17α-Estradiol prevents ovariectomy-mediated obesity and bone loss

Shivani N. Mann[a,b,c], **Kevin S. Pitel**[d], **Molly H. Nelson-Holte**[d], **Urszula T. Iwaniec**[e], **Russell T. Turner**[e], **Roshini Sathiaseelan**[a], **James L. Kirkland**[f], **Augusto Schneider**[g], **Katherine T. Morris**[h], **Subramaniam Malayannan**[d], **John R. Hawse**[d], **Michael B. Stout**[a,b,c,*]

[a]Department of Nutritional Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

[b]Oklahoma Center for Geroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

[c]Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

[d]Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA

[e]School of Biological and Population Health Sciences, Oregon State University, Corvallis, OR, USA

[f]Robert and Arlene Kogod Center on Aging, Rochester, MN, USA

[g]Faculdade de Nutrição, Universidade Federal de Pelotas, Pelotas, RS, Brazil

[h]Department of Surgery, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Abstract

Menopause is a natural physiological process in older women that is associated with reduced estrogen production and results in increased risk for obesity, diabetes, and osteoporosis. 17α-estradiol (17α-E2) treatment in males, but not females, reverses several metabolic conditions associated with advancing age, highlighting sexually dimorphic actions on age-related pathologies. In this study we sought to determine if 17α-E2 could prevent ovariectomy (OVX)-mediated detriments on adiposity and bone parameters in females. Eight-week-old female C57BL/6J mice were subjected to SHAM or OVX surgery and received dietary 17α-E2 during a six-week intervention period. We observed that 17α-E2 prevented OVX-induced increases in body weight and adiposity. Similarly, uterine weight and luminal cell thickness were decreased by OVX and

*Corresponding author at: 1200 N Stonewall Ave, Suite 3057, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73117, USA. michael-stout@ouhsc.edu (M.B. Stout).

prevented by 17α-E2 treatment. Interestingly, 17α-E2 prevented OVX-induced declines in tibial metaphysis cancellous bone. And similarly, 17α-E2 improved bone density parameters in both tibia and femur cancellous bone, primarily in OVX mice. In contrast, to the effects on cancellous bone, cortical bone parameters were largely unaffected by OVX or 17α-E2. In the non-weight bearing lumbar vertebrae, OVX reduced trabecular thickness but not spacing, while 17α-E2 increased trabecular thickness and reduced spacing. Despite this, 17α-E2 did improve bone volume/tissue volume in lumbar vertebrae. Overall, we found that 17α-E2 prevented OVX-induced increases in adiposity and changes in bone mass and architecture, with minimal effects in SHAM-operated mice. We also observed that 17α-E2 rescued uterine tissue mass and lining morphology to control levels without inducing hypertrophy, suggesting that 17α-E2 could be considered as an adjunct to traditional hormone replacement therapies.

1. Introduction

Aging is the primary risk factor for several chronic diseases. Interestingly, the rate of aging and emergence of specific diseases often differ between the sexes, which undoubtedly contributes to disparate life expectancies (Austad, 2006; Austad and Fischer, 2016). Numerous interventions that target pro-aging pathways also elicit sexually dimorphic responses (Austad and Bartke, 2015; Partridge et al., 2020). The molecular underpinnings responsible for these differential effects remain poorly understood, which often prevents the translation of these interventions into a clinical setting (Maklakov and Lummaa, 2013). One of the most robust sexually dimorphic responses to an interventional compound is observed with 17α-estradiol (17α-E2).

17α-E2 is a diastereomer of 17β-estradiol (17β-E2) (Ikeda et al., 2015; Toran-Allerand et al., 2005) that is naturally present in both mammalian sexes at very low levels (Dykens et al., 2005; Toran-Allerand, 2005; Courant et al., 2010). 17α-E2 has predominantly been studied as a neuroprotective hormone with mild to moderate effectiveness in models of ischemia, Alzheimer's, and Parkinson's diseases (Perez et al., 2005; Ozacmak and Sayan, 2009; Green and Simpkins, 2000; Levin-Allerhand et al., 2002). It was not until recently that the effects of 17α-E2 on systemic aging, longevity, and conditions that promote aging and reduce lifespan (e.g. obesity) were evaluated. In this timeframe, the NIA Interventions Testing Program (ITP) firmly established that 17α-E2 extends lifespan in male, but not female, mice at two different dietary doses (Harrison et al., 2014; Strong et al., 2016). Shortly thereafter, we reported that 17α-E2 treatment in aged male mice reverses several conditions associated with advancing age, including visceral adiposity, ectopic lipid accumulation, glucose intolerance, insulin resistance, and chronic low-grade inflammation (Stout et al., 2017a). We have also extended these studies into models of diet-induced obesity and genetically-induced hyperphagia and observed similar benefits, indicating links between the effects of 17α-E2 and hypothalamic anorexigenic pathways (Steyn et al., 2018). Garratt and colleagues have also reported similar findings in male mice, including improved glucose tolerance and

insulin sensitivity (Garratt et al., 2017), increased hepatic mTORC2 signaling (Garratt et al., 2017), and prolonged skeletal muscle preservation and physical function parameters (Garratt et al., 2019) with 17α-E2 treatment. Similar to the lifespan studies by the ITP, female mice failed to benefit from 17α-E2 in these studies, thereby highlighting the sex-specific nature of 17α-E2 actions.

