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# Gestational and lactational exposure to BPA or BPS has minimal effects on skeletal outcomes in adult female mice

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ABSTRACT

Bisphenol-A (BPA) and bisphenol-S (BPS) are estrogen disrupting chemicals (EDCs) found in the environment and common household items. Estrogen is a primary hormonal regulator of bone growth and development; however, the impact of gestational BPA or BPS exposure on skeletal health of offspring remains relatively unknown. In this longitudinal study, adult female mice were randomized into three groups: 200 μg BPA/kg BW (BPA), 200 μg BPS/kg BW (BPS) or control (CON). Animals in each group were further randomized to exercise treatment (EX) or sedentary (SED) control, resulting in six overall groups. BPA/BPS/CON and EX/SED treatment were initiated prior to mating and continued through mating, gestation, and lactation. One female offspring from each dam ($n$ = 6/group) was assessed at 17 weeks of age to evaluate effects of EDC exposure on the adult skeleton. Cortical geometry of the mid-diaphysis and trabecular microarchitecture of the distal femur were assessed via micro-computed tomography. Biomechanical strength and mineral apposition rate of the femoral diaphysis were assessed via three-point bending and dynamic histomorphometry, respectively. Sclerostin expression was measured using immunohistochemistry. Two-factor ANOVA or ANCOVA were used to determine the effects of maternal exercise and BPA or BPS exposure on trabecular and cortical bone outcomes, respectively. Consistent with prior studies, there were no significant differences in body weight, femoral length, cortical geometry, trabecular microarchitecture, or biomechanical strength between groups in female offspring. In conclusion, gestational BPA exposure and maternal exercise have minimal impact on skeletal outcomes in female adult offspring.

## 1. Introduction

Bisphenols are endocrine disrupting chemicals (EDCs) found in plastics that can have significant impacts on human health (Rochester, 2013; Rochester and Bolden, 2015).Human studies have associated bisphenol-A (BPA) and its analogs bisphenol-F (BPF) and bisphenol-S (BPS) exposure with several endocrine disorders, from low circulating sex hormones and decreased birth weight to metabolic diseases, such as obesity and cardiovascular disease (Rochester, 2013; Vom Saal and Vandenberg, 2021). The most common route of exposure of BPA and BPS in humans is food contamination (Geens et al., 2012; Qiu et al., 2019; Halden, 2010), but they can also be found in thermal paper, dust, and certain dental and medical equipment (Geens et al., 2012; Qiu et al., 2019). BPA exposure is essentially ubiquitous in humans, with BPA being detectable in almost 100% of the blood or urine samples tested (Rochester, 2013). Many industrialized countries, such as the United States, China, Germany, and Australia have high exposure rates, often times above the current tolerable daily intake (TDI) (Huang et al., 2017). In addition, the majority of epidemiological studies show that BPA exposure is associated with adverse effects on human health even at intakes below the current TDI (Rochester, 2013). However, despite the known anti-estrogenic effects of BPA and its ubiquitous exposure, little is known about the possible effects that exposure to BPA and its analogs can have on the skeleton.

Estrogen is one of the most influential hormones in growth and maintenance of the skeleton across the entire lifespan in both males and

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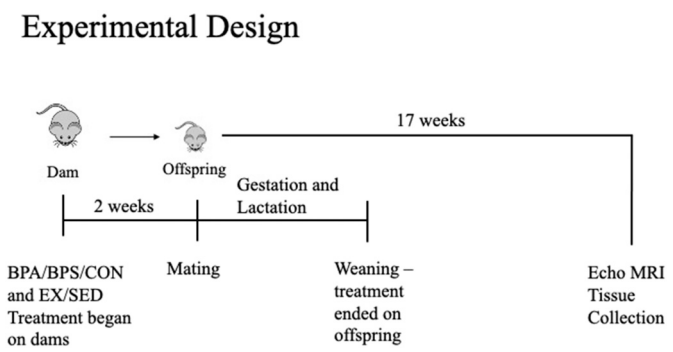


**Fig. 1.** Experimental design.

females due to its actions on each bone cell type (Cutler, 1997; Fortes et al., 2014; Yilmaz et al., 2005; Khosla et al., 2001; Cauley, 2015). Estrogen can block osteocyte and osteoblast apoptosis, induce osteoblast differentiation, increases osteoclast apoptosis, and reduce osteoclast differentiation (Khosla et al., 2012; Syed and Khosla, 2005; Shevde et al., 2000; Bord et al., 2003; Khalid and Krum, 2016). More recently, it has also been suggested that estrogen can downregulate the expression of sclerostin (Drake and Khosla, 2017; Delgado-Calle et al., 2012; Dirkes et al., 2020a). Sclerostin is a protein produced in the osteocyte which acts locally within the bone to inhibit bone growth. In adult women and female rodent models, estrogen status is inversely related to sclerostin expression, whether measured via serum sclerostin levels (Mödder et al., 2011; Kim et al., 2015; Mirza et al., 2010) or by sclerostin mRNA (Fujita et al., 2014) or protein in the bone (Kim et al., 2012a; Jia et al., 2014). However, whether gestational interruption of estrogen signaling could impact sclerostin expression is unknown.

Estrogen actions are mediated primarily by estrogen binding with the nuclear estrogen receptor (ER), which is found in two isoforms, ESR1 (i.e., ERα) and ESR2 (i.e., ERβ). Bone expresses both ESR1 and ESR2; however, ESR1 tends to be more prevalent in cortical bone, whereas ESR2 is more widely distributed in cancellous bone (Onoe et al., 1997). Bisphenols have structural features that allow them to bind with ERs and modify gene expression; however, different analogs appear to have different binding capabilities and anti-estrogenic effects (Wetherill et al., 2007; Routledge et al., 2000; Diel et al., 2000; Li et al., 2018; Molina-Molina et al., 2013; Le Fol et al., 2017). Despite the possible differences in receptor binding, there is evidence that both BPA and BPS can block estrogen binding in competitive assays (Molina-Molina et al., 2013), and that both BPA and BPS can stimulate cellular activity, although BPS binding usually leads to a weaker response than BPA binding (Rochester and Bolden, 2015; Molina-Molina et al., 2013; Le Fol et al., 2017; Maćczak et al., 2017). In addition to direct actions on ERs that interrupt estrogenic activity, BPA can affect epigenetic programming via DNA methylation or histone modification (Dolinoy et al., 2007; Yaoi et al., 2008; Doherty et al., 2010) particularly when exposure occurs during gestation (Kundakovic and Champagne, 2011).

