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Original Article

Simvastatin did not prevent nor restore ovariectomy-induced bone loss in adult rats

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Abstract

Current published results on whether statins have beneficial effects on bone metabolism have been conflicting so far. In order to further investigate if statins were promising candidates for the treatment for osteoporosis, we conducted a study in which rats were ovariectomized (OVX) at 6 months of age, allowed to lose bone for 60 days and followed by oral administration of simvastatin at the dose levels of 0.3-10 mg/kg/d for 60 days. PGE2 (6 mg/kg) was used as a positive control. Study endpoints included bone histomorphometry on the proximal tibial metaphysis (PTM) and the tibial diaphysis (TX), dual-energy X-ray absorptiometry on the right femur and micro computed tomography (μCT) on the 5th lumbar vertebra (LV). After 120 days of OVX, cancellous bone lost by 80% in the PTM and 18% in the LV accompanied by increased bone formation and resorption. Simvastatin at all dose levels did not affect bone volume, bone formation rate and bone erosion surface when compared to 120 day ovariectomized animals at all bone sites studied. By contrast, PGE2 restored cancellous and cortical bone area to sham control levels. In conclusion, this study demonstrated that unlike PGE2, oral administration of simvastatin did not have effects on cancellous or cortical bone formation and resorption; and consequently was not able to prevent further bone loss or restore bone mass in the osteopenic, OVX rats.

Keywords: Simvastatin, Ovariectomy, Bone Histomorphometry, DEXA, Micro-CT

Introduction

Current treatments for osteoporosis include supplements of calcium and vitamin D, calcitonin, bisphosphonates, estrogen replacement therapy (HRT) or the use of selective estrogen receptor modulators (SERMs)[1]. These treatments are efficient in the prevention of bone loss, but are not favored in the treatment of established osteoporosis where there is a need for an effective bone anabolic factor to increase bone volume. Unfortunately, except for clinical trials with parathyroid hormone, fluoride and growth hormone, anabolic agents such as prostaglandin E2 and fibroblast growth factor have not proceeded to clinic because of their significant adverse effects. Statins have been safely administered to patients to reduce serum cholesterol concentration for over a decade. Recently, it was reported that some of the statins might have the potential to promote bone formation and inhibit ovariectomy-induced bone loss in rats[2-4]. If this was the case, statins could serve as promising drugs to prevent the development of bone loss. In fact, many clinical trials showed that statins' administration were associated with decreased bone turnover markers with increased bone mineral density in the spine and/or associated with reduction of vertebral or hip fracture risks[5-8]. Some otherwise conflicting results were also reported[9-13]. Based on the substantial interests in statins, we carried out a study to investigate the prevention and restoration effects of simvastatin using an established osteopenia model, in which rats were ovariectomized at the age of 6 months and allowed to lose bone for 60 days before treating daily for 60 days. Bone histomorphometry, micro-CT and DEXA were used to evaluate multiple skeletal sites including the metaphysis and diaphysis of long and axial bones.

Authors Yao, Tian, Setterberg and Jee have no conflict of interest. Authors Farmer, Cooper, Chmielewski and Lundy have corporate appointments with Procter & Gamble Pharmaceuticals.

