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Clinical Significance of 1-year Treatment with Raloxifene on Bone and Lipid Metabolism in Japanese Postmenopausal Women with Osteoporosis

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Abstract. It has been well established that raloxifene (RLX) has beneficial effects on bone primarily in Caucasian women. However, to date, there is a dearth of data for Japanese postmenopausal women. In this study, we prospectively evaluated the effects of RLX on bone and lipid metabolism in fifty Japanese postmenopausal patients with untreated osteoporosis. We measured bone mineral density (BMD) by dual-energy X-ray absorptiometry at 7 sites including the lumbar spine, femoral neck, and distal radius. BMD was significantly increased at the lumbar spine both at 6 months and at 12 months compared with at baseline ($p<0.01$ for both), although the possibility could not be completely excluded that this increase may be partly explained by an apparent increase induced by degenerative changes in lumbar vertebrae since we had no control subjects to compare and be more certain of the findings in this study. Both bone-specific alkaline phosphatase (BAP) and serum N-terminal telopeptide of type I collagen (NTx) significantly decreased both at 6 months ($p<0.01$ for both) and at 12 months ($p<0.01$ for both) compared with at baseline, but not below the lower limit of the reference value. Total cholesterol and low-density lipoprotein cholesterol were significantly improved while triglycerides and high-density lipoprotein cholesterol were unaltered. Although longer and larger studies with fracture endpoints are needed to draw definite conclusions, our findings suggest the favorable effects of RLX on bone and lipid metabolism in Japanese postmenopausal women with osteoporosis as in Caucasian women.

Key words: Raloxifene, Osteoporosis, Bone mineral density, Bone turnover, Lipid

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RALOXIFENE (RLX) is one of the most potential selective estrogen receptor modulators, designed to have differential effects on estrogen receptors in various tissues. A number of clinical trials [1–6] evaluating the effects of RLX on bone, including MORE trial [1], the largest double-blind placebo-controlled one, have clearly demonstrated that RLX improves accelerated bone turnover, increases bone mineral density (BMD) at the lumbar spine (LS) and at the femoral neck (FN), and reduces the risk of new vertebral fractures. In addition, favorable effects on lipid metabolism have also been repeatedly reported in the literature [2, 3].

However, to date, clinical trials for RLX for postmenopausal osteoporosis, including MORE trial, have enrolled primarily Caucasian women [1–4]. Although osteoporosis is an emerging public health problem in Japan as well, the effects of RLX for Japanese women have not been rigorously examined thus far, except in one clinical trial reported by Morii *et al.* [5]. Thus, there is a dearth of data for the effects of RLX in Japa-

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nese postmenopausal women with osteoporosis. In addition, Kung *et al*. [6] reported that changes in serum lipids when using RLX and safety profile of RLX were somewhat different in different ethnic backgrounds, suggesting the possibility of different effects of RLX in Japanese women from in Caucasian women.

Therefore, the purpose of our study is to prospectively evaluate the effects of RLX 60 mg/day on bone turnover, BMD at several sites, and lipid metabolism in Japanese postmenopausal women with osteoporosis.

Subjects and Methods

Subjects

Fifty Japanese postmenopausal women (mean age 72.22 ± 9.5 years, from 54 to 91 years) with untreated osteoporosis, with their last menstrual period at least 2 years before, and without clinically significant postmenopausal symptoms, including hot flushing, at the beginning of this study, who initially attended the clinic of Rakuwakai Otowa Hospital between April 2004 and October 2005, were selected for this study. The diagnosis of osteoporosis was established on the basis of T-score of bone mineral density (BMD) at the LS (L2–L4) at least 2.5 SDs below the young adult mean, and along with the criteria for the diagnosis of osteoporosis in Japan. All patients were begun on treatment with RLX (60 mg/day). During the course of this study, the dose of RLX remained unchanged. This study involved a 12-month (at baseline, and 6 months and 12 months after the initial treatment) longitudinal examination of these 50 patients.

