

Sex-specific responses in trabecular and cortical microstructure of tibia due to repeated irradiation from micro-computed tomography in adult CD-1 mice

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ABSTRACT

While repeated *in vivo* micro-computed tomography (μ CT) allows for longitudinal measurement of bone outcomes in rodent models, it is important to determine that the resulting irradiation – dependent on the frequency and number of scans – does not exceed the effects of the intervention. The objective of this study was to determine whether repeated irradiation exposure from μ CT scans at 1-month intervals for a total of four scans would alter trabecular or cortical bone structure outcomes and/or bone mineral density in tibias from both male and female CD-1 mice. The right tibia of male ($n = 12$) and female ($n = 11$) CD-1 mice were scanned using μ CT at 2, 3, 4, and 5 months of age, while the contralateral left tibia served as a control and was scanned only at 5 months of age. All scans were performed at a resolution of $9 \mu\text{m}$ using a radiation dose of 460 mGy per scan. Some outcomes of trabecular bone structure were affected by repeated irradiation in both males and females. The bone volume fraction was lower in the irradiated right tibia compared to the non-irradiated left tibia in both males ($p < 0.05$) and females ($p < 0.01$) as a result of decreased trabecular number (males $p < 0.05$; females $p < 0.05$) and increased trabecular separation (males $p < 0.05$; females $p < 0.01$). Some cortical measures were also affected in females but not in males, including lower cortical bone periosteal perimeter ($p < 0.05$), lower total area ($p < 0.01$) and lower marrow area ($p < 0.05$) with repeated irradiation. Exposure to repeated radiation at intervals of 1 month, for a total of four scans, altered trabecular bone in both male and female CD-1 mice while outcomes of cortical bone structure were altered only in females.

1. Introduction

In vivo micro-computed tomography (μ CT) allows for the longitudinal visualization and assessment of bone structure and bone mineral density (BMD) in rodent models (Holdsworth and Thornton, 2002; Clark and Badaea, 2014). The advantage of performing *in vivo* analysis is the ability to track bone outcomes longitudinally within the same animal, reducing variation within measurements as well as the overall numbers of research animals required (Waarsing et al., 2004). μ CT can be used to monitor bone outcomes during ovariectomy (Boyd et al., 2006; Francisco et al., 2011; Longo et al., 2016; Waarsing et al., 2004) or states of disease (Johnson et al., 2011; Proulx et al., 2007), and in relation to a variety of interventions such as drugs (Tyagi et al., 2014; Proulx et al., 2007; Moverare-Skrtec et al., 2014), diet (Sacco et al., 2017; Sacco et al., 2018; Wakefield et al., 2019; Yumol et al., 2018; Longo et al., 2017), or exercise (Wallace et al., 2015). However,

an unavoidable limitation of *in vivo* μ CT is the exposure of animals to ionizing radiation, potentially damaging the tissues depending on the cumulative radiation dose (Holdsworth and Thornton, 2002; Laperre et al., 2011; Klinck et al., 2008). As a result, it is imperative to ensure that any modulation caused by irradiation exposure does not exceed the effect of the experimental intervention.

Image quality is modifiable by radiation dose, with higher resolution scans and resulting X-ray doses producing better images, however, this is not necessarily practical for *in vivo* imaging due to potential radiation exposure, prolonged anesthetic use, and long-term storage of large file sizes (6–7 GB per scan) (Longo et al., 2016; Sacco et al., 2017). Radiation dose must be considered within protocols for longitudinal scanning of the hindlimb using μ CT in live animals since residual radiation damage accumulates and can cause tissue damage in the trabecular and cortical bone compartments (Ford et al., 2003; Clark and Badaea, 2014). Previously, the effects of radiation exposure on bone

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tissue have been investigated using varying radiation doses, exposure frequencies, and total number of scans in rodents at various ages (Laperre et al., 2011; Klinck et al., 2008; Brouwers et al., 2007; Sacco et al., 2017; Longo et al., 2016; Mustafy et al., 2018). Both rats and mice are commonly used experimental models, but rats are less susceptible to ionizing radiation exposure than mice as they absorb less radiation due to their larger skeletal size (Klinck et al., 2008; Brouwers et al., 2007; Longo et al., 2016; Mustafy et al., 2018). In rats, repeated *in vivo* μ CT scans ranging from weekly to monthly intervals with radiation doses up to 939 mGy per scan did not affect tibia bone structure (Klinck et al., 2008; Brouwers et al., 2007; Longo et al., 2016). However, within rats there is a tolerable upper limit before bone structure is compromised; nine radiation exposures at weekly intervals either at 1650 mGy or 2470 mGy, but not at 830 mGy, resulted in compromised trabecular bone structure (Mustafy et al., 2018). As previously stated, mice are generally more sensitive to radiation exposure; three radiation exposures of 776 mGy per scan in adult male C57BL/6J mice at 2-week intervals (Laperre et al., 2011) and four radiation exposures to 846 mGy per scan in adult female mouse strains (C3H/HeJ, C57BL/6J, and BALB/cByJ) at 1-week intervals (Klinck et al., 2008) both impacted bone outcomes. In our lab, we followed up these studies by testing lower doses of radiation at 222 mGy and 460 mGy per scan with less frequent exposure during key stages of bone growth and development at 2, 4, and 6 months of age in male and female adult CD-1 mice. Based on previous studies, 2-month intervals were chosen since they were hypothesized to have no impact on bone structure. Regardless of radiation dose, trabecular and cortical bone outcomes were similar between the repeatedly irradiated tibia and non-irradiated control (Sacco et al., 2017). While the effect of repeated irradiation on bone outcomes has been reported using a variety of different mouse strains, it is important to recognize that these previous findings may not be fully translatable among strains.

