Polyethylene particles inserted over calvarium induce cancellous bone loss in femur in female mice

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ABSTRACT

Focal bone resorption (osteolysis) induced by wear particles contributes to long-term orthopedic joint failure. However, the impact of focal osteolysis on remote skeletal sites has received less attention. The goal of this study was to determine the effects of polyethylene particles placed over calvaria on representative axial and appendicular skeletal sites in female mice. Because recent work has identified housing temperature as an important biological variable in mice, response to particle treatment was measured in animals housed at room (22 °C) and thermoneutral (32 °C) temperature. Osteolysis was evident in skeletal tissue adjacent to particle insertion. In addition, cancellous bone loss was observed in distal femur metaphysis. The bone loss was associated with lower osteoblast-lined perimeter and lower mineralizing perimeter in distal femur, lower osteocalcin gene expression in tibia, and lower serum osteocalcin, suggesting the response was due, at least in part, to reduced bone formation. Mild cold stress induced by sub-thermoneutral housing resulted in cancellous bone loss in distal femur and lumbar vertebra but did not influence skeletal response to particles. In summary, the results indicate that focal inflammation induced by polyethylene particles has the potential to result in systemic bone loss. This is significant because bone loss is a risk factor for fracture.

1. Introduction

> 600,000 orthopedic hip and knee replacements are performed annually in the United States (Kurtz et al., 2007). These procedures are used to treat bone fracture and a variety of degenerative joint disorders. Typically, quality of life rapidly improves following joint replacement. Unfortunately, approximately 17% of orthopedic hip replacements and 8% of knee replacements eventually fail and require surgical revision (Kurtz et al., 2005). The failure rate of joint prostheses has remained largely unchanged over the last 30 years (Kurtz et al., 2005). However, the number of joint replacements performed annually has increased dramatically (Kurtz et al., 2005) and, as a consequence, it is likely that the number of joint replacement revisions performed will also increase (Kurtz et al., 2007). Additionally, some studies find accelerated age-related bone loss in the contralateral limb following total joint replacement, suggesting arthroplasty can negatively influence the skeleton at distant sites (Gundry et al., 2017; Meek et al., 2011).

The precise mechanisms mediating implant failure are not entirely known but focal bone resorption (osteolysis) associated with particle-induced inflammation contributes to long-term orthopedic joint failure (Dattani, 2007; Gallo et al., 2002). Friction between the implant and the bone surface is responsible for generation of orthopedic wear particles (Dattani, 2007; Gallo et al., 2002). Wear particles from all commonly used orthopedic material (e.g., polyethylene, metal, and ceramic) can stimulate osteolysis (Dattani, 2007; von Knoch et al., 2004a). Of these, ultra-high molecular weight polyethylene is of particular importance as it has been and continues to be widely used in joint replacements. The process of ultra-high molecular weight polyethylene breakdown in hip replacement is reported to begin at the articular surface of the cup (Witkiewicz et al., 1993). Interaction between the polyethylene particles and the host cells leads to oxidative changes of the polyethylene and to size reduction of the particles. Wear particles are commonly found in and around tissues resected during joint revision and in distal lymph nodes (Witkiewicz et al., 1993; Kobayashi et al., 1997), the latter indicating that there is transport of the particles from site of generation to remote sites.

The effects of wear particles on bone are commonly investigated using small rodent models (von Knoch et al., 2004b; Wedemeyer et al.,

2007; Nich et al., 2010; Kauther et al., 2010; Jin et al., 2011; Takahashi et al., 2011; von Knoch et al., 2005a; Darowish et al., 2009; Childs et al., 2001; von Knoch et al., 2005b; Ren et al., 2006). In mice, placement of particles over the calvarium induces osteolysis that is readily detectable using histology (von Knoch et al., 2004a; von Knoch et al., 2004b; Wedemeyer et al., 2007; Nich et al., 2010; Jin et al., 2011; Takahashi et al., 2011; von Knoch et al., 2005a; Darowish et al., 2009; Childs et al., 2001; von Knoch et al., 2005b; Ren et al., 2006; Yang et al., 2004; K. Ren et al., 2011) or micro-computed tomography (μCT) (Wedemeyer et al., 2007; Nich et al., 2010; Kauther et al., 2010; Darowish et al., 2009; Ren et al., 2006; K. Ren et al., 2011; Burton et al., 2013; Green et al., 2013). Animal studies to date have appropriately focused on the role of focal bone loss contributing to implant loosening. However, focal inflammation can also lead to systemic bone loss (Desimone et al., 1993) but few studies have evaluated the impact of polyethylene particle-induced local inflammation on bone mass, microarchitecture, or turnover at remote skeletal sites.