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Materials and methods

Experimental protocol. Seventy-two female 3-month-old Sprague Dawley rats were acclimated to local vivarium conditions (Simonsen Laboratories, Gilroy, GA). They were pair-fed in cages with the room temperature maintained at 72ºF and 12:12 light/dark cycles. The rats were allowed free access to water and pelleted commercial natural diet (Teklad Rodent Laboratory Chow #8604, Harlan Teklad, Madison, WI) that contains 1.46% calcium, 0.99% phosphorus and 4.96 IU/g of vitamin D_3. At 6 months of age, the rats were divided into 10 body weight-matched groups with 6-8 rats per group. One group was killed as baseline control (Basal), the others were sham or bilaterally ovariectomized. After 60 days of operation, pre-treatment sham (60-d Sham) or ovariectomized (60-d OVX) animals (6 per group) were euthanized as pre-treatment controls. The remaining rats were treated daily with 0.3, 3.0, 6.0 and 10.0 mg/kg of simvastatin by oral gavage (ACIC Fine Chemicals, Mississauga, Ontario, Canada) for 60 days or with vehicle of acetate buffers (physiologic saline, methylcellulose and polyoxyethylene sorbitan monooleate). A group of rats subcutaneously injected with 6 mg/kg/d of PGE2 (Cayman Chemicals, Ann Arbor, Michigan) served as a positive control. All the rats received 90 mg/kg of Xylenol orange before treatments and 10 mg/kg of Calcein (Sigma Chemical Co., St. Louis, MO) on 14 and 4 days before sacrifice. At necropsy the final sham (120d-Sham) and ovariectomized (120d-OVX) vehicle-treated and simvastatin-treated rats were anesthetized by an intraperitoneal injection of Ketamine (50 mg/kg) and Xylazine (10 mg/kg) and sacrificed by cardiac puncture. Changes of bone mass were measured in the tibia by bone histomorphometry and in the femur by dual energy X-ray absorptiometry (DEXA, Hologic QDR-2000 plus bone densitometer, Hologic, Inc., Waltham, MA) and in the 5th lumbar vertebra by micro-computed tomography system (μCT 20, serial # 96-2004, Scanco Medical, AG). Blood serum was taken during necropsy for determination of lipid levels. The above protocol was approved by the Institutional Animal Care Committee at Procter and Gamble Pharmaceuticals.

Bone histomorphometry. The proximal tibiae and the middle-third of the right tibiae were stained with Villanueva bone stain, dehydrated in graded concentrations of ethanol, defatted in acetone, and embedded in methyl methacrylate (Fisher Scientific, Fairlawn, NJ). Longitudinal sections of proximal tibiae (PT) and cross-sections at the tibio-fibular junction of the tibial shafts (TX) were cut to 230 μm thickness using a low speed metallurgical saw and then ground to 20 μm (PT) and 30 μm (TX) for histomorphometric measurements. Histomorphometry was done with a semi-automatic image analysis system (OsteoMeasure, OsteoMetrics Inc., Decatur, GA) linked to a microscope equipped with transmission and fluorescent light.

The region of the proximal tibial metaphysis that was studied was from 1 mm to 4 mm distal to the growth plate-metaphyseal junction. Static measurements included total tissue area (T.Ar), bone area (B.Ar) and bone perimeter (B.Pm). Dynamic measurements included single- (sL.Pm) and double-labeled perimeter (dL.Pm), eroded perimeter (E.Pm), and interlabel width (It.L.Wi). These indices were used to calculate percentage trabecular bone area (B.Ar/T.Ar), trabecular number (Tb.N), trabecular width (Tb.Wi), trabecular separation (Tb.Sp), percentage eroded perimeter (E.Pm/B.Pm), mineral apposition rate (MAR), mineralizing perimeter (Md.Pm), and bone formation rate per unit of bone area (BFR/B.Ar), of total tissue area (BFR/T.Ar), and of bone surface (BFR/B.Pm) according to Parfitt et al.[14,15].

Cortical bone measurements included total cross-sectional area (T.Ar), marrow area (Ma.Ar), eroded perimeter (E.Pm), single- and double-labeled perimeter (sL.Pm, dL.Pm), and interlabeled width (It.L.Wi). These parameters were used to calculate the cortical bone area (Ct.Ar), percentage cortical area (%Ct.Ar), percentage marrow area (%Ma.Ar), percentage mineralizing perimeter (L.Pm/B.Pm), mineral apposition rate (MAR) and bone formation rate per bone surface (BFR/B.Pm) of the periosteal (Ps) and endocortical (Ec) bone surfaces according to Jee et al.[16].

Micro-computed tomography. The 5th lumbar vertebral bodies were removed from all animals and were cleaned of soft tissue. The processes were removed and the vertebral bodies placed in 70% ethanol. Each lumbar vertebral body was imaged using a micro-computed tomography system (μCT 20, serial # 96-2004, Scanco Medical AG). The caudal end of the vertebra was placed on the left side of the holder alignment line to aid in consistent positioning of the bone. A sponge material moistened with 70% ethanol, which acts to secure the vertebra in position and keep the sample moist, separated the samples. Image acquisition parameters for the vertebra included standard resolution (300 projections), 26 μm slice increment, and 150 msec integration time. Approximately 186 slices were scanned per vertebra. Once acquisition was complete, the images were sent to a SGI Octane Workstation for all subsequent analyses. The image analysis involved: (a) setting threshold of the images to bone and background; (b) determining of the volume of interest (VOI); (c) separating of the cortical from the trabecular bone; and (d) measuring of structural parameters[17]. Measurements made on the 3-D datasets included trabecular bone volume, surface area, trabecular thickness, trabecular number, trabecular separation, connectivity density and cortical thickness.