Here, we focused on mice due to their susceptibility to radiation and as a research model they can be used to produce transgenic strains, for nutritional programming studies (Sacco et al., 2017; Sacco et al., 2018), and are typically more cost effective compared to rats (Bryda, 2013). Several studies in mice have incorporated longitudinal assessment of bone structure using *in vivo* μ CT to study the effect of diet interventions, with some studies identifying sex-specific responses to these interventions (Sacco et al., 2017; Sacco et al., 2018; Wakefield et al., 2019; Yumol et al., 2018). Therefore, it was important to test the sex-specific effects of repeated irradiation. While longitudinal μ CT at 2-month intervals for a total of three scans did not affect tibial trabecular or cortical bone outcomes (Sacco et al., 2017), it may be pertinent to perform more scans at a shorter frequency to assess bone structure and BMD. A shorter duration between scans may be especially important during periods of rapid alterations in bone, such as those during adolescence. Previously, bone structure and BMD across the lifespan was characterized in male and female C57BL/6J mice using *ex vivo* μ CT scans of the lumbar vertebra and distal femur. Trabecular bone volume peaks between 1 and 2 months of age followed by a rapid decline until about 6 months of age, while cortical bone cross sectional area and cortical thickness increase until 6 months of age before plateauing (Glatt et al., 2007). However, it is important to recognize that the changes in bone structure and BMD may vary among strains. Additionally, responses to interventions during the first months of life may be detectable with shorter intervals among scans due to the responsiveness of bone structure during development. Thus, the objective of this study was to determine whether repeated irradiation exposure from μ CT scans at 1-month intervals for a total of four scans would alter trabecular or cortical bone structure outcomes and/or BMD in both male and female CD-1 mice. We hypothesized that the repeated irradiation exposure would not affect either trabecular or cortical bone outcomes or BMD, based on our previous findings scanning at 2-month intervals using a radiation dose of 460 mGy (Sacco et al., 2017).

2. Materials and methods

2.1. Animals

All procedures that involved animals were in compliance with the Canadian Council on Animal Care (1993) and approved by the Brock University Animal Care Committee (AUP #17-06-01). Seven-week old male ($n = 12$) and female ($n = 11$) CD-1 mice were obtained from Charles River (St Constant, QC, Canada) and acclimatized for one week. Females were housed 4–5 per cage, while males were housed individually. Mice were kept on a standard 12-hour light:12-hour dark cycle and had *ad libitum* access to food and water. The standard AIN-93G diet (TD.94045, Harlan Teklad, Mississauga, ON) was provided to all mice.

2.2. Experimental design

In vivo hindlimb scans were performed to assess bone structure and BMD of the right tibia at 2, 3, 4, and 5 months of age, while the contralateral left tibia was only scanned at 5 months. This experimental design allows for within subject comparisons, the left tibia serving as a non-irradiated internal control that can be directly compared to the repeatedly irradiated right tibia (Sacco et al., 2017).

2.3. Micro-computed tomography of hindlimb

Scans were completed using μ CT (SkyScan 1176, Bruker-microCT, Kontich, Belgium) and host software (1176 version 1.1, Bruker-microCT, Kontich, Belgium) as previously described (Sacco et al., 2017; Longo et al., 2017). In brief, mice were anaesthetized in an induction chamber with isoflurane at a constant flow rate of 2–5% and transitioned to a nose cone housed inside the μ CT for maintenance of the anesthetic for the duration of the scan. Mice were provided with ophthalmic gel applied to their eyes to prevent dryness. On the scanning bed, the mouse was positioned on its back with the leg to be scanned extended and the foot secured in a foam holder lined with dental wax to prevent slippage. The foam was then inserted into the plastic holder attached to the scanning bed. The non-scanning leg and tail were secured away from the scanning region, towards the head (Longo et al., 2017).

The μ CT scans were set to an isotropic resolution of 9 μ m with a 1 mm aluminum filter. The scanning parameters consisted of 40 kV, 300 μ A, 0.8° rotation step, 3350 ms exposure time, over 180° with no frame averaging. Previously, these specific scanning parameters were determined using a MOSFET portable dosimeter (TN-502RD-H, Best Medical Canada, Ottawa, Canada) to yield a radiation dose of 460 mGy (Sacco et al., 2017). This radiation dose provides images that could be used to evaluate trabecular and cortical bone microarchitecture and vBMD. The total tibia length was measured *ex vivo* using digital calipers (Ex Cal 1P54 digital caliper, iGaging, California, United States).

2.4. Image processing and analysis

Cross-sectional images were reconstructed using GPUReconServer program (version 1.7.3.2, Bruker-microCT) and NRecon Reconstruction (version 1.7.3.1, Bruker-microCT). All images were reconstructed using variable post-alignment compensations within +5/–5, along with smoothing (4), beam hardening (40%), and ring artifact (6), while dynamic image range for the X-ray attenuation coefficient was set for all males and females (0.00–09392).

Reconstructed images were then reoriented using DataViewer (version 1.5.6.2, Bruker microCT), following which the region of interest (ROI) for the trabecular and cortical bone was selected using CTAnalyzer software (version 1.17.7.2+, Bruker-microCT). The transaxial slice at which the primary spongiosa of the proximal tibia metaphysis disconnects and the formation of the mineralized cartilage

“bridge” is formed was used as the landmark for ROI selection. To examine trabecular bone structural properties at the proximal tibia metaphysis, the top slice of ROI was set with a 75 slices (0.66 mm) separation from metaphyseal side of the growth plate. The total ROI consisted of 70 slices (0.62 mm) extending towards the ankle and was selected by manually contouring a few pixels within the endocortical shell. While cortical structure in the diaphysis was analyzed using a ROI consisting of 200 slices (1.76 mm) extending towards the ankle with an 850 slice (7.47 mm) separation from metaphyseal side of the growth plate. To select cortical bone for analysis, the marrow cavity was excluded by manually contouring a subtractive ROI and saved, followed by drawing a new ROI around the periosteal perimeter. Trabecular and cortical bone ROIs in relation to the described landmarking have been outlined in Fig. 4C. For segmentation a global threshold of 65–255 and 105–255 was used for trabecular and cortical bone, respectively, for all animals to binarize the bone tissue from non-bone tissue. Two standard phantoms with a known density of 0.25 g/cm³ and 0.75 g/cm³ were used to calibrate the μ CT for BMD analysis.