In humans, few studies have looked at relationships between BPA exposure and skeletal health. In adult women, serum or urinary BPA levels are not associated with bone mineral density (BMD) (Kim et al., 2012b; Zhao et al., 2012; Vitku et al., 2018) Similarly, in school-aged girls, there was no correlation between urinary BPA levels and height (Wang et al., 2018); however, there was a significant negative correlation between urinary BPA levels and height in school-aged boys (Wang et al., 2018). In another study, maternal urinary BPA levels in the first trimester were negatively correlated with offspring BMD at age 10, regardless of sex (van Zwol et al., 2020). While these studies suggest BPA exposure could negatively impact skeletal growth, particularly in males, the impact of BPA exposure across the lifespan on BMD or fracture incidence has not been investigated, nor have any studies looked at skeletal effects of bisphenols other than BPA. BPA exposure could have effects on either sex, but this study will be focusing on female offspring. In female animal models, the impact of BPA seems to be dependent on timing of exposure. For example, BPA exposure after ovariectomy (OVX) decreased cancellous BMD more than just OVX alone (Seidlová-Wuttke et al., 2004); however, when given during gestation, the majority of studies show no effects of BPA exposure on female offspring (Lejonklou et al., 2016; Lind et al., 2017; Xin et al., 2018; Pelch et al., 2012). Only one study in female offspring exposed to BPA during gestation showed a decrease in bone stiffness at 52-weeks of age, but it was not correlated with any morphological changes or changes in other measures of biomechanical strenght (Lind et al., 2019). While impact of BPA exposure in female animals seems to be limited, little is known about the effects of other bisphenol analogs, such as BPS, and there are still many unanswered questions about the mechanisms behind the morphological impairments seen in response to BPA exposure.

In addition to effects of gestational BPA or BPS exposure, little is known about the effects of maternal exercise on bone. One study shows that maternal exercise can increase the production of osteogenic genes in female offspring (Gaeini et al., 2017); however, another study indicated that maternal exercise could lead to decreases in BMD of the tibia in both male and female offspring (Rosa et al., 2013). Indirectly, maternal exercise has been shown to be protective against obesity in both male and female offspring (Harris et al., 2018; Wasinski et al., 2015), and obesity is considered detrimental to bone health, particularly for men (Nielson et al., 2011). Gestational BPA exposure is also associated with increased body weight in male mice (Van Esterik et al., 2014), so it is possible that maternal exercise could have a protective effect on skeletal health in the context of BPA exposure indirectly through improvements in body weight.

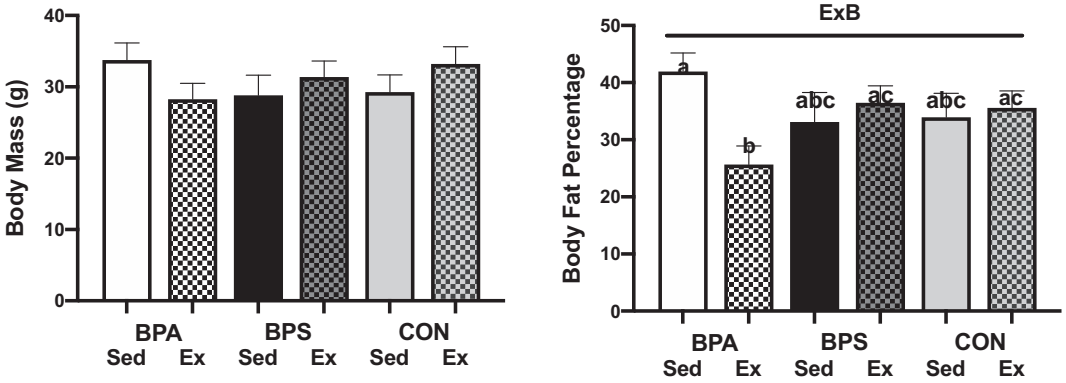


**Fig. 2.** Animal Characteristics. Data are means ± SEM. BPA: gestational bisphenol-A exposure; BPS: gestational bisphenol-S exposure; CON: control; Ex: dam had access to unlocked running wheel; Sed: dam had locked running wheel. B: main effect of gestational bisphenol exposure ($p < 0.05$); E: main effect of exercise status ($p < 0.05$). Different letters denote significance if a B*E interaction was present.