Bone densitometry. Whole bone mineral density (BMD) of the right femurs was determined *ex vivo* using DEXA. The scanning of small animal bones requires the use of the regional high-resolution software (with 0.0100 inch line spacing and 0.00499 inch point resolution). This software automatically selects a small X-ray source collimator (0.05 cm diameter) and employs a high-resolution protocol to scan the femur from the proximal end to the distal end.

Results are presented as means±SD. The statistical analyses were performed using SAS statistical software (SAS Institute Inc., Cary, NC) to perform analysis of variance with Fisher's protected two-sided Least Significance Difference (LSD) test for comparison between groups. $P<0.05$ was considered significant.

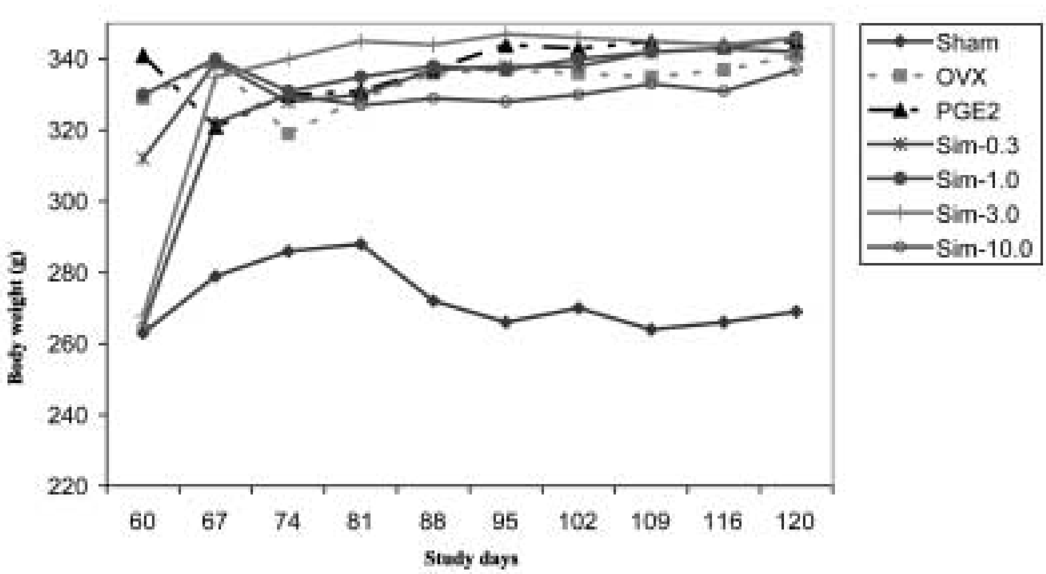

Figure 1. Body weight changes during the treatment peroid.

Results

Body weights (Figure 1). Body weights were 20% higher in the OVX animals than in sham animals. The OVX rats treated with simvastatin or PGE2 group had similar body weights as the OVX rats treated with vehicle.

Lipid evaluations (Table 1). At 120 days post-OVX, vehicle-treated OVX rats had a marginal increase in serum cholesterol compared with the sham controls ($p=0.1875$). Simvastatin did not ameliorate this marginal increase in cholesterol compared to the sham level, but at the 0.3 and 10.0 mg/kg doses significantly increased cholesterol compared to the 120d-OVX group. High-density lipoproteins (HDL) and triglyceride levels in the 120d-OVX group were not significantly different from the 120d-Sham group. Simvastatin significantly increased the HDL at the 0.3 mg/kg dose compared to the 120d-OVX group.

