Effect of Risedronate on the Cortical and Cancellous Bone Mass and Mechanical Properties in Ovariectomized Rats: A Comparison with the Effects of Alfacalcidol

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Summary The purpose of the present study was to compare the effects of risedronate (RIS) and alfacalcidol (ALF) on the cortical and cancellous bone mass and mechanical properties in ovariectomized rats in a head-to-head fashion. Forty female Sprague-Dawley rats, 7 mo of age, were randomized into six groups: the sham-operated control (Sham) group, and five ovariectomized groups: treated with vehicle, RIS (0.1, 1.0, or 2.5 mg/kg, p.o., daily), and ALF (0.5 μg/kg, p.o., daily). At the end of the 8-wk experimental period, bone histomorphometric analyses of the cancellous bone of the proximal tibial metaphysis and cortical bone of the tibial diaphysis was performed, and the mechanical properties of the bone were evaluated at the femoral distal metaphysis (FDM) and femoral diaphysis (FD). RIS prevented the decrease in the cancellous bone volume/total tissue volume (BV/TV) noted in ovariectomized rats in a dose-dependent manner, by suppressing increases in cancellous bone formation and resorption, without any apparent effect on the Ct Ar or maximum load of the FDM or FD. On the other hand, ALF increased the cancellous BV/TV, Ct Ar, and maximum load of the FDM or FD, by mildly decreasing cancellous bone formation and resorption, increasing periosteal and endocortical bone formation, and preventing an increase in endocortical bone resorption. Thus, the present study clearly showed that RIS and ALF had differential effects on the cortical and cancellous bone mass and mechanical properties in ovariectomized rats.

Key Words ovariectomy, osteopenia, risedronate, alfacalcidol, rat

Osteoporosis primarily affects postmenopausal women, because estrogen deficiency after menopause increases bone turnover and induces marked bone loss. Bisphosphonates and active vitamin D are widely used for the treatment of osteoporosis in Japan. Bisphosphonates such as alendronate and risedronate (RIS) inhibit osteoclast-mediated bone resorption, and the loss of osteoclast function and apoptosis of these cells are probably consequences of the loss of functions of one or more of the important signaling proteins (1). These bisphosphonates are not metabolized but can inhibit enzymes of the mevalonate pathway, thereby preventing the biosynthesis of isoprenoid compounds that are essential for the post-translational modification of small GTPases (2). Alendronate and RIS have been reported to increase the lumbar and femoral neck bone mineral density (BMD) and reduce the incidence of vertebral and nonvertebral fractures in postmenopausal women with osteoporosis (3–7). On the other hand, active vitamin D such as calcitriol and alfacalcidol (ALF) has been reported to maintain the lumbar and metacarpal BMD and reduce the incidence of vertebral fractures in patients with postmenopausal osteoporosis (8–11). It appears that RIS may be superior to ALF in increasing the BMD and decreasing the incidence of fractures in postmenopausal women with osteoporosis.

Several preclinical studies have reported the efficacy of RIS and ALF against osteopenia using a rat model of postmenopausal osteoporosis. RIS suppresses bone resorption and prevents cancellous bone loss in ovariectomized rats (12, 13). On the other hand, ALF suppresses bone resorption and yet maintains or even stimulates bone formation at both cancellous and cortical bone sites, thereby increasing the BMD and improving the mechanical properties of the bone (14). Although the efficacy of RIS or ALF for the cortical and cancellous bone mass and/or the mechanical properties has been reported in ovariectomized rats, the head-to-head comparison of the effects of the two drugs on both the bone mass and mechanical properties has not been reported. The purpose of the present study was to compare the effects of RIS and ALF on the cortical and cancellous bone mass and mechanical properties in ovariecto-
mized rats in a head-to-head fashion.

**MATERIALS AND METHODS**

*Treatment of animals.* Forty female Sprague-Dawley rats, 7 mo of age, were purchased from Charles River Japan (Kanagawa, Japan). They were fed a pelleted standard chow diet containing 1.25% calcium and 0.9% phosphorus (CRF-1: Oriental Yeast, Co., Ltd., Tokyo, Japan). The animals were housed under local vivarium conditions (temperature 23.3˚C, humidity 55%, and 12 h on/off light cycle), with free access to water. After allowing 1 wk for adaptation to the new environment, the rats were randomized by the stratified weight allowing 1 wk for adaptation to the new environment, and the animal experiment protocols were maintained according to the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals, and the animal experiment protocols were approved by the Laboratory Animal Care Committee of Hamri Co., Ltd.