Proximal tibia metaphyseal trabecular bone outcomes assessed using 3D analysis included: bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), degree of anisotropy (DA), structural model index (SMI), volumetric BMD (vBMD), and tissue mineral density (TMD) (Bouxsein et al., 2010). Cortical bone outcomes assessed using 2D analysis included: cortical area fraction (Ct.Ar/Tt.Ar), cortical thickness (Ct.Th), periosteal perimeter (Ps.Pm), endocortical perimeter (Ec.Pm), total area (Tt.Ar), medullary area (Ma.Ar), and tissue mineral density (TMD) (Bouxsein et al., 2010) (CTAnalyzer, version 1.17.7.2+, Bruker-microCT).

2.5. Statistical analysis

All values are presented as means \pm standard error (SEM). Comparisons were made using a paired *t*-test. All statistical analysis was done using GraphPad Prism (version 6.0 h) with statistical significance set to $p \leq 0.05$.

3. Results

3.1. Animal characteristics

Body weight at the beginning of the study (2 months of age) for males was 32.7 \pm 2.3 g and females was 28.0 \pm 1.5 g. Endpoint body weight (5 months of age) for males was 39.2 \pm 5.7 g and females was 35.7 \pm 4.5 g, resulting in similar weight gain over the course of the study between males (6.4 \pm 4.3 g) and females (7.7 \pm 3.8 g). All trabecular and cortical bone outcomes from *in vivo* scans of the right tibia at 2, 3, 4, and 5 months of age and the left tibia at 5 months have been summarized in Supplementary Table 1.

3.2. Trabecular bone outcomes

Tibial length was unaffected by the repeated irradiation in both males and females (Fig. 1). Proximal tibia metaphyseal trabecular bone was compromised with repeated irradiation in both male and female mice (Fig. 2). BV/TV (%) of the right tibia in males was 22% lower compared to the left ($p < 0.05$) and in females was 22% lower in the right tibia compared to the left ($p < 0.01$; Fig. 2A). Repeated irradiation also resulted in significant changes in other trabecular bone outcomes. Tb.N (mm⁻¹) was 22% lower in males ($p < 0.05$) and 18% lower in females ($p < 0.05$; Fig. 2B), while there was no difference in Tb.Th (mm) for either males or females (Fig. 2C), and a 14% increase in Tb.Sp (mm) in males ($p < 0.05$) and 5% increase in females ($p < 0.01$; Fig. 2D) in the repeatedly irradiated right tibia compared to the control left tibia. DA (no units) (Fig. 2E) and SMI (Fig. 2F) was unaffected by the repeated irradiation in both males and females. In

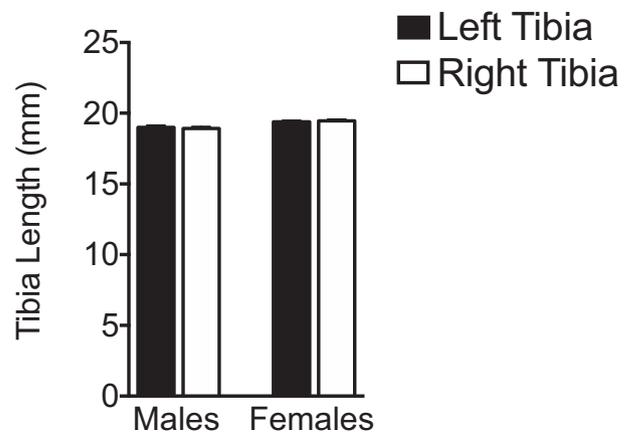


Fig. 1. Repeated irradiation from μ CT scans does not alter tibia length in both males and females. The right tibia was scanned at 2, 3, 4, and 5 months of age and the left tibia was scanned only at 5 months of age.

males, trabecular vBMD (g/cm³) was 9% lower in the right tibia compared to the left ($p < 0.05$) but did not differ between the left and right tibia in females (Fig. 2G).

3.3. Cortical bone outcomes

Cortical bone structure was compromised in females but not males with repeated irradiation. Both Ct.Ar/Tt.Ar (%) and Ct.Th (mm) were unaffected by the repeated irradiation as the left and right tibia had similar results in both males and females (Fig. 3A & B). Ps.Pm (mm) was unaffected in males but decreased by 3% in the right tibia compared to the left tibia in females ($p < 0.05$; Fig. 3C), while Ec.Pm (mm) was unaffected by repeated irradiation in both males and females (Fig. 3D). Tt.Ar (mm²) was unaffected in males but decreased 3% in the right tibia compared to the left in the females ($p < 0.01$; Fig. 3E) and Ma.Ar (mm²) was unaffected in males but decreased by 7% in the right tibia compared to the left in the females ($p < 0.05$; Fig. 3F). Cortical TMD (g/cm³) was unchanged in the right tibia compared to the left in males but increased by 2% in the right tibia compared to the left in females ($p < 0.01$; Fig. 3G).