Groups	Dose	Cholesterol	High Density Lipoproteins	Triglycerides
120 d-Sham	None	117.6±24.9	96.3±20.6	48.9±16.5
120 d-OVX	None	131.8±22.1	96.0±15.4	40.2±16.9
Simvastatin	0.3 mg/kg	*145.5±14.3	*116.0±16.1	59.0±20.0
Simvastatin	1.0 mg/kg	135.3±18.3	105.0±16.3	47.7±15.9
Simvastatin	3.0 mg/kg	136.2±14.6	97.8±13.8	39.8±21.6
Simvastatin	10.0 mg/kg	*140.7±18.2	106.5±15.2	49.7±14.1
*$p<0.05$ vs. 120d-OVX.				

Table 1. Lipid evaluations.

Proximal tibial metaphysis histomorphometry (Table 2). After 60 days of OVX, bone volume decreased significantly compared to the pre-treatment sham group due to a decrease in trabecular number. There was a continued loss of trabecular bone for an additional 60 days of OVX with significant decreases in both trabecular thickness and number. Ovariectomy increased mineral apposition rate, and bone formation rates compared to the sham-operated animals.

PGE2 completely restored bone area to 60d-Sham level accompanied by partially restored trabecular number, increased trabecular width and bone formation. Bone resorption was decreased. Although simvastatin had about 20-50% more bone area compared to the 120d-OVX group, the bone area varied for all dose levels was significantly less than the 60d-OVX group. Simvastatin did not significantly affect eroded perimeter and bone formation (mineralizing surface, mineral apposition rate, BFR/T.Ar, BFR/B.Ar and BFR/B.Pm) compared to the 120d- and 60d-OVX groups.

Tibia diaphysis histomorphometry (Table 3). At 120 days, ovariectomy increased total cross-sectional area and marrow area with a significant increase in endocortical mineralizing surface and bone formation rate compared to the sham groups. Periosteal bone formation and mineralizing surface were dramatically increased in the 60d-OVX group but returned to 60d-Sham control level at 120 days. At the 1 and 3 mg/kg doses, simvastatin significantly increased tissue area but had no changes in percentage cortical bone or marrow area compared to the OVX groups. PGE2 increased both periosteal and endocortical bone formation compared to the sham and OVX groups. At all doses, simvastatin had no significant effects on endocortical or periosteal bone formation and endocortical

Parameters Groups	B.Ar/T.Ar %	Tb.WI μm	Tb.N #/mm	Tb.SP μm	Md.Pm %	E.Pm %	MAR μm/d	BFR/T.Ar %/y	BFR/B.Ar %/y	BFR/B.Pm $\mu m^3/\mu m^3$/ d×100
Basal	#*12.3±3.3	43.3±4.4	#*2.8±0.5	#*322.4±70.7	#*17.6±3.5	#*4.7±0.9	0.7±0.0	23.1±9.9	#*187.5±48.1	#*13.2±3.2
60d-Sham	#13.0±2.2	45.7±2.6	#2.8±0.4	#311.9±52.3	#25.9±9.0	#3.2±1.0	0.5±0.4	26.5±21.0	#224.4±178.4	#16.7±13.2
120d-Sham	*10.6±3.3	*43.0±6.7	*2.4±0.5	*385.1±101.1	*30.4±4.2	*3.4±1.4	0.7±0.1	#35.3±11.3	*343.8±83.6	*23.7±4.5
60d-OVX	7.6±1.7	45.2±4.1	1.6±0.3	564.7±117.5	35.6±1.6	12.9±3.4	1.0±0.0	36.8±7.1	486.6±63.8	35.7±1.7
120d-OVX	#3.1±1.9	#35.2±6.8	#0.8±0.4	#1425.4±707.6	34.9±1.4	12.2±3.8	0.9±0.1	#16.3±8.6	559.8±112.7	31.5±3.4
PGE2	#*13.7±4.2	#*65.1±6.6	#2.0±0.4	*437.4±116.4	#*40.7±2.0	#*6.6±2.2	1.0±0.1	#*53.2±14.8	*393.4±40.8	#*41.7±2.4
Sim-0.3	#*4.0±1.4	*43.1±6.7	#0.9±0.2	#*1106.0±325.7	33.6±2.0	10.3±3.0	0.8±0.1	#15.6±3.8	403.9±79.0	27.9±2.0
Sim-1.0	#*3.7±1.5	*39.5±7.7	#0.9±0.2	#*1156.2±470.3	35.3±2.2	10.5±1.0	0.9±0.1	#17.3±5.8	484.5±94.6	30.6±2.9
Sim-3.0	#*4.6±1.8	*46.4±6.5	#0.9±0.2	#*1065.2±419.2	35.3±1.7	10.7±0.7	0.9±0.1	#19.1±6.5	418.4±56.5	31.4±1.5
Sim-10.0	#*3.8±2.1	*41.8±12.9	#0.8±0.3	#*1210.2±441.6	32.9±3.8	10.7±2.3	0.9±0.1	#15.1±5.0	453.8±157.7	28.7±5.9