*Preparation of specimens.* All the rats were labeled with 25 mg/kg tetracycline (Sigma Chemical, St. Louis, MO, USA) injected intramuscularly and 8 mg/kg calcein (Sigma Chemical) injected subcutaneously at 9 and 3 d, respectively, before death. Urine samples were collected over a 24 h period using metabolic cages before death, and the specimens were stored at −20˚C. At 8 wk after the start of the experiment, the animals were sacrificed by exsanguinations after being anesthetized by intraperitoneal injection of 25–30 mg/kg pentobarbital sodium. Serum specimens were collected from all the rats, and the right femur and right tibia were isolated.

The serum samples were stored at −20˚C. The urine and serum samples were used for the measurements of the biochemical markers as described below. The femurs were stored at −20˚C and then used for volumetric BMD measurement and biomechanical testing as described below. The tibiae were processed for bone histomorphometric analyses. The bones were fixed in cold 40% ethanol overnight, and then cut into three parts using an Isomet saw (Buehler, Lake Bluff, IL, USA). The proximal tibial metaphysis and tibial diaphysis with the fibular junction were cut at 40-μm thickness using a diamond wire Histo-Saw machine (Delaware Diamond Knives, Wilmington, DE, USA), and the thickness of each cross-sectional specimen was determined with an Inspectors' Dial Bench Gauge (L.S. Starrett, Athol, MA, USA). Frontal sections of the proximal tibial metaphysis were cut at 8-μm or 4-μm thickness using a microtome (Leica RM2155; Leica Inc., Nussloch, Germany). The 8-μm sections were then transferred onto chromalum-gelatin-coated slides and dried overnight under a press at 42˚C. All the sections were coverslipped with Eukitt (Calibrated Instruments, Hawthorne, NY, USA) for the static and dynamic histomorphometric analyses. For tartrate-resistant acid phosphatase (TRAP) histochemistry, the 8-μm sections of the proximal tibial metaphysis were deplasticized with three changes of 2-methoxyethylacetate for 30 min each, two changes of acetonitrile for 5 min each, and sequential changes of ethanol (95, 70, and 40%), then two changes of deionized water for 5 min each for rehydration. The deplasticized and rehydrated sections (8-μm thickness) were placed in 0.1 M acetic acid buffer at pH 5.0 for 5 min, and the TRAP reaction was subsequently performed using a leukocyte acid phosphatase kit (Sigma Chemical). Counterstaining of sections stained for TRAP was performed using Mayer's hematoxylin (1 min) and the sections were air-dried and mounted with a plastic UV mounting medium (Polysciences). For Goldner Trichrom staining to count osteoblast surface, the adjacent 4-μm sections of the proximal tibial metaphysis were deplasticized and rehydrated, then followed by the procedure of Goldner Trichrom stain and mounted with Eukitt (Calibrated Instruments).

*Urine and serum biochemical analyses.* Urinary deoxyppyridinoline (DPD) levels were measured by enzyme-immunoassay (EIA) using a Pyrilinks-D kit (Metra Biosystems Inc., CA, USA). Serum calcium and phosphorus levels were measured by an automated instrument.
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...IRMA kit (Immutopics, Inc., CA, USA). Serum osteocalcin (OC) levels were measured by immunoradiometric assay (IRMA) using a Rat Osteocalcin IRMA kit (Immutopics, Inc., CA, USA).

**Peripheral quantitative tomographic analysis.** The distal metaphysis and diaphysis of the femur were scanned by peripheral quantitative tomography (pQCT; Noldand/Stratec XCT Research SA; Stratec Medizintechnik GmbH, Pforzheim, Germany) in 50% ethanol/saline. The bone areas were placed horizontally inside a glass tube and scanned at a voxel size of 0.12 mm. The scan line was adjusted using the scout view of the pQCT system. Skeletal sites 3 mm and 14 mm proximal to the distal growth plate were scanned. For analysis, a threshold of 395 mg/cm³ at contour mode 2 was used to separate the bone areas from the marrow regions. To separate the cortical areas from the trabecular areas, we used a constant threshold of 690 mg/cm³. Volumetric BMD (mg/cm³) was calculated.

