Featured Article

Effects of the Steroidal Aromatase Inhibitor Exemestane and the Nonsteroidal Aromatase Inhibitor Letrozole on Bone and Lipid Metabolism in Ovariectomized Rats

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ABSTRACT

Purpose: **Exemestane (EXE) and letrozole (LET) are third-generation aromatase inhibitors currently prescribed for postmenopausal hormone-dependent breast cancer. The impact on end organs of estrogen depletion in menopausal women is of significant clinical importance. We studied the effects of EXE, its principal metabolite, 17-hydroexemestane (17-H-EXE), and LET on bone and lipid metabolism in ovariectomized (OVX) rats.**

Experimental Design: **OVX rats were treated by weekly intramuscular injection for 16 weeks with 20, 50, and 100 mg/kg EXE, 20 mg/kg 17-H-EXE, and daily oral gavage of 1 mg/kg LET. At the end of the treatment period, bone mineral density (BMD), the bone resorption marker serum pyridinoline, the bone formation marker serum osteocalcin, bone mechanical properties, histomorphometry, and serum lipid concentrations were determined.**

Results: **Lumbar vertebral and femoral BMD, bending strength of the femur, compressive strength of the fifth lumbar vertebra, and trabecular bone volume were significantly higher in OVX animals given EXE and 17-H-EXE than in OVX controls. EXE and 17-H-EXE significantly reduced an ovariectomy-induced increase in serum pyridinoline and serum osteocalcin. EXE and 17-H-EXE given to OVX rats caused significant reductions of serum cholesterol and low-density lipoprotein cholesterol. In contrast, OVX rats treated with LET had BMD, bone biomarkers, mechanical failure properties, and lipid levels similar to those of OVX controls.**

Conclusions: **EXE and 17-H-EXE significantly prevent bone loss, enhance bone mechanical strength, and lower serum cholesterol and low-density lipoprotein levels in OVX rats. These protective effects on end-organ function are not seen with the nonsteroidal inhibitor LET.**

INTRODUCTION

Antagonizing the growth-stimulatory effects of estrogen is an established clinical approach to patients with hormone-dependent breast cancer. Tamoxifen competes with estrogen for binding to the estrogen receptor and, in the presence of tumor and tissue-specific coactivators and corepressors, exerts either an antiestrogenic or estrogen agonist effect via the estrogen response element. The estrogen agonist effects are beneficial on bone metabolism and lipid metabolism but are thought to be responsible for an unopposed estrogen-like effect on the endometrium leading to endometrial thickening, bleeding, and, in rare cases, cancer (1, 2). The antiestrogenic effect arrests growth in hormone-dependent breast cancer cells, but adaptation and mutation by the tumor cells may lead to tamoxifen resistance or dependence.

The aromatase inhibitors act by inhibiting estrogen synthesis and deplete estrogen concentrations in the circulation, tumor, and peritumoral tissue and are thus a useful alternative antiestrogenic strategy to tamoxifen.

This inhibition is incomplete in premenopausal women, and monotherapy with the inhibitors is thus confined to postmenopausal women. These women are already at risk of bone loss and osteoporosis, as well as cardiovascular disease, in part due to adverse changes in lipid metabolism associated with low estrogen levels. Further or complete reduction in circulating estrogen by aromatase inhibition is therefore of potential importance in women receiving long-term therapy for early-stage breast cancer and even more so if aromatase inhibitors are contemplated for prevention of the disease in otherwise healthy women.

Two classes of inhibitors are in current clinical practice, the irreversible steroidal aromatase inhibitors, exemplified by exemestane (EXE; ref. 3), and the reversible imidazoles, letrozole (LET; ref. 4) and anastrozole (5, 6). EXE is structurally related to androstenedione and has some affinity for the androgen receptor (3, 7). The principal metabolite of EXE is 17-hydroexemestane (17-H-EXE), which has a short half-life but, like the parent compound, is androgenic (7). Androstenedione has been shown to protect against the development of osteopenia in estrogen-deficient ovariectomized (OVX) rats, and it mediates

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this effect through its androgenicity and not through conversion to estrogens. These effects are also abrogated in the presence of the pure antiandrogen casodex, confirming the action through the androgen receptor (8). We have hypothesized that EXE and its principal metabolite, 17-H-EXE, exert a positive androgenic effect on bone metabolism that is distinct from the nonsteroidal inhibitors and that this is of potential significance in menopausal women on long-term therapy with this class of agents.