All subjects completed a questionnaire administered by the doctor or the nurse prior to entry into the study, and underwent laboratory blood and urinary tests. We excluded subjects who had a history of deep venous thrombosis or other diseases (type 1 diabetes mellitus, liver disease, renal dysfunction, malignancy, hyperthyroidism, hyperparathyroidism, hypercorticoidism, or hypogonadism), those who could not walk well by themselves, and those taking medications that could influence bone metabolism, such as bisphosphonates, calcitonin, estrogens, testosterones, steroids, thyroid hormones, diuretics, heparin or anticonvulsants except for active Vitamin D_3. If, at study entry, it had been administered for more than 3 years, alfacalcidol (ALF), 1,25-dihydroxyvitamin D analog (1.0 μg/day), was allowed, provided that the dose was not changed during this study. All the subjects underwent plain x-ray (antero-posterior and lateral views) of the LS, and those found to have scoliosis, compression fractures of all the lumbar vertebrae among L2–L4, or ectopic calcifications that could interfere with the bone mineral results were excluded. We also excluded those with triglycerides (TG) level >500 mg/dl, because their low-density cholesterol (LDL) cannot be calculated adequately by using the Friedewald equation. None of the subjects were smokers or drug abusers.

This study was performed in accordance with the recommendations of the Declaration of Helsinki and approved by the Ethical Committee of Rakuwakai Otowa Hospital, and all participants provided informed consent.

BMD measurements

BMD was measured at 7 sites, including LS (L2–L4), FN, trochanter, total neck, Ward's triangle, ultra distal radius, distal 1/3 radius, by means of dual energy X-ray absorptiometry (DXA) (Hologic QDR 4500c; Hologic Inc., Waltham, MA, USA) at baseline, and 6 months and 12 months after the initial treatment. To eliminate technical discrepancies, the same operator measured all the subjects. The reproducibility was calculated as the coefficient of variation obtained by daily measurements of a standard phantom over a period of 2 years. The CV of our instrument is 0.43% with the standard phantom. Values of BMD at the LS were expressed as the mean of those at the L2–L4. T-scores and Z-scores were calculated on the basis of the normal reference values of the age- and gender-matched Japanese group provided by the DXA system manufacturer.

Biochemical measurements

All subjects underwent laboratory blood tests at baseline, at 6 months, and at 12 months s. Serum samples were obtained before 8:00 AM after an overnight fast, and were immediately processed and kept frozen at –20°C until the assays were carried out. Serum total cholesterol (TC), TG, high-density cholesterol (HDL), calcium (Ca), phosphate (P), and alkaline phosphatase (ALP) were measured with standard laboratory methods. LDL was calculated by the Friedewald equation (LDL = TC – [HDL + TG/5]). Serum bone-specific alkaline phosphatase (BAP) was measured with an

enzyme immunoassay kit (Osteolinks-BAP; Sumitomo Pharmaceuticals Inc., Tokyo, Japan; reference range: 9.6–35.4 U/L) as a marker of bone formation. Serum N-terminal telopeptide of type I collagen (NTx) was measured by means of an enzyme-linked immunosorbent assay (Osteomark; Mochida Pharmaceutical Co., Tokyo, Japan; reference range: 7.5–16.5 nmolBCE/L for premenopausal women, and 10.7–24.0 nmolBCE/L for postmenopausal women) as a marker of bone resorption.

Statistical analysis

Data were analyzed by paired t-test for longitudinal differences between at baseline and at 6 months, and between at baseline and at 12 months, and by Pearson's correlation test for determining correlations. Statistics were calculated with StatView version 5.0 (Abacus Concepts, Inc., Berkeley, CA, USA). A P value <0.05 was considered statistically significant.

Results

Of the 50 patients enrolled, 16 discontinued this study. Among them, one patient reported increased blood pressure, one reported muscle pain for the entire body, and two reported leg cramps. All of these adverse events were resolved spontaneously with cessation of RLX. Other three patients discontinued due to change of address. The other nine patients discontinued because they could not adhere to the protocol of this study during the course. No case of venous thromboembolic event, clinical bone fracture, or other serious treatment-emergent events was reported. In the result, 34 patients (mean age 71.47 ± 9.1 years) completed this study. Among them, 21 patients were treated with RLX alone, while the other 13 patients with RLX in addition to ALF. Table 1 shows their baseline and longitudinal characteristics.