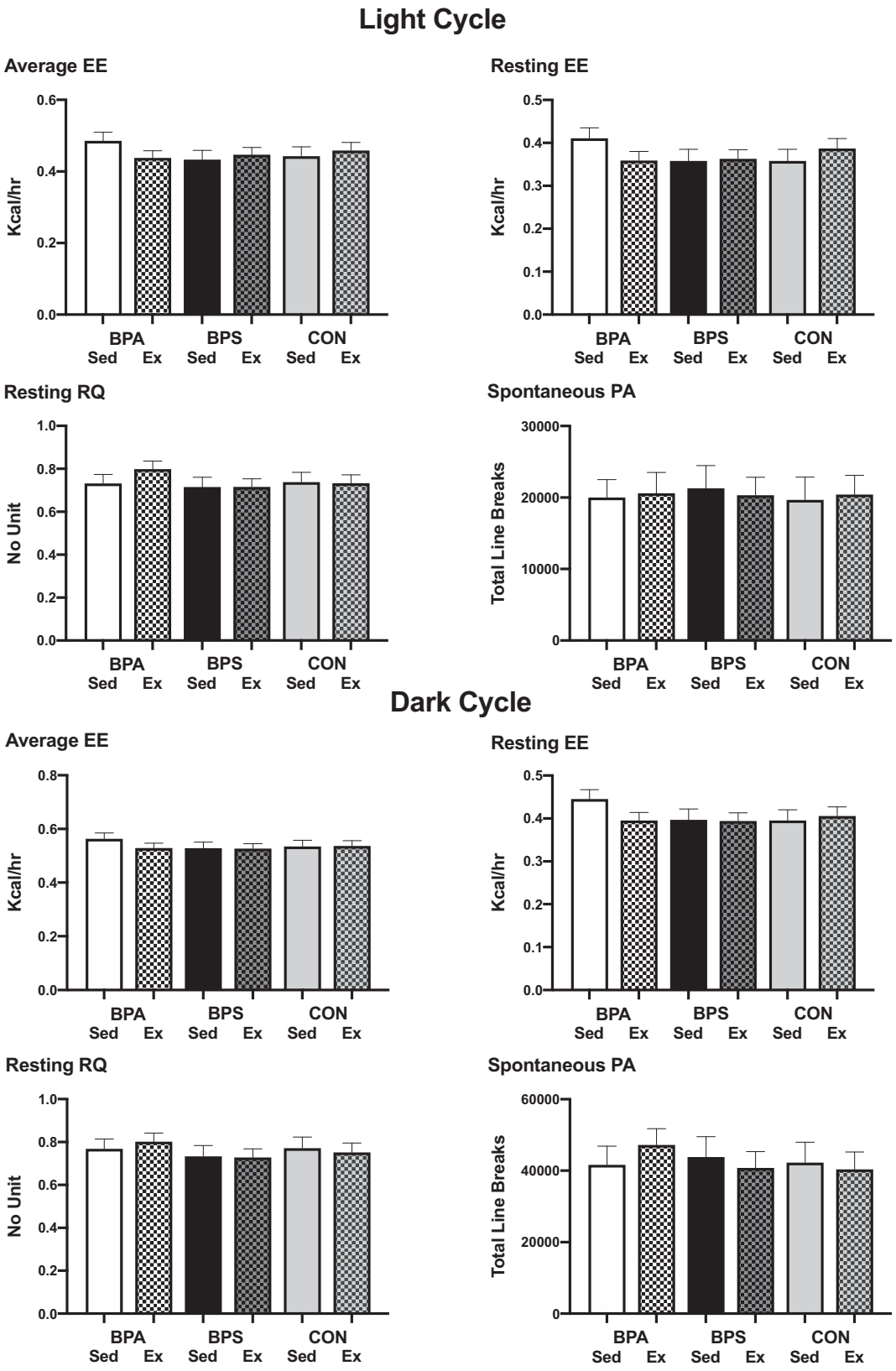


**Fig. 3.** Metabolic Chamber Data. Data are means ± SEM. BPA: gestational bisphenol-A exposure; BPS: gestational bisphenol-S exposure; CON: control group; Ex: dam had access to unlocked running wheel; Sed: dam had locked running wheel; EE: energy expenditure; RQ: respiratory quotient, PA: physical activity. E: main effect of exercise status ($p < 0.05$). There was a main effect of exercise on average energy expenditure [(kcal/h) Ex: 0.54 ± 0.01; Sed: 0.57 ± 0.01; $p = 0.037$].