Despite the collective evidence outlined above, it must be noted that most of the studies to date have evaluated health parameters that deteriorate to a greater degree in male mammals with advancing age (Garratt and Stout, 2018), including nutrient-sensing pathway perturbations, systemic metabolic parameters, and pro-inflammatory stress (Stout et al., 2017a; Steyn et al., 2018; Garratt et al., 2017; Mann et al., 2020; Garratt et al., 2018). In fact, it is well-established that female mammals possess an inherent advantage as compared to their male counterparts with regard to metabolic plasticity, immunological responses, and DNA damage (Austad and Fischer, 2016; Moran et al., 2013). This is known to be at least partially mediated by endogenous 17β-E2 due to its ability to beneficially effect a myriad of pathways and processes including glucose homeostasis, insulin sensitivity, immune cell migration and activation, and the mTOR signaling pathway in an estrogen receptor α (ERα)-dependent manner (Mauvais-Jarvis et al., 2013; Bian et al., 2019). Despite 17α-E2 having lesser binding affinity for ERα than 17β-E2 (Edwards and McGuire, 1980; Anstead et al., 1997), we have recently determined that ERα likely plays an important role in mediating 17α-E2 effects on health parameters in male mice (Mann et al., 2020). In light of these recent findings, coupled with the fact that no studies to date have explored the effects of 17α-E2 on female-dominant age-related conditions (e.g. bone loss), we hypothesized that the beneficial effects of 17α-E2 would be observed in models of menopause, specifically ovariectomy (OVX).

Menopause is a natural physiological process in older women due to cessation of ovulation leading to the loss of endogenous estrogen production (Gold, 2011), which is associated with an increasing incidence of obesity, diabetes, and osteoporosis (Khosla and Monroe, 2018; Carr, 2003). The greatly reduced estrogen production in OVX mice (Rogers et al., 2009) and postmenopausal humans (Nuutila et al., 1995; Lovejoy et al., 2008) abolishes the 17β-E2-mediated protective effects on metabolic and bone health. From a metabolic perspective, OVX mice and postmenopausal humans begin to display phenotypes reminiscent of age-matched males, including increased visceral adiposity, insulin resistance, and peritoneal inflammation (Mauvais-Jarvis et al., 2013; Mauvais-Jarvis, 2011), all of which promote type 2 diabetes onset. From a bone health perspective, 17β-E2 maintains bone density by modulating osteoclast and osteoblast activity in a manner that favors bone formation (Khosla et al., 2012). In the context of OVX and menopause, the balance between osteoclast and osteoblast homeostasis is disrupted, thereby promoting bone resorption.

The studies outlined herein aimed to determine if 17α-E2 could prevent OVX-mediated detriments on adiposity and bone parameters in mice. We found that 17α-E2 prevented OVX-induced increases in adiposity and deleterious alterations on bone density in the femur, tibia, and LV_5 vertebrae with minimal to no effects in SHAM-operated mice. We also determined that the dietary dose of 17α-E2 often used in males is not hyperproliferative in uterine tissues, thereby suggesting that 17α-E2 could conceivably be used as an adjunctive

therapy in humans displaying adverse health outcomes with traditional hormone replacement therapies.

2. Methods

2.1 Animal diets

TestDiet, a division of Purina Mills (Richmond, IN), prepared the diets for these studies. We used TestDiet 58YP (66.4% CHO, 20.5% PRO, 13.1% FAT) ± 17α-E2 (14.4 ppm; Steraloids, Newport, RI).

2.2 Animals and experimental design

Eight-week old female C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and housed five per cage at 22 ± 0.5 °C on a 12:12-hour light-dark cycle. Following a two-week facility acclimation, mice were randomized by body mass and adiposity to one of four groups: SHAM surgery (SHAM) + control (CON) diet; SHAM + 17α-E2 diet; OVX + CON diet; OVX + 17α-E2 diet. At ten weeks of age, mice were anesthetized (ketamine:xylazine [87 mg/kg:15.5 mg/kg]; 0.1 ml/20 g mouse wt. IP) and were prepared for surgery by removing the fur along the dorsal midline and swabbing with a 10% iodine solution. A dorsal midline skin incision was made caudal to the posterior border of the ribs. A second small incision was made through the posterior abdominal wall on each side of the animal to enter the abdominal cavity. For excision, each ovary was grasped gently using forceps and lifted from the abdominal cavity through the incisions and mosquito forceps were used to crush the fallopian tube and cranial-most part of the uterine horn distal to the ovary. The ovary was then removed by cutting above the clamped area. The uterine horn was returned into the abdomen cavity and the abdominal wall incision was closed using 6–0 Prolene stitches. The skin incision was closed using wound clips, which were removed 10–14 days following surgery. Mice were then returned to clean cages on heating pads and were immediately provided their respective treatment diets. Sham surgeries were performed in an identical fashion with the exception of crushing the fallopian tube/uterine horn and removing the ovaries. All mice had ad libitum access to food and water throughout the 6-week intervention. Body mass and composition were measured every other week throughout the intervention. Body composition was assessed by quantitative magnetic resonance using an EchoMRI-100H analyzer (Houston, TX) as previously described (Chen et al., 2016). Mice were euthanized using CO_2 prior to dissection. Tissues were excised, weighed, flash-frozen, and stored at −80 °C unless otherwise noted. The uteri were fixed in 4% PFA prior to being paraffin-embedded for future analyses. The left femur and 5th lumbar vertebrae (LV_5) were removed and fixed in 10% neutral-buffered formalin overnight. Formalin-fixed bones were transferred to 70% ethanol and stored at 4 °C until μCT scans were performed as outlined below. All studies were approved by the appropriate Institutional Animal Care and Use Committees (IACUC).

2.3 Assessing uterine luminal epithelial cell height

Immediately following sacrifice, uteri were collected, wet weights were recorded and tissue was subsequently fixed as described above. Following fixation, uteri were embedded in paraffin and processed following standard procedures. Longitudinal 5 micron sections were

prepared, placed on glass microscope slides and stained with hematoxylin and eosin (H&E). The height of luminal epithelial cells was determined at 10 different locations across each tissue section using a light microscope. Mean values were calculated for each individual animal and subsequently averaged among the indicated treatment groups.

2.4   pQCT analyses

pQCT of tibia was performed as previously described (Hawse et al., 2014). In brief, prior to sacrifice, mice were anesthetized and placed in supine position on a gantry using the Stratec XCT Research SA+, software version 6.20C (Stratec Medizintechnik Gmbh, Pforzheim, Germany). Tibia slice images were measured at 1.9 mm (corresponding to the proximal tibial metaphysis) and 9 mm (corresponding to the tibial diaphysis) from the proximal end of the tibia to obtain trabecular and cortical parameters, respectively.

