The effect of estrogen on bone requires ERα in nonhematopoietic cells but is enhanced by ERα in hematopoietic cells

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Henning P, Ohlsson C, Engdahl C, Farman H, Windahl SH, Carlsten H, Lagerquist MK. The effect of estrogen on bone requires ERα in nonhematopoietic cells but is enhanced by ERα in hematopoietic cells. Am J Physiol Endocrinol Metab 307: E589–E595, 2014. —The effects of estrogen on bone are mediated mainly via estrogen receptor (ERα). ERαs in osteocytes (hematopoietic origin) are involved in the trabecular bone-sparing effects of estrogen, but conflicting data are reported on the role of ERα in osteoblast lineage cells (nonhematopoietic origin) for bone metabolism. Because Cre-mediated cell-specific gene inactivation used in previous studies might be confounded by nonspecific and/or incomplete cell-specific ERα deletion, we herein used an alternative approach to determine the relative importance of ERα in hematopoietic (HC) and nonhematopoietic cells (NHC) for bone mass. Chimeric mice with selective inactivation of ERα in HC or NHC were created by bone marrow transplantations of wild-type (WT) and ERα-knockout (ERα−/−) mice. Estradiol treatment increased both trabecular and cortical bone mass in ovariec-tomized WT/WT (defined as recipient/donor) and WT/ERα−/− mice but not in ERα−/−/WT or ERα−/−/ERα−/− mice. However, estradiol effects on both bone compartments were reduced (~50%) in WT/ERα−/− mice compared with WT/WT mice. The effects of estradiol on fat mass and B lymphopoiesis required ERα specifically in NHC and HC, respectively. In conclusion, ERαs in NHC is required for the effects of estrogen on both trabecular and cortical bone, but these effects are enhanced by ERαs in HC.

estrogen; estrogen receptor-α; bone marrow transplantation; trabecular bone; cortical bone

ESTROGEN REGULATES VARIOUS BIOLOGICAL PROCESSES, and one important target tissue is bone. The reduction in estrogen levels seen at menopause is strongly associated with postmenopausal osteoporosis, and low estrogen levels have also been associated with low bone mass and increased fracture risk in elderly men (8, 17, 26).

The effects of estrogen are mediated primarily via the nuclear estrogen receptors (ERs) ERα and ERβ. ERα is the main mediator of the trabecular and cortical bone protective effects of estrogen (2, 23). ERα expression is found both in nonhematopoietic cells (NHC), including the bone-forming osteoblasts and osteocytes, which are of mesenchymal origin, and in hematopoietic cells (HC), including the bone-resorbing osteoclasts (27). Treatment with estrogen has beneficial effects on bone mass but is associated with several side effects. Therefore, it is important to determine the target cell(s) mediating the positive ERα-mediated estrogenic effects on bone to be able to separate the positive estrogen effects on bone from the negative side effects.

To determine the primary ERα target cell for the effects of estrogen on bone, Cre-mediated cell-specific inactivation of ERαs in osteoblasts and osteoclast lineage cells have been used in several studies. Deletion of ERα in osteoclasts using cathepsin-Cre mice showed that ERαs in osteoclasts is important for the maintenance of trabecular bone in female mice, whereas cortical bone is unaffected (19). A subsequent study showed that deletion of ERα in myeloid cells, including monocytes, macrophages, and granulocytes in addition to osteoclasts, using LysM-Cre mice, also affects trabecular but not cortical bone in female mice (15). Collectively, these two studies demonstrate clearly that ERαs in osteoclasts is involved in the regulation of trabecular bone in females.

