Non-invasive measurement of osteoporotic bone loss in mice after corticosteroid treatment of ovariectomy using high resolution microCT

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1 Abstract

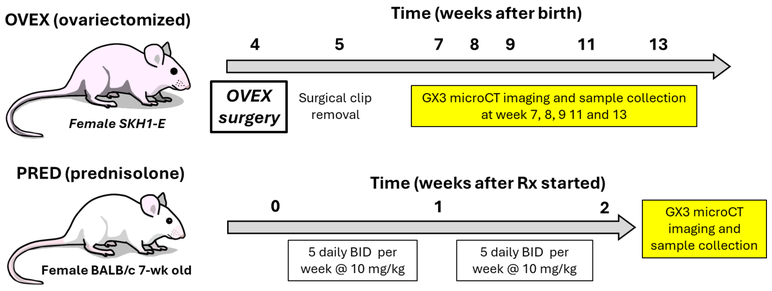
Osteoporosis (OP) is a chronic bone condition that weakens bone mass and density, commonly occurring in post-menopausal women as estrogen levels decrease. There are also acute causes of OP, including corticosteroid-induced OP resulting from extended use of prednisolone (PRED) in immunosuppressive therapies. Growth plate thickness and trabecular degeneration are key aspects of bone loss due to acute or chronic OP. While microCT is a key preclinical imaging modality for bone measurements, often the assessments are only done using excised bones at terminal timepoints, requiring high mouse numbers for longitudinal measurements. In this study we monitored trabecular degeneration and growth plate thickness in two models of bone loss: chronic, ovariectomy-induced (OVEX) OP, and acute PRED associated OP. Both models were assessed at different timepoints using the Quantum™ GX3 microCT (Revvity, Inc.) to image and measure bone loss in the spine and femurs. High resolution images were captured both in living animals and post-mortem for analyses across the two models.

OVEX OP SKH-1E female mice (n=3 per timepoint) were ovariectomized at 4 weeks of age, and OVEX and control mice were imaged *in* vivo at 7, 8, 9, 11 and 13 weeks of age. Bone samples (femurs and spines) were collected after each timepoint for *ex vivo* imaging. Corticosteroid-induced OP BALB/c female mice (n=3 per timepoint) were given PRED (10 mg/kg, bid) for two weeks (or no treatment for controls), then imaged live and bone samples were collected for *ex vivo* bone imaging. For *in vivo* imaging, both femurs and lumbar spine were scanned separately [4 mins, 80 kV (160 µA), FOV 36 mm, 0.5 mm Al filter], limiting radiation exposure to allow 3-4 additional longitudinal acquisitions. Bone microCT images were acquired for high resolution *ex vivo* validation [4 mins, 80 kV (50 µA), FOV 8 mm, 0.5 mm Al filter]. The microCT images were then subject to bone microarchitecture analysis (BMA) using the Analyze 15 software.

BMA analyses of OVEX microCT images shows bone loss in both density and volume. Bone density reduction was seen in *in vivo* (FOV36) scans of the spine cortex and femur trabeculae as early as 9 weeks. Trabecular volume suppression can also be observed throughout the course of imaging. High-resolution *ex vivo* (FOV8) scans of bone samples confirm our *in vivo* findings and provide better volumetric assessments of OVEX spines. Similarly, two weeks of short-term PRED treatments is sufficient to cause loss in both bone density (-6% in cortex and -9% in intertrabecular) and volume (-15% in trabeculae), as shown by *in vivo* and *ex vivo* femur scans. Interestingly, the short-term exposure of high dose PRED seems to slightly increase trabecular volume (+6%), which is in contrast with the OVEX model. Furthermore, the OVEX and PRED models differed significantly in the magnitude of trabecular bone loss, with dramatic loss seen in OVEX mice and mild or little trabecular bone loss seen with PRED. Assisted by high-resolution e*x vivo* imaging and quantification, the pixel size improved 4.4-fold to 2.86 µm. Thus, the Quantum GX3 allows assessment of the fine structural details within trabecular bone and growth plate regions, confirming non-invasive observations, and offering enhanced quantification and visualization capabilities. Future studies may address mechanistic difference is lower-dose, chronic PRED treatment.