2.5   μCT analyses

Micro-CT (μCT) was used for nondestructive three-dimensional evaluation of cortical and cancellous bone volume and architecture as previously described (Hawse et al., 2014). In brief, femurs and LV_5 were scanned in 70% ethanol at a voxel size of $12 \times 12 \times 12$ μm using a Scanco μCT40 scanner (Scanco Medical AG, Brüttisellen, Switzerland). Total femur lengths (mm) were determined and cortical bone was evaluated in 20 slices (0.24 mm) in the femoral midshaft. Direct cortical measurements included cross-sectional tissue volume (TV, mm^3), cortical volume (Ct.V, mm^3), marrow volume (Ma.V, mm^3), cortical thickness (Ct.Th, μm), and polar moment of inertia (I_{Polar}, mm^4). Total bone volume was also determined for LV_5. This was followed by evaluation of cancellous bone at the femur metaphysis and in the vertebral body. Cancellous bone measurements included bone volume/tissue volume (BV/TV, %), trabecular number (Tb.N, mm^{-1}), trabecular thickness (Tb.Th, μm), trabecular spacing (Tb.Sp, μm), and connectivity density (Conn.D, mm^{-3}).

2.6   Statistical analyses

Analyses of differences between groups were performed by two-way ANOVA or 2-way repeated measures ANOVA with Holm-Sidak post-hoc tests where appropriate using SigmaPlot 12.5 Software. All tests were two-tailed and values are presented as mean ± SEM with $p < 0.05$ considered significantly different.

3.   Results

3.1   17α-E2 prevents OVX-mediated increases in adiposity and uterine atrophy

We evaluated the effects of 17α-E2 on adiposity and uterine morphology in young female mice following SHAM or OVX surgeries. As expected, female mice subjected to OVX displayed robust increases in body mass, adiposity, and lean mass as compared to SHAM mice receiving the CON diet (Fig. 1a–c). These changes were completely attenuated by 17α-E2 administration in OVX females. Interestingly, both SHAM and OVX mice receiving 17α-E2 displayed similar body mass and adiposity levels as the SHAM CON group, thereby suggesting that 17α-E2 treatment elicits similar effects as endogenous estrogens (likely 17β-E2), yet is not particularly synergistic. This suggests 17α-E2 and 17β-E2 are likely competing for the same receptors in vivo, which we provide evidence for in other reports

(Mann et al., 2020). We also evaluated intra- (periovarian [POV]) and extra-peritoneal (inguinal [ING]) white adipose tissue (WAT) masses because a redistribution of lipid to ectopic sites occurs and intra-peritoneal WAT depots increase with aging (Stout et al., 2014; Stout et al., 2017b). We found that OVX nearly doubled POV and ING WAT masses in only six weeks and that 17α-E2 completely prevented these changes (Fig. 1d–e). As anticipated, uterine mass was also severely reduced by OVX, which was mirrored by uterine luminal epithelial cell height (Fig. 1f–h), a marker of GSM (Balica et al., 2017; Kim et al., 2015). 17α-E2 also prevented OVX-mediated decreases in uterine tissue, which is consistent with other studies in both young and middle-aged mice (Strong et al., 2016). Contrary to what we observed in mass and adiposity variables, SHAM mice receiving 17α-E2 did display a mild uterine hypertrophy phenotype. This suggests that endogenous estrogens and 17α-E2 may signal in the uterus through several receptors, thereby synergistically inducing growth.

3.2 17α-E2 prevents OVX-induced changes in cancellous bone in tibia and femur

The primary objective of this study was to determine if 17α-E2 can mitigate bone changes in mice following OVX surgeries. As expected, mice subjected to OVX displayed significant declines in total bone content and density at the tibial metaphysis (Fig. 2a–d). All of these changes were completely prevented in OVX mice receiving 17α-E2. In an effort to evaluate bone parameters with greater resolution, we also performed μCT analyses in the distal metaphysis region of femurs from these mice. The μCT scans revealed that OVX mice receiving 17α-E2 displayed higher BV/TV ratios (Fig. 2e). 17α-E2 treatment also resulted in higher trabecular number and lower trabecular spacing in OVX mice, with no changes in trabecular thickness (Fig. 2f–h). 17α-E2 prevented the deleterious effects of OVX on trabecular number and spacing. Furthermore, 17α-E2 also resulted in significantly higher connectivity density in both SHAM and OVX mice (Fig. 2i). Representative μCT images demonstrate differences in cancellous bone at the metaphyseal region of the femur following 17α-E2 treatment (Fig. 2j). These data clearly demonstrate that short-term 17α-E2 treatment can effectively attenuate bone changes associated with OVX.

3.3 17α-E2 elicits minimal effects on cortical bone in OVX female mice

In addition to evaluating cancellous bone, we also assessed cortical bone at the diaphysis region of the tibia and femur. In contrast to the effects described in Section 3.2 of OVX and 17α-E2 treatment on cancellous bone, cortical bone parameters were largely unaffected by surgery or treatment in this study. Neither OVX nor 17α-E2 treatment significantly altered the diaphyseal total content, cortical content, or cortical density within the tibia (Fig. 3a, c–d). Interestingly, OVX did reduce diaphyseal total density, which was prevented by 17α-E2 treatment (Fig. 3b). We also performed μCT analyses in the diaphyseal region of femurs from these mice. The only variable found to be altered by surgery or treatment in these analyses was femur length, which was significantly increased by OVX and prevented by 17α-E2 (Fig. 3e). No other variables in the femoral diaphysis were found to be altered by surgery or treatment in these analyses, including cross sectional tissue volume, cortical thickness, cortical volume, marrow volume, and polar moment of inertia (an index of bone strength in torsion) (Fig. 3f–j). This lack of differences among groups can be visually appreciated in representative μCT images (Fig. 3k). These data indicate that the short-term

effects of OVX and 17α-E2 elicit minimal changes in cortical bone parameters at the femur diaphysis.