Housing temperature has emerged as an important biological variable that may influence experimental outcomes in mouse studies (Eng et al., 2015; Kokolus et al., 2013; Rosania, 2014; Stemmer et al., 2015; Ganeshan and Chawla, 2017). Mice are generally housed at 18–23 °C, a temperature range that is well below the thermoneutral zone (temperature range where basal rate of heat production is in equilibrium with heat loss) for this species (~32 °C). Importantly, housing temperature affects age-related bone loss (Iwaniec et al., 2016). Specifically, mild cold stress induced by room temperature housing leads to premature cancellous bone loss in mice. Although the precise mechanism is not fully established, the bone loss is associated with increased sympathetic outflow accompanying adaptive thermogenesis. Additionally, housing temperature influences the immune system (Kokolus et al., 2013), an important mediator of particle-induced osteolysis. Therefore, the purpose of this study was to (1) evaluate whether focal osteolysis induced by placement of polyethylene particles over the calvarium alters bone metabolism at remote skeletal sites and (2) determine whether the response is influenced by housing temperature.

2. Methods

2.1. Experimental protocol

Four-week-old female C57BL/6 (B6) mice were purchased from Jackson Laboratory (Bar Harbor, MN). The mice were housed individually in climate-controlled rooms on a 12 h light dark cycle. All mice were fed standard rodent chow (Teklad 8604, Harlen Laboratories, Indianapolis, IN). The animals were maintained in accordance with the National Institutes of Health Guide for the Care and the Use of Laboratory Animals. The Oregon State University Institutional Animal Care and Use Committee approved all protocols.

2.1.1. Experiment 1: effects of particle-induced calvarial osteolysis and housing temperature on bone in femur and lumbar vertebra

Mice (n = 43) were maintained at room temperature (22 °C) from 4 to 10 weeks of age. At 10 weeks of age, the mice were randomized by weight into one of four treatment groups: (1) 22 °C control (sham surgery, n = 11), (2) 22 °C particles (n = 11), (3) 32 °C control (sham surgery, n = 10), or (4) 32 °C particles (n = 11). Particle treatment was initiated 1 week following randomization to allow groups 3 and 4 to adapt to thermoneutral housing. The animals were sacrificed 2 weeks following particle implantation (at 13 weeks of age). The 2-week duration was selected based in part on precious work by von Knoch et al. (2005a) showing a robust bone response to particles two weeks following implantation. For tissue collection, mice were anesthetized (2% isoflurane delivered in oxygen), weighed, and killed by decapitation. Calvariae, femora, and the 5th lumbar vertebrae were excised and placed in 10% formalin for 24 h and then transferred to 70% ethanol for

storage prior to evaluation.

2.1.2. Experiment 2: effects of particle-induced calvarial osteolysis on bone in femur, gene expression in tibia, and biochemical markers of bone turnover in serum

4-week-old mice (n = 19) were placed in a 32 °C room upon arrival at Oregon State University. At 6 weeks of age, the mice were randomized by weight into one of two treatment groups: (1) control (n = 10) or (2) particles (n = 9). Particles were implanted over calvaria and the animals sacrificed 2 weeks later (at 8 weeks of age). The fluorochrome calcein (15 mg/kg; Sigma Chemical, St Louis, MO) was administered by subcutaneous injection to label mineralizing bone at 4 days and 1 day prior to sacrifice. As in Experiment 1, calvariae and femora were excised for evaluation of bone. In addition, trunk blood was collected for evaluation of serum osteocalcin, a global marker of bone turnover, and tibiae were flash frozen in liquid nitrogen for evaluation of gene expression. Serum and tibia were stored at −80 °C until analysis.

2.2. Particle implantation

A polyethylene particle stock solution was prepared to deliver 2.5 mg of particles in 15 μl of solution. Polyethylene particles (S-395 *N1*, Shamrock Technologies Inc., Newark NJ), mean diameter 5 μm, were washed 6 times in 70% ethanol. 2 ml of wet particles were suspended in 95% ethanol. For particle placement, mice were anesthetized (2% isoflurane delivered in oxygen), and particles implanted using a model described by von Knoch et al. (2004b). A one cm skin incision was made over the calvarium. The skin was retracted and 15 μl of particle solution delivering 2.5 mg of particles were applied by pipette on top of the exposed calvarial surface between bregma and lambda. The incision was closed with 7 mm surgical staples (Reflex 7 Wound Closure System). Sham-operated controls underwent the same procedure excluding particles.

2.3. Micro-computed tomography

Calvaria, femora, and 5th lumbar vertebra were imaged using microcomputed tomography (μCT; μCT40 scanner, Scanco Medical AG, Bassersdorf, Switzerland) at 55 kVp x-ray voltage, 145 μA intensity, and 200 ms integration time using cubic voxels, 12 μm on a side. Filtering parameters sigma and support were set to 0.8 and 1, respectively. All samples were scanned immersed in 70% ethanol. All μCT data are reported using standard 3 dimensional nomenclature (Bouxsein et al., 2010).

