 232	1719 ± 274	1986 ± 499	1908 ± 392
Protein (g/day)	77 ± 20	77 ± 20	70 ± 9	71 ± 15	80 ± 24	81 ± 23
Calcium (mg/day)	495 ± 188	486 ± 132	529 ± 228	482 ± 137	472 ± 155	488 ± 132
Vitamin D (μg/day)	10.2 ± 2.0	10.2 ± 2.0	10.3 ± 2.2	10.2 ± 2.4	10.1 ± 2.0	10.2 ± 1.8
Vitamin K (μg/day)	256 ± 118	236 ± 113	261 ± 134	222 ± 124	252 ± 108	246 ± 106

Data are expressed as the means ±SD. An unpaired *t* test was used to compare parameters between the two groups. ANOVA with repeated measurements was used to examine the longitudinal changes in parameters in all participants. Two-way ANOVA with repeated measurements was used to examine differences in changes in parameters between the two groups. There were no significant differences in either baseline parameters or changes in any parameters between the two groups. 25-OHD, 25-hydroxyvitamin D; ucOC, undercarboxylated osteocalcin; FSH, follicle stimulating hormone; TSH, thyroid stimulating hormone.

### ***Compliance with running and jumping and muscle-strengthening exercises***

The 1-year running distance (mean ±SD) was 1344.0 ± 876.0 km in the exercise group and 1103 ± 1033.1 km in the control group. There was no significant difference in the 1-year running distance between the two groups. Although the participants in the exercise group were instructed to perform the jumping and muscle-strengthening exercise 3 days per week, or 156 days per 52 weeks (1 year), the jumping exercise was only performed on 26.5 days (17%) and the muscle-strengthening exercise was performed on 78.0 days (50%) during the year.

### ***Changes in BMD and bone turnover markers***

In all participants, the lumbar spine and total hip BMD had significantly increased from the baseline values after 1 year (**Fig. 2** and **Table 3**, one-way ANOVA with repeated measurements). The percentage increases in the lumbar spine and total hip BMD from the baseline values were 1.31% and 1.54%, respectively. However, there were no significant differences in the increases in the lumbar spine and total hip BMD between the two groups (**Fig. 2** and **Table 3**, two-way ANOVA with repeated measurements).

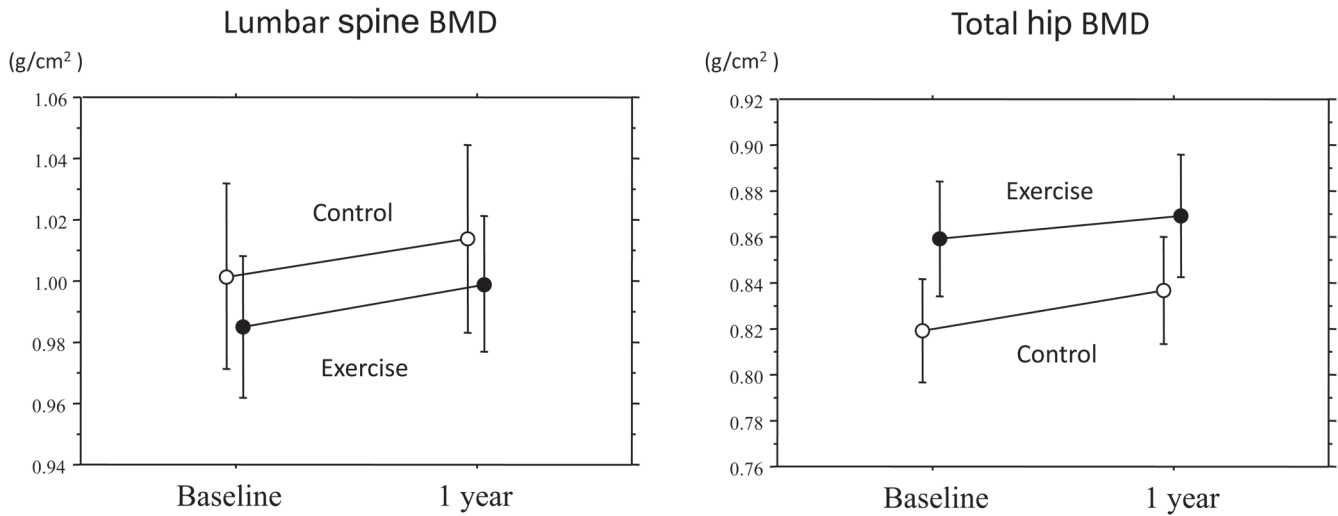
In all participants, the serum BAP, osteocalcin, and TRAP5b levels had significantly increased from the