We therefore selected the OVX Sprague Dawley rat to evaluate the effects of the aromatase inhibitors on bone metabolism (9, 10). This is a validated postmenopausal model for the study of bone-sparing drugs. For example, at 35 weeks after ovariectomy, lumbar spine bone mineral density (BMD) has been shown to be 19% lower than in intact animals, whereas OVX animals given the selective estrogen receptor modulator (SERM) EM-800 (1 mg/kg) or raloxifene (1 mg/kg) had 93% and 90%, respectively, of the BMD values observed in intact rats (11). The model is also useful to study the lipid profile resulting from treatment with various endocrine therapies, another end-organ effect of interest to us in evaluating the two classes of aromatase inhibitors (12). Our goal here was to determine whether there are differences on these two important end-organ functions between the two classes of aromatase inhibitors.

MATERIALS AND METHODS

Animal Protocol. All experimental procedures were performed under the guidelines established by the Canadian Council on Animal Care. Ten-month-old female Sprague Dawley rats were obtained from Harlan (Indianapolis, IN). The rats were housed 2 animals per cage on a 12-hour light/dark cycle, with room temperature set at 22°C. The animals were allowed to acclimatize for 4 weeks, with *ad libitum* access to both food (TD 89222 diet, 0.5% calcium and 0.4% phosphorus; Teklad, Madison, WI) and tap water. All animals were matched according to body weight and assigned to nine experimental groups of 12 animals each, as follows: group 1, intact controls (intramuscular injection); group 2, OVX controls (intramuscular injection); groups 3–5, OVX + EXE (20, 50, or 100 mg/kg; intramuscular injection); group 6, OVX + 20 mg/kg 17-H-EXE (intramuscular injection); group 7, intact controls (oral gavage); group 8, OVX controls (oral gavage); group 9, OVX + 1 mg/kg LET (oral gavage). The intact animals were subjected to the same general surgical procedure as OVX animals, except the ovaries were not excised. EXE and 17-H-EXE (Fig. 1) were provided by Phar-
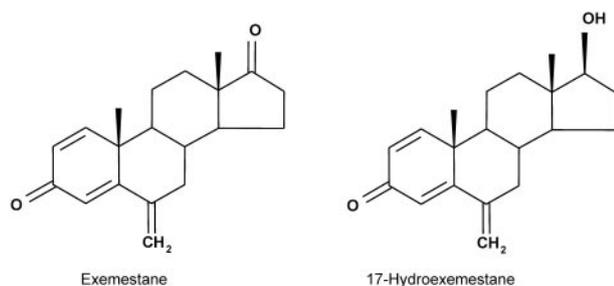
macia Oncology (Peapack, NJ). The administration of the compound was initiated a day after OVX. EXE, 17-H-EXE, and vehicle (for EXE and 17-H-EXE controls) were given by intramuscular injection once weekly, and the dosing sites for all animals were alternated between the left and right sides. LET and vehicle (for LET controls) were given by oral gavage daily in a volume of 0.1 mL/100 g of body weight. After 16 weeks of treatment, the animals were euthanized by cardiac puncture under ketamine anesthesia. All animals were fasted overnight before blood collection for the bone biochemical marker and lipid assays. The whole lumbar spine and femora from each animal were excised for analysis.

Bone Densitometry. The lumbar spine and left femur of individual animals were scanned by dual-energy X-ray absorptiometry (Hologic QDR; 4500 A) using the regional high-resolution scan mode (0.311 × 0.311-mm pixels) with a line spacing of 0.0311 cm and a point resolution of 0.0311 cm. Whole left femur and lumbar vertebrae (the first through sixth vertebrae) were used in the analysis. The bone mineral content and area were measured, and BMD was calculated automatically as bone mineral content/area.

Bone Biochemical Markers. Bone turnover markers were measured using commercially available kits as specified by the manufacturers. All assays were performed in duplicate.

Bone resorption marker serum pyridinoline (PYD) was measured by competitive enzyme immunoassay using a serum PYD kit (Metra Biosystems, Inc., Mountain View, CA). Briefly, serum samples were filtrated by a 30k MWCO Spin filter and incubated at 4°C for 22 hours in the dark. Bone formation marker serum osteocalcin (OC) was measured using a rat OC enzyme immunoassay kit (Biomedical Technologies, Stoughton, MA). Briefly, serum samples were diluted 1:10 with sample buffer and incubated at 4°C for 22 hours.

Mechanical Tests. The mechanical failure properties of the femora and vertebrae were conducted using an Instron 8501 material testing system (Instron Corp., Canton, MA). In both experiments, force and deformation data were collected at a rate of 25 Hz using a 12-bit data acquisition card (National Instruments, Austin, TX), Labview 5.0 data acquisition software (National Instruments), and a Pentium II computer (Compaq, Toronto, Canada).