Bone segmentation in femur and lumbar vertebra was conducted at a threshold of 245 (scale, 0–1000) determined empirically. Femora (cortical + cancellous bone) were evaluated for total femur bone volume (mm³). Femur length was measured as the distance between the proximal end of the femoral head and distal end of the femoral condyles. Cortical bone architecture was evaluated in a 0.24 mm (20 slices) region of the diaphysis that started 60% distal from the top of the femoral head. Cross-sectional volume (mm³, cortical bone volume + marrow volume), cortical bone volume (mm³), marrow volume (mm³), cortical thickness (μm), and polar moment of inertia (mm⁴, an index of bone strength in torsion) were measured. Cancellous bone architecture was evaluated in the femoral metaphysis and in 5th lumbar vertebra. For the femoral metaphysis, 42 slices (0.50 mm) of bone were measured in a region that began 45 slices (0.54 mm) proximal to the growth plate. The entire cancellous compartment was evaluated in the vertebral body. Direct cancellous bone measurements included cancellous bone volume fraction (%, ratio of the segmented bone volume to the total volume of the region of interest), connectivity density (mm⁻³, measure of the degree of connectivity of trabeculae), trabecular thickness (μm), trabecular number (mm⁻¹, number of trabeculae intercepted per unit length), and trabecular spacing (μm).
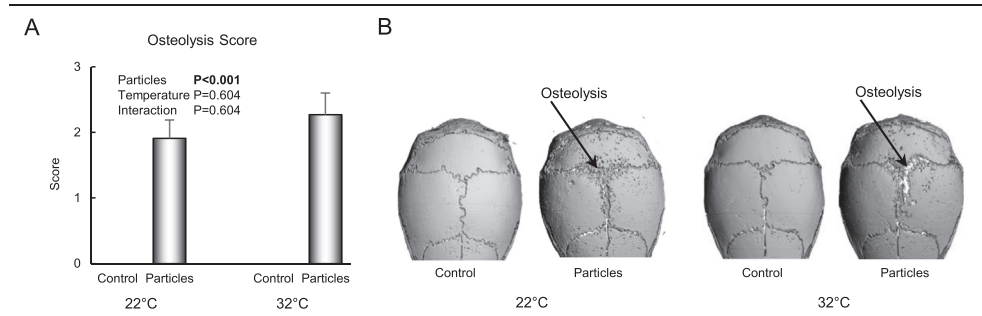
Fig. 1. Effects of polyethylene particle placement over calvaria and housing temperature on calvarial osteolysis in 13-week-old female B6 mice. (A) Placement of particles over calvaria resulted in comparable osteolysis in mice housed at 22 °C or 32 °C. Osteolysis was not detected in control mice housed at either temperature. (B) Representative μCT images of calvaria from sham or particle-treated mice housed at 22 °C or 32 °C. Osteolysis is readily appreciated in the particle-treated mice by extensive pitting of the calvarial surface. Data are mean ± SE; n = 10–11/group.

2.4. Pathology (osteolysis) score

Calvarial osteolysis was evaluated using a semi-quantitative (osteolysis score) assay. Scanned calvaria were imaged at a threshold of ≥ 235 (scale of 0–1000) and the reconstructed 3-dimensional images used for visual assessment of bone response to particle challenge. Bone response was scored on a scale from 0 (normal bone) to 4 (extensive focal osteolysis) by two blinded independent observers.

2.5. Histomorphometry

Distal femora were dehydrated in a graded series of ethanol and xylene, and embedded undecalcified in modified methyl methacrylate as described (Iwaniec et al., 2008). Frontal sections (4 μm thick) were cut with a vertical bed microtome (Leica 2065) and affixed to gel-coated slides. One section per bone was stained with tartrate resistant acid phosphatase and counter stained with toluidine blue and used for cell-based measurements. A second section was left unstained for visualization of fluorochrome labels. Histomorphometric data were collected in cancellous bone in distal femur metaphysis using the Osteo-Measure System (OsteoMetrics, Inc., Atlanta, GA). The region of interest was located 0.25 mm proximal to the growth plate and extended 1.2 mm. Cellular measurements collected included osteoblast perimeter (osteoblast perimeter/bone perimeter, %) and osteoclast perimeter (osteoclast perimeter/bone perimeter, %). Osteoblasts were identified morphologically as cuboidal cells adjacent to a layer of osteoid in direct contact with the bone surface. Osteoclasts were identified as multinucleated (two or more nuclei) cells with acid phosphatase-positive (red-stained) cytoplasm in contact with the bone surface. Fluorochrome-based measurements of bone formation included mineralizing perimeter (mineralizing perimeter/bone perimeter, %), mineral apposition rate (the mean distance between two calcein markers divided by the 3-day interlabel interval, μm/day), and bone formation rate adjusted for bone perimeter (bone formation rate/bone perimeter, μm²/μm/year). All bone histomorphometric data are reported using standard 2-dimensional nomenclature (Parfitt et al., 1987).

2.6. Gene expression

Whole tibiae were pulverized with a mortar and pestle in liquid nitrogen and then further homogenized in Trizol (Invitrogen; Valencia, CA). Total RNA was isolated according to the manufacturer's protocol, and mRNA was reverse transcribed into cDNA using SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen). The expression of 84 genes related to osteogenic differentiation was determined using the Mouse Osteogenesis RT² Profiler™ PCR Array (Qiagen; Carlsbad, CA) according to the manufacturer's protocol. Gene expression was normalized to an average of GusB and Hsp90 and relative quantification was determined using the ΔΔCt method using RT² Profiler PCR Array Data Analysis software version 3.5 (Qiagen).