baseline values after 1 year (**Fig. 3** and **Table 3**, one-way ANOVA with repeated measurements). The urinary NTX level increased from the baseline value, but this change was not statistically significant (**Fig. 3** and **Table 3**, one-way ANOVA with repeated measurements). The percentage increases in the serum BAP, osteocalcin, TRAP5b, and urinary NTX levels from the baseline values were 17.8%, 13.2%, 27.8%, and 11.7%, respectively. However, no significant differences in these increases were observed between the two groups (**Fig. 3** and **Table 3**, two-way ANOVA with repeated measurements).

### ***Changes in biochemical markers (other than bone turnover markers), physical functions, and nutritional intakes***

In all participants, the serum 25-OHD, ucOC, FSH, and TSH levels did not significantly change from the baseline values after 1 year (**Table 2**, one-way ANOVA with repeated measurements). There were no significant differences in the changes in any biochemical markers between the two groups (**Table 2**, two-way ANOVA with repeated measurements).

In all participants, there were no significant changes in the knee extension and flexion strength and leg press power from the baseline values after 1 year of study (**Table 2**, one-way ANOVA with repeated measurements). There were no significant differences in the changes in any



**Fig. 2** Changes in lumbar spine and total hip BMD.

Data are expressed as means  $\pm$ SE. For all participants, the lumbar spine and total hip BMD had significantly increased from the baseline values after 1 year (Table 3, one-way ANOVA with repeated measurements). The percentage increases in the lumbar spine and total hip BMD from the baseline values were 1.31% and 1.54%, respectively. However, there were no significant differences in the increases in the lumbar spine (left panel) and total hip BMD (right panel) between the two groups (Table 3, two-way ANOVA with repeated measurements).

**Table 3** One- and two-way ANOVA with repeated measurements (*P* values)

	One-way ANOVA All participants	Two-way ANOVA Exercise group vs. control group
Lumbar spine BMD	0.0012	N S
Total hip BMD	0.0043	N S
Serum BAP	0.0022	N S
Serum osteocalcin	<0.0001	N S
Serum TRAP	<0.0001	N S
Urinary NTX	N S	N S

One-way ANOVA with repeated measurements was used to examine the longitudinal changes in parameters in all participants.

In all participants, changes in the lumbar spine and total hip BMD, serum BAP, osteocalcin, and TRAP5b levels were statistically significant.

Two-way ANOVA with repeated measurements was used to examine differences in changes in parameters between the two groups. There were no significant differences in changes in any parameters between the two groups.