**Biomechanical testing.** Just after pQCT analysis of the femur, the mechanical properties of the diaphysis of this bone were evaluated by the three-point bending test. Load was applied midway between two supports placed 15 mm apart. The femur was positioned so that the loading point was at the center of the femoral diaphysis and bending occurred about the medial-lateral axis. The specimens were tested in a saline bath at 37°C. Each specimen was submerged in the saline bath for about 3 min before the testing, to allow temperature equilibration. Load-displacement curves were recorded at a crosshead speed of 20 mm/min using a materials-testing machine (MZ500D; Maruto, Co., Ltd., Tokyo, Japan). The parameters analyzed were the maximum load, stiffness, and breaking energy.

Just after the three-point bending test of the right femoral diaphysis, the distal metaphysis was isolated over a length of 10 mm from the joint surface of the femoral condyle. The mechanical properties of this segment were then measured by the compression test. Compressive load was applied by the rectangular parallelepiped crosshead (length 2 cm, width 2 cm, and height 1 cm) on the specimen from the lateral aspect to the medial aspect. The specimens were tested in a saline bath at 37°C. Each specimen was submerged in the saline bath for about 3 min before the testing, to allow for temperature equilibration. Load-displacement curves were recorded at a crosshead speed of 10 mm/min and compression depth of 2.5 mm, using a materials-testing machine (MZ500D; Maruto, Co., Ltd.). The parameters analyzed were the maximum load, stiffness, and breaking energy.

**Bone histomorphometry of the tibia.** A digitizing morphometry system was used to measure the bone histomorphometric parameters. The system consisted of an epifluorescence microscope (Nikon E-400, Osteometrics, Atlanta, GA, USA), an Osteomeasure High Resolution Color Subsystem (Osteometrics), and a digitizing pad (Numonics 2206; Numonics Corp., Montomerville, PA, USA) coupled to an IBM computer and a morphometry program (Osteometrics). The measured parameters for cancellous bone included the total tissue volume (TV), bone volume (BV), bone surface (BS), eroded surface (ES), single- and double-labeled surfaces (sLS and dLS, respectively), and osteoblast surface (ObS). These data were used to calculate the percent cancellous bone volume (BV/TV), trabecular number (Tb N), trabecular thickness (Tb Th), trabecular separation (Tb Sp), ES/BS, MS/BS [(sLS/2+dLS)/BS], mineral apposition rate (MAR), bone formation rate (BFR/BS, BFR/BV, and Obs/BS, in accordance with the standard nomenclature described by Parfitt et al. (17). In the present study, the region of cancellous bone measured was 1–4 mm distal to the lower margin of the growth plate in the proximal tibia, which consists of secondary spongiosa. Cells showing positive staining for TRAP were counted in the region from the distal end of the growth plate to 0.2 mm from the growth plate, and the number of osteoclasts (N.Oc) and the osteoclast surface (OcS) per BS were calculated. The measured parameters for cortical bone were the total tissue area (Tt Ar), cortical bone area (Ct Ar), endocortical ES, periosteal and endocortical BS, sLS, dLS, and the interlabel width. These data were used to calculate the marrow area (Ma Ar), endocortical ES/BS, and periosteal and endocortical MS/BS [(sLS/2+dLS)/BS], MAR, and BFR/BS.

**Statistical analysis.** All the data were expressed as means and standard deviation (SD). Multiple comparisons of data among the groups were performed by analysis of variance (ANOVA) with Fisher’s protected least significant difference (PLSD) test. All statistical analyses were performed using the Stat View J-5.0 program on a Macintosh computer. A significance level of \( p < 0.05 \) was used for all the comparisons.

**RESULTS**

**Body weight and biochemical markers (Table 1 and Fig. 1)**

The initial body weight did not differ significantly among the six groups. OVX was associated with an increase in the body weight of the animals.

OVX increased the serum OC and urinary DPD levels, and decreased the serum calcium levels. RIS (1.0 and 2.5 mg/kg) prevented the elevation of both the serum OC and urinary DPD levels similarly, but RIS (0.1 mg/kg) did not affect the urinary DPD levels. On the other hand, ALF enhanced the elevation of both the markers, attenuated the decrease of the serum calcium levels, and increased the serum phosphorus levels.

**pQCT analysis of the femur (Fig. 2)**

OVX decreased the cancellous BMD of the femoral distal metaphysis, but had no effect on the BMD of the femoral diaphysis. RIS (0.1 and 1.0 mg/kg) attenuated and RIS (2.5 mg/kg) prevented the decrease of the cancellous BMD of the femoral metaphysis. RIS (at any dose) did not affect the BMD of the femoral diaphysis. ALF prevented the decrease in the cancellous BMD of the femoral metaphysis, but had no effect on the BMD of the femoral diaphysis.