BMD was significantly increased at the LS both at 6 months and at 12 months compared with at baseline ($p = 0.018$, and $p<0.001$, respectively). On the contrary, BMD was not increased at the any other sites measured, either at 6 months or at 12 months. Percentage change of BMD compared with the baseline was also significant only at the LS both at 6 months (2.77 ± 7.2%, $p = 0.020$) and at 12 months (5.44 ± 7.1%, $p<0.001$), but not at the any other sites (Fig. 1).

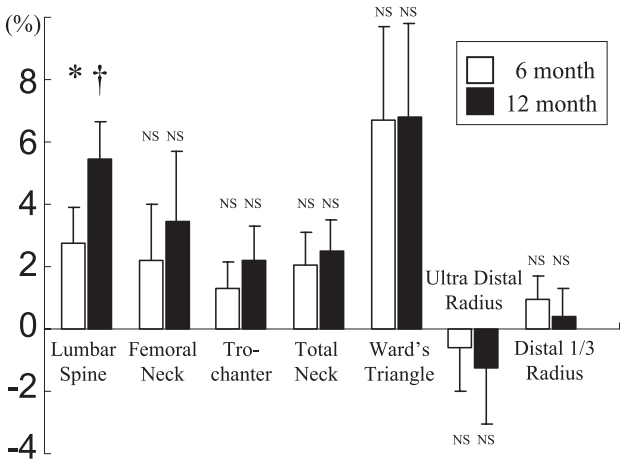

Fig. 1. Percentage change in BMD compared with initial values at the lumbar spine, at the femoral neck, at the trochanter, at the total neck, at the Ward's triangle, at the ultra distal radius, and at the distal 1/3 radius, evaluated after 6 months (white columns) and 12 months (black columns) of treatment. Each column represents mean ± SEM. NS, $P \geq 0.05$; †, $P<0.01$ *vs*. baseline.

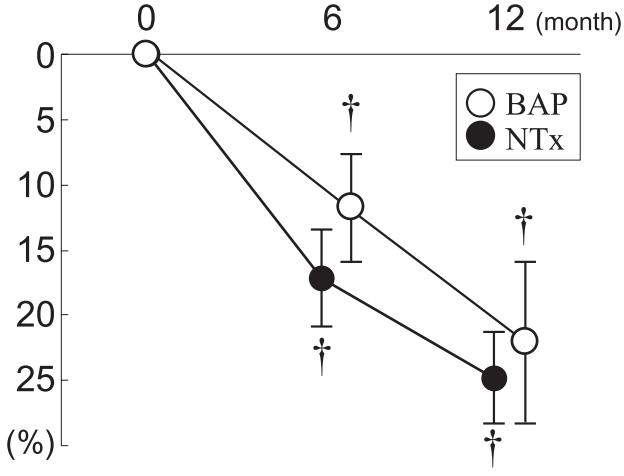

Fig. 2. Percentage change of BAP (white circles) and NTx (black circles), compared with baseline values, evaluated after 6 and 12 months of treatment. Data are shown as mean ± SEM. †, $P<0.01$ *vs*. baseline.

On the other hand, both BAP and NTx were significantly reduced by –11.73 ± 26.2% ($p = 0.008$) and –17.20 ± 25.1% ($p<0.001$), respectively, at 6 months, and by –21.91 ± 32.8% ($p<0.001$), and –24.79 ± 19.4% ($p<0.001$), respectively, at 12 months, compared with those at baseline (Fig. 2). In all of the patients, both BAP and NTx remained above the lower limit of their reference values in Japanese premenopausal women (9.6 U/L, and 7.5 nmolBCE/L, respectively) during the