2.7. Serum chemistry

Serum osteocalcin was measured using mouse Gla-osteocalcin High Sensitive EIA kit from Clontech (Mountain View, CA).

2.8. Statistics

Experiment 1 was performed according to a 2 × 2 factorial design with categorical variables for treatment group (control and particle group) and temperature (22 °C and 32 °C). Two-factor analysis of variance with an interaction between treatment and temperature was used to compare mean values for bone parameters. A linear model with different variances across the four groups was used when variances were distinct. In Experiment 2, mean responses were compared using t-tests or the Wilcoxon-Mann-Whitney test. Goodness of fit was evaluated based on Levene's test for homogeneity of variance, plots of residuals versus fitted values, normal quantile plots, and Anderson-Darling tests of normality. The Benjamini and Hochberg (1995) method for maintaining the false discovery rate at 5% was used to adjust for multiple comparisons. Differences were considered significant at p ≤ 0.05. All data are presented as mean ± SE. Data analysis was performed using R version 3.2.2.

3. Results

3.1. Experiment 1: effects of particle-induced calvarial osteolysis and housing temperature on bone in femur and lumbar vertebra

The effects of polyethylene particle placement over calvaria and housing temperature on calvarial osteolysis are shown in Fig. 1. Placement of particles over the calvarium resulted in higher osteolysis score, irrespective of housing temperature (Fig. 1A). The differences in particle-induced osteolysis can be readily appreciated in μCT images of calvaria from representative mice in each treatment group (Fig. 1B).

The effects of polyethylene particle placement over calvaria and housing temperature on body weight, femur length, total femur bone volume, and cortical microarchitecture in the femur diaphysis are shown in Table 1. Significant differences in body weight, femur length, total femur bone volume, or femur diaphysis cross-sectional volume, cortical volume, cortical thickness, or polar moment of inertia were not detected with calvarial particle treatment. However, placement of particles over the calvarium resulted in greater marrow volume. Thermoneutral housing resulted in a tendency (p < 0.1) for greater total femur bone volume but had no effect on other endpoints evaluated including femur length, cross sectional volume, cortical volume, marrow volume, cortical thickness, or polar moment of inertia. Significant interactions between particle treatment and housing temperature were not detected for any of the endpoints evaluated.

The effects of polyethylene particle placement over calvaria and housing temperature on cancellous bone microarchitecture and cellular indices of bone turnover in the distal femur metaphysis are shown in Fig. 2. Placement of particles over calvaria resulted in lower cancellous

Table 1

Effects of polyethylene particle placement over calvaria and housing temperature on body weight, femur length, total femur bone volume, and cortical microarchitecture in the femur diaphysis in 13-week-old female B6 mice.

Treatment	22 °C		32 °C		FDR adjusted p-value		
	Control	Particles	Control	Particles	Particles	Temperature	Interaction
Body weight (g)	20.1 ± 0.3	20.5 ± 0.3	20.6 ± 0.5	21.3 ± 0.5	0.366	0.243	0.820
Femur							
Length (mm)	14.9 ± 0.1	15.0 ± 0.1	14.8 ± 0.1	15.0 ± 0.1	0.366	0.926	0.692
Bone volume (mm^3)	16.3 ± 0.3	16.1 ± 0.3	16.9 ± 0.4	17.0 ± 0.4	0.894	0.064	0.799
Femur diaphysis (cortical bone)							
Cross-sectional volume (mm^3)	0.38 ± 0.00	0.39 ± 0.00	0.38 ± 0.01	0.40 ± 0.01	0.108	0.600	0.600
Cortical volume (mm^3)	0.17 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.744	0.463	0.478
Marrow volume (mm^3)	0.21 ± 0.00	0.22 ± 0.00	0.21 ± 0.00	0.22 ± 0.00	0.012	0.926	0.913
Cortical thickness (μm)	175 ± 2	171 ± 2	176 ± 3	178 ± 3	0.653	0.366	0.493
Polar moment of inertia (mm^4)	0.28 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.31 ± 0.01	0.354	0.519	0.463

Data are mean ± SE; n = 10–11/group.

bone volume fraction (Fig. 2A), connectivity density (Fig. 2B) and trabecular number (Fig. 2D), and higher trabecular separation (Fig. 2E). The changes in architecture were associated with lower osteoblast perimeter (Fig. 2F), higher osteoclast perimeter (Fig. 2G), lower osteoblast perimeter to osteoclast perimeter ratio (Fig. 2H), and lower mineralizing perimeter (Fig. 2I) and bone formation rate (Fig. 2K). Thermoneutral housing resulted in higher cancellous bone volume fraction, connectivity density and trabecular number, and lower trabecular spacing. In addition, thermoneutral housing resulted in lower osteoclast perimeter and higher mineralizing perimeter and bone formation rate. Significant treatment differences were not detected for trabecular thickness (Fig. 2C). Significant interactions between particle treatment and housing temperature were not detected for any of the endpoints evaluated, with the exception of mineral apposition rate. However, post hoc analysis did not detect significant differences in mineral apposition rate among treatment groups.