2 Animal models and imaging methods

Animal models for osteoporosis

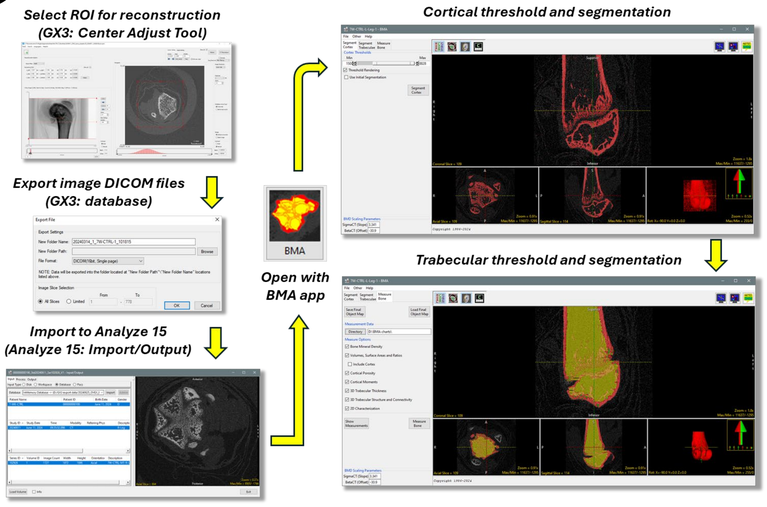


Quantum GX3 imaging settings

	In vivo (Live animals)	*Ex vivo (Bone samples)*
Field-of-view (FOV)	36 mm (FOV36)	8 mm (FOV8)
X-ray source filter, kV, uA	Aluminum 0.5 mm, 80kV, 160uA	Aluminum 0.5 mm, 80kV, 50uA
Scan mode, duration	HighRes, 4min	HighRes, 4min
Reconstruction mode, pixel size	SuperHigh, 12.87um	SuperHigh, 2.86um

Two osteoporosis models were used for this study. The first was ovariectomized SKH1-E mice (Charles River). Surgeries were performed 4 weeks after birth, and the surgical clips were removed on week 5. The GX3 microCT imaging were performed on weeks 7, 8, 9,11 and 13. The second model was induced by short-term exposure of prednisolone (10mg/kg BID, Sigma Aldrich) for two weeks. Each week, the treatments consisted of 5 daily BID via oral gavage. Two different settings, as listed in the table above, were used for *in vivo* (live animal) and *ex vivo* (bone sample) microCT imaging, respectively.

3 Workflow of BMA analysis



BMA output metrics and their description (3D-related)

Bone Mineral Density

BoneMean BMD	Mean BMD of bone
BoneStd BMD	Standard deviation of bone
BoneVolume	Volume of bone
CortexMean BMD	Mean BMD of cortex
CortexStd BMD	Standard deviation of cortex
CortexVolume	Volume of cortex
IntraTrabecularMean BMD	Mean BMD of intratrabecular
IntraTrabecularStd BMD	Standard deviation of intratrabecular
IntraTrabecularVolume	Volume of intratrabecular
TrabeculaeMean BMD	Mean BMD of trabecular
TrabeculaeStd BMD	Standard deviation of trabecular
TrabeculaeVolume	Volume of trabecular
TrabecularTissueMean BMD	Mean BMD of trabecular tissue
TrabecularTissueStd BMD	Standard deviation of trabecular tissue
TrabecularTissueVolume	Volume of trabecular tissue
WholeMean BMD	Mean BMD of whole ROI
WholeStd BMD	Standard deviation of whole ROI
WholeVolume	Volume of whole ROI

Volume, Surface and Ratios

TV	Total Volume
BV	Bone Volume
BS	Bone Surface
BV/TV	Bone Volume Fraction
BS/TV	Bone Surface Density
BS/BV	Specific Bone Surface

Cortical Porosity

Ct.Po	Cortical Porosity
Po.N	Pore Number
Po.V	Total Pore Volume
AvgPo.V	Average Pore Volume
Po.V.SD	Standard Deviation of Pore Volume
Po.Dn	Pore Density