4. Discussion

In this study, we investigated the effects of longitudinal *in vivo* μ CT scans on male and female CD-1 mouse tibia trabecular and cortical bone structure and vBMD. Trabecular bone structure in both male and female CD-1 mice was affected by repeated irradiation from longitudinal μ CT scans at 1-month intervals at 2, 3, 4, and 5 months of age for a total of four scans. More specifically, the repeatedly irradiated right tibia had a lower BV/TV compared to the non-irradiated left tibia as a result of lower Tb.N and increased Tb.Sp, however, vBMD was only lower in the irradiated tibia in the males but not females. Tibia length growth was not compromised by repeated irradiation. Furthermore, these results suggest that females were more affected, as both trabecular and cortical outcomes, including Ps.Pm, Tt.Ar, and Ma.Ar were impacted.

The longitudinal radiation dose had a consistent effect on the trabecular findings for both males and females, highlighting the susceptibility of the trabecular bone regardless of sex. The differential response of cortical bone between males and females to the repeated irradiation highlights the need to study sex differences and that different types of bone may respond differently by sex. Though, it is important to note that for outcomes of cortical bone structure, some but not all measures were affected by repeated irradiation in females. Previously reported in C57BL/6J mice, males and females follow slightly different age-related changes in trabecular bone structure during adolescence from 1

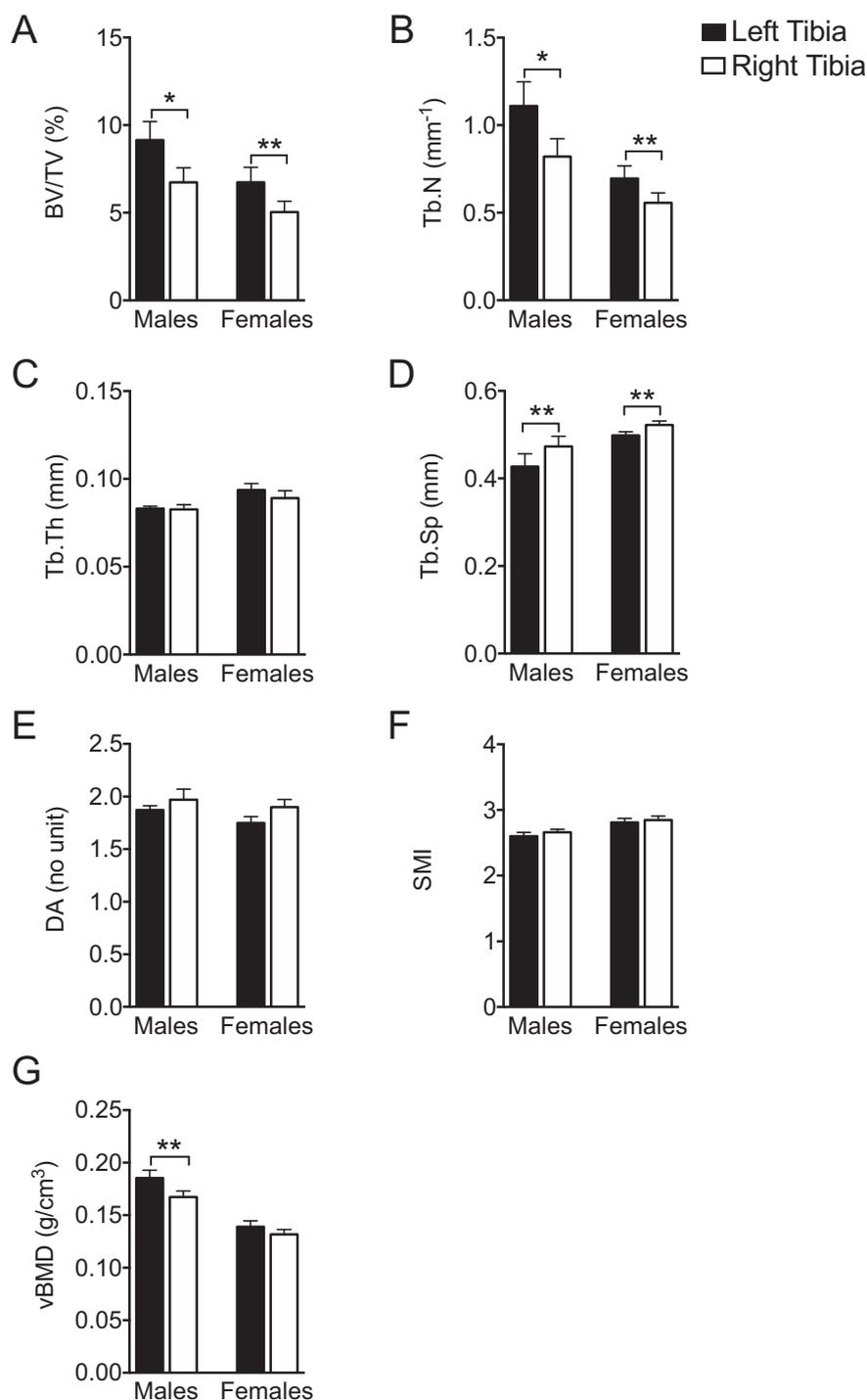


Fig. 2. Repeated irradiation from μ CT scans alters some measures of trabecular bone structure at the proximal tibia in both males and females. Trabecular bone structure and volumetric bone mineral density (vBMD) of the non-irradiated left tibias (filled bars) and irradiated right tibias (empty bars) of male ($n = 12$) and female ($n = 11$) CD-1 mice. (A) Bone volume fraction (BV/TV) of the right tibia was lower compared to the left in males ($p < 0.05$) and females ($p < 0.01$). (B) Trabecular number (Tb.N) of the right tibia was lower compared to the left in males ($p < 0.05$) and females ($p < 0.01$). (C) Trabecular thickness (Tb.Th) was unchanged in the right tibia compared to the left in both males and females. (D) Trabecular separation (Tb.Sp) was increased in the right tibia compared to the left in males ($p < 0.01$) and females ($p < 0.01$). (E) Degree of anisotropy (DA) was unchanged in the right tibia compared to the left in both males and females. (F) Structural model index (SMI) was unchanged in the right tibia compared to the left in both males and females. (G) Trabecular vBMD (g/cm^3) of the right tibia was lower compared to the left in males ($p < 0.01$) but not females. Data represented as mean \pm SEM, statistical significance denoted as * $p < 0.05$, and ** $p < 0.01$.