3.4 17α-E2 prevents OVX-induced changes in cancellous bone in lumbar vertebra

Given that vertebral bone fractures are quite prevalent in post-menopausal women (Lips et al., 1999), we sought to determine if 17α-E2 could also elicit beneficial effects in the LV_5. As expected, 17α-E2 improved cancellous BV/TV ratios in the vertebrae of both SHAM and OVX mice (Fig. 4a). Mice subjected to OVX displayed significant declines in trabecular thickness, which was prevented by 17α-E2 treatment (Fig. 4c). Interestingly, OVX failed to modulate trabecular number and spacing in vertebral bone, although trabecular number displayed trending increases and trabecular spacing significantly decreased with 17α-E2 treatment regardless of surgical status (Fig. 4b, d). Connectivity density within the vertebrae was increased by OVX (Fig. 4e), but this may be due to an initial compensatory effect caused by estrogen deficiency (Yao et al., 2005). 17α-E2 treatment did prevent this phenotype from further increasing in the context of OVX, and 17α-E2 was able to increase connectivity density under intact-SHAM conditions, suggesting that 17α-E2 is able prevent OVX-induced reductions in bone density in LV_5 similarly to our observations in the femur. Representative μCT images depict vertebral trabecular bone following SHAM or OVX and CON or 17α-E2 treatment (Fig. 4f). These data demonstrate that short-term 17α-E2 treatment does, indeed, prevent some deleterious effects associated with OVX in vertebral bone, which is aligned with our results from the tibia and femur.

4. Discussion

17α-E2 has recently been shown to induce considerable benefits on healthspan and lifespan in male mice (Harrison et al., 2014; Strong et al., 2016; Stout et al., 2017a; Garratt et al., 2017; Mann et al., 2020; Garratt et al., 2018), although little benefit has been observed in females receiving the compound (Garratt et al., 2017; Garratt et al., 2018). We have postulated that the lack of beneficial effects of 17α-E2 in females may be due to the high levels of endogenous 17β-E2, which may outcompete the less-potent 17α-E2 at estrogen receptors (Mann et al., 2020). In this report we sought to determine if 17α-E2 could elicit beneficial outcomes on age-related phenotypes associated with ovariectomy in female mice, with a specific emphasis on bone loss. In human females, bone loss is mechanistically linked to a lack of endogenous estrogen production that occurs during the menopausal transition, and is a significant contributor to morbidity (Kim et al., 2015). Given that it elicits such robust sexually dimorphic responses in mice, studies employing 17α-E2 can provide critical insight into the mechanisms underlying the differences in aging biology between the sexes. To our knowledge, the effects of 17α-E2 on bone dynamics has not been previously evaluated, although our recent work shows that 17α-E2 induces metabolic benefits through ERα (Mann et al., 2020), a receptor known to be highly involved in bone homeostasis (Khosla and Monroe, 2018). This led us to hypothesize that 17α-E2 would induce beneficial effects in OVX mice, which is a model for post-menopausal bone loss in humans.

The most prominent effect observed in the current study was the ability of 17α-E2 to prevent OVX-related reductions in cancellous bone density. To our knowledge, this is the

first report to demonstrate that 17α-E2 can mitigate a female dominant age-related disease condition. This finding also supports our previous observations that 17α-E2 acts through ERα (Mann et al., 2020) due to the significant role of ERα in modulating bone turnover rates (Rooney and van der Meulen, 2017; Windahl et al., 2013; Kondoh et al., 2014). Studies utilizing multiple bone cell specific ERα knockout mouse models displayed decreased bone mass in females and males (Rooney and van der Meulen, 2017). ERα is involved in osteoblast proliferation (Galea et al., 2013) and also promotes osteoclast apoptosis through FasL cleavage by Mmp3 (Garcia et al., 2013; Krum et al., 2008). Similarly, osteocyte ERα is known to mediate trabecular bone formation through modulation of WNT signaling, a critical mediator of skeletal homeostasis. These studies, coupled with our observations, suggest that 17α-E2 may be signaling at least partially through ERα in bone to ameliorate OVX-induced dyssynchronous osteoclast and osteoblast activity. Contrary to the prominent effects of 17α-E2 on cancellous bone, we observed a general lack of effects of 17α-E2 in diaphyseal cortical bone. This observation was not incredibly surprising because we also found that cortical bone was essentially unaffected by OVX in this study. The remodeling rate for cortical bone is significantly lower than that of cancellous bone due to having largely reduced surface area per unit bone, which renders it less responsive to short-term alterations following interventions such as OVX or drug treatments (Burghardt et al., 2010; Seeman, 2013). A few notable cortical bone parameters were altered in this study. For instance, diaphyseal total density was reduced with OVX and this was prevented by 17α-E2. This decreased density was consistent with other short-term OVX studies (Fanti et al., 1998) and demonstrates that 17α-E2 is able to induce beneficial effects on diaphyseal bone. We also found that OVX resulted in greater femur length, which is commonly observed following OVX due to endogenous estrogens modulating osteoblast activity at the growth plate (Minematsu et al., 2001). Importantly, 17α-E2 prevented increases in femur length, which suggests that 17α-E2 may be acting through ERα similarly to 17β-E2 to regulate growth plate closure (Borjesson et al., 2012); a possibility that will need to be explored through future studies. Collectively, these data indicate that 17α-E2 can beneficially modulate bone turnover dynamics when endogenous estrogens are reduced or eliminated. Given the strong involvement of ERα on bone homeostasis, coupled with our recent report demonstrating the importance of ERα for 17α-E2 signaling, we surmise that 17α-E2 also modulates bone parameters through ERα. Future studies employing OVX in ERα knockout models will be needed to confirm this speculation.