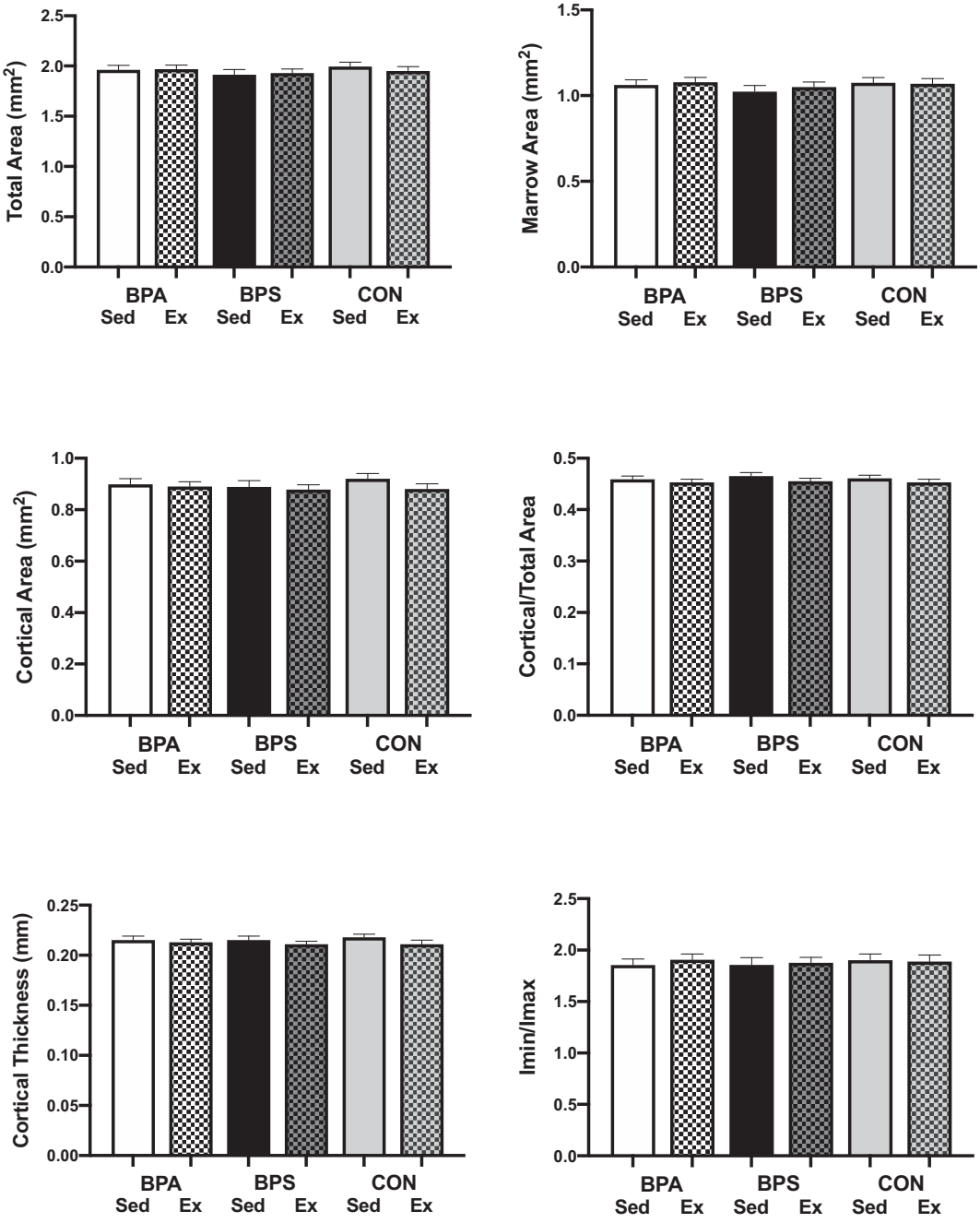


**Fig. 4.** Cortical Geometry of the Femur. Data are adjusted means ± adjusted SEM. BPA: gestational bisphenol-A exposure; BPS: gestational bisphenol-S exposure; CON: control; Ex: dam had access to unlocked running wheel; Sed: dam had locked running wheel.

Here, we explored the effects of gestational BPA or BPS exposure of the offspring via the mother on skeletal outcomes in adult female offspring. Based on previous literature, we hypothesized that gestational exposure to BPA would minimally impact morphological or biomechanical outcomes in the female offspring. Considering that BPS often has weaker effects than BPA, we also hypothesized that gestational BPS exposure would minimally impact morphological or biomechanical outcomes in female offspring.

## 2. Methods

### 2.1. Experimental design

This project was part of a larger parent study designed to examine the effects of gestational BPA/BPS exposure on adipose tissue and metabolic function in offspring.

Female, sexually mature, C57B6/J (Jackson Labs, Bar Harbor, ME) mice were randomly assigned to three treatment groups: BPA exposure (BPA), BPS exposure (BPS), or control (CON). Within those treatment groups, they were further randomized into two groups – an exercising treatment (EX) that had an unlocked running wheel in the cage, or a sedentary control (SED) that had a locked running wheel in the cage, resulting in six treatment groups ($n = 6$/group). All animals were given one small vanilla wafer (Nilla Wafer, Nabisco, East Hanover, NJ) daily. The animals in the BPA group had the equivalent of 200 μg BPA/kg body weight dissolved in ethanol and pipetted onto the wafer that was provided daily to the mice. The animals in the BPS group had the equivalent of 200 μg BPS/kg body weight dissolved in ethanol and pipetted onto the

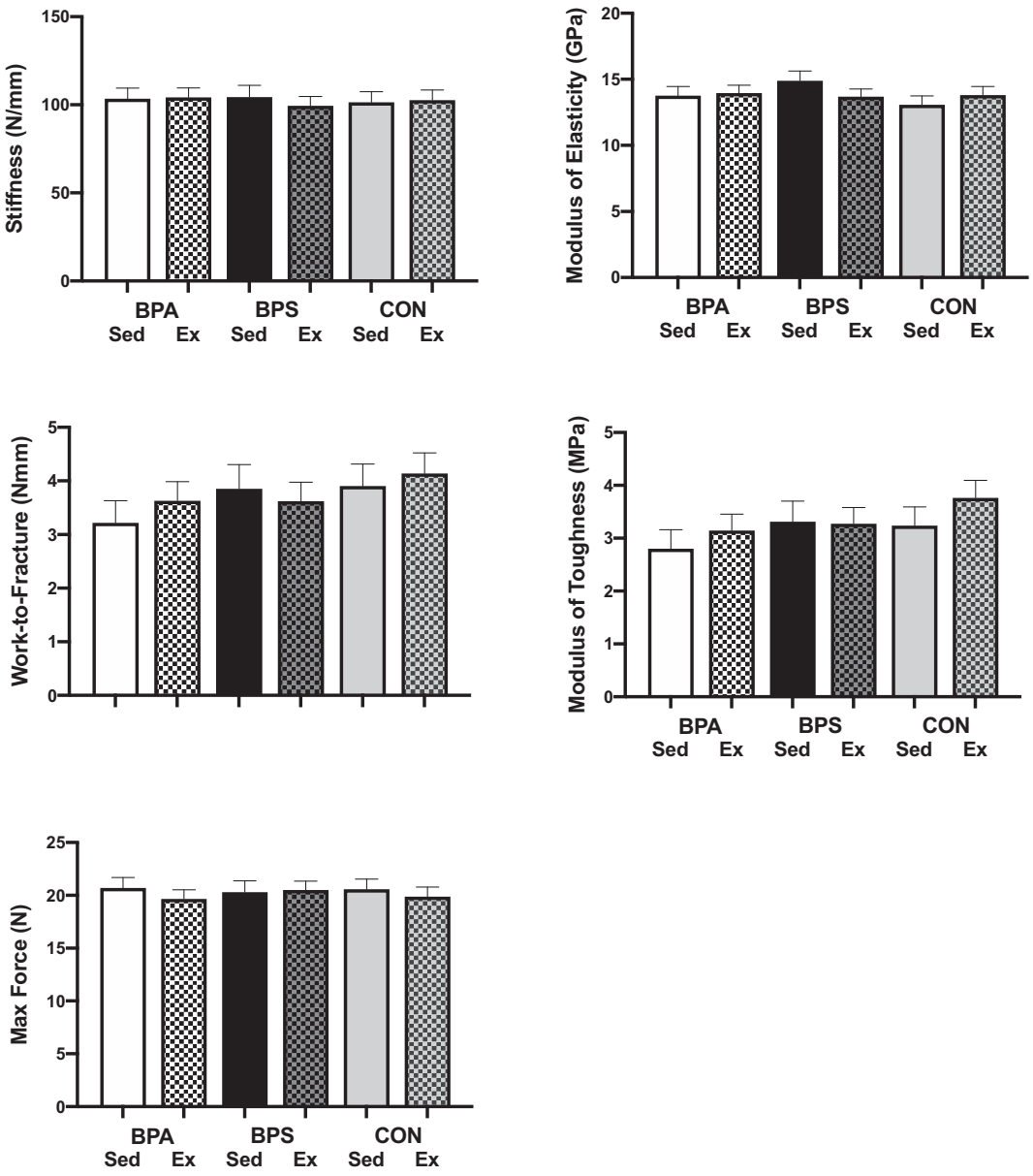


**Fig. 5.** Biomechanical Strength of the Femur. Data are adjusted means ± adjusted SEM. BPA: gestational bisphenol-A exposure; BPS: gestational bisphenol-S exposure; CON: control; Ex: dam had access to unlocked running wheel; Sed: dam had locked running wheel.