through 6 months of age, with females undergoing a more rapid decline than males (Glatt et al., 2007). As previously mentioned, mice are more susceptible to radiation exposure than rats due to their smaller size and lower bone volume (Klinck et al., 2008). This may explain the sex-differences reported here, as female CD-1 mice are generally smaller than males and as a result, may be absorbing a slightly higher amount of radiation. Here, the cortical bone from the females was affected to a lesser degree (differences of 2–7%) compared to the trabecular bone (differences of 5–22%) with some inconsistencies. Repeated irradiation of the right tibia resulted in a decreased Ps.Pm, Tt.Ar, and Ma.Ar compared to the non-irradiated left tibia suggestive of smaller bones, and this can explain the increase in TMD, though Ct.Ar/Tt.Ar, a main outcome of cortical structure, remained unaffected. A previous study

used 12-week old female C3H/HeJ and C57BL/6J mice scanning at 1-week intervals using scanning parameters generating 846 mGy per scan. Similar to our results, this study also reported alterations in cortical bone outcomes due to repeated μ CT scans to be less severe and more inconsistent (differences of 3–6%) in comparison to the trabecular bone outcomes (differences of 8–20%) (Klinck et al., 2008). Trabecular and cortical bone both contribute to overall bone strength and follow different trajectories over the lifespan. Trabecular bone peaks around 1 to 2 months of age followed by a rapid age associated bone loss until 6 months of age, while cortical bone continues to develop up until about 6 months of age (Glatt et al., 2007). Capturing alterations in bone structure may require more frequent scans, especially during critical time points of rapid development in which an intervention may incur a