Trabecular Thickness

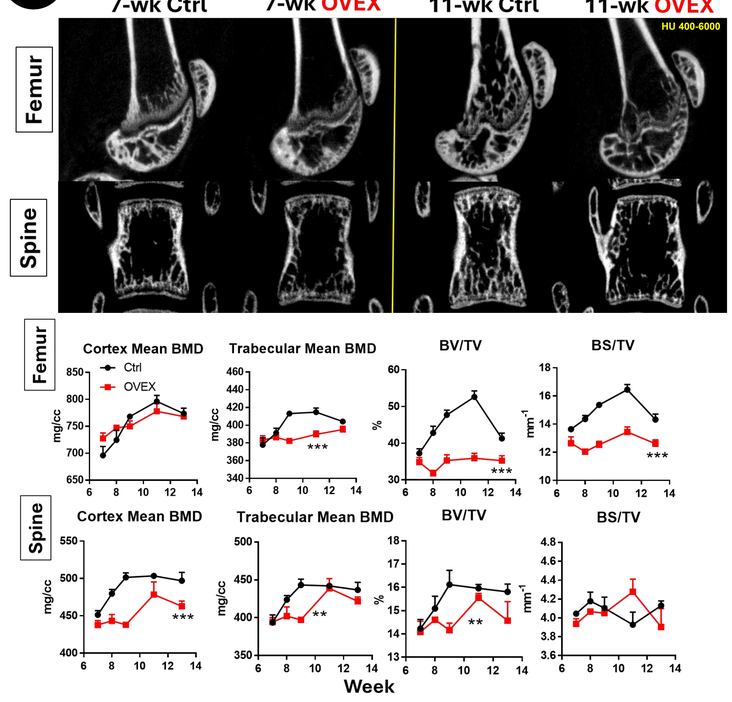
Tb.Th	Trabecular Thickness
Tb.Sp	Trabecular Separation
Tb.Th.SD	Standard Deviation of Trabecular Thickness
Tb.Sp.SD	Standard Deviation of Trabecular Separation

Trabecular Structure and Connectivity

SMI	Structure Model Index
Conn.D	Connectivity Density

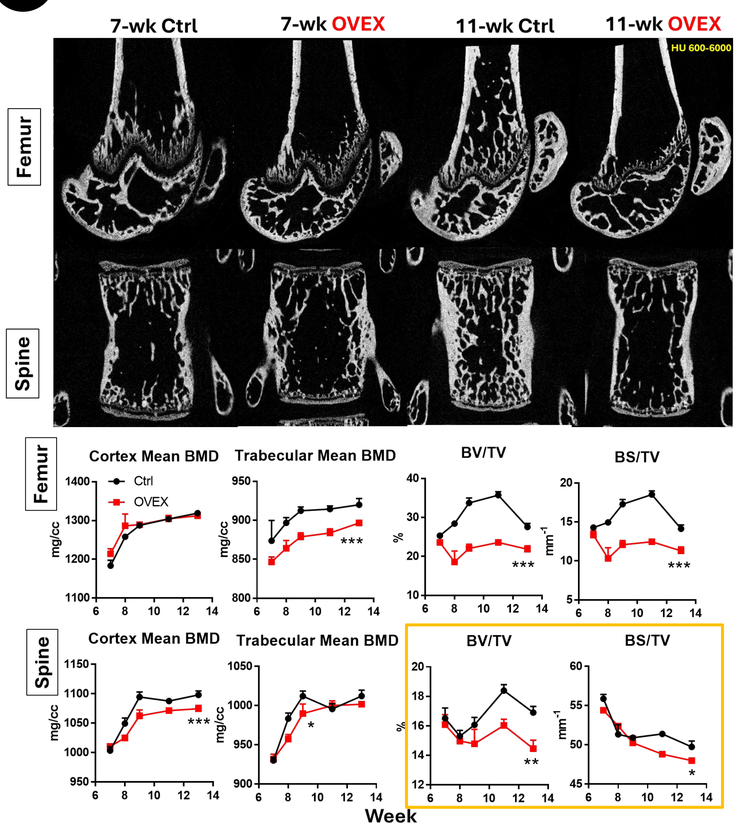
The workflow of BMA analysis starts with ROI selection on the GX3 software. Using the [Center Adjust Tool], the region of interest is centered and cropped. High-resolution 3D images are exported in the DICOM format and then imported into the Analyze 15 database. For BMA analyses, the BMA app within the Analyze 15 software was called. After setting the cortical and trabecular threshold values, the results were shown and saved in corresponding .csv files. The tables above show all 3D-related metrics produced by the BMA module.