Not only does menopause adversely affect bone homeostasis, it also promotes an increase in overall body fat and central adiposity (Carr, 2003; Christensen and Pike, 2015). As alluded to above, we have previously reported that 17α-E2 dramatically reduces adiposity in male mice (Stout et al., 2017a; Steyn et al., 2018; Mann et al., 2020). In this study, we also found that 17α-E2 can prevent OVX-related gains in adiposity in females. The reduction in periovarian WAT is of particular importance due to intraabdominal adiposity being closely associated with systemic declines in metabolic homeostasis. Several studies have clearly demonstrated that menopausal-related increases in central adiposity promote glucose intolerance, insulin resistance, dyslipidemia, and increased risk for type 2 diabetes (Carr, 2003). Interestingly, all of these metabolic perturbations are male dominant prior to menopause in females, suggesting that estrogens serve a protective role not only in

metabolic homeostasis, but also potentially on systemic aging processes that are not limited to specific organ systems. Our current findings also support the idea that 17α-E2 likely competes with endogenous 17β-E2 in female mice due to the minimal beneficial effects of 17α-E2 in intact and/or unchallenged females. ERα knockout female mice are known to display increased fat pad masses and adipocyte size (Heine et al., 2000), and we previously demonstrated that ERα is required for fat loss by 17α-E2 in intact male mice (Mann et al., 2020). This suggests that 17α-E2 may beneficially modulate adiposity in OVX females through ERα-mediated mechanisms, which could potentially be related to estrogenic upregulation of antioxidant enzymes and suppression of mitochondrial reactive oxygen species production (Borras et al., 2010; Bonaccorsi et al., 2018; Yang et al., 2014). Although we did not directly evaluate oxidative stress or systemic metabolic parameters in this study, we surmise that longer term studies including a battery of mitochondrial assessments and/or metabolic-related outcomes would prove fruitful in determining if 17α-E2 can mitigate OVX-related metabolic declines similarly to the observed adiposity reductions. However, it must be noted that Garratt et al. recently reported that long-term treatment with 17α-E2 failed to reduce adiposity or improve glucose homeostasis parameters in female mice subjected to OVX (Garratt et al., 2017; Garratt et al., 2019). This suggests that competition between 17α-E2 and 17β-E2 may not completely explain the lack of effects of 17α-E2 in intact females, or that lifelong ablation of endogenous estrogen production induces compensatory physiological responses that limits 17α-E2 signaling capabilities. Future studies evaluating metabolic parameters in a longitudinal fashion following OVX and 17α-E2 treatment are necessary for definitive conclusions to be drawn.

The loss of endogenous estrogen production in females is also known to induce deleterious changes in uterine morphology. These include reductions in uterine mass and thinning of the uterine epithelial lining, promoting the genitourinary syndrome of menopause (GSM) which is characterized by vaginal bleeding and discomfort, increased urinary tract infections, and declines in quality of life (Carr, 2003; Kim et al., 2015). We found that 17α-E2 treatment prevents both the loss of uterine mass and thinning of the uterine luminal epithelia. Similarly, other lifespan extending treatments, including caloric restriction (CR) and rapamycin, are also known to preserve ovarian morphology and function and endogenous estrogen production in female mice (Garcia et al., 2019; Sukur et al., 2014). Importantly, 17α-E2 did not induce hyperproliferation of uterine tissue in intact or OVX mice, which has been observed with 17β-E2 administration (Zhu and Pollard, 2007). It is known that 17β-E2 effects uterine luminal epithelial cells through non-genomic actions of membrane-bound ERα and activation of PI3K/AKT signaling (Kazi et al., 2009; O'Brien et al., 2006). Although we recently established that 17α-E2 elicits similar genomic activity through ERα to that of 17β-E2 (Mann et al., 2020), it remains unclear if any of the effects of 17α-E2 can be attributed non-genomic actions. Future studies utilizing membrane-only ERα (MOER) and nuclear-only ERα (NOER) models (Allard et al., 2019) would provide further insight into the functionality of 17α-E2 in the uterus. Given that CR is known to protect against age-related deleterious changes in the uterus, it must be noted that 17α-E2 does moderately reduce food intake by modulating hypothalamic feeding mechanisms (Stout et al., 2017a; Steyn et al., 2018). Therefore, some of the uterine benefits may be mediated through secondary mechanisms that are unrelated to direct signaling in OVX mice. Additional future

studies employing OVX and pair-feeding paradigms will be needed to unravel these intersecting mechanisms.

There are a few of notable limitations to this study. First, we used young mice, which limits the scope of interpretation because they were growing animals at the time of OVX and treatment initiation. Second, this was a relatively short interventional period, which may explain the lack of effects observed in diaphyseal cortical bone. Longer studies have demonstrated changes in cortical bone parameters following OVX (Edwards et al., 1992; Jee et al., 1990; Rosales Rocabado et al., 2018), therefore it cannot be definitively concluded that 17α-E2 has limited effects in cortical bone. Third, this study does not provide clear mechanistic insight into how 17α-E2 modulates uterine and/or bone parameters in female mice, therefore additional studies will be needed to explore the involvement of ERα or other signaling mechanisms. Future studies would also benefit greatly by implementing a 17β-E2-treated positive control group and by employing histomorphometry assessments to identify potential differences between osteoblast, osteoclast, and osteocyte activity following 17α-E2 or 17β-E2 treatment. In contrast, the strengths of the study include the demonstration that 17α-E2 is able to prevent OVX-related obesity and deleterious effects in bone and uterus. Perhaps more importantly, this report is the first to establish that 17α-E2 can improve age-related health parameters that are female-dominant in the context of OVX, thereby supporting the idea that endogenous estrogens may curtail 17α-E2 actions and explain sexually-divergent responsiveness to the compound.