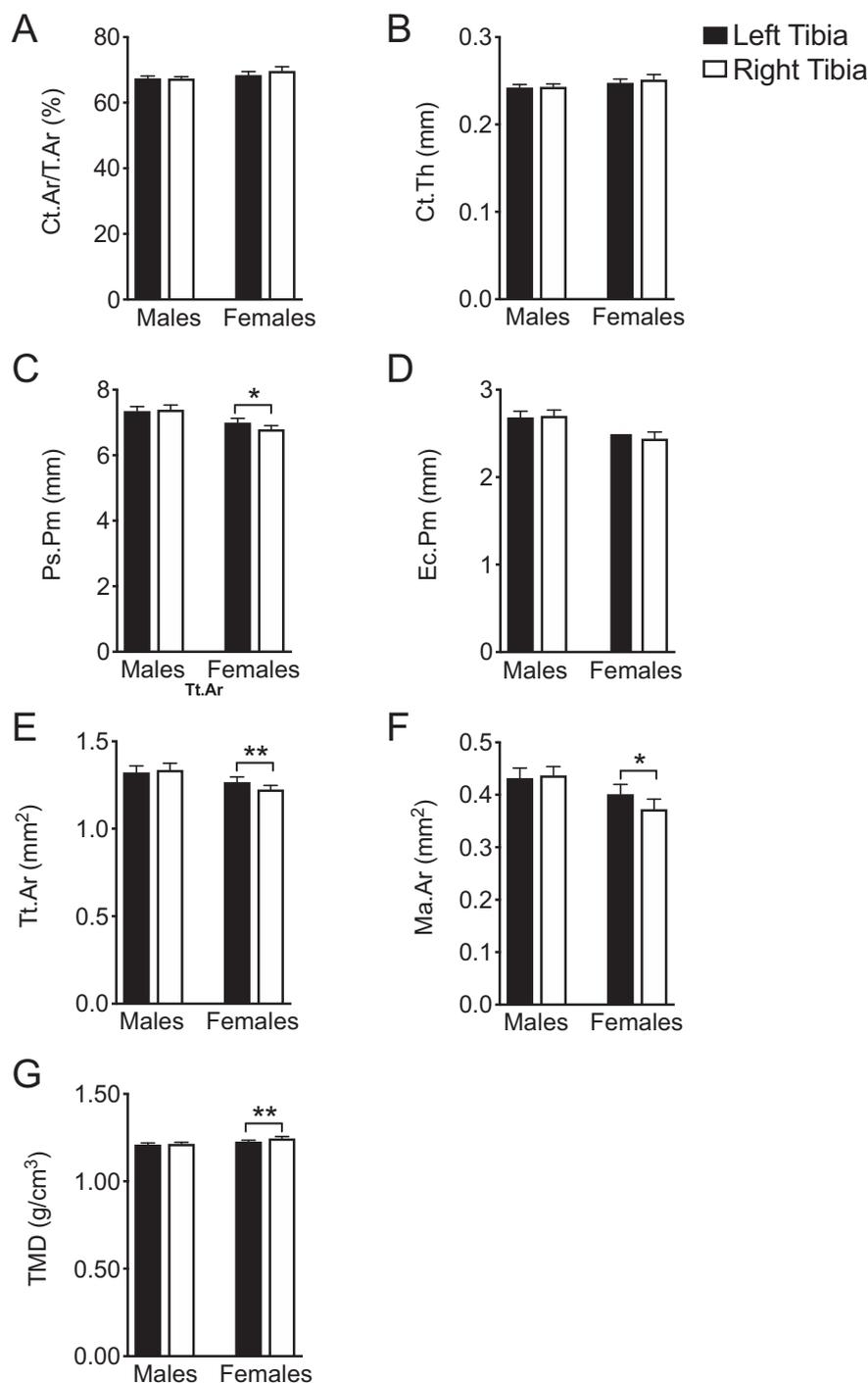


Fig. 3. Repeated irradiation from μ CT scans alters some measures of cortical bone structure in females but not males. Cortical bone structure and tissue mineral density (TMD) of the non-irradiated left tibias (filled bars) and irradiated right tibias (empty bars) of male ($n = 12$) and female ($n = 11$) CD-1 mice. (A) Cortical area fraction (Ct.Ar/Tt.Ar) was unchanged in the right tibia compared to the left in both males and females. (B) Cortical thickness (Ct.Th) was unchanged in the right tibia compared to the left in both males and females. (C) Periosteal perimeter (Ps.Pm) of the right tibia was lower compared to the left in females ($p < 0.05$) but not males. (D) Endocortical perimeter (Ec.Pm) was unchanged in the right tibia compared to the left in both males and females. (E) Total area (Tt.Ar) of the right tibia was lower compared to the left in females ($p < 0.01$) but not males. (F) Medullary area (Ma.Ar) of the right tibia was lower compared to the left in females ($p < 0.05$) but not males. (G) Cortical TMD (g/cm^3) of the right tibia was greater compared to the left in females ($p < 0.01$) but not males. Data represented as mean \pm SEM, statistical significance denoted as * $p < 0.05$ and ** $p < 0.01$.

specific response, and possibly in a sex-specific manner. Indeed, a previous study showed that maternal exposure to citrus flavonoids resulted in improved bone structure at 4 and 6 months of age in male offspring while female offspring experienced a detrimental response at 2 and 4 months of age that disappeared by 6 months of age (Sacco et al., 2017; Sacco et al., 2018). Since scans in the above-mentioned study were performed at 2-month intervals, more frequent scanning would have provided a more comprehensive time-course and insight into potential opportunities to intervene with other dietary components to positively impact bone structure.

Bone marrow houses osteoprogenitor cells that can differentiate into osteoblasts, which are integral to bone formation. During a μ CT scan of the leg, bone marrow is inevitably irradiated, which could alter bone

formation rates and potentially affect overall bone structure (Mustafy et al., 2018; Dare et al., 1997). *In vitro* osteoblast differentiation and activity was previously reported to be unaffected by a single radiation exposure up to 400 mGy (Dare et al., 1997). Furthermore, a study in female Wistar rats, exposed to 939 mGy of radiation exposure for eight *in vivo* μ CT scans at 1 week intervals, reported having an effect on bone marrow cell viability (Brouwers et al., 2007). A limitation of this study is that we cannot distinguish whether the impact of repeated irradiation on trabecular and cortical bone outcomes is due to altered osteoblast or osteoclast activities.

A major strength of the present study was the study design, which allowed for the left leg to serve as an internal control to the repeatedly irradiated right leg. Additionally, the *in vivo* μ CT analyses allowed for