wafer that was provided daily to the mice. The control animals had ethanol alone placed on the wafer. Cookies from all groups were air dried before feeding to animals. Dams were weighed weekly and chemical doses were adjusted according to body weight. The BPA dose falls below the diet-administered maximum nontoxic dose for rodents (200 mg/kg body weight per day), is within the presumptive no-observed-adverse-effect level, and yields serum concentrations comparable with those identified in human populations unknowingly exposed to this chemical (Mao et al., 2020). Two weeks after treatment started, all mice were mated to control C57B6/J male mice which had not been exposed to BPA or BPS. BPA/BPS/CON and EX/SED treatment continued during gestation and lactation. Once weaned, one female offspring was randomly selected from each litter and included in the bone studies detailed below (Fig. 1). Dams were singly housed and kept on a 12-hour light-dark cycle in a temperature-controlled room.

After weaning, female offspring were placed on a high-fat diet (18.6% protein, 37.7% carbohydrate, and 43.8% fat by kcal; Envigo, Madison WI). Mice were pair-housed and maintained on a 12-hour light-dark cycle in a temperature-controlled room until 16 weeks of age. One week before sacrifice, body composition was measured via EchoMRI and daily activity and energy expenditure was measured via metabolic chamber. At sacrifice, body weight was measured, and hind limbs were collected, flash frozen, and stored at −80 °C for further analysis. All procedures were approved in advance by the University of Missouri Institutional Animal Care and Use Committee (Protocol #8693).

### 2.2. Femoral cortical geometry and trabecular microarchitecture

Micro-computed tomographic (μCT) imaging of the right femur was performed using a high-resolution imaging system (Xradia 520 Versa, ZEISS, Oberkochen, Germany), as previously described (Dirkes et al., 2020b). The methods used were in accordance with guidelines for the use of μCT in rodents (Bouxsein et al., 2010). Scans were acquired using an isotropic voxel size of 0.012 mm, a peak X-ray tube potential of 60 kV, and a 2-s exposure time. Trabecular bone microarchitecture was evaluated in a 0.5-mm region of interest directly above the growth plate of