**Bone histomorphometric analysis of the cancellous bone of the proximal tibial metaphysis (Fig. 3 and Table 2)**

OVX decreased the cancellous BV/TV and Tb N and
Table 1. Body weight and serum calcium and phosphorus.

<table>
<thead>
<tr>
<th></th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Calcium (mg/dL)</th>
<th>Phosphorus (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>364±33</td>
<td>341±22</td>
<td>10.5±0.7</td>
<td>5.5±0.9</td>
</tr>
<tr>
<td>O VX Vehicle</td>
<td>364±29</td>
<td>404±26\textsuperscript{a}</td>
<td>9.6±0.5\textsuperscript{a}</td>
<td>5.7±0.5</td>
</tr>
<tr>
<td>RIS (0.1 mg/kg)</td>
<td>367±27</td>
<td>400±35\textsuperscript{a}</td>
<td>9.7±0.4\textsuperscript{a}</td>
<td>5.5±0.9</td>
</tr>
<tr>
<td>RIS (1.0 mg/kg)</td>
<td>362±23</td>
<td>372±38</td>
<td>9.6±0.3\textsuperscript{a}</td>
<td>5.8±0.8</td>
</tr>
<tr>
<td>RIS (2.5 mg/kg)</td>
<td>378±54</td>
<td>408±26\textsuperscript{d}</td>
<td>9.6±0.3\textsuperscript{a}</td>
<td>5.6±0.4</td>
</tr>
<tr>
<td>ALF (0.5 μg/kg)</td>
<td>369±29</td>
<td>378±23\textsuperscript{a}</td>
<td>10.0±0.2\textsuperscript{d}</td>
<td>7.0±0.5\textsuperscript{e}</td>
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Data are expressed as mean±SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups.
\textsuperscript{a}Significant vs. Sham, \textsuperscript{b}significant vs. Vehicle, \textsuperscript{c}significant vs. RIS (1.0 mg/kg), \textsuperscript{d}significant vs. RIS (2.5 mg/kg), \textsuperscript{e}significant vs. RIS (all doses).

Fig. 1. Bone markers. Data are expressed as mean±SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups. Sham: Sham-operated control, Vehicle: OVX+Vehicle, 0.1: OVX+RIS (0.1 mg/kg), 1.0: OVX+RIS (1.0 mg/kg), 2.5: OVX+RIS (2.5 mg/kg), ALF: OVX+ALF. a: significant vs. Sham, b: significant vs. Vehicle, c: significant vs. RIS (all doses).

Fig. 2. Femoral BMD measured by pQCT. Data are expressed as mean±SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups. Sham: Sham-operated control, Vehicle: OVX+Vehicle, 0.1: OVX+RIS (0.1 mg/kg), 1.0: OVX+RIS (1.0 mg/kg), 2.5: OVX+RIS (2.5 mg/kg), ALF: OVX+ALF. a: significant vs. Sham, b: significant vs. Vehicle, c: significant vs. RIS (all doses).
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increased the Tb Sp, as a result of increased bone resorption (N.Oc/BS, OcS/BS) and bone formation (ObS/BS, MS/BS, MAR, BFR/BS, BFR/BV). RIS (0.1 mg/kg) attenuated, and RIS (1.0 and 2.5 mg/kg) prevented these changes of the structural parameters, primarily by suppressing bone resorption (N.Oc/BS, OcS/BS) and bone formation (ObS/BS, MS/BS, MAR, BFR/BS, BFR/BV). The suppression of bone turnover by RIS (1.0 and 2.5 mg/kg) was greater than that by RIS (0.1 mg/kg). ALF increased the cancellous BV/TV to beyond the value obtained in the sham-operated controls, and prevented the alterations in the Tb N and Tb Sp, primarily by mildly suppressing bone resorption (N.Oc/BS, OcS/BS) and bone formation (MS/BS, BFR/BS, BFR/BV). The effect of ALF on cancellous BV/TV was greater than that of RIS, because the decrease of BFR/BS by ALF was comparatively milder, and ObS/BS and MAR were even maintained by ALF.