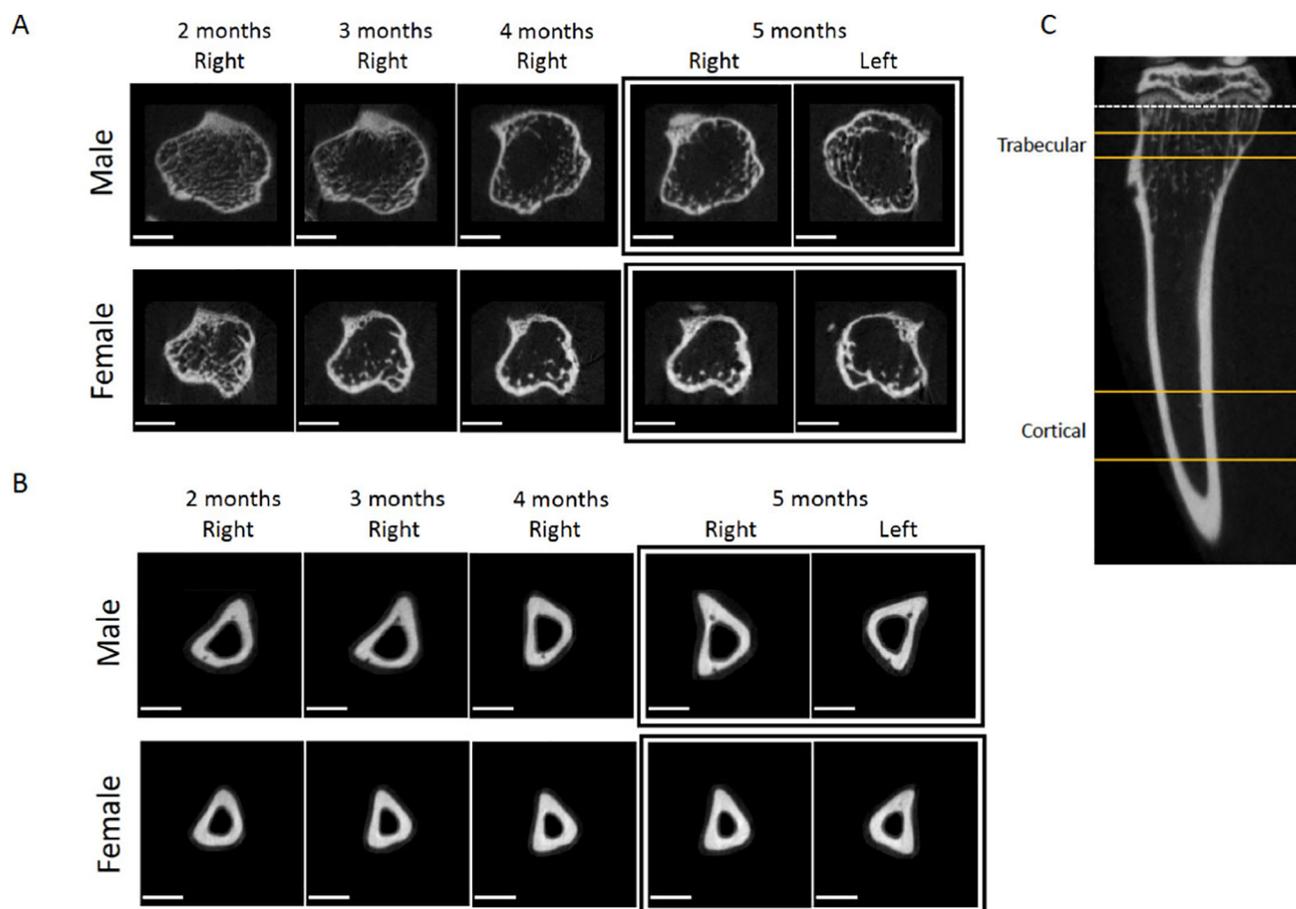


Fig. 4. Representative grey scales images of (A) trabecular and (B) cortical trans-axial cross-sections from a male and female mouse that most closely represented the average of the BV/TV for both the right and left proximal tibia. The same mouse was used for both trabecular and cortical bone images. White lines in panels (A) and (B) are 1 mm for the tibial trabecular and cortical bone images. (C) Representative tibia landmarking. The dashed line represents the reference point at which the primary spongiosa of the proximal tibia metaphysis disconnects. Using this reference point an offset of 75 (0.66 mm) slices to the trabecular region of interest and 850 slices (7.47 mm) to the cortical region of interest was set. The trabecular region of interest was set at 70 (0.62 mm) slices and cortical was set at 200 slices (1.76 mm), both extending into the diaphysis outlined by the solid lines.

bone to be tracked longitudinally within the same animal. While, a limitation of this study was that bone strength was unable to be tested since the right and left tibias were stored under different conditions for future follow-up within a larger study.

5. Conclusions

Repeated irradiation exposure at 1-month intervals for a total of four scans affects trabecular bone in both male and female CD-1 mice. Specifically, some but not all outcomes of cortical bone structure are affected in females while males show no differences in these same outcomes. The results of this study highlight the importance of studying sex differences and careful study design to best optimize μ CT scan frequency and number while still capturing any potential effect of an intervention. Establishing the effects of radiation exposure becomes more pertinent for interventions that induce more subtle alterations such as diet (Sacco et al., 2017; Sacco et al., 2018; Yumol et al., 2018; Wakefield et al., 2019), whereas ovariectomy (Boyd et al., 2006; Francisco et al., 2011; Longo et al., 2017) and drug interventions (Moverare-Skrtec et al., 2014; Tyagi et al., 2014) may result in more profound effects on bone. Although repeated μ CT scans at 1-month intervals resulted in decreased BV/TV and Tb.N along with increased Tb.Sp, this frequency may be usable as long as the magnitude of the intervention is greater than that of the irradiation. Another consideration is that these results are from CD-1 mice only and whether other mouse strains respond similarly should be verified.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bonr.2019.100232>.

Transparency document

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

Author contribution

K.N.B. conceived the study idea along with S.J.P. and W.E.W. K.N.B. conducted the *in vivo* trial and K.N.B. and J.L.Y. performed *in vivo* micro-computed tomography scans. K.N.B. performed image analyses and was responsible for data collection and statistical analyses. S.J.P. and W.E.W. contributed the materials and infrastructure. S.J.P. and W.E.W. supervised the project. K.N.B. wrote the manuscript that was reviewed and approved by all authors.

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Declaration of competing interest

KN Bott, JL Yumol, SJ Peters, and WE Ward have no conflicts of interest.