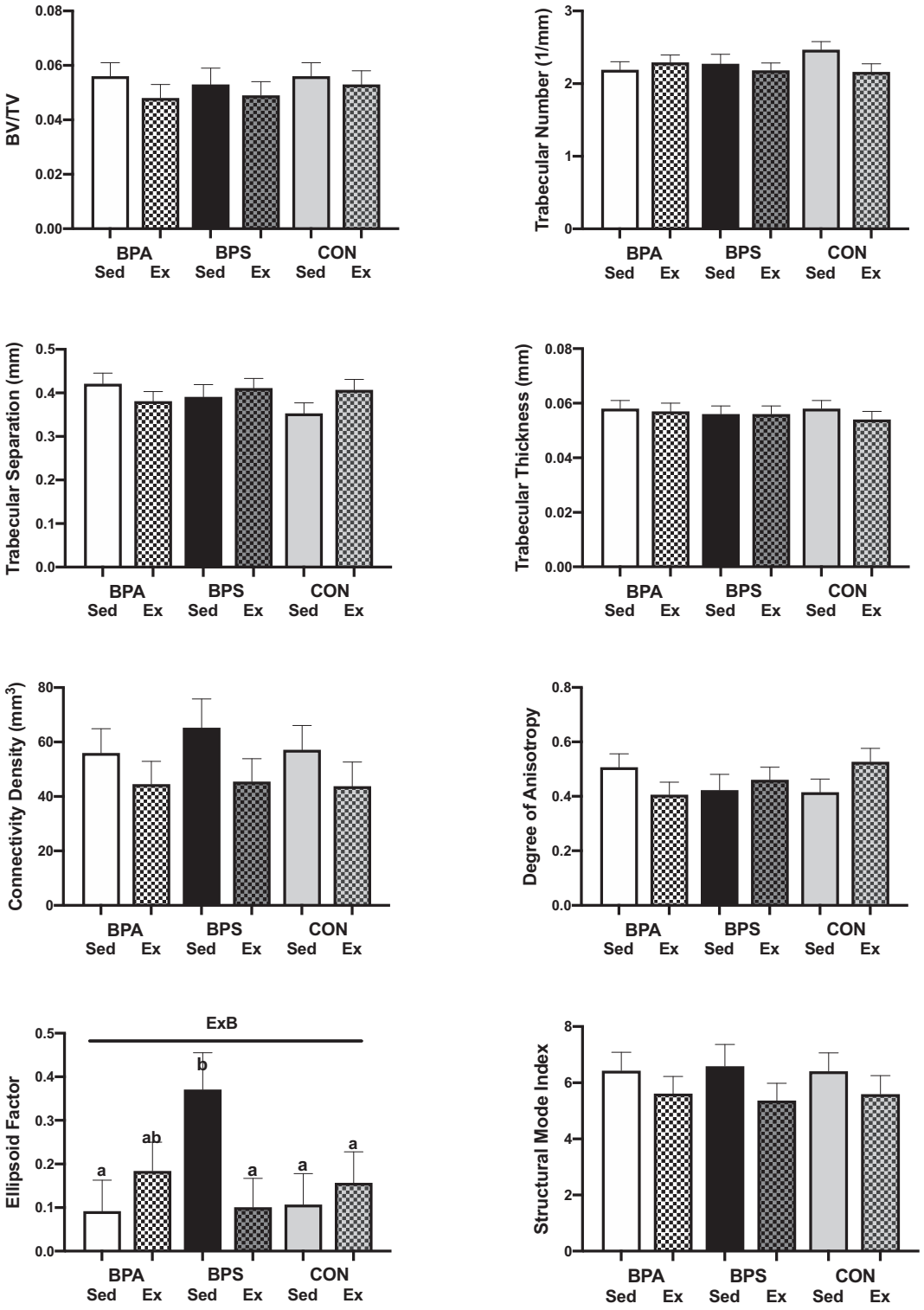


**Fig. 6.** Trabecular Microarchitecture of the Femur. Data are means ± SEM. BPA: gestational bisphenol-A exposure; BPS: gestational bisphenol-S exposure; CON: control; Ex: dam had access to unlocked running wheel; Sed: dam had locked running wheel. B: main effect of gestational bisphenol exposure ($p < 0.05$); E: main effect of exercise status ($p < 0.05$). Different letters denote significance if a B*E interaction was present.

the distal femur, as previously described (Ortinau et al., 2017a; Ortinau et al., 2017b). Cortical bone cross-sectional geometry was evaluated at a 1-mm region of interest at the mid-diaphysis of the femur as previously described (Ortinau et al., 2017a; Ortinau et al., 2017b). The optimized threshold function was used to delineate mineralized bone from soft tissue. Scans were analyzed using BoneJ software (Doube et al., 2010) (NIH public domain), and measures of cortical geometry and trabecular microarchitecture were collected. Outcomes for cortical geometry included: femur length (Le), total cross-sectional area inside the periosteal envelope (Tt.Ar), marrow area (Ma.Ar), cortical bone area (Ct.Ar), cortical area fraction (Ct.Ar/Tt.Ar), mean cortical thickness (Ct.Th), and robustness (R, total bone area over length calculated as R = Tt.Ar/

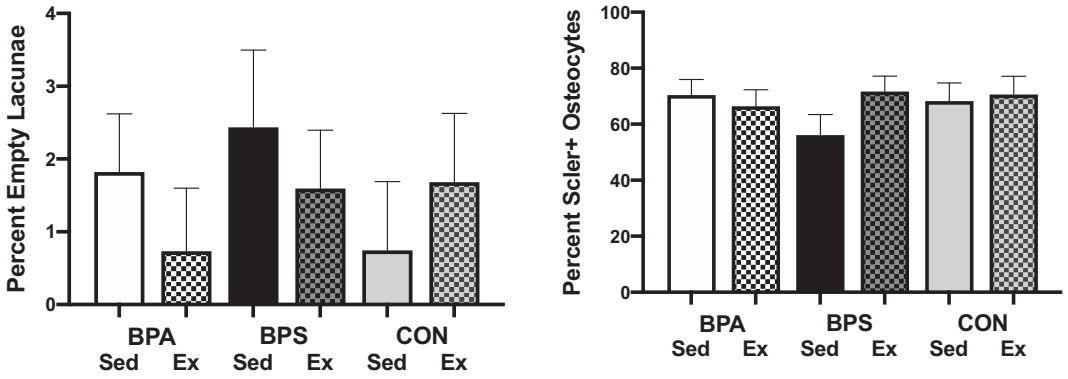


**Fig. 7.** Sclerostin Expression of the Tibia. Data are means ± SEM. BPA: gestational bisphenol-A exposure; BPS: gestational bisphenol-S exposure; CON: control; Ex: dam had access to unlocked running wheel; Sed: dam had locked running wheel.

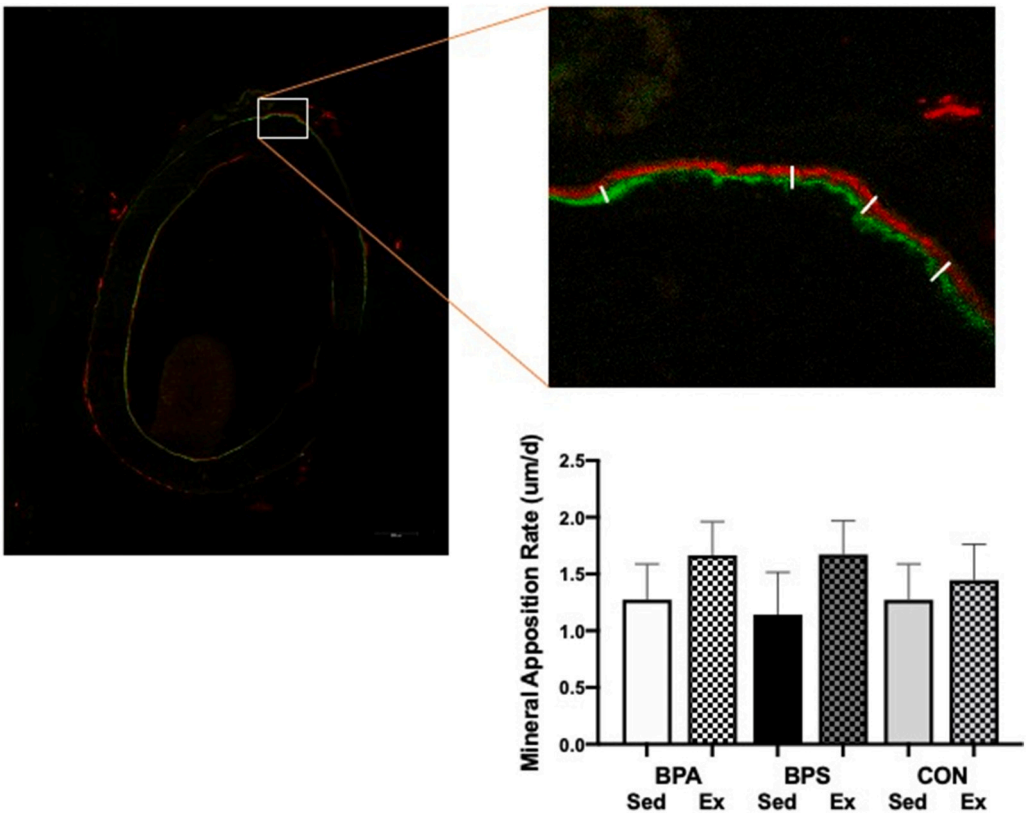


**Fig. 8.** Mineral Apposition Rate of the Femur. Data are means ± SEM. BPA: gestational bisphenol-A exposure; BPS: gestational bisphenol-S exposure; CON: control; Ex: dam had access to unlocked running wheel; Sed: dam had locked running wheel.