Bone histomorphometric analysis of cortical bone of the tibial diaphysis (Fig. 4 and Table 3)

OVX did not affect the Tt Ar, Ct Ar, or Ma Ar, despite increased periosteal bone formation (MS/BS, MAR, BFR/BS) and endocortical bone resorption (ES/BS). RIS (at any dose) also did not increase the Tt Ar and Ct Ar, but prevented an increase of endocortical bone resorption (ES/BS), and the Tt Ar and Ct Ar were higher in the
RIS (0.1 and 2.5 mg/kg) group than in the sham-operated control group. ALF increased the Tt Ar and Ct Ar, and the Tt Ar, Ct Ar, and Ma Ar were all higher in the ALF group than in the sham-operated control group because of the acceleration of periosteal bone formation (MS/BS, BFR/BS), the increase in endocortical bone for-
mation (MS/BS, MAR, BFR/BS), and prevention of an increase in endocortical bone resorption (ES/BS).

**Biomechanical test of the femur (Table 4)**

OVX decreased the maximum load and stiffness of the femoral distal metaphysis, without any effect on the mechanical properties of the femoral diaphysis. RIS (at any dose) did not affect any of the mechanical properties of the femoral distal metaphysis or diaphysis, whereas ALF increased the maximum load and breaking energy of the femoral distal metaphysis and the maximum load and stiffness of the femoral diaphysis beyond the values obtained in the sham-operated controls.

**DISCUSSION**

The present study demonstrated that RIS prevented the decreases in the cancellous BV/TV in a dose-dependent manner without any apparent effect on Ct Ar, while ALF increased both the cancellous BV/TV and Ct Ar, and that ALF, but not RIS, increased the maximum load of the femoral distal metaphysis and femoral diaphysis. Thus, the present study clearly showed the differential preventive effects of RIS and ALF on the cortical and cancellous bone mass and mechanical properties in ovariectomized rats.

Previously, we reported the therapeutic effects of alendronate and ALF on cancellous and cortical bone mass and mechanical properties in ovariectomized osteopenic rats (18). Alendronate increased only cancellous bone mass, while alfacalcidol increased cancellous and cortical bone mass and the maximum load of the femoral distal metaphysis, suggesting the differential therapeutic effects of alendronate and alfacalcidol on cancellous and cortical bone mass and mechanical properties in ovariectomized osteopenic rats. The results regarding the effects of bisphosphonates and ALF were similar in the two studies except for the effect of bisphosphonates on cortical bone mass. The differential effect of alendronate and RIS on cortical bone mass in these two studies may partly be associated with the animal model (OVX rats with/without established osteopenia) and the degree of suppression of endocortical bone turnover by alendronate and RIS.

Rat models have been useful for predicting the efficacy of drugs in humans, especially in terms of their effects on cancellous bone; nevertheless some significant differences in bone physiology exist between rats and humans. In particular, spontaneous bone fractures in association with declining estrogen levels or aging do not occur in rats. Additionally, the bones of rats do not stop growing, although female rats do show slowing of growth after about 1 y of age (19–21) and some proliferation of the growth plate is seen after about 9 mo of age (22). Finally, the cortical bones of rats lack the Haversian-based remodeling observed in human cortical bone. Therefore, pharmacological efficacy of drugs in ovariectomized rats may not be exactly translatable into clinical efficacy in postmenopausal women.

In the present study, OVX increased bone turnover, resulting in the loss of the cancellous BMD and cancellous BV/TV and Tb N. Bone loss following OVX was observed primarily in regions rich in cancellous bone. Perforations rather than thinning of the trabecular bone developed with the deterioration of the mechanical properties of the femoral distal metaphysis. However, neither the loss of the cortical BMD, Tt Ar, and Ct Ar or deterioration of the mechanical properties of the femoral diaphysis was observed following OVX.

RIS prevented the decreases in the cancellous BMD and cancellous BV/TV in a dose-dependent manner in ovariectomized rats by suppressing bone turnover, without any apparent effect on the cortical BMD, Ct Ar, or maximum load of the femoral distal metaphysis or femoral diaphysis, despite the suppressed endocortical bone resorption. Available evidence suggests that osteoclasts on the trabecular surface may be more responsive to bisphosphonates than those on the endocortical surface (23). It has also been reported that hindlimb-bandage (immobilization) affected only the metaphysis, and not the diaphysis of long bones, and that RIS could prevent immobilization-induced loss of bone mass and bone strength at the metaphysis (15). These findings suggest that the effects of RIS on the long bones of the hindlimbs may be site-specific in immobilized rats, and cancellous bone, which is richer in metaphyseal bone, may be more responsive to RIS than cortical bone, which is richer in the diaphyseal bone. In the present study, RIS was effective against the loss of the bone mass of sites rich in cancellous bone.