Several in vitro studies have suggested that ERαs in osteoblasts/osteocytes is also of importance in the regulation of bone metabolism (9, 11, 26), but it was only recently that the in vivo role of ERαs in osteoblast lineage cells was evaluated using in total six different Cre-mice in five independent studies (1, 14, 18, 22, 29). Almeida et al. (1) demonstrated that mice with inactivation of ERαs using Prx1-Cre mice (recombination in mesenchymal progenitors) or osterix1-Cre mice (recombination in osteoblast progenitors) had reduced cortical but not trabecular bone mass, whereas these two bone compartments were not affected when Col1α1-Cre mice (recombination in mature osteoblasts) was used for the inactivation of ERα, suggesting that osteoblast progenitors mediate the effect of estrogens on cortical but not trabecular bone. In contrast, three other studies demonstrated that the trabecular bone was reduced in mice with osteoblast-specific inactivation of ERα when both Runx2-Cre mice [recombination in early osteoblasts (22)] and osteocalcin-Cre mice [recombination in mature osteoblasts, (14, 18)] were used for the inactivation. In a fifth study, ERα was inactivated in late osteoblasts/osteocytes using Dmp1-Cre mice (29), and although gonadal intact female mice had normal trabecular bone mass, the estrogenic response to estradiol (E2) in ovariec-tomized (O VX) mice was reduced for this mouse strain. These studies demonstrate that there is an inconsistency regarding the role of ERα in osteoblast lineage cells for the regulation of bone homeostasis. It is possible that nonspecific or incomplete cell-specific inactivation of ERα for some of the used Cre-mouse models might have contributed to these conflicting results.

Herein, we used an alternative approach to determine the relative importance of ERαs in HC and NHC for the effect of estrogen on both trabecular and cortical bone. Chimeric mice with selective inactivation of ERαs in HC or NHC were created

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by bone marrow transplantations of wild-type (WT) and ERα-knockout (ERα−/−) mice and the estrogenic response in OVX mice was evaluated (Fig. 1A).

MATERIALS AND METHODS

Animals

All experimental procedures involving animals were approved by the Ethics Committee of the University of Gothenburg. Female total ERα-inactivated mice (ERα−/−) and WT littermates were used. ERα−/− mice have a deletion in exon 3 of the ERα gene and do not express any of the isoforms of the ERα protein. The total ERα−/− mice and corresponding WT (ERα+/+) littermates were inbred C57BL/6 mice and generated by breeding male ERα+/+ with female ERα+/+ mice that were obtained as described previously (3). Ovariectomy was performed at 10 wk of age through a midline incision of the skin and flank incisions of the peritoneum (Fig. 1). Surgery was performed under anesthesia with isoflurane (Pfizer, Täby, Sweden). Carprofen (OrionPharma, Espoo, Finland) was used postoperatively as an analgesic.

Preparation of Cells for Transplantation

Donor WT and ERα−/− mice were treated with enrofloxacin antibiotics (0.6 mg/ml drinking water; Baytril Vet, Bayer HealthCare, Copenhagen, Denmark) for 1 wk prior to euthanization. Bone marrow (BM) cells were obtained from freshly isolated femur and tibia by flushing from and hip/pelvic bone by crushing with mortar and pestle. Cells were placed in α-MEM medium and filtered through a 100-μm cellstrainer. Hematopoietic stem cells (HSC) were purified after centrifugation (1,500 rpm, 10 min) using the EasySep Mouse Hematopoietic Progenitor Enrichment Kit (Stemcell Technologies, Grenoble, France) according to kit instructions. Enriched HSCs were resuspended in PBS and adjusted to 1.25 × 10^6 cells/ml.

BM Transplantation

BM transplantation was performed 2 wk after ovariectomy (Fig. 1). OVX ERα−/− mice and WT littermates treated with enrofloxacin antibiotics in the drinking water (0.6 mg/ml; Baytril Vet) for 1 wk were lethally irradiated with 8.5 Gy and intravenously reconstituted with enriched HSCs (250,000 cells) from donor ERα−/− and WT mice, resulting in four groups: WT recipients receiving WT BM (WT/WT; n = 19), WT recipients receiving ERα−/− BM (WT/ERα−/−; n = 20), ERα−/− recipients receiving WT BM (ERα−/−/WT; n = 20), and ERα−/− recipients receiving ERα−/− BM (ERα−/−/ERα−/−; n = 18). Reconstituted mice were treated with enrofloxacin (0.6 mg/ml) and placed in new autoclaved cages every day for 2 wk to prevent infection during the aplastic phase.