Le). Outcomes for trabecular microarchitecture included: bone volume fraction (BV/TV), connectivity density (Conn.D, degree of trabeculae connectivity normalized to total bone volume), mean trabecular thickness (Tb.Th), trabecular separation (Tb.Sp, distance between trabeculae), trabecular number (Tb.N, average number of trabeculae per unit length calculated as 1/(Tb.Th + Tb.Sp) (Bruker-microCT, 2012)), structural model index (SMI), and degree of anisotropy (DA).

### 2.3. Femoral biomechanical strength

Biomechanical strength of the right femur was performed using three-point bending (Jepsen et al., 2015). Briefly, femurs were cleaned of all soft tissue and placed in the three-point bending apparatus with a span of 6-mm. Femurs were loaded via a materials testing machine (Instron 5942; Instron, Inc., Norwood, MA) at a rate of 10 mm/min at the midpoint of the tibia until fracture. Outputs from the Instron machine were used to produce a load-displacement curve. The slope of the load-displacement curve was used to estimate material stiffness, and the area under the load-displacement curve was used to estimate work-to-fracture (Turner and Burr, 2001). Maximal load was measured as the highest force applied to the bone before fracture (Turner and Burr, 2001). Load-displacement data were converted into stress and strain to produce a stress-strain curve using the geometric measurements of the bone and following the equations of Turner and Burr (Turner and Burr, 2001). The slope of the stress-strain curve was used to estimate Young's modulus of elasticity, and the area under the curve was used to estimate the modulus of toughness (Turner and Burr, 2001).

### 2.4. Tibial osteocyte sclerostin expression

Sclerostin expression was evaluated using immunohistochemistry as previously described (Dirkes et al., 2020b). Briefly, Right tibiae were fixed in 10% formalin for 48 h at 4 °C, and then decalcified in 14% EDTA at 4 °C. Decalcified tibiae were embedded in paraffin wax blocks, and 5-μm sections were taken transversely at the mid-diaphysis of the tibia for measures of cortical bone. The sections were deparaffinized and underwent heat-induced epitope retrieval overnight at 60 °C using a 10 mM sodium citrate buffer, followed by blocking of endogenous avidin and biotin expression (Avidin Biotin Blocking Solution, Thermo Scientific, Waltham, MA). Sections were then incubated in anti-sclerostin primary antibodies (Abcam, Cambridge, UK) overnight at 4 °C, followed by blocking of endogenous peroxidase activity (3% $H_2O_2$, Ricca Chemical, Arlington, TX) and secondary antibody application. Secondary antibody binding and detection were accomplished using the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA), with diaminobenzidine (ImmPACT DAB, Vector Laboratories, Burlingame, CA) as the chromogen. Sections were counterstained with hematoxylin (Fisher Scientific, Hampton, NH), dried, and mounted. Sections were analyzed at 20× for sclerostin expression. Sclerostin positive (Sost+) osteocytes were defined as osteocytes exhibiting brown staining, and sclerostin negative (Sost-) osteocytes were defined as osteocytes exhibiting blue (hematoxylin) staining. Data are reported as percent Sost+ osteocytes. In addition to Sost+ and Sost- osteocytes, empty osteocytic lacunae revealed by hematoxylin staining were counted, and data are reported as percent empty lacunae, as previously described (Pereira et al., 2017).

### 2.5. Dynamic histomorphometry of the femur

Dynamic histomorphometry of the left femur was analyzed using calcein and alizarin fluorescent labeling. Caclein (15 mg/kg BW) and alizarin (20 mg/kg BW) were administered via intraperitoneal injection 7 and 3 days before sacrifice, respectively. Femora were cleaned of all soft tissue, fixed in 10% formalin overnight at 4 °C, dehydrated in graded alcohols, and dried overnight. Samples were then embedded in low-viscosity epoxy (Epo-Thin, Beuehler Ltd., Lake Bluff, IL) under a vacuum and allowed to cure overnight. A 1-mm slice was taken from the mid-diaphysis using a low-speed saw. Sections were mounted on slides and polished to smooth the bone surface. Slides were imaged using a fluorescent confocal microscope (Leica GSD 3D, Leica Biosystems, Buffalo Grove, IL) with excitations at 560 nm and 642 nm for calcein and alizarin, respectively. Images were analyzed in ImageJ. Mineral apposition rate (MAR) was calculated as the distance between the corresponding edges of the two consecutive labels divided by the time between injections (um/day), as recommended by ASBMR (Dempster et al., 2013).

### 2.6. Statistical analysis

Two-way ANOVA was used to assess the main and interactive effects of gestational BPA or BPS exposure and exercise status of the dam on metabolic outcomes, trabecular microarchitecture, and percentage of sclerostin+ osteocytes. Body weight is a strong predictor of cortical bone size and strength, so cortical geometry and biomechanical strength outcomes were assessed by two-way ANCOVA with final body weight included as a covariate (Jepsen et al., 2015). If an interaction was present, one-way ANOVA or ANCOVA based on dam group was used as necessary to determine the location of the interaction. Data are presented as means ± SEM or adjusted means ± SEM. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS software (SPSS/25.0, SPSS, Chicago, IL, USA).