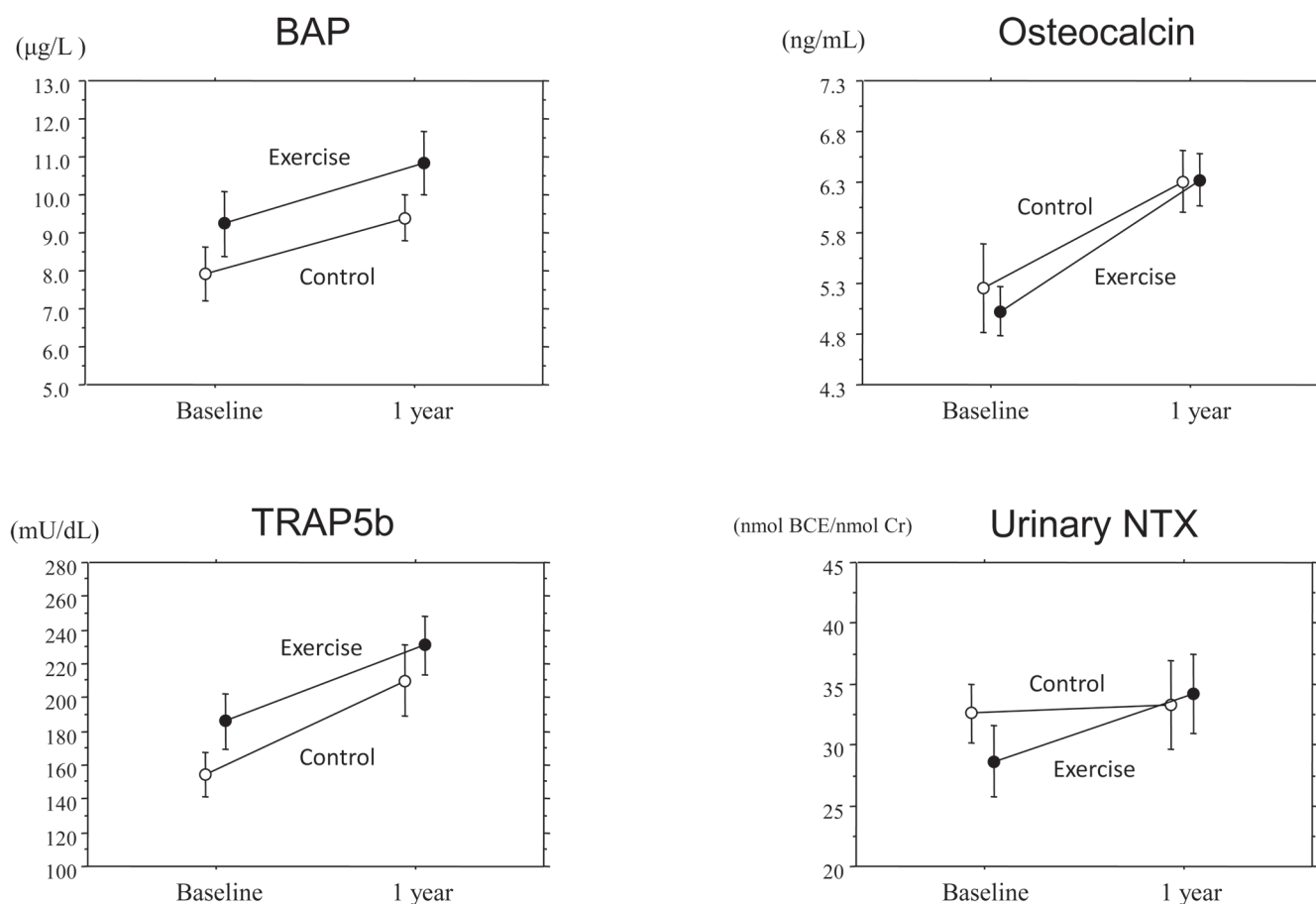
physical functions between the two groups (Table 2, two-way ANOVA with repeated measurements).

In all participants, there were no significant changes in the energy, protein, calcium, vitamin D, and vitamin K intakes from the baseline values after 1 year (Table 2, one-way ANOVA with repeated measurements). Also, there were no significant differences in the changes in any nutritional intakes between the two groups (Table 2, two-way ANOVA with repeated measurements).

### Discussion

Peak bone mass is reached by age 30 years in women<sup>3</sup> and bone mass remains stable until menopause, when the

loss of estrogen in conjunction with aging is associated with a decline in BMD.<sup>4</sup> A prospective study was conducted to clarify the 1-year changes in the lumbar spine and total hip BMD and bone turnover markers in premenopausal amateur runners (mean age: 37.9 years) and to determine whether jumping plus muscle-strengthening exercises would have positive effects on the bone parameters in these runners. For all participants, the average lumbar spine and total hip BMD modestly increased, together with modest increases in the average serum BAP, osteocalcin, and TRAP5b levels. However, the jumping plus muscle-strengthening exercises did not significantly influence the bone parameters, possibly because of poor compliance. These results suggest that long-distance run-



**Fig. 3** Changes in bone turnover markers.

Data are expressed as means  $\pm$ SE. In all participants, serum BAP, osteocalcin, and TRAP5b levels had significantly increased from the baseline values after 1 year (**Table 3**, one-way ANOVA with repeated measurements). The urinary NTX level increased from the baseline value, but this change was not statistically significant (**Table 3**, one-way ANOVA with repeated measurements). The increases in the serum BAP, osteocalcin, TRAP5b, and urinary NTX levels from the baseline values were 17.8%, 13.2%, 27.8%, and 11.7%, respectively. However, there were no significant differences in these increases between the two groups (**Table 3**, two-way ANOVA with repeated measurements). BAP, bone-specific alkaline phosphatase; TRAP5b, tartrate-resistant acid phosphatase 5b; NTX, cross-linked N-terminal telopeptides of type I collagen.

ning at the recreational level may be useful for maintaining bone health in premenopausal women.