Acknowledgements

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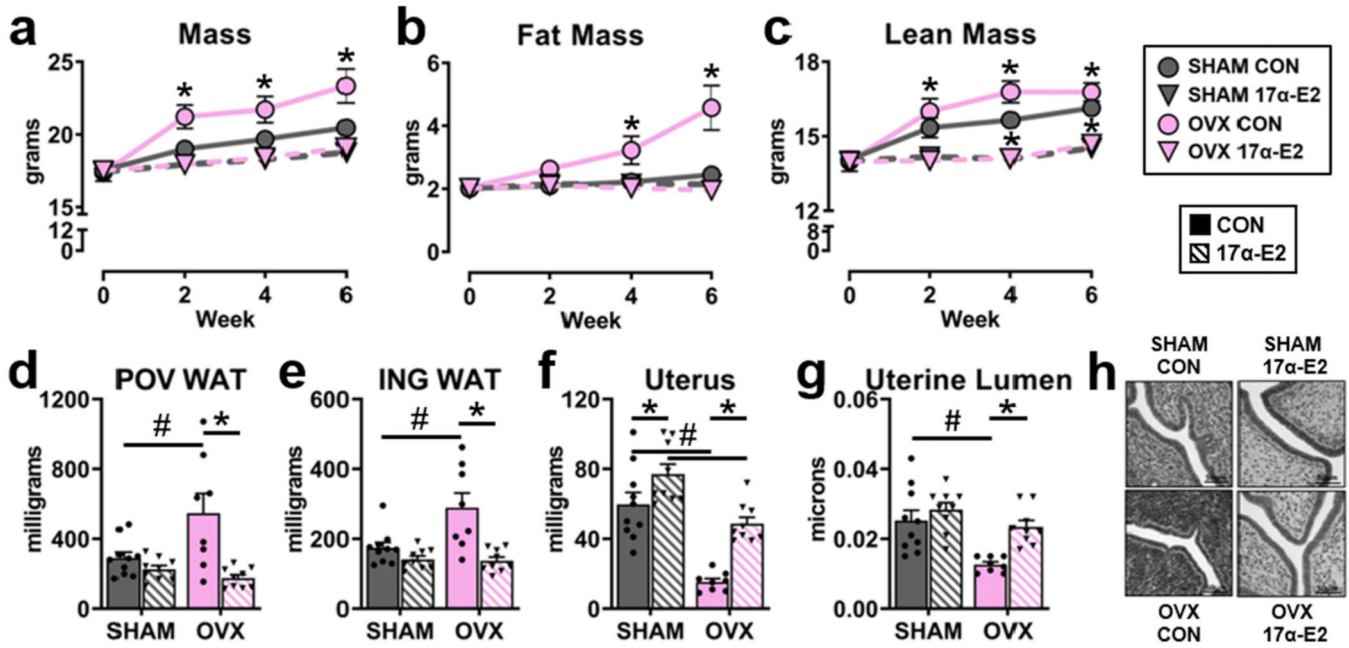
Fig. 1.

17α-E2 prevents OVX-mediated changes in adiposity and uterine morphology. (a) Total body mass, (b) fat mass, and (c) lean mass over 6 weeks of female SHAM and OVX mice treated with CON or 17α-E2 post-surgery. (d) Peri-ovarian white adipose tissue (WAT) mass and (e) Inguinal WAT mass at 6 weeks post-surgery. (f) Total uterine mass and (g) luminal cell height at 6 weeks post-surgery. (h) Representative images of H&E stained uteri at 6 weeks post-surgery and treatment with CON or 17α-E2. All data are presented as mean ± SEM and were analyzed by 2-way repeated measures ANOVA (a–c) or 2-way ANOVA (d-g) with Holm-Sidak post-hoc tests. *#p < 0.05. * indicates significance between CON and 17α-E2 within the same surgical condition. # indicates significance between SHAM and OVX within the same dietary treatment. n = 10 (SHAM CON), 9 (SHAM 17α-E2), 8 (OVX CON), 9 (OVX 17α-E2).
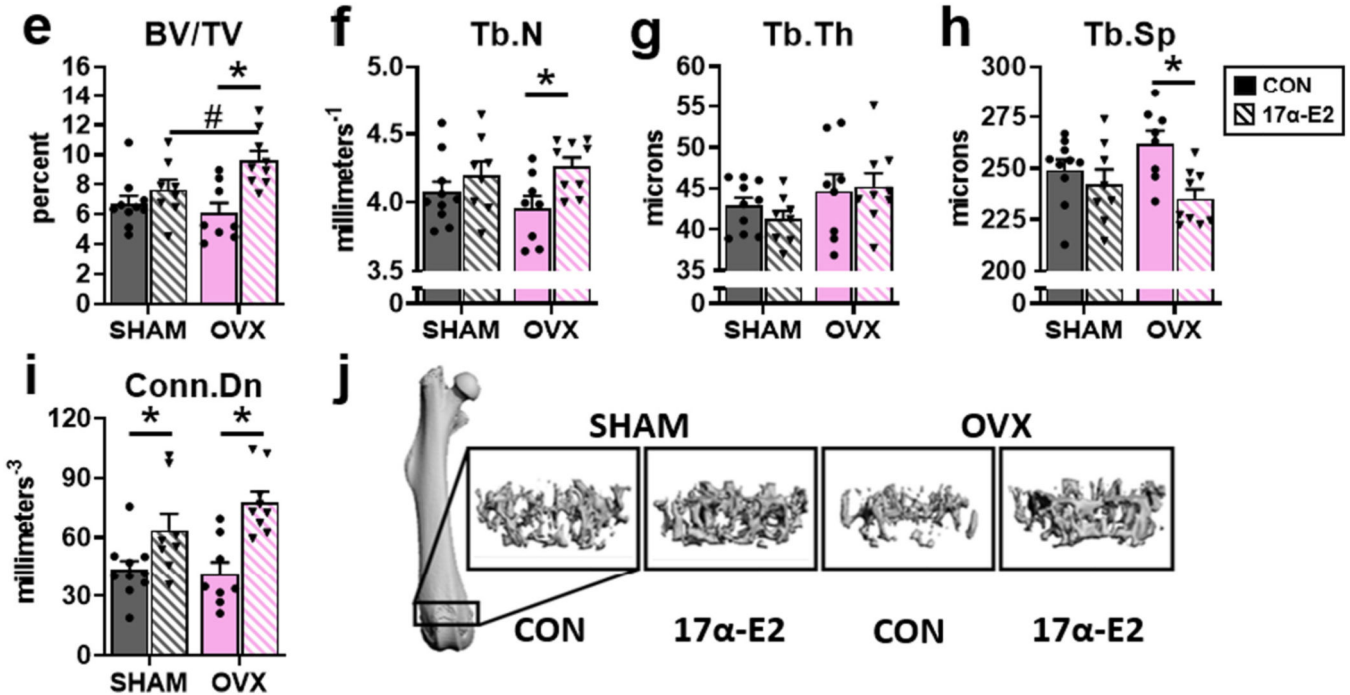
Tibia Cancellous pQCT
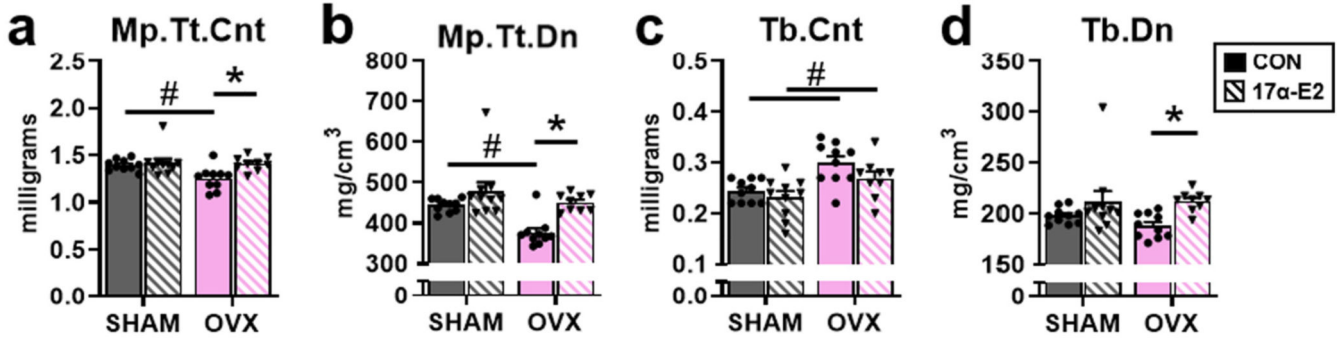
Femur Cancellous μCT