4 In vivo longitudinal imaging of OVEX mice



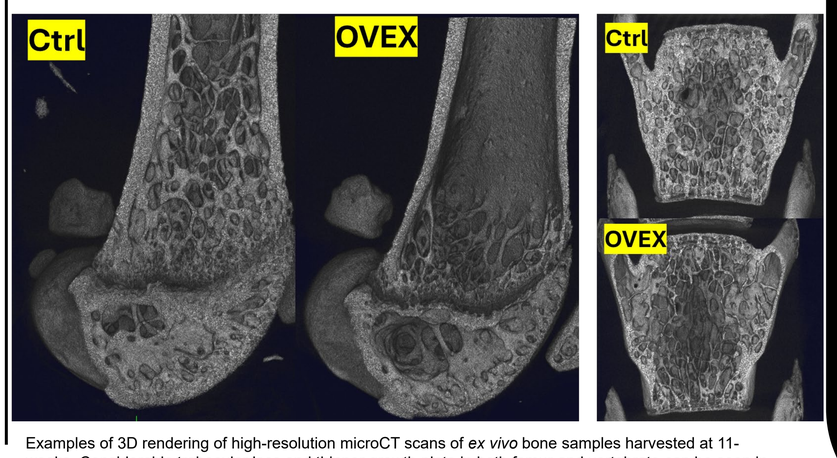
In vivo scans were performed at a larger field-of-view (FOV 36 mm) for both femurs (left and right) and spine (the segment between chest and pelvis). The same bone samples (femurs and spine) were collected after *in vivo* imaging. The 7-week microCT images represent the early stage of OVEX and the 11-week images show the later stage of the disease. Quantitative analysis of BMA metrics indicated significant trabeculae growth suppression in the femurs, resulting in lower BV/TV and BS/TV ratios (trabeculae volume to total; trabeculae surface to total, respectively). In the spine, we also observed bone density loss in the cortex. [*p<0.05, **p<0.01, ***p<0.001, Two-way ANOVA, femur: n=6 (for both left and right), spine: n=3].

5 Ex vivo high-resolution OVEX bone imaging



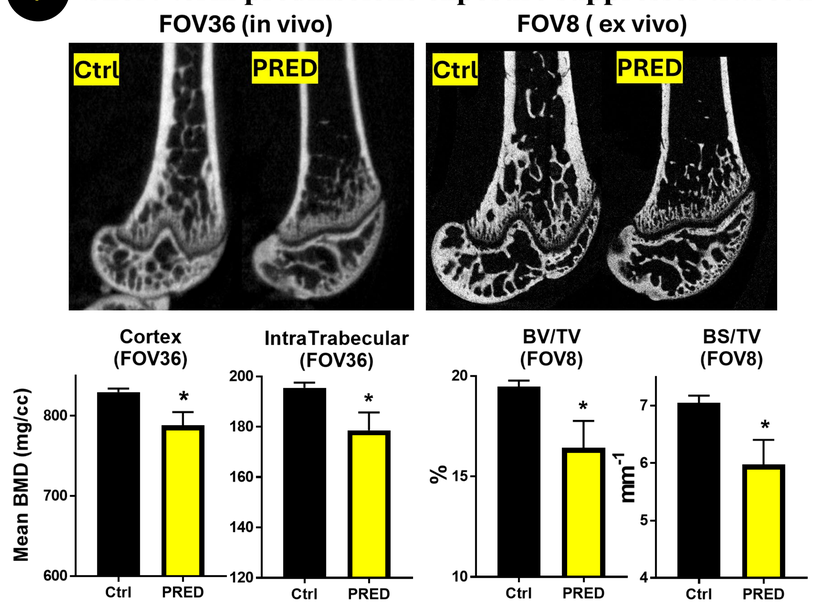
Ex vivo bone scans were performed in a smaller FOV8 (8 mm). Both femurs close to the knees, and the second segment of the vertebrate above the pelvis were scanned. A lower X-ray power (4W) was used to reduce light source size and therefore achieve better image quality. The overall quantitative assessments are consistent with the *in vivo* findings. In addition to the significant improvement in resolution over the FOV36 scans, the smaller pixel size (2.9um) at FOV8 improves volumetric and surface measurement precision and therefore provides better curve separation as shown in the golden box. [*p<0.05, **p<0.01, ***p<0.001, Two-way ANOVA, femur: n=6, spine: n=3].