The effects of polyethylene particle placement over calvaria and housing temperature on cancellous bone microarchitecture in 5th lumbar vertebra are shown in Table 2. Placement of particles over calvaria had no significant effect on any of the cancellous endpoints evaluated. Thermoneutral housing resulted in higher cancellous bone volume fraction, trabecular thickness and trabecular number, and lower trabecular spacing. Connectivity density was not significantly affected by housing temperature. Significant interactions between particle treatment and housing temperature were not detected for any of the endpoints evaluated.

All mice were ambulatory throughout the study. Food consumption was reduced in mice housed at thermoneutral temperature but was not influenced by particle treatment (data not shown).

3.2. Experiment 2: effects of particle-induced calvarial osteolysis on bone in femur, gene expression in tibia, and biochemical markers of bone turnover in serum

As in Experiment 1, all mice were ambulatory and insertion of particles had no effect on body weight or food intake (data not shown).

The effect of polyethylene particles on local (calvarium) and remote (femur) skeletal sites is shown in Fig. 3. Placement of particles over calvaria resulted in higher osteolysis score (Fig. 3A). Significant differences in femur length (Fig. 3B) or total femur bone volume (Fig. 3C) were not detected with treatment. Placement of particles over calvaria resulted in lower cancellous bone volume fraction (Fig. 3D) and lower connectivity density (Fig. 3E) in distal femur metaphysis. There were no significant changes in trabecular thickness (Fig. 3F), trabecular number (Fig. 3G), or trabecular separation (Fig. 3H) with treatment.

Placement of particles over calvaria resulted in lower levels of osteocalcin, a global marker of bone turnover (Fig. 3I). Gene profiling identified 12 differentially expressed genes in particle-treated compared to control mice, including lower expression for osteocalcin (Bglap) (Table 3). Other differentially expressed genes important to bone metabolism include genes related to growth factor signaling (Igf1r, Tgfb1, Tgfbr2, Tgfbr3), transcription factors (Nfkb1 and Sox9), integrins (Itgam, Itga2b, Itgam), fatty acid translocase Cd36 and metalloproteinase Mmp8. Tibial expression of Cd36 and Tgfbr3 was higher in mice with placement of particles over calvaria whereas expression levels for the remaining 10 differentially expressed genes were lower.

4. Discussion

Placement of polyethylene particles over calvaria resulted in osteolysis adjacent to the site of particle insertion. In addition to this focal bone resorption, cancellous bone loss was observed at remote skeletal sites, specifically distal femur metaphysis. Particle-induced cancellous bone loss in the distal femur metaphysis was associated with increased osteoclast-lined bone perimeter, and decreased osteoblast-lined bone perimeter, mineralizing perimeter and bone formation rate. Furthermore, mRNA levels for osteocalcin in tibia and osteocalcin levels in serum were lower in polyethylene particle-treated mice. Mild cold stress induced by sub-thermoneutral (room temperature) housing resulted in cancellous bone loss in distal femur and lumbar vertebra. However, housing temperature did not influence the skeletal response to polyethylene particles.

Tissues resected during orthopedic joint revision have increased levels of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β (Holding et al., 2006; Horiki et al., 2004). In rodent models, insertion of polyethylene particles over calvaria results in focal inflammation and pitting of the bone (von Knoch et al., 2004b). The particles induce the expression of chemokines (Kaufman et al., 2008; Nakashima et al., 1999; Yaszay et al., 2001) that act to attract immune cells to the site of particle challenge (Maitra et al., 2010; P.G. Ren et al., 2011; St Pierre et al., 2010). Immune cells contribute to particle-induced osteolysis by increasing tissue levels of cytokines that increase bone resorption (e.g., RANKL, TNF-α, IL-1β, IL-17).