Table 1. Means ± SD of the variables assessed in examined subjects

	Postmenopausal women with osteoporosis		
	Baseline (n = 50)	6 months (n = 39)	12 months (n = 34)
Age (years)	72.22 ± 9.5	72.18 ± 9.4	71.47 ± 9.1
Height (cm)	150.66 ± 6.2	150.55 ± 6.3	150.58 ± 6.2
Weight (kg)	49.28 ± 7.4	49.17 ± 7.3	49.20 ± 7.0
BMI (kg/m^2)	21.69 ± 2.9	21.70 ± 3.0	21.70 ± 2.9
Ca (mg/dL)	9.62 ± 0.5	9.41 ± 0.4†	9.48 ± 0.5*
P (mg/dL)	3.76 ± 0.6	3.60 ± 0.5*	3.53 ± 0.5*
TC (mg/dL)	204.44 ± 32.4	198.08 ± 33.8*	191.94 ± 30.9†
TG (mg/dL)	122.90 ± 65.6	132.97 ± 60.2^{NS}	122.44 ± 62.9^{NS}
HDL (mg/dL)	55.14 ± 13.1	55.26 ± 13.2^{NS}	53.97 ± 11.0^{NS}
LDL (mg/dL)	124.72 ± 33.0	116.23 ± 27.7*	113.48 ± 26.8*
BAP (U/L)	33.06 ± 16.2	27.70 ± 11.6†	23.55 ± 9.4†
NTx (nmolBCE/L)	19.29 ± 6.1	15.52 ± 4.1†	14.22 ± 2.9†
Lumbar spine			
BMD (g/cm^2)	0.674 ± 0.138	0.704 ± 0.140*	0.724 ± 0.129†
T score (SD)	–3.143 ± 1.317	–2.854 ± 1.330*	–2.663 ± 1.231†
Z score (SD)	–0.517 ± 0.947	–0.316 ± 0.943*	–0.201 ± 0.847†
Femoral neck			
BMD (g/cm^2)	0.544 ± 0.104	0.553 ± 0.097^{NS}	0.556 ± 0.100^{NS}
T score (SD)	–2.232 ± 0.953	–2.145 ± 0.894^{NS}	–2.124 ± 0.918^{NS}
Z score (SD)	–0.424 ± 1.091	–0.330 ± 0.996^{NS}	–0.338 ± 0.925^{NS}
Trochanter			
BMD (g/cm^2)	0.436 ± 0.091	0.446 ± 0.087^{NS}	0.446 ± 0.091^{NS}
T score (SD)	–4.012 ± 1.633	–3.844 ± 1.561^{NS}	–3.848 ± 1.629^{NS}
Z score (SD)	–0.028 ± 1.435	0.099 ± 1.412^{NS}	0.107 ± 1.257^{NS}
Total neck			
BMD (g/cm^2)	0.597 ± 0.122	0.611 ± 0.118^{NS}	0.613 ± 0.127^{NS}
T score (SD)	–2.418 ± 1.107	–2.287 ± 1.069^{NS}	–2.272 ± 1.156^{NS}
Z score (SD)	–0.385 ± 1.095	–0.268 ± 1.051^{NS}	–0.285 ± 1.050^{NS}
Ward's triangle			
BMD (g/cm^2)	0.315 ± 0.108	0.338 ± 0.096^{NS}	0.339 ± 0.096^{NS}
T score (SD)	–3.244 ± 0.823	–3.072 ± 0.730^{NS}	–3.061 ± 0.735^{NS}
Z score (SD)	–0.638 ± 1.788	–0.327 ± 1.499^{NS}	–0.417 ± 1.433^{NS}
Ultra distal radius			
BMD (g/cm^2)	0.286 ± 0.075	0.287 ± 0.068^{NS}	0.294 ± 0.060^{NS}
T score (SD)	–3.223 ± 1.629	–3.189 ± 1.477^{NS}	–3.042 ± 1.314^{NS}
Z score (SD)	–0.182 ± 1.474	–0.151 ± 1.367^{NS}	–0.072 ± 1.189^{NS}
Distal 1/3 radius			
BMD (g/cm^2)	0.451 ± 0.090	0.456 ± 0.085^{NS}	0.454 ± 0.078^{NS}
T score (SD)	–2.874 ± 1.424	–2.796 ± 1.342^{NS}	–2.826 ± 1.240^{NS}
Z score (SD)	–0.516 ± 1.622	–0.426 ± 1.577^{NS}	–0.512 ± 1.344^{NS}

Data represent mean ± SD.

BMI, body mass index; ALP, alkaline phosphatase; Ca, calcium; P, phosphate; TC, total cholesterol; TG, triglycerides; HDL, high density cholesterol; LDL, low density cholesterol; BAP, bone type alkaline phosphatase; NTx, N-terminal telopeptide of type I collagen

P-values for comparisons for the parameters vs. baseline: ^{NS} $P>0.05$; * $P<0.05$; † $P<0.01$.

course of this study (Fig. 3).