Sim, Simvastatin 0.3, 1.0, 3.0, 10.0 mg/kg/d, respectively; B.Ar, bone area; T.Ar, total tissue area; Tb.Wi, trabecular width; Tb.N, trabecular number; Tb.Sp, trabecular separation; Md.Pm, mineralizing perimeter; E.Pm, eroded parameter; MAR, mineral apposition rate; BFR, bone formation rate; B.Pm, bone perimeter. Among OVX groups and other groups: $^{\#}p<0.05$ vs. 60d-OVX; $^{*}p<0.05$ vs. 120d-OVX.

Table 2. Selected histomorphometric changes of the proximal tibial metaphysic (PTM).

Parameters Groups	T.Ar mm^2	Ma.Ar mm^2	Ct.Ar %	Ct.Wi μm	Ps-Md.Pm %	Ps-MAR μm/d	Ps-BFR μm/d×100	Ec-Md.Pm %	Ec-MAR μm/d	Ec-BFR μm/d×100	Ec-E.Pm %
Basal	#*4.4±0.4	#*0.7±0.1	83.2±1.9	3.6±0.4	#*20.7±14.6	0.8±0.2	#18.9±17.9	#*2.8±1.7	#*0.0±0.0	#*0.0±0.0	#*2.2±2.6
60d-Sham	4.8±0.3	0.8±0.1	82.3±1.6	4.0±0.2	#28.7±12.3	#0.5±0.4	#18.3±16.8	#9.8±5.6	#0.2±0.5	#3.3±8.1	#3.9±3.8
120d-Sham	*4.7±0.1	*0.8±0.1	83.0±1.7	3.9±0.1	*22.8±14.8	0.4±0.4	15.4±19.0	*18.5±5.7	*0.2±0.5	*6.6±12.7	*5.5±2.7
60d-OVX	4.7±0.2	0.8±0.1	82.0±2.0	3.9±0.2	67.9±14.5	1.0±0.2	74.9±28.0	18.8±5.9	1.0±0.1	19.4±6.9	14.1±4.3
120d-OVX	5.0±0.3	0.9±0.1	81.8±1.2	4.1±0.2	#39.4±19.4	0.7±0.1	#30.5±23.2	28.2±10.1	1.0±0.1	#28.1±11.8	#9.4±6.4
PGE2	5.2±0.2	0.8±0.1	83.8±2.0	#*4.3±0.2	*63.0±15.5	1.1±0.2	*72.3±33.3	35.3±15.5	1.1±0.2	#35.3±15.5	#*2.2±2.2
Sim-0.3	5.3±0.3	0.9±0.1	82.7±0.9	4.4±0.3	40.2±6.7	0.7±0.1	#30.1±4.3	23.7±6.2	0.8±0.1	20.2±6.9	10.4±1.8
Sim-1.0	#*5.6±0.4	1.0±0.1	81.6±1.8	4.5±0.3	45.2±14.5	0.7±0.1	#37.0±18.1	32.1±5.5	1.07±.15	34.4±7.5	#7.5±2.2
Sim-3.0	#*5.4±0.4	0.9±0.2	82.9±2.5	4.5±0.2	30.1±10.2	0.4±0.2	#13.5±10.4	28.7±7.3	0.9±0.2	27.9±8.5	#9.1±3.2
Sim-10.0	5.3±0.2	0.9±0.1	82.6±2.6	4.4±0.3	40.5±5.2	0.6±0.1	#25.8±5.4	25.8±5.6	0.9±0.1	24.7±8.9	11.3±8.1

Sim, Simvastatin 0.3, 1.0, 3.0, 10.0 mg/kg/d, respectively; T.Ar, total cross-sectional area; Ma.Ar, marrow area; Ct.Ar, cortical bone area; Ct.Wi, cortical bone width; Ps, periosteal surface; Ec, endorcortical surface; Md.Pm, mineralizing perimeter; MAR, mineral apposition rate; BFR, bone formation rate; E.Pm, eroded perimeter. Among OVX groups and other groups: $^{\#}p<0.05$ vs. 60d-OVX; $^{*}p<0.05$ vs. 120d-OVX.