The diaphysis of the right femur was tested to failure in three-point bending according to a procedure previously described (13). Briefly, samples were subjected to a preload of 2 N and then deformed at a rate of 2 mm/min until failure. The point of failure was defined as a successive drop in load greater than 10%. The body of the fifth lumbar vertebra was tested to failure in unconfined compression using a similar procedure described previously (14). Briefly, a preload of 1 N was applied to the sample, and the sample was then deformed at a rate of 1 mm/min until failure occurred. In these experiments, the point of failure was defined as a successive drop in load greater than 5%.

Specimen Processing. Left femora from the rats were cleaned of soft tissue and fixed in 70% EtOH. The femora were bisected using an Isomet 1000 slow-speed saw (Buehler, Lake Bluff, NY) and further fixed in 70% EtOH before plastic processing. Both halves of the femora were dehydrated in ascending grades of acetone and processed in ascending grades of Spurr (resin)/acetone before being embedded and polymerized in
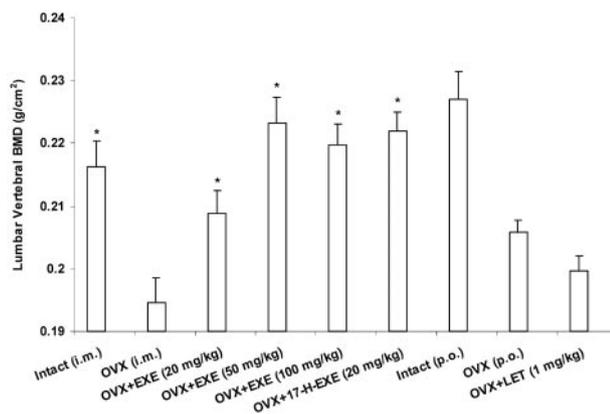

Fig. 1 Molecular structure of EXE and 17-H-EXE.

Fig. 2 BMD of the lumbar spine after 16 weeks of treatment with EXE, 17-H-EXE, and LET. *Scale bars* represent the mean ± SE (n = 12). ∗, $P < 0.005$ *versus* OVX controls.

Spurr resin at 50°C. Five-micrometer sections were cut serially using a Leica RM 2165 rotary microtome (Leica, Richmond Hill, Canada) equipped with a tungsten carbide knife. The 5-μm sections were stained with toluidine blue and Goldner's trichrome for static histomorphometric assessment.

Histomorphometric Analysis. Static histomorphometry was performed on a 5-μm undecalcified Goldner's trichrome-stained section of each proximal femur. All quantitative assessments were performed by a single trained technician using a semiautomated image analysis system (Bioquant; R&M Biometrics, Nashville, TN). A Metalux microscope (Leica) equipped with a ×10 objective (total magnification, ×125) was used to assess the stained slides. Measurements of bone were taken from a 15-mm^2 area in the central region beginning 0.2-mm distal to the growth plate. The area selected for measurement covered most of the trabecular bone available for measurement. For each sample, the following variables were measured: trabecular bone volume, mineralized trabecular bone volume, osteoid volume, osteoid surface, and eroded surface. In addition, from the above measurements, trabecular thickness, osteoid thickness, trabecular number, and trabecular separation were calculated. All measurements and calculations were done following the American Society for Bone and Mineral Research nomenclature and guidelines (15) and the evaluation methods previously described (16–19).

Serum Lipid Assays. Blood samples were allowed to clot at 4°C for 2 hours and then centrifuged at 2,000 × g for 10 minutes. The serum was transferred to new tubes for lipid assays. Total serum cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride levels were measured using the Bayer Advia Reagent Packs and assayed on an ADVIA 1650 chemistry system analyzer (Bayer Diagnostics, Bayer Inc.).

Statistical Analysis. Data are expressed as the mean ± SE. All of the data were analyzed using a one-way analysis of variance with statistical software (Analyze-it Software, Ltd., United Kingdom). Pairwise comparisons between groups were performed using Fisher's protected least significant difference

post hoc test. P values of <0.05 were considered statistically significant.