Ca and P were also significantly reduced at 6 months ($p<0.001$, $p = 0.047$, respectively), and at 12 months ($p = 0.015$, 0.030, respectively), compared with those at baseline, which did not result in clinically relevant hypocalcemia or hypophosphatemia.

TC and LDL also significantly reduced at 6 months ($p = 0.017$, 0.034, respectively), and at 12 months

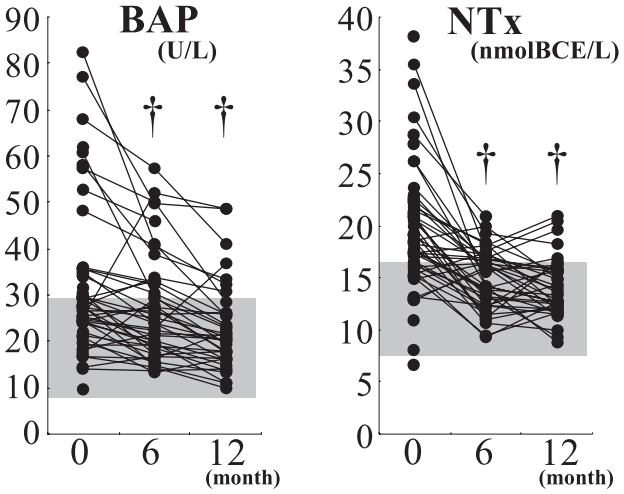

Fig. 3. Longitudinal changes of bone turnover markers in this 1-year prospective study. BAP and NTx levels at baseline, at 6 months, and at 12 months in our patients with osteoporosis are plotted. Shaded areas represent reference range of Japanese premenopausal women. *P*-values for comparison of the parameters *vs.* baseline. †, $P<0.01$ *vs*. baseline.

($p = 0.001$, 0.040, respectively), compared with those at baseline. On the other hand, neither TG nor HDL changed significantly during the course.

Discussion

In this one-year prospective study examining the effects of RLX on Japanese postmenopausal women with osteoporosis, we found a significant increase of BMD at the LS both at 6 months and at 12 months, suggesting that its therapeutic effects on BMD can be seen as early as at 6 months, as has been reported in the previous studies examining mainly Caucasian women [1–4]. Furthermore, because our patients were somewhat older than those in the previous studies [1–6], our results also suggest that RLX could be efficacious regardless of age. Indeed, younger age used to be considered to increase the efficacy of RLX based on the results of univariate analyses of the MORE study [7], but from the multivariate model in the same population, younger age was no longer a significant factor in increasing its efficacy [8], which is consistent with our findings.

However, unexpectedly, the increase rate of BMD of LS was somewhat higher than that observed in the previous studies [1–6]. This apparent difference may be partly due to the variability in BMD values resulting from the small sample size in our study, or because about one third of our patients were treated with combined use of RLX and ALF, suggesting that RLX in combination with ALF may be potentially more beneficial for bone health than RLX alone. Actually, several clinical studies [9, 10] previously showed that ALF reduced bone turnover and prevented vertebral fractures although its effectiveness may be relatively low, and animal-model studies also showed that ALF inhibited bone resorption [11, 12] and stimulated formation [11] to increase BMD. Moreover, although native vitamin D plus calcium, but not activated vitamin D, was supplemented in both patients and controls of the previous clinical studies for RLX [1–6], Nuti *et al*. [13] recently reported superiority of ALF compared to native vitamin D plus calcium in increasing lumbar BMD. Additionally, recently published meta-analyses also showed the advantageous efficacy of ALF versus native vitamin D in preventing bone loss and subsequent fractures [14]. In general, it is tempting to consider the possibility that combination therapies might provide benefits over what could be expected from either one alone. However, although there seems to be possible benefits for BMD or bone turnover in some combination therapies [15, 16] including those of bisphosphonate plus ALF [15] and estrogen plus calcitriol [16], the efficacy of the combination therapy of RLX and ALF has not been reported yet. In addition, about the efficacy of preventing the fracture, the data are not established in any combination therapies [16]. Further studies are thus needed to clarify whether the combination therapy of RLX and ALF are clinically more effective than that with RLX alone.