Hormone Treatment

Two weeks after the BM transplantation (Fig. 1), the mice were inserted with a subcutaneous slow-release pellet (Innovative Research of America) with E2 (167 ng · mouse−1 · day−1) or placebo during isoflurane anesthesia. Four weeks after the start of treatment, the mice were anesthetized with Ketalar/Domitor (Pfizer), bled, and euthanized by cervical dislocation. Serum was stored individually at −20°C until use. Uterus, gonadal fat, and thymus were collected and weighed.

Assessment of Bone Parameters

High-resolution microcomputed tomography. High-resolution microcomputed tomography (μCT) analyses were performed on the distal femur by using an 1172 model μCT (Bruker MicroCT, Aartselaar, Belgium). The femurs were imaged with an X-ray tube voltage of 50 kV and current of 201 μA, with a 0.5-mm aluminum filter. The scanning angular rotation was 180° and the angular increment 0.7°. The voxel size was 4.48 μm isotropically. The NRRecon (version 1.6.9) was employed to perform the reconstruction following the scans. The cortical measurements in the femur were performed in the diaphyseal region of femur starting at a distance of 5.23 mm from the growth plate and extending a further longitudinal distance of 135 μm in the proximal direction. The trabecular bone proximal to the distal growth plate was selected for analyses within a conforming volume of interest (cortical bone excluded), commencing at a distance of 426 μm from the growth plate and extending a further longitudinal distance of 135 μm in the proximal direction. For bone mineral density analysis, the equipment was calibrated with ceramic standard samples.

Histomorphometry. Bone histomorphometry was applied to analyze trabecular bone in the vertebral body of undecalcified lumbar

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**Fig. 1.** Selective inactivation of estrogen receptor-α (ERα) expression in hematopoietic and nonhematopoietic cellular types. A: schematic presentation of the experimental setup. Lethally irradiated ovariectomized (OVX) wild-type (WT) and ERα-knockout (ERα-KO; ERα−/−) mice were reconstituted with hematopoietic progenitor cells from WT and ERα−/− mice, generating 4 groups, WT/WT (recipient/donor), WT/ERα−/−, ERα−/−/WT, and ERα−/−/ERα−/−, and treated with estradiol (E2; 167 ng · mouse−1 · day−1) or placebo for 4 wk. B and C: RNA was prepared from bone marrow (B) and spleen (C), and ERα expression was determined using real-time PCR. Values are presented as means ± SE; n = 9–10. ***p < 0.001 vs. WT/WT. Student’s t-test. BMT, bone marrow transplantation.
vertebrae L₃ stored in 70% EtOH. Each sample was dehydrated in an increasing series of EtOH concentrations, defatted in xylene, and embedded in methyl methacrylate (Technovit 9100 New; Heraeus Kulzer, Hanau, Germany). The embedded sample was sectioned longitudinally in a coronal plane using a fully motorized rotary microtome and a tungsten carbide knife. Plastic sections were obtained from three standardized sites of vertebral body cavity with a distance of 100 μm between adjacent sites. These three sites were named site A, site B, and site C. In each site, static parameters were determined in a 4-μm-thick section stained in Masson-Goldner’s Trichrome (3a). In each section, trabecular bone was analyzed using two adjacent fields with a total area of 1.5 mm². Images were captured using a Nikon Eclipse 80i light microscope connected to a Sony DXC-S500 video camera and analyzed using OsteoMeasure histomorphometry system (OsteoMetrics, Atlanta, GA), following the guidelines of the American Society for Bone and Mineral Research (2a).