RESULTS

Bone Mineral Density. The effect of 16 weeks of treatment with EXE, 17-H-EXE, and LET on lumbar spine BMD is illustrated in Fig. 2. Sixteen weeks after ovariectomy, BMD of the lumbar spine was 10.0% lower in OVX rats than in intact controls ($P < 0.005$). At 16 weeks after treatment, lumbar spine BMD was 7.4%, 14.8%, 12.9%, and 14.0%, higher in OVX animals given 20, 50, and 100 mg/kg EXE and 20 mg/kg 17-H-EXE, respectively, than in OVX controls (all P values < 0.001). Similar effects were observed on femoral BMD (Fig. 3). Sixteen weeks after ovariectomy, femoral BMD had decreased by 7.4% ($P < 0.01$ *versus* intact controls). Femoral BMDs were 5.9%, 7.7%, 7.9%, and 7.9% higher in OVX animals given 20, 50, and 100 mg/kg EXE and 20 mg/kg 17-H-EXE, respectively, than in OVX controls (all P values < 0.02). No significant changes were observed in lumbar spine and femoral BMD in OVX rats treated with LET compared with OVX controls.

Bone Resorption Marker Serum Pyridinoline. The preventive effects on bone loss of EXE were also correlated with serum PYD excretion, a biochemical marker of bone resorption. In the present experiment, ovariectomy resulted in a significant increase in serum PYD, suggesting an increase in bone loss. Sixteen weeks after ovariectomy, the serum PYD excretion was 44% higher in OVX rats than in intact controls ($P < 0.001$), suggesting excessive bone resorption in OVX rats; 20, 50, and 100 mg/kg EXE and 20 mg/kg 17-H-EXE reduced the ovariectomy-induced increase of PYD by 85.4%, 89.9%, 95.5%, and 100%, respectively (all P values < 0.001). No significant change was observed in PYD excretion in OVX rats with LET treatment compared with levels in OVX controls (Fig. 4).

Bone Formation Marker Serum Osteocalcin. Ovariectomy resulted in a significant increase in serum OC, a biochemical marker of bone formation. As seen in Fig. 5, 16 weeks after ovariectomy, serum OC was significantly increased by 21% in OVX rats compared with intact controls ($P < 0.001$). In OVX animals 20, 50, and 100 mg/kg EXE and 20 mg/kg 17-H-EXE
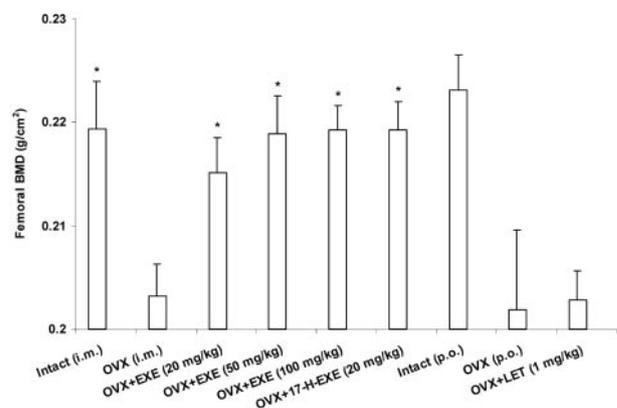

Fig. 3 BMD of the femora after 16 weeks of treatment with EXE, 17-H-EXE, and LET. *Scale bars* represent the mean ± SE (n = 12). ∗, $P < 0.05$ *versus* OVX controls.
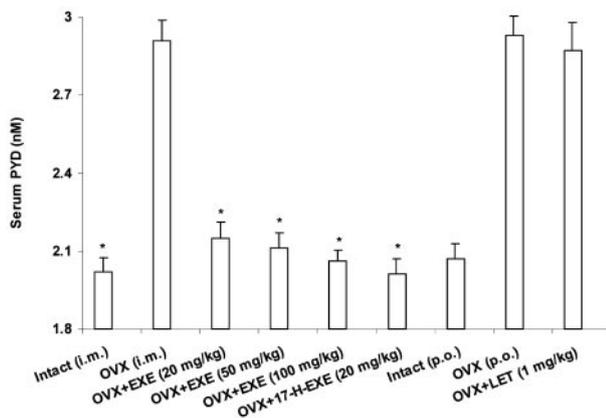
Fig. 4 Serum PYD levels after 16 weeks of treatment with EXE, 17-H-EXE, and LET. *Scale bars* represent the mean ± SE (*n* = 12). ∗, *P* < 0.001 *versus* OVX controls.
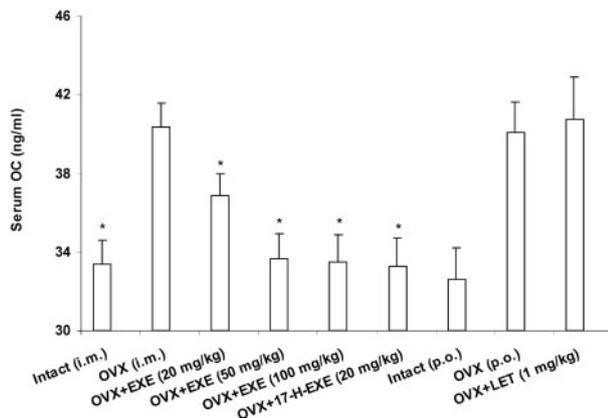