Insertion of polyethylene particles over calvaria in mice results in an acute inflammation response that resolves in approximately three weeks (Langlois et al., 2016). In the present study and in agreement with von Knoch et al. (2005a), we observed robust calvarial osteolysis two weeks following particle challenge. Additionally, bone resorption was elevated and bone formation decreased in distal femur metaphysis, indicating a sustained negative impact of particles inserted over calvaria on bone turnover at this remote location. Ross et al. (2014) evaluated intra-articular (knee joint) application of LPS-doped polyethylene particles in adult (6-month-old) male rats with titanium implants in femurs. These investigators identified elevated serum biochemical markers of bone turnover and bone loss near the site of particle delivery (tibia) but did not detect cortical bone loss in humerus
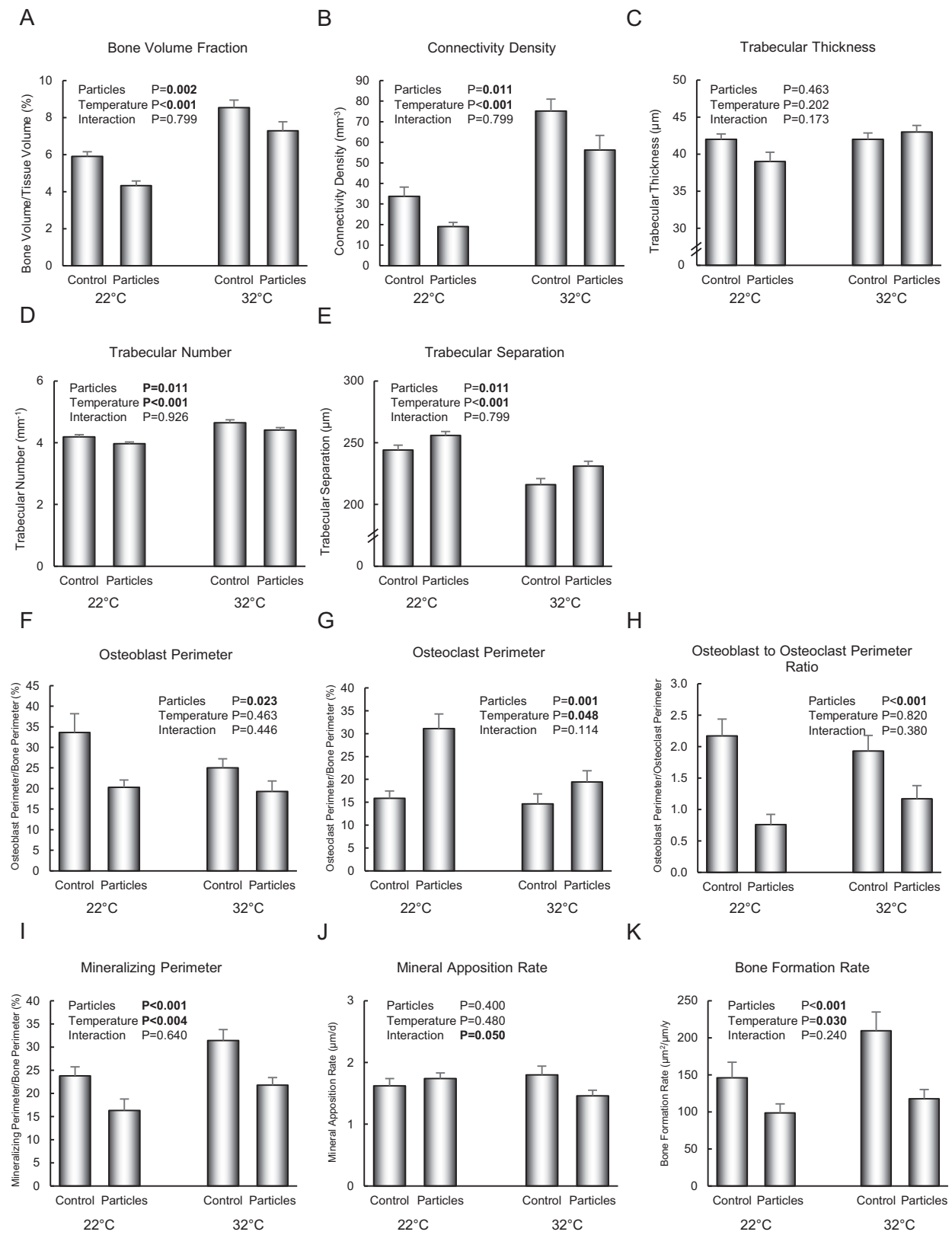
Fig. 2. Effects of polyethylene particle placement over calvaria and housing temperature on (A) cancellous bone volume fraction, (B) connectivity density, (C) trabecular thickness, (D) trabecular number, (E) trabecular separation, (F) osteoblast perimeter, (G) osteoclast perimeter, (H) osteoblast to osteoclast perimeter ratio, (I) mineralizing perimeter, (J) mineral apposition rate, and (K) bone formation rate in the distal femur metaphysis of 13-week-old female B6 mice. Data are mean ± SE; n = 10–11/group.

Table 2
Effects of polyethylene particle placement over calvaria and housing temperature on cancellous microarchitecture in the 5th lumbar vertebra in 13-week-old female B6 mice.

Treatment	22 °C		32 °C		FDR adjusted p-value		
	Control	Particles	Control	Particles	Particles	Temperature	Interaction
Bone volume/tissue volume (%)	15.9 ± 0.4	14.9 ± 0.4	18.3 ± 0.6	18.3 ± 0.6	0.519	0.000	0.519
Connectivity density (mm^{-3})	161.3 ± 3.2	156.5 ± 7.9	158.8 ± 7.4	163.3 ± 4.8	0.974	0.820	0.603
Trabecular number (mm^{-1})	4.2 ± 0.1	4.0 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	0.463	0.012	0.600
Trabecular thickness (μm)	43 ± 0	43 ± 0	46 ± 1	46 ± 1	0.974	0.000	0.600
Trabecular spacing (μm)	241 ± 4	252 ± 4	229 ± 6	231 ± 6	0.377	0.012	0.600

Data are mean ± SE; n = 10–11/group.

or cancellous bone loss in lumbar vertebra. In concordance with Ross et al. (2014), we also observed an increase in serum osteocalcin, a biochemical marker of bone turnover. Similarly, we failed to detect cortical bone loss in femur or cancellous bone loss in the lumbar vertebra. However, we did detect cancellous bone loss in femur metaphysis. These findings suggest that particle-induced osteolysis results in bone-specific and bone compartment-specific effects. Cancellous compartments that undergo high rates of bone turnover (e.g., distal femur metaphysis) may be more sensitive to bone loss than sites that undergo lower rates of turnover (e.g., lumbar vertebra) (Turner et al., 2013).