Whether long-distance running at the recreational level enhances bone health in young adult women is uncertain. Female athletes, especially long-distance runners, have a risk of developing the female athlete triad, i.e., low energy availability, functional hypothalamic amenorrhea, and osteoporosis.<sup>17</sup> Low energy availability leads to a reduction in fat mass, resulting in a decrease in leptin secretion. Leptin is synthesized by adipocytes and regulates bone formation via the sympathetic nervous system.<sup>18</sup> Amenorrhea, low body mass index, low calcium intake (<1300 mg/day), and low energy intake (<2000 kcal/day) are all risk factors for increased bone resorption.<sup>19</sup> Young adult women with the female athlete triad may lose bone as a result of an increase in bone resorption because of

estrogen deficiency and a decrease in bone formation because of low leptin secretion, leading to the uncoupling of bone resorption and formation. The participants in the present study did not have any menstruation problems, but their body mass index (20.8 kg/m<sup>2</sup>) was on the low side (standard value for Japanese population: 22.0 kg/m<sup>2</sup>). Furthermore, the calcium and energy intakes were also considered to be low. However, although we did not evaluate the serum levels of leptin, the BMD was not low and bone turnover markers such as BAP, osteocalcin, TRAP5b, and urinary NTX were within the normal ranges at baseline. Thus, the bone health of the premenopausal amateur runners did not appear to be impaired at baseline.

The average lumbar spine and total hip BMD for all participants increased modestly (1.31% and 1.54%, re-

spectively) together with increases in the average bone turnover markers. Although the percentage increases in the serum BAP, osteocalcin, TRAP5b, and urinary NTX levels from the baseline values were 17.8%, 13.2%, 27.8%, and 11.7%, respectively, modest increases in the bone turnover markers were confirmed by consistent changes in the bone formation and resorption markers. Generally, sports and exercise contribute to acquisition or maintenance of BMD.<sup>5</sup> However, comparisons of the BMD among collegiate female athletes revealed that long-distance runners had a lower BMD than athletes performing weight-bearing activities.<sup>6</sup> In elite female endurance athletes, a significant correlation was observed between the number of hours of training per week and decreases in BMD.<sup>20</sup> The study participants in the present study were premenopausal amateur runners without any menstruation problems, and the mean annual running distance was 1103–1344 km (92–112 km per month), suggesting running activity at the recreational level. The running activities of our study participants were considered to be within the recommended levels for developing and maintaining health set by the American College of Sports Medicine.<sup>21</sup> Running activity at the recreational level appeared to be useful for enhancing bone health at clinically relevant skeletal sites without causing an elevation in the stress hormone cortisol.<sup>22</sup> However, the calcium and vitamin D nutritional status would likely need to be improved to obtain further BMD increases.

The type of sports activity is an important determinant of bone health: strength-based and high-impact sports are associated with a higher BMD.<sup>6</sup> In young women, high-impact exercises such as jumping increase the femoral neck BMD, and muscle-strengthening exercises increase the lumbar spine BMD.<sup>8</sup> In particular, brief high-impact exercises (<30 min) improve the BMD at the hip but not at the lumbar spine.<sup>7</sup> Thus, a jumping exercise was added with the aim of enhancing the increase in the hip BMD and back muscle-strengthening exercises were added with the aim of increasing the lumbar spine BMD in premenopausal amateur runners. However, no significant effect of jumping plus muscle-strengthening exercises on the BMD or bone turnover markers was obtained. This result may be mainly attributable to the poor compliance with the exercise protocol. According to our survey, the main reasons for the poor compliance were a lack of appropriate facilities where the runners could perform the jumping and muscle-strengthening exercises and busy schedules for daily activities or attending school. The participants should have been motivated by thinking about their bone health in later life in terms of the primary prevention of osteoporosis.