Three-point bending. The femurs were fixed in formaldehyde for 2 days and after that stored in 70% ethanol. The biomechanics were analyzed with the three-point bending test (span length 55 mm, loading speed 0.155 mm/min) for the midfemur using the Instron universal testing machine (Instron 3366; Instron, Norwood, MA). The loading speed 0.155 mm/min) for the midfemur using the Instron universal testing machine (Instron 3366; Instron, Norwood, MA). The biomechanical parameters were calculated based on the recorded load deformation curves.

BM Cell Preparation and Flow Cytometry

BM cells were harvested from one femur using a syringe with 5 ml of PBS. Pelleted cells were resuspended in Tris-buffered 0.83% NH₄Cl solution to lyse erythrocytes and washed in PBS. The total number of leukocytes was analyzed using an automated cell counter (Sysmex, Hamburg, Germany). Cells were stained with V450-conjugated anti-B220 and analyzed using a FACSCanto instrument (Becton Dickinson, Oxford, UK). FlowJo software version 8.5.2 (Tree Star, Ashland, OR) was used for data analysis.

Real-Time PCR

RNA was isolated from BM and spleen using the RNaseasy kit (Qiagen, Chatswort, CA). The amplifications were performed using the StepOnePlus Real-Time PCR System (PE Applied Biosystems, Stockholm, Sweden), using Assay-on-Demand primer and probe sets (PE Applied Biosystems) labeled with the reporter fluorescent dye VIC. Designed primers and a probe labeled with the reporter fluorescent dye VIC, which was specific for 18S ribosomal RNA, were included as an internal standard. The assay identification number was ERα Mm00433147_m1.

Statistical Analyses

Values are given as means ± SE. The statistical difference between OVX + placebo and OVX + E₂ was calculated using Student’s t-test. The statistical differences in E₂ response between the WT/WT and the WT/ERα⁻/⁻ group were calculated by the interaction P value from a two-way-ANOVA that included donor genotype (WT or ERα⁻/⁻) and treatment (OVX + placebo or OVX + E₂).

RESULTS AND DISCUSSION

Generation of Chimeric Mice With Selective Inactivation of ERα Expression in HC and NHC

To create chimeric mice with selective inactivation of ERα in HC or NHC, WT and ERα⁻/⁻ mice were lethally irradiated and reconstituted with hematopoietic progenitor cells from WT and ERα⁻/⁻ mice, generating four groups: WT/WT (recipient/donor), WT/ERα⁻/⁻, ERα⁻/⁻/WT, and ERα⁻/⁻/ERα⁻/⁻ (Fig. 1A). ERα⁻/⁻ mice have increased circulating sex steroid levels due to disturbed negative feedback (12). Since sex steroids have been shown to protect HC against irradiation (5, 24), all mice were OVX prior to irradiation to prevent unequal protection of HC in WT and ERα⁻/⁻ recipients.

To verify successful BM transplantations, ERα expression was measured in BM and spleen, two hematopoietic tissues. ERα expression was decreased substantially in WT/ERα⁻/⁻ mice compared with WT/WT mice in both BM and spleen.

Fig. 2. Estrogenic response in uterus and gonadal fat is dependent on ERα expression in nonhematopoietic cells, whereas the effect on B cell frequency in bone marrow (BM) is dependent on ERα expression in hematopoietic cells. Lethally irradiated OVX WT and ERα⁻/⁻ mice were reconstituted with hematopoietic progenitor cells from WT and ERα⁻/⁻ mice, generating four groups: WT/WT (recipient/donor), WT/ERα⁻/⁻, ERα⁻/⁻/WT, and ERα⁻/⁻/ERα⁻/⁻ and treated with E₂ (167 ng·mouse⁻¹·day⁻¹) or placebo for 4 wk. A and B: uteri (A) and gonadal fat pads (B) were dissected and weighed at termination. C: B cell frequency (%B220-positive cells) was analyzed in BM using flow cytometry. Values are presented as means ± SE; n = 9–10. ***P < 0.01 and ****P < 0.001 vs. placebo, Student’s t-test.
Estrogenic Response in Uterus and Gonadal Fat is Dependent on ERα Expression in NHC, Whereas the Effect on B-Cell Frequency in BM is Dependent on ERα Expression in HC