Fig. 5 Serum OC levels after 16 weeks of treatment with EXE, 17-H-EXE, and LET. *Scale bars* represent the mean ± SE (*n* = 12). ∗, *P* < 0.05 *versus* OVX controls.

reduced the ovariectomy-induced increase in serum OC by 50%, 96%, 98%, and 100% (all *P* values < 0.05), respectively. No significant change was observed in serum OC levels in OVX rats with LET treatment compared with OVX controls.

Mechanical Properties. Ovariectomy or administration of EXE and 17-H-EXE significantly affected the failure properties of the femur in the three-point bending test (Fig. 6). Ovariectomy caused a 14.8% decrease in three-point bending strength (*P* < 0.01 *versus* intact controls). The OVX animals given EXE and 17-H-EXE recovered the failure property that was diminished by ovariectomy. The OVX animals given 20, 50, and 100 mg/kg EXE and 20 mg/kg 17-H-EXE had a 15.4%, 18.8%, 17.3%, and 17.0% increase, respectively, in three-point bending strength compared with OVX controls (all *P* values < 0.01).

The effects of EXE and 17-H-EXE on the compressive strength of the fifth lumbar vertebra are shown in Fig. 7. Ovariectomy caused a 14.6% decrease in compressive strength (*P* < 0.05 *versus* intact controls), which recovered with administration of EXE and 17-H-EXE. Significant increases in compressive strength

of the fifth lumbar vertebra by 6.8%, 14.1%, 14.8%, and 14.7% (all *P* values < 0.05) were seen in the OVX rats given 20, 50, and 100 mg/kg EXE and 20 mg/kg 17-H-EXE, respectively.

No significant changes were observed in mechanical failure properties in OVX rats treated with LET compared with OVX controls (Fig. 6 and 7).

Bone Histomorphometry. A summary of the structural measures from the static histomorphometry is shown in Table 1. The OVX rats had a significantly lower trabecular bone volume, mineralized trabecular bone volume, and trabecular number and a higher trabecular separation than the intact controls (all *P* values < 0.001). OVX rats given 20, 50, and 100 mg/kg EXE and 20 mg/kg 17-H-EXE had a significantly higher trabecular bone volume, mineralized trabecular bone volume, and trabecular number and a lower trabecular separation than the OVX controls (all *P* values < 0.001). The eroded surface measure was significantly lower in OVX rats given 50 and 100 mg/kg EXE and 20 mg/kg 17-H-EXE than in OVX controls (all *P* values < 0.05). No significant difference was observed on the measures of trabecular thickness between the groups.

The osteoid results, a measure of bone formation, are also shown in Table 1. The osteoid volume and osteoid surface of the OVX rats were significantly higher than in the intact controls or in OVX rats given any of the doses of EXE or 17-H-EXE (all *P*
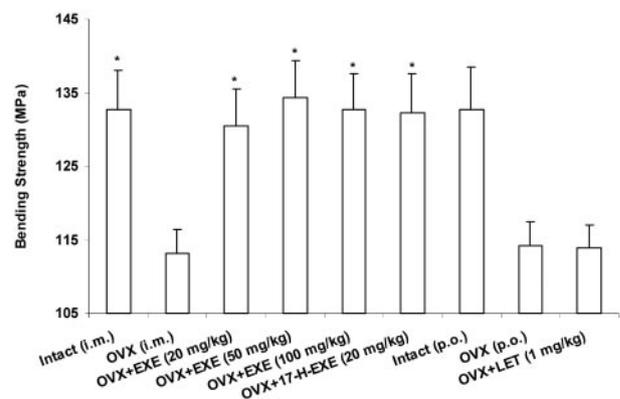

Fig. 6 Three-point bending strength of the femora after 16 weeks of treatment with EXE, 17-H-EXE, and LET. *Scale bars* represent the mean ± SE (*n* = 12). ∗, *P* < 0.01 *versus* OVX controls.
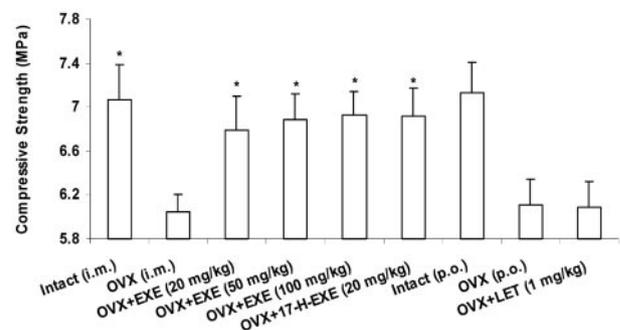

Fig. 7 Compressive strength of the fifth lumbar vertebrae after 16 weeks of treatment with EXE, 17-H-EXE, and LET. *Scale bars* represent the mean ± SE (*n* = 12). ∗, *P* < 0.05 *versus* OVX controls.