Sun exposure significantly influences vitamin D nutritional status, and vitamin D may improve athletic performance in athletes.<sup>23</sup> In all participants of the present study, outdoor long-distance running had been performed at baseline, which means they might have been exposed

to sun longer than age-matched non-running premenopausal women. However, they were considered to have vitamin D insufficiency according to the mean serum 25-OHD level at baseline. Changes in the serum 25-OHD levels did not differ significantly between the running plus exercise and the running only group. Vitamin D nutritional status did not appear to influence bone parameter changes between the two groups. Further analyses may be needed to determine the reasons for the non-significant effect of jumping plus muscle-strengthening exercises on bone parameters.

The strength of the present study was its prospective design for clarifying 1-year changes in the lumbar spine and total hip BMD and bone turnover markers in premenopausal amateur runners. The crucial limitations of the present study include the absence of a non-exercise control group, the small number of participants, the poor compliance with the jumping plus muscle-strengthening exercises, and the absence of calcium and vitamin D supplementation. Both the calcium intake (495 mg/day) and the serum 25-OHD level (21.3 ng/mL) were relatively low in our study subjects. Further studies are needed, particularly to examine whether jumping and/or muscle-strengthening exercises can enhance bone health in premenopausal amateur runners.

In conclusion, the present prospective study clarified the 1-year changes in the lumbar spine and total hip BMD and bone turnover markers in premenopausal amateur runners. The lumbar spine and total hip BMD modestly increased, together with modest increases in bone turnover markers, indicating mild effects of running activity on bone turnover and the BMD of clinically relevant skeletal sites. The results of the present study suggest that long-distance running at the recreational level is useful for maintaining bone health in premenopausal women.

### Acknowledgements

We would like to thank Dr. Hiroyuki Nakajima and the staff at the Yokohama Sports Medical Center (Kanagawa, Japan) for the measurement of BMD and biochemical marker testing, and Mr. Masahiko Yamamoto in the Unit of Sports and Health Science, Tokyo Polytechnic University (Kanagawa, Japan), for evaluation of the physical functions. This study was funded by the Nakatomi Foundation, the Bone and Joint Decade Japan, and the Keio University Doctoral Student Grant-in-Aid Program.

### References

1. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy: Osteoporosis prevention, diagnosis, and therapy. *JAMA* 2001; **285**: 785–795. [Medline] [CrossRef]
2. Cauley JA, Thompson DE, Ensrud KC, Scott JC, Black D: Risk of mortality following clinical fractures. *Osteoporos Int* 2000; **11**: 556–561. [Medline] [CrossRef]