ALF prevented the decrease of the cancellous BMD and increased the cancellous BV/TV and Ct Ar in ovariectomized rats by mildly decreasing the histomorphometric cancellous bone turnover parameter, maintaining trabecular bone formation, increasing periosteal and endocortical bone formation, and preventing an increase of endocortical bone resorption. ALF also increased the maximum load of the femoral distal metaphysis and femoral diaphysis. ALF was reported to cause a dose-dependent suppression of bone resorption and yet maintain or even stimulate bone formation, as reflected by the increases in the serum OC levels and BFR/BS at both cancellous and cortical bone sites, resulting in an increase in the BMD and improvement of the mechanical strength (14), consistent with our results. However, ALF increased bone turnover when it was evaluated by the urinary DPD and serum OC levels. There seems to be a discrepancy between bone histomorphometric parameters and serum and urinary bone turnover markers, especially when bone resorption is evaluated. The main reason for this may be that urinary DPD and serum OC (markers of general bone metabolism) do not always reflect local bone metabolism in the tibia. Because of coupling of bone resorption and bone formation in bone remodeling, increased bone formation markers could be followed by increased bone resorption markers. Thus, the elevated urinary levels of DPD might be associated with the increased serum levels of OC 8 wk after OVX. However, probably because alterations of bone turnover markers are time-dependent and bone formation and bone resorption may
increase, reach an early plateau, and later return to baseline during treatment with anabolic drugs (24). We surmise that a reduction in urinary DPD levels by the anti-resorptive action of ALF could have been observed, if the duration of observation had been longer.

In regard to the effects of RIS and ALF on skeletal sites rich in cancellous bone in ovariectomized rats, the effect of ALF (0.5 μg/kg) on the cancellous BV/TV was greater than that of RIS (2.5 mg/kg), and only ALF increased the maximum load of the femoral distal metaphysis, because of the milder decrease of BFR/BS following ALF administration as compared with that following RIS administration. ALF prevented the decrease in the serum calcium levels, without causing hypercalcemia. Conversely, bisphosphonates, such as alendronate (5 mg/d), have been reported to be more efficacious than ALF (1 μg/d) in increasing the lumbar BMD and preventing vertebral fractures in postmenopausal women with osteoporosis (6, 7). This discrepancy may be due to the differential response of cancellous bone to ALF between ovariectomized rats and postmenopausal women (human); the beneficial effects of ALF might be greater on rat bones than on human bones (11, 14). In ovariectomized rats, ALF, but not RIS, increased the Ct.Ar and maximum load of the femoral diaphysis by increasing periosteal and endocortical bone formation and decreasing endocortical bone resorption. This result may possibly suggest the potential efficacy of ALF for cortical bone in postmenopausal women. The present study clearly showed the differential effects of RIS and ALF on cortical and cancellous bone mass, bone formation and resorption, and the mechanical properties of the bone in ovariectomized rats.

The effects of ALF and RIS on the cancellous bone microarchitecture evaluated with a micro-computed tomography have been reported in OVX animals (25, 26). ALF (0.1 and 0.2 μg/kg) vigorously increased the interconnections, plate-like structures, and cancellous BV/TV in OVX rats (25). RIS (2.5 mg/kg) increased the cancellous BV/TV, maximum load, and stiffness in OVX minipigs (26). According to the results of the cancellous BV/TV, ALF treatment in OVX rats seems to be more effective in increasing cancellous bone mass than RIS in OVX minipigs (25, 26). Therefore, it might be of interest to compare the effects of ALF and RIS on the cancellous bone microarchitecture in a head-to-head fashion in the present study. Further studies are needed to confirm the differential effects of RIS and ALF on the three-dimensional cancellous bone microarchitecture in OVX animals.

In conclusion, the present study demonstrated that RIS prevented the decrease in cancellous bone mass observed in ovariectomized rats in a dose-dependent manner by suppressing bone turnover, without any apparent effect on the cortical bone mass. On the other hand, ALF increased both the cancellous and cortical bone mass by mildly decreasing trabecular bone turnover, increasing periosteal and endocortical bone formation, and preventing an increase of endocortical bone resorption. ALF, but not RIS, increased the maximum load of the bone at both sites rich in cortical and cancellous bone. Thus, the present study clearly showed the differential effects of RIS and ALF on cortical and cancellous bone mass and mechanical properties in ovariectomized rats.

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