Table 3. Selected histomorphometric changes of the tibial diaphysis (TX).

bone resorption compared to the 120d-OVX group; these indices were all lower than those of the 60d-OVX group.

Lumbar vertebral mCT (Table 4). Ovariectomy significantly decreased vertebral bone volume, trabecular number, trabecular and cortical bone thickness compared to the sham groups. PGE2 restored cancellous bone volume, increased cancellous and cortical bone thickness. Simvastatin caused no significant change of vertebral bone volume and architectural changes at the doses tested compared to the 120d- and 60d-OVX groups.

Femur DEXA (Figure 2). Ovariectomy significantly decreased whole femur aBMD compared to the sham groups. However, in this study the 120d-OVX group had slightly but not significantly higher values than the 60d-OVX group. Simvastatin did not cause significant changes in aBMD compared to the 120d-OVX group.

Discussion

The results of this study indicated that daily oral administration of simvastatin, one of the 3-hydroxy-3-methylglutaryl co-enzyme A (HMG Co-A) reductase inhibitors used to reduce serum cholesterol, was not able to prevent bone losses following ovariectomy at the dose levels of 0.3, 1, 3, 10 mg/kg/d for 60 days in the tibia, femur and lumbar vertebra of the 8-month-old rats.

We did not see a decrease in serum lipid levels but an increase of cholesterol with 0.3 and 10.0 mg/kg doses of simvastatin. Simvastatin has been shown to lower cholesterol in the patients with hydroxycholesterol[18]. However, in animal studies, simvastatin increased serum cholesterol up to 235% in the rat between nine and twelve hours post-dosing[19]. Since

Groups	Bone volume /tissue volume %	Trabecular thickness µm	Trabecular number 1/mm	Trabecular separation µm	Connectivity Density mm^{-3}	Cortical Thickness µm
Basal	#*41.4±2.0	#*75.7±2.0	*5.4±0.2	#*107.2±8.5	#*92.9 ±14.0	#*188.0 ±13.8
60 d-Sham	#41.4±2.6	#78.1±5.0	5.3±0.1	110.5±6.1	#82.9±16.2	#198.0±20.6
120 d-Sham	*41.2±1.9	*81.7±4.3	*5.0±0.2	*116.8±8.0	*67.6±10.7	*201.5±17.1
60 d-OVX	34.8±2.8	71.5±3.0	4.8±0.2	134.4±11.4	83.6±5.0	172.1±17.2
120 d-OVX	33.5±2.1	77.1±1.4	4.3±0.2	153.7±12.9	56.6±5.0	177.6±6.3
PGE2	#*44.0±2.9	#*87.2±5.8	*5.0±0.4	#*110.0±13.7	*74.0±22.8	#*195.3±6.5
Sim-0.3	33.2±1.8	76.2±2.4	4.3±0.2	154.0±13.2	60.5±6.3	175.6±2.8
Sim-1.0	31.5±2.4	74.3±2.8	4.2±0.2	162.2±13.4	60.8±5.9	167.6±12.7
Sim-3.0	33.0±2.1	76.8±2.3	4.3±0.2	156.7±14.0	59.1±9.4	174.1±11.7
Sim-10.0	33.9±1.3	75.1±3.6	4.5±0.2	146.6±8.7	67.1±9.6	174.5±8.7

Sim, Simvastatin 0.3, 1.0, 3.0, 10.0 mg/kg/d, respectively. Among OVX groups and other groups: #$p<0.05$ vs. 60d-OVX; *$p<0.05$ vs. 120d-OVX.

Table 4. Selected mCT changes of the lumbar vertebra (LV).