3. National Institutes of Health Osteoporosis and Related Bone Diseases National Resource Center: Osteoporosis: peak bone mass in women. National Institutes of Health, Bethesda, MD, 2012.
4. Warming L, Hassager C, Christiansen C: Changes in bone mineral density with age in men and women: a longitudinal study. *Osteoporos Int* 2002; **13**: 105–112. [Medline] [CrossRef]
5. Iwamoto J, Sato Y, Takeda T, Matsumoto H: Role of sport and exercise in the maintenance of female bone health. *J Bone Miner Metab* 2009; **27**: 530–537. [Medline] [CrossRef]
6. Mudd LM, Fornetti W, Pivarnik JM: Bone mineral density in collegiate female athletes: comparisons among sports. *J Athl Train* 2007; **42**: 403–408. [Medline]
7. Babatunde OO, Forsyth JJ, Gidlow CJ: A meta-analysis of brief high-impact exercises for enhancing bone health in premenopausal women. *Osteoporos Int* 2012; **23**: 109–119. [Medline] [CrossRef]
8. Martyn-St James M, Carroll S: Effects of different impact exercise modalities on bone mineral density in premenopausal women: a meta-analysis. *J Bone Miner Metab* 2010; **28**: 251–267. [Medline] [CrossRef]
9. Nikander R, Kannus P, Rantalainen T, Uusi-Rasi K, Heinonen A, Sievänen H: Cross-sectional geometry of weight-bearing tibia in female athletes subjected to different exercise loadings. *Osteoporos Int* 2010; **21**: 1687–1694. [Medline] [CrossRef]
10. Wilks DC, Winwood K, Gilliver SF, Kwiet A, Chatfield M, Michaelis I, Sun LW, Ferretti JL, Sargeant AJ, Felsenberg D, Rittweger J: Bone mass and geometry of the tibia and the radius of master sprinters, middle and long distance runners, race-walkers and sedentary control participants: a pQCT study. *Bone* 2009; **45**: 91–97. [Medline] [CrossRef]
11. National Health and Nutrition Examination Survey: Dual Energy X-ray Absorptiometry (DXA) Procedures Manual. [http://www.cdc.gov/nchs/data/nhanes/nhanes\\_07\\_08/manual\\_dexa.pdf](http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_dexa.pdf) (accessed January 18, 2014).
12. Fuchs RK, Bauer JJ, Snow CM: Jumping improves hip and lumbar spine bone mass in prepubescent children: a randomized controlled trial. *J Bone Miner Res* 2001; **16**: 148–156. [Medline] [CrossRef]
13. Mitsubishi Kagaku Iyodo: INFORMATION 2009; Vol. 09–07 C04. <http://www.medience.co.jp/information/pdf/09-07.pdf> (accessed April 6, 2013).
14. Nishizawa Y, Ohta H, Miura M, Inaba M, Ichimura S, Shiraki M, Takada J, Chaki O, Hagino H, Fujiwara S, Fukunaga M, Miki T, Yoshimura N: Guidelines for the use of bone metabolic markers in the diagnosis and treatment of osteoporosis (2012 edition). *J Bone Miner Metab* 2013; **31**: 1–15. [Medline] [CrossRef]
15. Okazaki R, Sugimoto T, Kaji H, Fujii Y, Shiraki M, Inoue D, Endo I, Okano T, Hirota T, Kurahashi I, Matsumoto T: Vitamin D insufficiency defined by serum 25-hydroxyvitamin D and parathyroid hormone before and after oral vitamin D<sub>3</sub> load in Japanese subjects. *J Bone Miner Metab* 2011; **29**: 103–110. [Medline] [CrossRef]
16. Uenishi K, Ishida H, Nakamura K: Development of a simple food frequency questionnaire to estimate intakes of calcium and other nutrients for the prevention and management of osteoporosis. *J Nutr Sci Vitaminol (Tokyo)* 2008; **54**: 25–29. [Medline] [CrossRef]
17. Nattiv A, Loucks AB, Manore MM, Sanborn CF, Sundgot-Borgen J, Warren MP, American College of Sports Medicine: American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc* 2007; **39**: 1867–1882. [Medline]
18. Takeda S: Osteoporosis: a neuroskeletal disease? *Int J Biochem Cell Biol* 2009; **41**: 455–459. [Medline] [CrossRef]
19. Barrack MT, Van Loan MD, Rauh MJ, Nichols JF: Physiologic and behavioral indicators of energy deficiency in female adolescent runners with elevated bone turnover. *Am J Clin Nutr* 2010; **92**: 652–659. [Medline] [CrossRef]
20. Pollock N, Grogan C, Perry M, Pedlar C, Cooke K, Morrissey D, Dimitriou L: Bone-mineral density and other features of the female athlete triad in elite endurance runners: a longitudinal and cross-sectional observational study. *Int J Sport Nutr Exerc Metab* 2010; **20**: 418–426. [Medline]
21. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, Nieman DC, Swain DP, American College of Sports Medicine: American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc* 2011; **43**: 1334–1359. [Medline] [CrossRef]
22. Cooper MS, Syddall HE, Fall CH, Wood PJ, Stewart PM, Cooper C, Dennison EM: Circulating cortisone levels are associated with biochemical markers of bone formation and lumbar spine BMD: the Hertfordshire Cohort Study. *Clin Endocrinol (Oxf)* 2005; **62**: 692–697. [Medline] [CrossRef]
23. Cannell JJ, Hollis BW, Sorenson MB, Taft TN, Anderson JJ: Athletic performance and vitamin D. *Med Sci Sports Exerc* 2009; **41**: 1102–1110. [Medline] [CrossRef]