The uterus is a primary estrogen organ, and unopposed estrogen treatment results in uterine cell proliferation and

(-93 and -82%, respectively, P < 0.05, Student's t-test), confirming that WT/ERα-/- mice were successfully reconstituted with ERα-/- hematopoietic progenitor cells (Fig. 1, B and C). Furthermore, ERα expression was not significantly altered between ERα-/-/WT mice and WT/WT mice in BM and spleen, demonstrating successful reconstitution of WT hematopoietic progenitor cells in the ERα-/- mice (Fig. 1, B and C).

Fig. 3. ERα in nonhematopoietic cells is required for the effects of estrogen on both cortical and trabecular bone, but these effects are enhanced by ERα in hematopoietic cells. Lethally irradiated OVX WT and ERα-/-/mice were reconstituted with hematopoietic progenitor cells from WT and ERα-/-/mice, generating 4 groups, WT/WT (recipient/donor), WT/ERα-/-, ERα-/-/WT, and ERα-/-/ERα-/-, and treated with E2 (167 ng·mouse·day) or placebo for 4 wk. A–C: the cortical bone parameters cortical area (Crt.Ar.; A), cortical thickness (Crt.thk.; B), and cortical density (Crt. Den.; C) were analyzed in a middiaphyseal scan of the femur using high-resolution CT. D: the maximal load at failure (Fmax) was analyzed by 3-point bending of the femur. E: representative images of the middiaphyseal part of the femur by high-resolution microcomputed tomography (μCT). F-H: the trabecular bone parameters bone volume over total volume (Trab. BV/TV; F), trabecular number (Trab.N.; G), and trabecular separation (Trab.Sp.; H) were analyzed by histomorphometry in vertebrae L4. I: representative images of the metaphyseal part of the distal femur by high-resolution μCT. Values are presented as means ± SE; n = 9–10. ***P < 0.001 vs. placebo, Student’s t-test; ##P < 0.05 and ###P < 0.01, 2-way ANOVA (interaction P value).
increased risk of endometrial cancer (7). The proliferative effect on the uterus has been shown to be mediated via ERα (13), and we demonstrated recently that the AF-1 domain of the EРα is important for this effect (2). Four weeks of treatment with E2 resulted in a 12-fold increase in uterine weight in both WT/WT mice and mice lacking ERα in HC (WT/ ERα−/−) compared with placebo treatment (Fig. 2A). In contrast, mice lacking ERα in NHC (ERα−/−/WT and ERα−/−/ ERα−/−) did not respond to E2 regarding uterine growth, demonstrating, as expected, that estrogenic effects on the uterus are mediated via ERα in NHC.

Estrogen regulates adipose tissue mass, and this regulation is mediated via ERα (4, 20). HC in the adipose tissue have been implicated in obesity (28), and we evaluated the importance of ERα expression in HC vs. NHC for the fat-reducing effect of E2 in OVX mice. E2 treatment resulted in a 2.2-fold decrease in gonadal fat weight in OVX WT/WT mice and a 2.5-fold decrease in OVX WT/ERα−/− mice compared with placebo treatment (Fig. 2B). In contrast, treatment with E2 did not have an effect on gonadal fat weight in ERα−/−/WT or ERα−/−/ ERα−/− mice, demonstrating that ERα expression in NHC is required for the estrogenic effects on fat mass in OVX mice. This finding is consistent with a report describing that hypothalamic EРα is crucial for the fat-reducing effect of estrogen in OVX mice (30). In contrast, it has been reported that lack of ERα in HC of gonadal intact female mice results in increased adipose tissue mass, suggesting that although ERα in NHC is crucial for the effect of estrogen on fat mass, ERα in HC might in some situations contribute to this effect (21).