Table 1 Histomorphometric measures

Group	BV (%)	Md.V (%)	Tb.N (mm⁻¹)	Tb.Sp (μm)	Tb.Th (μm)	OV (%)	OS (%)	O.Th (μm)	*ES (%)*
Intact	16.93 ± 0.23	16.88 ± 0.24	2.83 ± 0.07	294.9 ± 8.0	60.02 ± 1.06	0.28 ± 0.05	3.85 ± 0.60	2.18 ± 0.13*	1.05 ± 0.25†
OVX	10.49 ± 0.78‡	10.11 ± 0.75‡	1.80 ± 0.09‡	502.3 ± 27.2‡	58.03 ± 2.29	3.52 ± 0.64‡	20.86 ± 2.62‡	4.89 ± 0.58	2.73 ± 0.63
OVX + EXE (20 mg/kg)	13.44 ± 0.67	13.52 ± 0.59	2.12 ± 0.10	411.8 ± 19.6	64.27 ± 1.33	0.77 ± 0.11	6.83 ± 0.86	4.03 ± 0.61	1.72 ± 0.61
OVX + EXE (50 mg/kg)	17.92 ± 0.90	17.84 ± 0.90	3.00 ± 0.17	277.8 ± 18.8	59.83 ± 0.73	0.43 ± 0.05	4.09 ± 0.62	3.32 ± 0.26*	1.11 ± 0.27†
OVX + EXE (100 mg/kg)	17.09 ± 1.11	17.04 ± 1.10	2.89 ± 0.14	289.7 ± 17.4	59.09 ± 2.69	0.31 ± 0.02	3.58 ± 0.33	2.81 ± 0.47*	1.02 ± 0.24†
OVX +17-H-EXE (20 mg/kg)	17.64 ± 0.92	17.56 ± 0.91	2.69 ± 0.16	311.72 ± 21.1	66.21 ± 3.92	0.40 ± 0.08	4.09 ± 0.51	3.27 ± 0.38*	1.14 ± 0.21†

NOTE. Data are mean ± SE (*n* = 5).

Abbreviations: BV, bone volume; Md.V, mineralized trabecular bone volume; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; OV, osteoid volume; OS, osteoid surface; O.Th, osteoid thickness; ES, eroded surface.

* $P < 0.005$ *versus* OVX rats.

† $P < 0.05$ *versus* OVX rats.

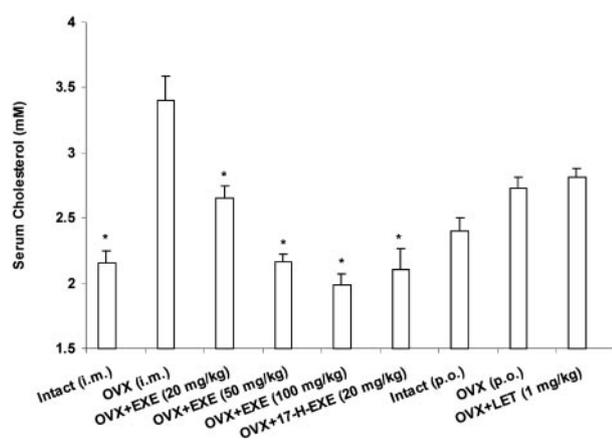‡ Significant difference from all other groups ($P < 0.001$).

Fig. 8 Serum cholesterol levels after 16 weeks of treatment with EXE, 17-H-EXE, and LET. *Scale bars* represent the mean ± SE (*n* = 12). *, $P < 0.005$ *versus* OVX controls.

values < 0.001). The osteoid thickness in the OVX rats was significantly higher than that in the intact controls or OVX rats given 50 and 100 mg/kg EXE and 20 mg/kg 17-H-EXE (all *P* values < 0.005).