On the other hand, although significant increase of BMD at the non-vertebral sites has been repeatedly reported in the previous studies for RLX [1, 5, 6], RLX in our study did not significantly increase BMD at the non-vertebral sites including femur and radius, either at 6 months or at 12 months. However, BMD at those sites showed a tendency to increase to some extent, although not statistically significant, suggesting that the tendency might reach significance in longer observation or larger number of subjects. Additionally, there is increasing evidence that the effects of RLX to reduce the risk of fractures hardly depend on the increase of BMD [17–19]. Therefore, we do not think that our findings of no increase of BMD at the non-vertebral sites rule out the potential effects of RLX to reduce the risk of non-vertebral fractures. Actually, its effects on

prevention of hip fractures in those patients, mostly Caucasian, with high risk of fractures, have already been demonstrated in MORE study [20], although not in all of the patients. A large-scale double-blinded prospective randomized study to evaluate its effects on prevention of fractures, vertebral or non-vertebral, is warranted for Japanese postmenopausal women.

Recent data from RLX trials have demonstrated that RLX reduces the relative risk of incident vertebral fractures to a level comparable to that observed with bisphosphonates [1, 17–19], although RLX induces smaller increase in BMD compared with bisphosphonates [16]. Therefore, RLX has been considered to exert its beneficial effects on bone not by increasing BMD as mentioned above, but rather mainly by improving 'bone quality', [17, 18, 22] indicating bone turnover markers as one of the best ways to estimate the therapeutic effects of RLX on bone at a clinical level [18, 22, 23]. In our study, both BAP and NTx significantly decreased, and the decrease of NTx was earlier and greater than that of BAP as reported in the previous studies [5]. Moreover, interestingly, in none of our patients, either BAP or NTx reduced below the lower limit of the reference value in Japanese premenopausal women, suggesting that RLX could restore accelerated bone turnover to levels that closely resembled those of healthy premenopausal women. Consistent with this finding of ours, recently published histomorphometry data [24] described that RLX maintained healthy bone structure without findings of woven bone resulting from abnormalities in collagen structure, or osteomalacia, which could occur due to an impairment of primary mineralization. On the other hand, in using bisphosphonates, although there is no hard evidence thus far except for animal data [25], there is a matter of concern about excessive reduction of bone turnover to result in bone brittleness, microdamage accumulation, and an increase in the homogeneity of the tissue matrix, which may lead to a reduction of the biomechanical competence of bone to increase the risk of subsequent fractures [26]. Our findings, therefore, support the possibility that RLX may be more beneficial than bisphosphonates in improving bone quality and potentially in increasing bone strength and subsequent fracture protection.

RLX also decreased Ca and P significantly in the present study as reported in the previous studies [5], although some controversies exist [27]. However, the pathomechanism of the decrease in Ca and P is unclear from our study, although it is probably due to the skeletal antiresorptive effects of RLX [5].

As has also repeatedly been reported in the literature [2, 3], RLX significantly improved lipid profile in our study as well, although the possibility could not be excluded that dietary and exercise advice, given when lipid profiles of the patients were abnormally high, exerted positive effects on the lipid profiles in our study. On the other hand, the potential relationship between hyperlipidemia and osteoporosis has often been referred to by some investigators [28, 29]. Epidemiological or observational clinical studies have repeatedly reported that osteoporosis coexists with arteriosclerotic vascular diseases [30], and lipids have been suggested as a common underlying pathogenetic factor affecting both of these two diseases [28, 29]. Indeed, whether lipids affect bone metabolism or not remains unclear thus far. However, Koshiyama *et al.* [29] has recently reported the possibility that hypercholesterolemia may be the main cause of abnormal bone metabolism in type 2 diabetes mellitus [31]. In addition, both *in vitro* [32] and *in vivo* animal model studies [33] have also demonstrated some detrimental effects of lipids on bone metabolism. These findings suggest that abnormal lipid metabolism itself could have an adverse impact on bone. Therefore, it is likely that RLX could have indirect but additive beneficial effects on bone health by improving lipid metabolism and possibly even subsequent arteriosclerotic diseases.

In conclusion, our results demonstrated the favorable effects of RLX on bone and lipid metabolism also in Japanese postmenopausal women with osteoporosis as previously reported mainly in Caucasian women. For the future, a large-scale double-blinded prospective randomized study for Japanese postmenopausal women is warranted to evaluate its effects on prevention of fractures as reported in Caucasian women.

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