## 3. Results

### 3.1. Animal characteristics

There were no main effects of gestational BPA or BPS exposure or exercise status of the dam on final body weight or body fat percentage between groups. There was a significant interaction between gestational exposure and exercise status on offspring body fat percentage ($p = 0.018$), in that exercise decreased body fat percentage only in the BPA exposure group. **(**Fig. 2**)** There were no main effects of gestational BPA or BPS exposure or exercise status of the dam on resting energy expenditure, respiratory quotient, or spontaneous physical activity as measured by the metabolic chamber. **(**Fig. 3**)**

### 3.2. Femoral cortical geometry

There were no main or interactive effects of gestational exposure or exercise status on femur length, Tt.Ar, Ma.Ar, Ct.Ar, Ct.Ar/Tt.Ar, Ct.Th, or Imax/Imin ratio. **(**Fig. 4**)**

### 3.3. Femoral biomechanical strength

There were no main or interactive effects of gestational exposure or exercise status on maximum force, stiffness, young's modulus of elasticity, work-to-fracture, or modulus of toughness. **(**Fig. 5**)**

### 3.4. Femoral trabecular microarchitecture

There were no main effects of gestational exposure or exercise status on bone volume ratio, trabecular thickness, trabecular separation, trabecular number, connectivity density, structural mode index, degree of anisotropy, or ellipsoid factor. There was a significant interaction ($p = 0.037$) between gestational exposure and exercise status on ellipsoid factor in that exercise increased ellipsoid factor in the BPA and CON groups, but not the BPS group. **(**Fig. 6**)**

### 3.5. Cortical osteocyte sclerostin expression of the tibia

There were no main or interactive effects of gestational exposure or exercise status on percent empty lacunae or present sclerostin positive osteocytes of the mid-diaphysis of the tibia. **(**Fig. 7**)**

### 3.6. Dynamic histomorphometry of the femur

There were no main or interactive effects of gestational exposure or exercise status on marrow apposition rate of the mid-diaphysis of the femur. **(**Fig. 8**)**

## 4. Discussion

Here, we showed that gestational exposure to environmentally relevant doses of BPA or BPS had minimal impacts on the skeleton of adult female offspring independent of maternal exercise. There were interactions between gestational exposure and maternal exercise on final body fat percentage and ellipsoid factor of cancellous bone, but no main effects of either experimental treatment on any outcomes.

These minimal impacts of gestational BPA exposure in adult female offspring are supported by previous studies (Lejonklou et al., 2016; Lind et al., 2017; Xin et al., 2018; Pelch et al., 2012; Lind et al., 2019). However, we were the first to examine gestational exposure to BPS and compare its effects to that of BPA. Considering the often weaker activity of BPS compared to BPA (Rochester and Bolden, 2015; Molina-Molina et al., 2013; Le Fol et al., 2017; Maćczak et al., 2017), we hypothesized that BPS would also have minimal effects on the skeleton of the adult female offspring, and that hypothesis was supported by the results of this study. There are a few possible explanations as to why BPA and other

bisphenol analogs are not disruptive to the skeleton in female animals, but they remain speculative. For one, alpha-fetoprotein plays a significant role in the female fetal brain to regulate endogenous estrogen exposure during neural development (McHenry et al., 2014); however, whether that role extends to exogenous estrogens or skeletal development is unknown. In addition, placentas associated with female offspring appear to be more resilient and adaptable to adverse maternal environments than placentas associated with male offspring (Clifton, 2010), implying that there could be alterations in gene or protein expression that would protect the female offspring from BPA exposure. Finally, because BPA has a significantly lower binding affinity for the ESRs than estradiol (Acconcia et al., 2015), it is a possibility that it was out-competed by the high circulating levels of estradiol seen in females. While we saw no main effects of BPA or BPS exposure on skeletal outcomes, there were some interesting interactions between BPA or BPS and maternal exercise.

Maternal exercise increased ellipsoid factor of the cancellous bone in BPA and CON animals but decreased ellipsoid factor in BPS animals. Ellipsoid factor is a measure of the plate- or rod-like nature of the 3D structure of individual trabeculae (Doube, 2015), which can have direct effects on the material properties of cancellous bone (Stauber et al., 2006; Liu et al., 2006). Ellipsoid factor ranges from −1 (purely plate-like) to +1 (purely rod-like). More plate-like trabeculae increases the elasticity of trabecular bone compared to more rod-like structures (Liu et al., 2006), so results would indicate that exercise decreases the elasticity of the individual trabeculae, except in the presence of BPS. However, we did not have the capacity to measure material properties of cancellous bone, and thus further studies are warranted to explore these possible effects of maternal exercise and the interaction with BPS exposure.

Few other studies have explored the possible impact of maternal exercise on skeletal development in the offspring. One study compared the effects of maternal exercise before pregnancy, during pregnancy, or both on osteogenic gene expression of the femur. In animals that exercised both before and during pregnancy, osteoprotegerin (OPG) increased and receptor activator of nuclear factor-κB ligand (RANKL) decreased, which significantly decreased the OPG/RANKL ratio (Gaeini et al., 2017), an indicator of osteoclast formation and activity (Chen et al., 2018). This would indicate a decrease in bone resorption; however, this study only looked at gene expression, and thus it is unknown whether this decrease in bone resorption resulted in more bone mass. Another study showed that moderate maternal resistance exercise during pregnancy resulted in lower cortical, but not cancellous, BMD of the tibia, independent of offspring sex (Rosa et al., 2013). This is in contrast with our study, which showed no morphological effects of maternal exercise. These differences could possibly be explained by animal model, since this study was done in rats, or type or intensity of exercise.

One limitation of this study was the power of the study. Post hoc power analysis revealed the power to detect differences between BPA, BPS, and CON groups was low (0.25–0.30) for primary cortical and cancellous bone outcomes. Despite low power, our results are consistent with prior studies in the literature. In conclusion, we showed that skeletal outcomes in adult, female offspring were minimally impacted by gestational BPA or BPS exposure or by maternal exercise, but more studies are warranted to further explore the impact that maternal exercise could have on skeletal outcomes in offspring.

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## CRediT authorship contribution statement

**Rebecca K. Dirkes:** Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Rebecca J. Welly:** Formal analysis, Investigation. **Jiude Mao:** Formal analysis, Investigation. **Jessica Kinkade:** Investigation. **Victoria J. Vieira-Potter:** Conceptualization, Supervision, Project administration. **Cheryl S. Rosenfeld:** Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition. **Pamela S. Bruzina:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

All authors have nothing to disclose and no conflicts of interest.

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