Estrogen has profound effects on the immune system and is known to regulate B lymphopoiesis (16). This regulation is mediated via ERα, and it has been shown in males, using a BM transplantation approach, that the effect of estrogen on the regulation of B lymphopoiesis is mediated mainly via ERα expression in HC (25). We detected a significant E2-induced decrease in the frequency of B lymphocytes, estimated by measurement of B220-positive cells in BM, in mice with an intact ERα expression in HC (WT/WT and ERα−/−/WT mice), whereas no estrogenic response was detected in mice lacking ERα expression in HC (WT/ERα−/− and ERα−/−/ERα−/− mice) (Fig. 2C). Thus, our data demonstrated that ERα expression in HC is also required for the effect on B-cells in female mice.

**ERα Expression in NHC is Required for the Effects of Estrogen Treatment on Both Trabecular and Cortical Bone**

As described above, conflicting data have been reported previously on the role of ERα in NHC vs. HC for bone mass (1, 6, 14, 15, 18, 19, 22, 29). To determine the importance of ERα in HC vs. NHC, the bone phenotype was characterized thoroughly in our chimeric mice. Since deletion of ERα expression in specific cell types has been shown to affect trabecular and cortical bone differentially (1, 15, 19, 29), we used high-resolution μCT of the femur diaphysis and histomorphometry of the vertebrae (L4) to be able to separate the effects on the two bone compartments.

Cortical bone comprises >80% of the skeleton and has been suggested to be the main skeletal target for the physiological effects of estrogen (9). We investigated cortical bone parameters using high-resolution μCT, and E2 treatment resulted in increased cortical bone area and thickness in WT/WT (+82 and +111%, P < 0.001, respectively) and WT/ERα−/− mice (+44 and +52%, P < 0.001, respectively), whereas no E2 response was detected in ERα−/−/WT or ERα−/−/ERα−/− mice (Fig. 3, A and B). This finding of a crucial role of ERα in NHC for cortical bone mass in female mice is consistent with recent reports on cell-specific ERα inactivation in osteoblast lineage cells using three different Cre-mouse models (osterix-Cre, Prx1-Cre, and osteocalcin-Cre) (1, 14, 18), whereas this cortical bone effect was not seen when ERα was inactivated using the Col1α1 or DMP1-Cre mice (1, 29). Collectively, these studies provide strong evidence that ERα in early but not late osteoblasts is crucial for cortical bone in female mice. The augmenting effect of estrogen on cortical bone thickness in adult OVX female mice is mainly the result of an enhanced endosteal bone apposition (10). E2 treatment decreased the endosteal circumference in WT/WT (−28%, P < 0.001) and WT/ERα−/− mice (−12%, P < 0.05), whereas no effect on endosteal bone apposition was detected in mice lacking ERα expression in NHC (ERα−/−/WT and ERα−/−/ERα−/− mice) (Table 1). Periosteal circumference was unaffected by E2.
ERα in Nonthematopoietic Cells and Bone Mass

The cortical bone mineral density was unaffected by E2 treatment regardless of ERα expression in HC or NHC (Fig. 3C). The increase in cortical bone mass was associated with an increase in the maximal load at failure (F\text{max}) in WT/WT (+29%, \(P < 0.001\)) and WT/ERα\textsuperscript{-/-} (+22%, \(P < 0.001\)) mice, as measured by three-point bending, whereas no effect on the biomechanical properties of the bone was detected in mice lacking ERα expression in NHC (Fig. 3D). We also analyzed the cortical porosity and found it to be increased after E2 treatment in WT/WT (+369%, \(P < 0.001\)) and WT/ERα\textsuperscript{-/-} (+284%, \(P < 0.001\)) mice, whereas no E2 effect was detected in ERα\textsuperscript{-/-}/WT or ERα\textsuperscript{-/-}/ERα\textsuperscript{-/-} mice (Table 1). However, a subanalysis of the cortical porosity of the outer 50 μm of the bone shaft revealed unchanged porosity after E2 treatment between all four groups (Table 1). This may be explained by the fact that E2 treatment results in endosteal accumulation of trabecularized bone in the cortical bone shaft, leading to apparent increased cortical porosity (Fig. 3E and Table 1).