Polyethylene particle treatment lowered cellular (osteoblast-lined bone perimeter in distal femur metaphysis), dynamic (mineralizing perimeter in distal femur metaphysis), molecular (osteocalcin gene expression in tibia), and biochemical (serum osteocalcin) indices of bone formation. Taken together, these findings strongly suggest that reduced bone formation contributes to bone loss in response to polyethylene particle challenge. Additionally, the increase in osteoclast-lined bone perimeter suggests that there was an increase in bone
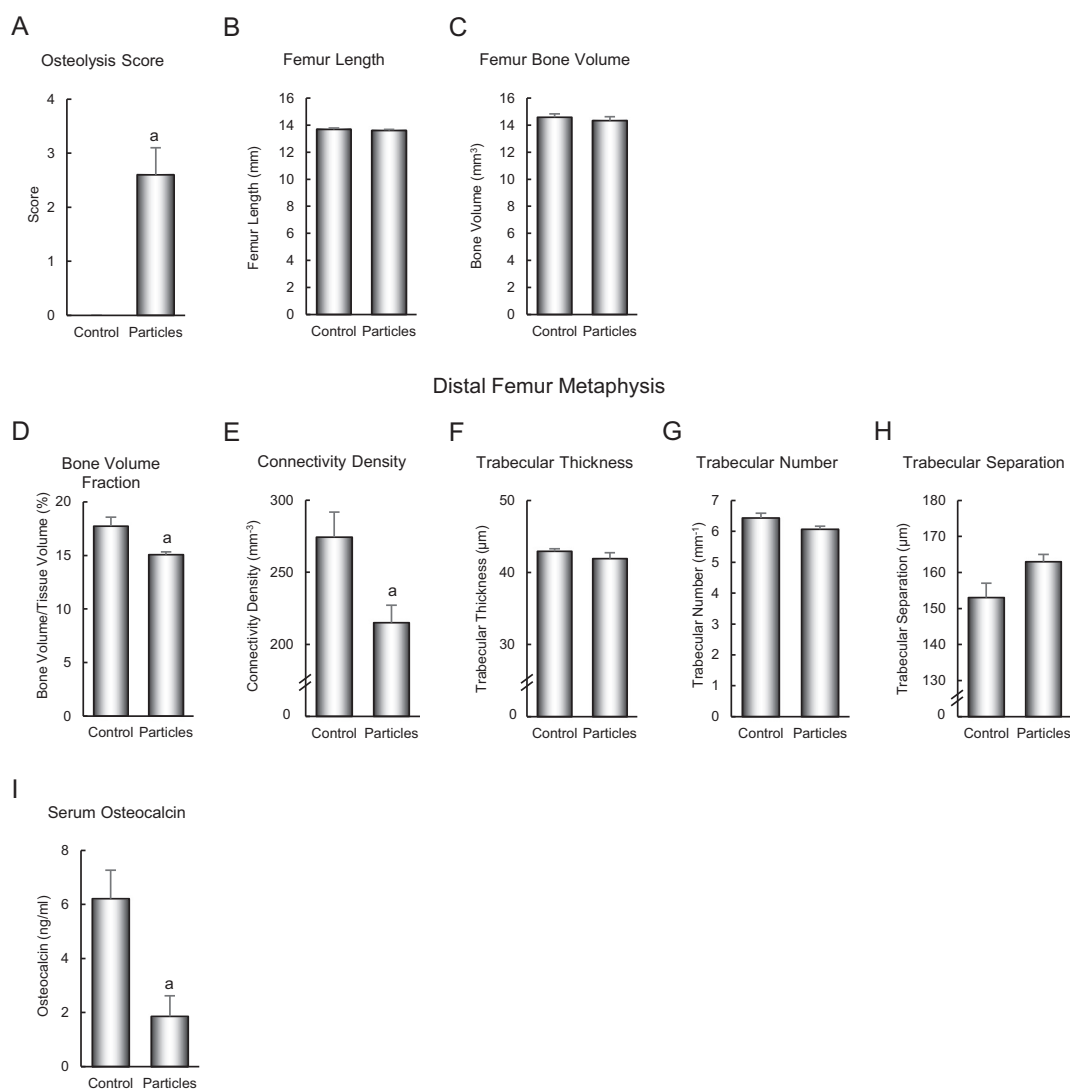

Fig. 3. Effects of polyethylene particle placement over calvaria on (A) calvarial osteolysis, (B) femur length, (C) total femur bone volume, and on (D) cancellous bone volume fraction, (E) connectivity density, (F) trabecular thickness, (G) trabecular number, and (H) trabecular separation in the distal femur metaphysis, and on (I) serum osteocalcin in 8-week-old female B6 mice. Data are mean ± SE; n = 9–10/group, [a]different from control, p ≤ 0.05.