Fig. 2.
17α-E2 prevents OVX-mediated loss of trabecular bone in the metaphysis region of leg bones. (a) Metaphysis total content [Mp.Tt.Cnt], (b) metaphysis total density [Mp.Tt.Dn], (c) trabecular content [Tb.Cnt], and (d) trabecular density [Tb.Dn] measured via pQCT in tibia metaphysis cancellous bone at 6 weeks post-SHAM (grey) or OVX (pink) surgery and CON (solid) or 17α-E2 (striped) treatment. (e) Bone volume in relation to tissue volume [BV/TV], (f) average number of trabeculae per unit length [Tb.N], (g) average trabecula thickness [Tb.Th], (h) spacing between trabecula [Tb.Sp], and (i) connectivity density of trabeculae [Conn.Dn] measured via μCT in femur metaphysis at 6 weeks post-SHAM or OVX surgery and CON or 17α-E2 treatment. (j) Representative μCT images of cancellous bone in each treatment group. All data are presented as mean ± SEM and were analyzed by 2-way ANOVA with Holm-Sidak post-hoc tests. *#p < 0.05. * indicates significance

between CON and 17α-E2 within the same surgical condition. [#] indicates significance between SHAM and OVX within the same dietary treatment. n = 10 (SHAM CON), 8–10 (SHAM 17α-E2), 8–10 (OVX CON), 9 (OVX 17α-E2).
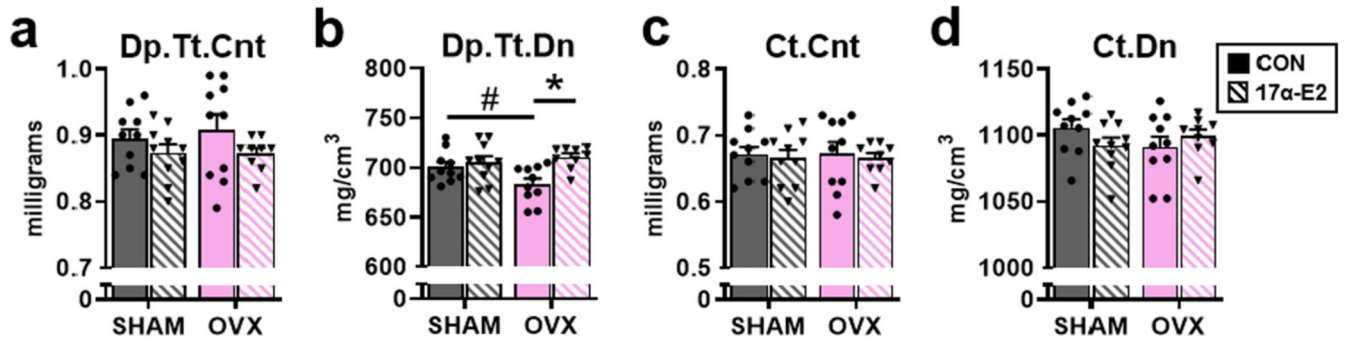
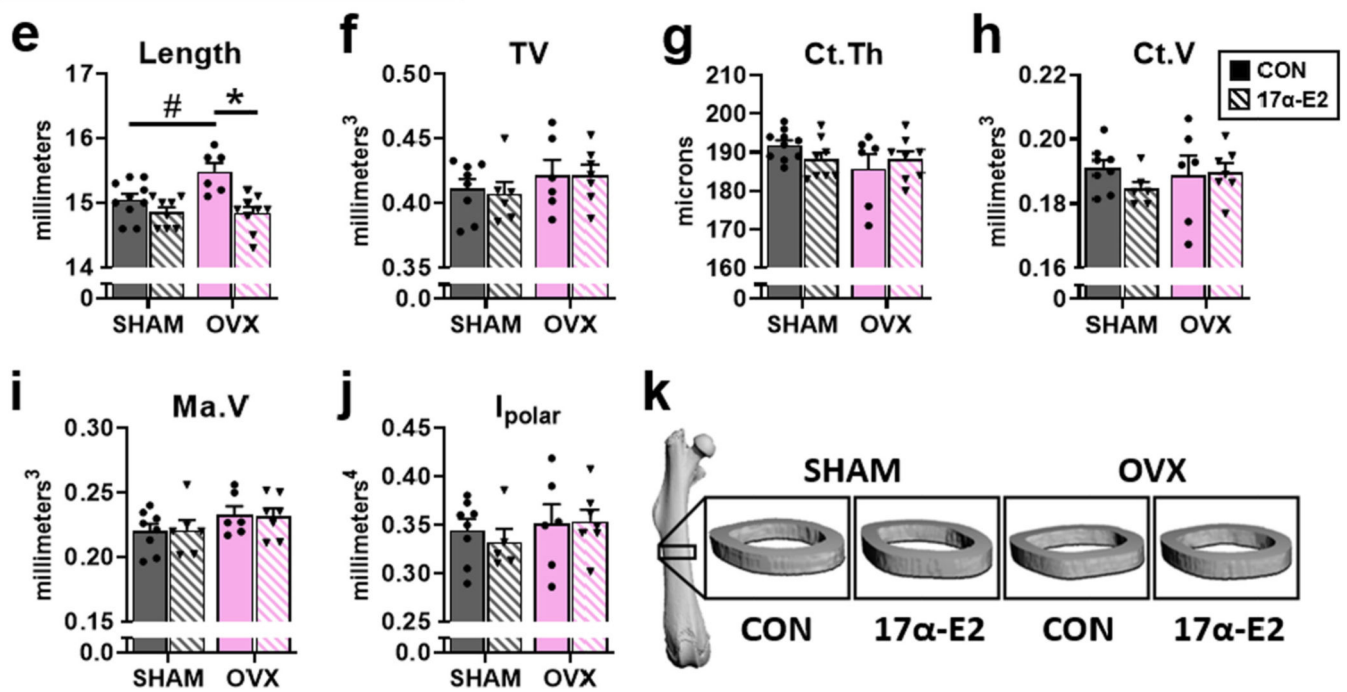# Tibia Cortical pQCT