Serum Lipids. The effects of EXE, 17-H-EXE, and LET on serum cholesterol are illustrated in Fig. 8. Sixteen weeks after ovariectomy, a 58% increase in total serum cholesterol was observed in OVX rats compared with levels in intact controls (*P* < 0.001). After 16 weeks of treatment, the administration of 20, 50, and 100 mg/kg EXE and 20 mg/kg 17-H-EXE to OVX rats caused a 22%, 36%, 42%, and 38% inhibition, respectively, of serum cholesterol levels compared with those in OVX controls (all *P* values < 0.002). The effects of EXE, 17-H-EXE, and LET on serum LDL levels are shown in Fig. 9. Sixteen weeks after ovariectomy, a 392% increase in LDL was observed in OVX rats compared with levels in intact controls (*P* < 0.001). The administration of 20, 50, and 100 mg/kg EXE and 20 mg/kg 17-H-EXE to OVX rats reduced LDL by >68% compared with OVX controls (all *P* values < 0.02). No significant change was observed in serum triglyceride levels in OVX rats treated with EXE or 17-H-EXE

(Table 2), nor were there significant changes in serum cholesterol, LDL, and triglyceride levels in OVX rats treated with LET compared with those in OVX controls (Figs. 8 and 9; Table 2).

DISCUSSION

Our results confirm the loss of BMD when OVX is performed. The rat has no peripheral aromatase; therefore, once
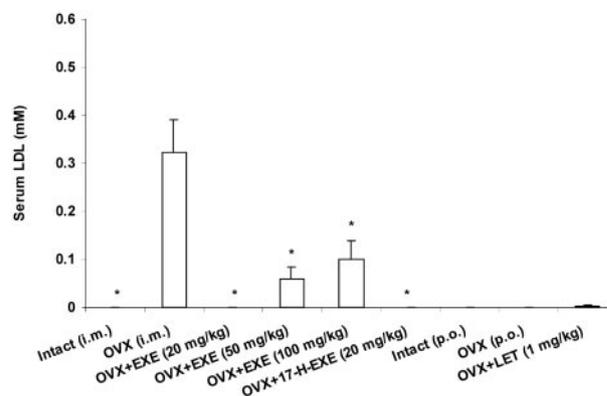

Fig. 9 Serum LDL levels after 16 weeks of treatment with EXE, 17-H-EXE, and LET. *Scale bars* represent the mean ± SE (*n* = 12). *, $P < 0.05$ *versus* OVX controls.

Table 2 Serum HDL and triglyceride levels

Group	HDL (mmol/L)	Triglycerides (mmol/L)
Intact (i.m.)	2.29 ± 0.06[a]	0.55 ± 0.03
OVX (i.m.)	2.65 ± 0.10	0.83 ± 0.07
OVX + EXE (20 mg/kg)	2.56 ± 0.08	0.77 ± 0.04
OVX + EXE (50 mg/kg)	1.82 ± 0.04[a]	0.87 ± 0.07
OVX + EXE (100 mg/kg)	1.64 ± 0.06[a]	0.71 ± 0.04
OVX +17-H-EXE (20 mg/kg)	2.04 ± 0.11[a]	0.91 ± 0.05
Intact (p.o.)	2.40 ± 0.09	0.70 ± 0.08
OVX (p.o.)	2.29 ± 0.08	0.67 ± 0.04
OVX + LET (1 mg/kg)	2.54 ± 0.06	0.87 ± 0.04

NOTE. Data are mean ± SE (*n* = 12).

Abbreviations: i.m., intramuscular injection; p.o., oral gavage.

* $P < 0.001$ *versus* OVX (i.m.) controls.

castrated, it is devoid of all estrogen. In this regard, the model is different from postmenopausal women, who have residual estrogen production from aromatization in skin, fat, muscle, and other organs. Thus, when giving a pure aromatase inhibitor to an animal that has undergone ovariectomy, one would not expect any further decrease in BMD compared with ovariectomy alone. Indeed this was the case for LET in our experiment. In contrast, EXE administration to the castrated animals demonstrated a dose-dependent protection against BMD reduction, as did its 17-H-EXE metabolite.

Estrogen withdrawal in the animals results in a marked increase in bone resorption, which is responsible for the reduction in BMD. EXE and its metabolite virtually abolished this ovariectomy-induced increase in bone resorption, as evidenced by the changes seen in serum PYD. It is known that increased bone turnover results in a decrease in BMD and is the cause of bone loss in postmenopausal osteoporosis (10).

In response to the increase in bone resorption induced by ovariectomy, one would expect to see an increase in bone formation in an attempt to prevent a fall in bone density. In our experiment, we saw the increase in bone formation marker induced by ovariectomy, which has been shown previously (20, 21). EXE reduced bone turnover, as reflected mainly by a decrease in the bone resorption marker in OVX rats. LET had no effect on this, again confirming a clear distinction between the two classes of drugs in OVX rats.