6 3D rendering of OVEX bone sample images (FOV8)



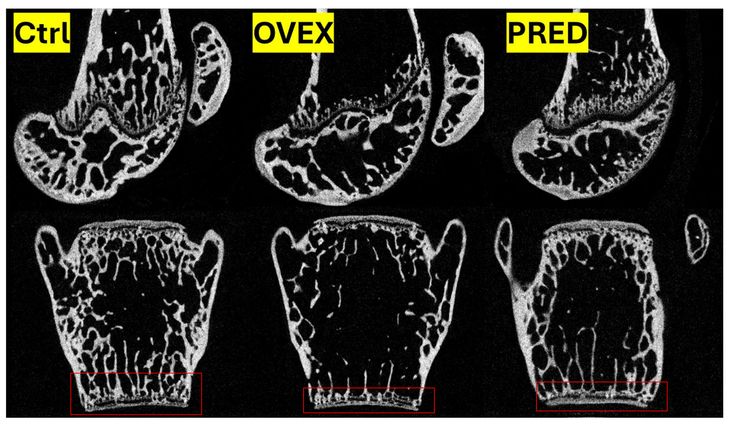
Examples of 3D rendering of high-resolution microCT scans of *ex vivo* bone samples harvested at 11-weeks. Considerable trabecular loss and thinner growth plate in both femur and vertebrate can be seen in the OVEX mice.

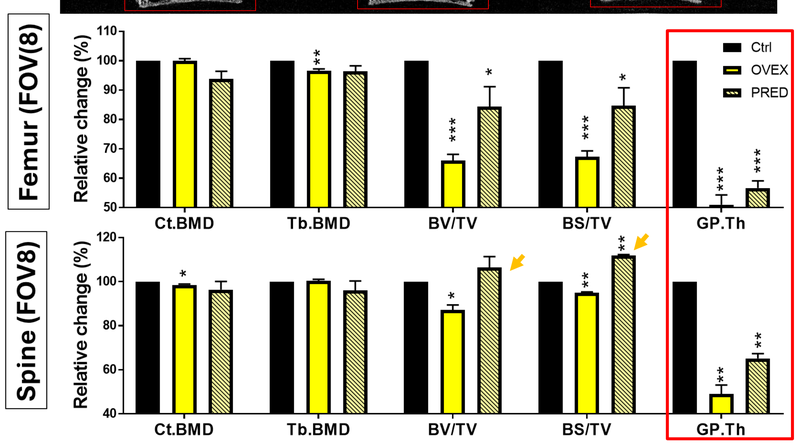
7 Short-term prednisolone exposure suppresses trabeculae



After two weeks of PRED treatment (10 mg/kg BID), *in vivo* FOV36 scans revealed lower cortex and intratrabecular density in the femurs. Furthermore, high-resolution *ex vivo* FOV8 scans provide better volume/surface measurements such as the BV/TV and BS/TV ratio. [*p<0.05, t-test, femur: n=6].

8 General comparison of OVEX and PRED models





In comparison with the PRED femurs, OVEX femurs (11-week) show more extensive bone loss in trabecular volume and surface. However, PRED femurs could have lost more cortical density after 2 weeks of exposure to the steroid. Interestingly, PRED spines show increase in trabecular volume and surface, which are in great contrast to the OVEX spines (golden arrows). Furthermore, we noticed reduction in growth plate thickness (GP.Th) in both OVEX and PRED mice (red boxes in both images and charts). Please note that the Ctrl bone images shown here are from the OVEX study. [*p<0.05, **p<0.01, ***p<0.001, t-test, femur: n=6, spine: n=3].

10 Summary

The present studies provide evidence for the utility of Quantum GX3 microCT imaging system to detect bone loss in two osteoporosis mouse models. These models were established by ovariectomy (OVEX) or shot-term (2 weeks) exposure of prednisolone (PRED). We used a larger FOV36 to non-invasively scan the femurs and lumbar spine in living animals and then collect the bone samples for high-resolution *ex vivo* FOV8 imaging. We found that imaging at FOV36 is a rapid and high-resolution method to measure small changes in both density and volume for the assessment of longitudinal bone loss. At terminal timepoints even greater detail can be quantified and visualized with *ex vivo* FOV8 imaging due to a 4.4-fold improvement in pixel size to 2.86 µm allowing assessment of the fine structural details within trabecular bone and growth plate regions, confirming non-invasive observations, and offering improved quantification and visualization capabilities.

In conclusion, the OVEX model mimics chronic bone loss and has a larger magnitude of trabecular bone loss in later stages of OP. Although bone loss is milder after short-term PRED dosing, *in vivo* FOV36 mode indeed detects a small but consistent reduction in femur density (<10%). *Ex vivo* scans of the PRED femur further reveal trabecular volume reduction by ~15%. In the spine, we saw an interesting increase in trabecular volume after two weeks in the PRED-treated mice.

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