Femur Cortical μCT

Fig. 3.
OVX and 17α-E2 have minimal effects on diaphysis bone parameters in leg bones.
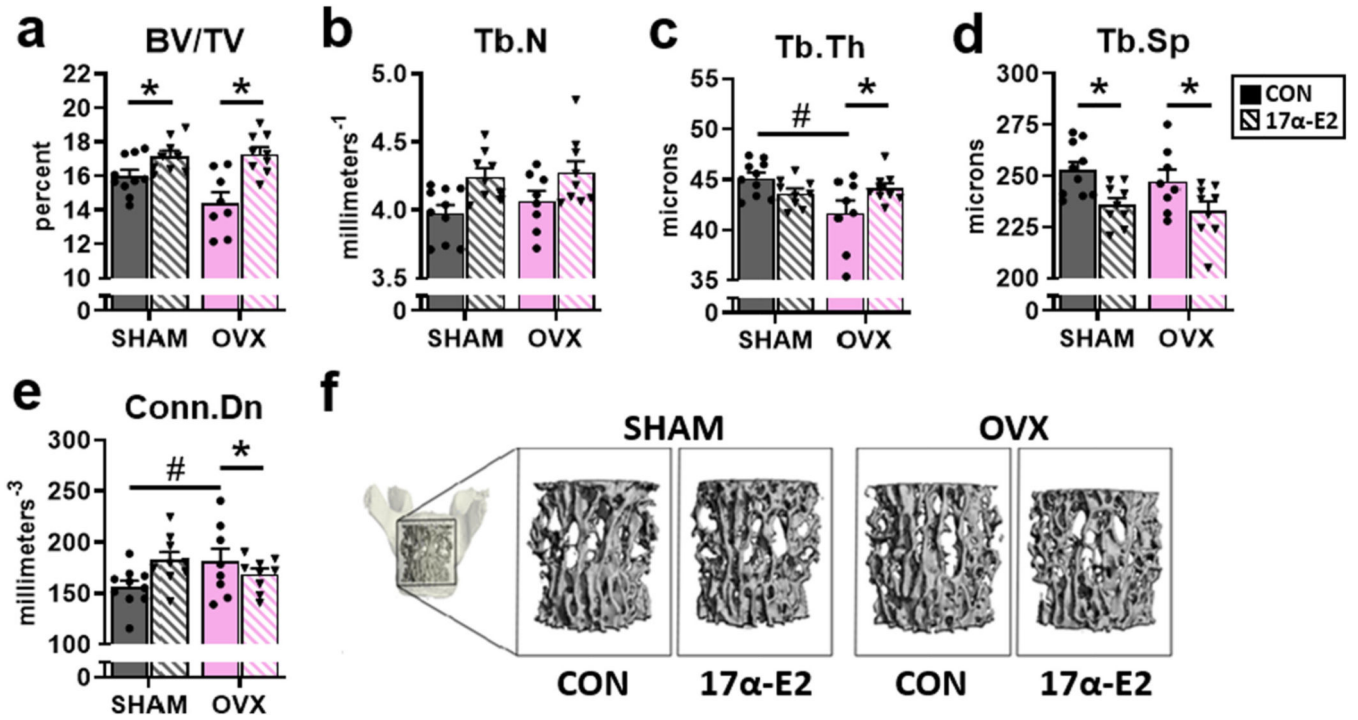
Fig. 4.

17α-E2 prevents OVX-mediated loss of vertebral trabecular bone. (a) Bone volume in relation to tissue volume [BV/TV], (b) trabecular number [Tb.N], (c) trabecular thickness [Tb.Th], (d) trabecular spacing [Tb.Sp], and (e) connectivity density [Conn.Dn] of LV_5 measured via μCT at 6 weeks post-SHAM (grey) or OVX (pink) surgery and CON (solid) or 17α-E2 (striped) treatment. (f) Representative μCT images of cancellous bone in LV_5. All data are presented as mean ± SEM and were analyzed by 2-way ANOVA with Holm-Sidak post-hoc tests. *#$p < 0.05$. * indicates significance between CON and 17α-E2 within the same surgical condition. # indicates significance between SHAM and OVX within the same dietary treatment. n = 10 (SHAM CON), 9 (SHAM 17α-E2), 8 (OVX CON), 9 (OVX 17α-E2).