In humans, some discrepancy has been noted (for example, with raloxifene) between BMD changes and clinical fracture risk, suggesting that BMD is only in part a marker of actual bone strength and risk of fracture (22, 23). The maintenance of bone strength, as measured by the three-point bending test and vertebral compressive test, is confirmatory that EXE and its metabolite not only attenuate the loss of BMD and reduce bone turnover but also improve bone strength. The histomorphometric findings in the OVX *versus* OVX + EXE-treated animals confirm that trabecular bone volume and mineralized trabecular bone volume are preserved in the drug-treated animals, compatible with the findings mentioned above. It is known that androgens have a protective effect on bone metabolism (24–26). It would thus appear that EXE exerts its effects on bone via its androgenic properties. Dehydroepiandrosterone sulfate, another androgen, has a positive effect on bone metabolism in the female Sprague Dawley model used in this experiment, and these effects can be reversed with the androgen receptor blocker flutamide (8, 24–27).

In the present study, OVX animals given EXE and 17-H-EXE had a marked decrease in total serum cholesterol and LDL levels compared with OVX controls. Also, as shown in Table 2, OVX animals given EXE and 17-H-EXE had a significant reduction in serum HDL levels compared with OVX controls. To understand the changes in HDL in rats, one has to contrast the changes seen in the animals with those in humans. In both humans and rodents, estrogen lowers cholesterol by up-regulating the hepatic LDL receptor, thus resulting in increased removal of serum cholesterol from the circulation (28). This effect results in a preferential reduction of LDL cholesterol in humans. However, in the rat, both HDL and LDL cholesterol are reduced because rat HDL contains apoprotein E (not found in human HDL), which also binds to the hepatic LDL receptor (29). Thus, in the rat, as opposed to humans, HDL cholesterol is a predom-

inant form of circulating cholesterol, and estrogen therapy lowers both HDL cholesterol and LDL cholesterol. Thus the reduction in both HDL and LDL seen with EXE and 17-H-EXE in the OVX rat is in the desired direction.

Concern has been raised that estrogen deprivation may increase cardiovascular events in women treated with long-term aromatase inhibitors. Both serum cholesterol and LDL levels are raised in OVX rats. These rises are attenuated considerably by treatment with EXE and its metabolite. The mechanism for this is unclear, but in postmenopausal women treated with EXE, a fall in triglycerides without any change in other measures of lipid metabolism has been noted (30, 31). Improvement in this rat model in lipid measures has previously been demonstrated with the SERM EM-800 and, in that experiment, was thought to be related to the estrogenic action of the SERM on this pathway. No estrogenic effect of EXE has previously been demonstrated (32–34), and in a recent experiment by Chang *et al.* (35) from Duke University using a luciferase reporter gene attached to the estrogen receptor, no inherent estrogenic or progestogenic activity was demonstrated. The exact mechanism of this therefore remains to be elucidated. It is possible that the drug and its metabolite interfere with the peroxisome proliferator-activated receptor system, but additional studies are needed to confirm this. In human studies with letrozole of up to 4 months, two showed no significant effect (36, 37) and one showed increases in total cholesterol (TCHOL), LDL, TCHOL/HDL and LDL/HDL (38). Thus our findings here need to be more fully evaluated in postmenopausal women to determine whether they demonstrate a distinction from the nonsteroidal inhibitors because LET showed no protective effect against the lipid variables we measured in this experiment. In this regard, results of a placebo-controlled study of changes induced by EXE on lipid and bone measures in low-risk postmenopausal node-negative receptor-positive breast cancer patients conducted in Norway will be reported at the 2004 American Society of Clinical Oncology meeting in New Orleans in June 2004.

In summary, EXE produced a dose-dependent improvement in bone metabolism and bone strength, which was not the case with the nonsteroidal inhibitor LET. These changes were replicated by administration of the metabolite 17-H-EXE. Similarly, apparent dose-dependent protective effects were noted for EXE and its metabolite on some measures of lipid metabolism. These findings are of significant importance and need confirmation in humans. If, indeed, they are similar in postmenopausal women, they could influence the choice of aromatase inhibitor for these women. This apparently favorable therapeutic index of the steroidal aromatase inhibitor would be important, if confirmed, because the aromatase inhibitors are currently the most important new class of agents for treatment and possible prevention of breast cancer.

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Effects of the Steroidal Aromatase Inhibitor Exemestane and the Nonsteroidal Aromatase Inhibitor Letrozole on Bone and Lipid Metabolism in Ovariectomized Rats

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