The trabecular bone volume per total volume (BV/TV) was increased significantly after E2 treatment in vertebrae of WT/WT mice (+208%, \(P < 0.001\)) and WT/ERα\textsuperscript{-/-} mice (+151%, \(P < 0.001\)), whereas no estrogenic effect was detected in mice lacking ERα expression in NHC (ERα\textsuperscript{-/-}/WT and ERα\textsuperscript{-/-}/ERα\textsuperscript{-/-} mice) (Fig. 3, F and I). Analysis of trabecular number (Trab.N.) and trabecular separation (Trab.Sp) revealed a similar pattern, with a significant increase in Trab.N. and decrease in Trab.Sp after E2 treatment in WT/WT (\(P < 0.001\)) and WT/ERα\textsuperscript{-/-} (\(P < 0.001\)) mice compared with placebo, whereas mice lacking ERα expression in NHC (ERα\textsuperscript{-/-}/WT and ERα\textsuperscript{-/-}/ERα\textsuperscript{-/-} mice) did not respond to E2 treatment (Fig. 3, G and H). These data demonstrate that ERα expression in NHC is required for E2 to be able to elicit a stimulatory response in trabecular bone. Cells within the osteoblast lineage are strong candidate NHC types for mediating the estrogenic effects on trabecular bone since it has been demonstrated in some (14, 18, 22, 29), but not all (1) recent reports, using Cre-mediated cell-specific ERα inactivation, that ERα in osteoblasts/osteocytes is involved in the regulation of trabecular bone mass.

ERα in HC Enhances the Stimulatory Effect of E2 on Both Trabecular and Cortical Bone

Our data demonstrate that ERα expression in NHC is required for E2 effects on both cortical and trabecular bone, whereas ERα expression solely in HC is not sufficient to generate an estrogenic response in either cortical or trabecular bone. Interestingly, in contrast to the similarity between the E2 effect in WT/WT and WT/ERα\textsuperscript{-/-} mice on uterine growth and gonadal fat reduction, the E2 response in WT/ERα\textsuperscript{-/-} mice was significantly decreased compared with the E2 response in WT/WT mice for both cortical (cortical thickness: −53%, \(P < 0.05\)) and trabecular (BV/TV: −27%, \(P < 0.01\)) bone parameters (Fig. 3). This suggests that ERα expression in HC enhances but is not required for estrogenic effects on both cortical and trabecular bone in female mice. Thus, ERα expression in HC potentiates the estrogenic bone effects, but this enhancing effect requires ERα expression in HC. Our result that the estrogenic response in trabecular bone is enhanced by ERα in HC is supported by the finding that female mice lacking ERα expression in osteoclast lineage cells have reduced trabecular bone mass (15, 19). However, we show that ERαs in HC enhance the E2 response not only on trabecular but also cortical bone, whereas the previous studies detected only protective effects of ERαs in myeloid cells/osteoclasts on trabecular bone. These conflicting results might be explained by the fact that other, nonmyeloid, HC are involved in the estrogenic effects on cortical bone or that the cell-specific inactivation of ERαs, using Cre-mediated inactivation, was partly incomplete.

Conclusion

ERα expression in NHC, but not in HC, is required for the effects of estrogen treatment on both trabecular and cortical bone in female mice. However, ERα in HC enhances the stimulatory effect of E2 on both bone compartments in the presence of ERα in NHC.

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DISCLOSURES

The authors have no conflicting financial interests.

Author Contributions


References