References

- Bouxsein, M.L., Boyd, S.K., Christiansen, B.A., Guldborg, R.E., Jepsen, K.J., Muller, R., 2010. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J. Bone Miner. Res.* 25, 1468–1486.
- Boyd, S.K., Davison, P., Muller, R., Gasser, J.A., 2006. Monitoring individual morphological changes over time in ovariectomized rats by in vivo micro-computed tomography. *Bone* 39, 854–862.
- Brouwers, J.E., van Rietbergen, B., Huiskes, R., 2007. No effects of in vivo micro-CT radiation on structural parameters and bone marrow cells in proximal tibia of wistar rats detected after eight weekly scans. *J. Orthop. Res.* 25, 1325–1332.
- Bryda, E.C., 2013. The mighty mouse: the impact of rodents on advances in biomedical research. *Mo. Med.* 110, 207–211.
- Canadian Council on Animal Care, 1993. Guide to the care and use of experimental animals. http://www.cac.ca/Documents/Standards/Guidelines/Experimental_Animals_Vol1.pdf, Accessed date: 20 November 2016.
- Clark, D.P., Badea, C.T., 2014. Micro-CT of rodents: state-of-the-art and future perspectives. *Phys Med* 30, 619–634.
- Dare, A., Hachisu, R., Yamaguchi, A., Yokose, S., Yoshiki, S., Okano, T., 1997. Effects of ionizing radiation on proliferation and differentiation of osteoblast-like cells. *J. Dent. Res.* 76, 658–664.
- Ford, N.L., Thornton, M.M., Holdsworth, D.W., 2003. Fundamental image quality limits for microcomputed tomography in small animals. *Med. Phys.* 30, 2869–2877.
- Francisco, J.I., Yu, Y., Oliver, R.A., Walsh, W.R., 2011. Relationship between age, skeletal site, and time post-ovariectomy on bone mineral and trabecular microarchitecture in rats. *J. Orthop. Res.* 29, 189–196.
- Glatt, V., Canalis, E., Stadmeier, L., Bouxsein, M.L., 2007. Age-related changes in trabecular architecture differ in female and male C57BL/6J mice. *J. Bone Miner. Res.* 22, 1197–1207.
- Holdsworth, D.W., Thornton, M.M., 2002. Micro-CT in small animal and specimen imaging. *Trends Biotechnol.* 20, S34–S39.
- Johnson, L.C., Johnson, R.W., Munoz, S.A., Mundy, G.R., Peterson, T.E., Sterling, J.A., 2011. Longitudinal live animal micro-CT allows for quantitative analysis of tumor-induced bone destruction. *Bone* 48, 141–151.
- Klinck, R.J., Campbell, G.M., Boyd, S.K., 2008. Radiation effects on bone architecture in mice and rats resulting from in vivo micro-computed tomography scanning. *Med. Eng. Phys.* 30, 888–895.
- Laperre, K., Depypere, M., van Gastel, N., Torrekens, S., Moermans, K., Bogaerts, R., Maes, F., Carmeliet, G., 2011. Development of micro-CT protocols for in vivo follow-up of mouse bone architecture without major radiation side effects. *Bone* 49, 613–622.
- Longo, A.B., Sacco, S.M., Salmon, P.L., Ward, W.E., 2016. Longitudinal use of micro-computed tomography does not alter microarchitecture of the proximal tibia in sham or ovariectomized Sprague-Dawley rats. *Calcif. Tissue Int.* 98, 631–641.
- Longo, A.B., Sacco, S.M., Ward, W.E., 2017a. Proper positioning and restraint of a rat hind limb for focused high resolution imaging of bone micro-architecture using in vivo micro-computed tomography. *J. Vis. Exp.* 129.
- Longo, A.B., Sullivan, P.J., Peters, S.J., LeBlanc, P.J., Wohl, G.R., Ward, W.E., 2017b. Lifelong intake of flaxseed or menhaden oil to provide varying n-6 to n-3 PUFA ratios modulate bone microarchitecture during growth, but not after OVX in Sprague-Dawley rats. *Mol. Nutr. Food Res.* 61.
- Moverare-Skrtic, S., Borjesson, A.E., Farman, H.H., Sjogren, K., Windahl, S.H., Lagerquist, M.K., Andersson, A., Stubelius, A., Carlsten, H., Gustafsson, J.A., Ohlsson, C., 2014. The estrogen receptor antagonist ICI 182,780 can act both as an agonist and an inverse agonist when estrogen receptor alpha AF-2 is modified. *Proc. Natl. Acad. Sci. U. S. A.* 111, 1180–1185.
- Mustafy, T., Benoit, A., Londono, I., Moldovan, F., Villemure, I., 2018. Can repeated in vivo micro-CT irradiation during adolescence alter bone microstructure, histomorphometry and longitudinal growth in a rodent model? *PLoS One* 13, e0207323.
- Proulx, S.T., Kwok, E., You, Z., Papuga, M.O., Beck, C.A., Shealy, D.J., Ritchlin, C.T., Awad, H.A., Boyce, B.F., Xing, L., Schwarz, E.M., 2007. Longitudinal assessment of synovial, lymph node, and bone volumes in inflammatory arthritis in mice by in vivo magnetic resonance imaging and microfocal computed tomography. *Arthritis Rheum.* 56, 4024–4037.
- Sacco, S.M., Saint, C., LeBlanc, P.J., Ward, W.E., 2017a. Maternal consumption of hesperidin and naringin flavanones exerts transient effects to tibia bone structure in female CD-1 offspring. *Nutrients* 9.
- Sacco, S.M., Saint, C., LeBlanc, P.J., Ward, W.E., 2018. Nutritional programming of bone structure in male offspring by maternal consumption of citrus flavanones. *Calcif. Tissue Int.* 102, 671–682.
- Sacco, S.M., Saint, C., Longo, A.B., Wakefield, C.B., Salmon, P.L., LeBlanc, P.J., Ward, W.E., 2017b. Repeated irradiation from micro-computed tomography scanning at 2, 4 and 6 months of age does not induce damage to tibial bone microstructure in male and female CD-1 mice. *Bonekey Rep* 6, 855.
- Tyagi, A.M., Mansoori, M.N., Srivastava, K., Khan, M.P., Kureel, J., Dixit, M., Shukla, P., Trivedi, R., Chattopadhyay, N., Singh, D., 2014. Enhanced immunoprotective effects by anti-IL-17 antibody translates to improved skeletal parameters under estrogen deficiency compared with anti-RANKL and anti-TNF-alpha antibodies. *J. Bone Miner. Res.* 29, 1981–1992.
- Waarsing, J.H., Day, J.S., van der Linden, J.C., Ederveen, A.G., Spanjers, C., De Clerck, N., Sasov, A., Verhaar, J.A.N., Weinans, H., 2004. Detecting and tracking local changes in the tibiae of individual rats: a novel method to analyse longitudinal in vivo micro-CT data. *Bone* 34, 163–169.
- Wakefield, C.B., Yumol, J.L., Sacco, S.M., Sullivan, P.J., Comelli, E.M., Ward, W.E., 2019. Bone structure is largely unchanged in growing male CD-1 mice fed lower levels of vitamin D and calcium than in the AIN-93G diet. *Bone Rep* 10, 100191.
- Wallace, I.J., Judex, S., Demes, B., 2015. Effects of load-bearing exercise on skeletal structure and mechanics differ between outbred populations of mice. *Bone* 72, 1–8.
- Yumol, J.L., Wakefield, C.B., Sacco, S.M., Sullivan, P.J., Comelli, E.M., Ward, W.E., 2018. Bone development in growing female mice fed calcium and vitamin D at lower levels than is present in the AIN-93G reference diet. *Bone Rep* 8, 229–238.