Table 3
Tibia osteogenesis array showing fold changes for differentially expressed genes in particle-treated compared to control 8-week-old female B6 mice.

Differentially expressed genes		
Particles versus control		
Symbol	Fold change	p <
Bglap	−1.4	0.011
Cd36	1.3	0.005
Icam1	−1.1	0.008
Igf1r	−1.1	0.024
Itga2b	−1.3	0.001
Itgam	−1.2	0.000
Mmp8	−1.3	0.000
Nfkb1	−1.1	0.000
Sox9	−1.3	0.022
Tgfb1	−1.1	0.020
Tgfbr2	−1.1	0.036
Tgfbr3	1.3	0.000

N = 8/group.

resorption in the femur metaphysis. The molecular mechanism for bone loss in distal femur remains to be determined. However, the differential expression of genes related to bone metabolism observed in tibia in the present study implicate disturbed growth factor signaling.

Polyethylene wear particles could influence bone metabolism at sites remote from their origin by several non-mutually exclusive mechanisms. These mechanisms could involve transport of particles to remote sites or involve generation of pro-inflammatory cytokines and growth factors at the site of wear particle generation. Wear particles detected at sites remote from the prosthesis indicate they undergo transport (Park et al., 2013). Thus, it is plausible for transported particles to interact directly with bone cells and induce bone loss at sites distant from their initial production. Alternatively, there is evidence that local inflammation induces systemic bone loss; systemic bone loss has been reported in rodents following subcutaneous injection of talcum and cotton wool (Minne et al., 1984), and following subcutaneous implantation of controlled release pellets containing PGE_2 (Desimone et al., 1993). The role of inflammatory mediators such as PGE_2 in the pathogenesis of osteolysis is well established (Tsutsumi et al., 2009). However, further research is required to more fully delineate the precise mechanisms by which polyethylene particles induce bone loss at sites remote from their implantation.

A potential limitation of this study is that we did not measure physical activity; a major reduction in weight bearing could contribute to osteopenia in long bones (Iwaniec and Turner, 2016). However, daily inspection revealed that the particle-treated mice were fully ambulatory and particles did not influence body weight gain or food consumption. These findings argue against reduced physical activity as a co-morbidity.

An additional potential limitation of the present studies is that the young age of the animals does not ideally model the patient population most likely to undergo joint replacement therapy. In contrast to humans, mice exhibit rapid cancellous bone loss in long bones prior to cessation of linear growth resulting in low cancellous bone volume fraction prior to skeletal maturity (Glatt et al., 2007). We recently identified thermoregulation as the culprit for the premature cancellous bone loss in mice. Because the thermoneutral range for mice is ~10 °C warmer than for humans, mice housed at room temperature are subjected to chronic mild cold stress. The higher femur total bone volume and higher cancellous bone volume fraction in femur and lumbar vertebra in mice housed at 32 °C in the present study indicates that a change in housing temperature can lead to changes in bone mass and architecture in as little as three weeks. In the future, it may be feasible to use older mice housed at thermoneutral to model the local and remote effects of wear particles on bone in aging humans.

In the present studies, we observed minimal interactions (mineral apposition rate only) between housing temperature and polyethylene particles in bone. However, as previously indicated, mice housed at room temperature had lower cancellous bone volume fraction than mice housed at thermoneutral. This suggests that the mechanisms mediating osteolysis-induced and cold stress-induced bone loss differ. Adaptation to cold stress is a complex physiological process mediated by sensory and sympathetic signaling. Bone is highly innervated, and sympathetic signaling and sensory signaling contribute to the skeletal response to inflammation (Hill et al., 1991). Thus, when modeling interventions to mitigate wear particle-induced osteolysis it is important to recognize that treatments affecting sensory and/or sympathetic signaling in room temperature-housed mice (e.g., gonadal hormones, β-blockers, leptin) have the potential to influence bone mass and architecture independent of the effects of wear particles (von Knoch et al., 2004b).

In summary, our studies confirm the importance of housing temperature and skeletal site as biological variables in mice. Furthermore, the results support the concept that, in addition to inducing focal osteolysis, polyethylene particles generated during wear have the potential to induce a negative bone turnover balance and bone loss at distal sites. Bone loss is an important risk factor for fractures. Additional research is required to determine the contribution of debris particles, if any, to the accelerated bone loss and increased fracture rate noted in patients following total joint arthroplasty (Gundry et al., 2017).

Authors' roles

Conception and design: KP, AB, RT, and UI
Data acquisition: KP, CW, AK, and DO
Data analysis: AB
Drafting manuscript: KP, RT and UI
Revising manuscript: KP, CW, AK, DO, AB, RT, and UI
Approving final version: KP, CW, AK, DO, AB, RT, and UI.

UI takes responsibility for the integrity of the data.

Data availability

All data presented in this manuscript are available from the corresponding author upon reasonable request.

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Conflicts of interest

None.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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