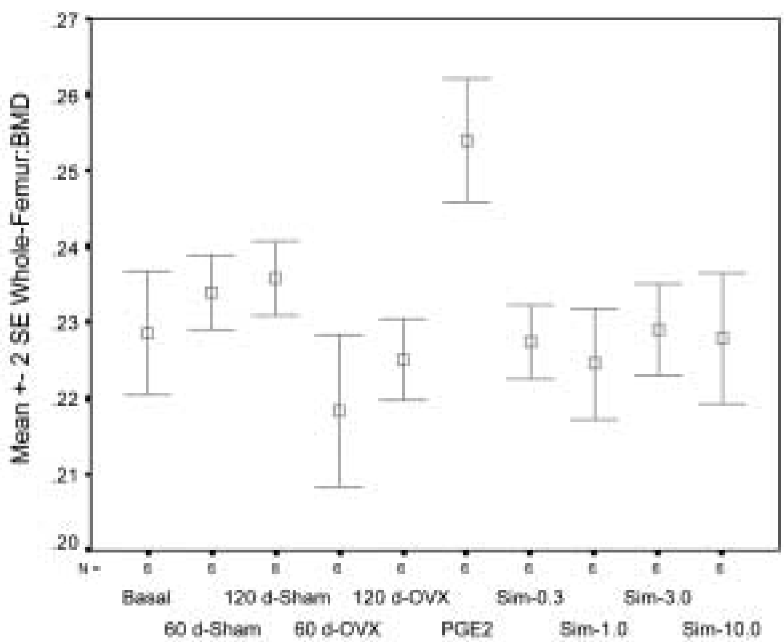

Figure 2. Femur - DEXA.

the blood samples were collected twenty-four hours or more from the final dosing, the lipid results being equal or higher than OVX, are reasonable. However, simvastatin effects on other tissues may not be solely related to their cholesterol-lowering action. Statins were reported to potentially promote osteoblastic bone formation and inhibiting osteoclast formation[20-23]. More extensive studies are needed to substantiate this hypothesis.

The results of clinical trials have not clearly demonstrated the beneficial effects of statins on bone metabolism. While some studies have suggested small increases in bone mineral density and lower hip or vertebral fracture risks in patients treated with statins[5-8,24-26], other studies have concluded that use of currently marketed statins had no relevant effects on reducing bone remodeling and the risk of osteoporotic fractures[9-13,27-29]. In animal studies, statins were reported to increase cancellous bone volume in 3-month-old female rats[2] and increase vertebral cancellous bone mass and compressive strength in 12-month-old female rats given simvastatin (10 mg/kg) orally[3]. In addition, statin-treated ovariectomized rats had higher cancellous bone mass and higher cortical bone formation than the OVX-alone animals when simvastatin was administered at a higher level (20 mg/kg, twice/day) and treated for a longer period (90 days)[2,4,32]. Statins may mediate their effects by increasing expression of bone morphogenetic protein-2 and therefore increasing osteoblast number and function; decreased osteoclastic number and activity might also account for their actions[2,23,33]. However, the lack of proper baseline and sham-operated control data made it difficult to interpret if statins could actually prevent or restore OVX-induced bone loss. In our current study in established osteoporosis rats, simvastatin showed minimal or absence of effects in preventing further bone loss induced by estrogen deficiency. Mundy et al.[2], found that simvastatin was effective in increasing cancellous bone mass up to 89% compared to OVX in the proximal tibial metaphysis of 3-month-old rats. The far less pronounced effects of statins in the present study may be due to the fact that rats we used were 8 months of age at the beginning of treatment, whose longitudinal growth rate was about 90% lower than that of 3-month-old rats[34,35]. In our study, we found that simvastatin did not affect the longitudinal growth rate (data not shown). The different findings between our study and that of Mundy's suggest that statins might promote bone growth (bone modeling) but their effects on bone development and bone maintenance (bone remodeling) warrant further investigation.

It is known that the absorption of the ingested doses of statins is between 40-75%[36]. All statins have high first-pass extraction by the liver, 95% of the statins are metabolized to inactive metabolites and leave a small amount to be absorbed into the blood stream and to reach bone. Therefore, the lack of skeletal effects of simvastatin observed in this study may be in part due to the low drug exposure in bone tissues following oral administration. Alternative routes of administration, which bypass the liver, or use other statins that target bone cells specifically may provide a better opportunity to further assess the potential effects of statins on bone. Consistent with this hypothesis, Mundy et al. have reported that statins cause greater increases in bone formation if administered by dermal application or via subcutaneous implantation[37].

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