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Parametric Response Mapping of CT Images Provides Early Detection of Local Bone Loss in a Rat Model of Osteoporosis

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Abstract

Loss of bone mass due to disease, such as osteoporosis and metastatic cancer to the bone, is a leading cause of orthopedic complications and hospitalization. Onset of bone loss resulting from disease increases the risk of incurring fractures and subsequent pain, increasing medical expenses while reducing quality of life. Although current standard CT-based protocols provide adequate prognostic information for assessing bone loss, many of the techniques for evaluating CT scans rely on measures based on whole-bone summary statistics. This reduces the sensitivity at identifying local regions of bone resorption, as well as formation. In this study, we evaluate the effectiveness of a voxel-based image post-processing technique, called the Parametric Response Map (PRM), for identifying local changes in bone mass in weight-bearing bones on CT scans using an established animal model of osteoporosis. Serial CT scans were evaluated weekly using PRM subsequent to ovariectomy or sham surgeries over the period of one month. For comparison, bone volume fraction and mineral density measurements were acquired and found to significantly differ between groups starting 3 weeks post-surgery. High resolution *ex vivo* measurements acquired four weeks post-surgery validated the extent of bone loss in the surgical groups. In contrast to standard methodologies for assessing bone loss, PRM results were capable of identifying local decreases in bone mineral by week 2, which were found to be significant between groups. This study concludes that PRM is able to detect changes in bone mineral with higher sensitivity and spatial differentiation than conventional techniques for evaluating CT scans, which may aid in clinical decision making for patients suffering from bone loss.

Keywords

ovariectomy; osteoporosis; imaging; CT; response; biomarker

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Introduction

Osseous bone is a dynamic system naturally altering its microenvironment through osteolytic and osteoblastic processes throughout an individual's life. Factors that include lifestyle changes, pregnancy, hormonal status, disease and age can alter the homeostasis of bone resulting in increasing bone loss. This will lead to bone weakening and possibly orthopedic complications, such as fractures, that will have a direct effect on a patient's quality of life. Approximately 50% of women and 25% of men over age 50 will have osteoporosis-related fractures in their remaining lifetime [1] with 71% of patients over 90 years old showing signs of osteoporosis [2]. Osteoporosis is both a clinical as well as a financial burden with costs from osteoporosis-related fractures projected to reach 25.3 billion by 2025 [3]. Current clinical management of these patients is to continually evaluate bone mineral content in an effort to identify weakened limbs and avoid skeletal-related events (SRE).

Dual-energy x-ray absorptiometry (DXA), a planar imaging technique, is employed as a quantitative imaging tool for the diagnosis of bone mineral insufficiency [4–7]. This imaging method provides a highly accurate measure of bone mineral density with subjective spatial context. Quantitative measurements from DXA are typically obtained through summary statistics from a region of interest where inclusion of healthy bone with those showing weakening will attenuate the sensitivity at identifying early bone loss. Three-dimensional imaging techniques, such as computed tomography (CT), have led to the development of protocols to quantify local bone changes over time [8]. Although CT provides higher spatial detail over DXA, as of yet no real clinical gains have been made as CT suffers from the same deficiencies when acquiring quantitative measurements of bone loss as DXA. With increasing therapeutic options for treating osteoporosis such as bisphosphonates and RANKL inhibitors, there is a clear unmet clinical need to identify early and locally weakened bones to circumvent the onset of SREs. With the correct tools, treatment decisions could be made quickly, based on imaging results, to individually optimize therapy and orthopedic correction surgeries.

Since its introduction in 2005, the parametric response map (PRM) when applied to quantitative imaging has been shown to improve the sensitivity at identifying early treatment response in cancer patients and is predictive of overall survival over what can be achieved using summary-statistical methods [9–15]. In brief, the PRM method involves spatially aligning serial pre- and mid-therapy quantitative images then individually classifying voxels based on the extent of change in the quantitative metric within the voxel. As such, the quantitative value within a tumor voxel may increase, decrease, or remained unchanged following treatment. Relative volumes of the three classifications are determined and used as outcome measures of response. At present, PRM has been demonstrated on a different MRI-based quantitative imaging approaches, such as diffusion and perfusion MRI (in the case of diffusion MRI, PRM was referred to as the functional diffusion map) [9, 11–18], as well as a variety of tumor types and locations [9–15, 17].

Although PRM analysis has been used exclusively for assessing therapeutic response in cancer, this technique may also offer a sensitive measure of bone tissue changes in patients suffering from bone loss, such as osteoporosis. To test its efficacy at identifying local changes in bone density resulting from an intervention (e.g. disease or treatment), we applied the PRM method to serially acquired CT data from a well-established rat model of osteoporosis [19, 20]. Animals were subject to ovariectomy (OVX) procedures, or sham for control, and legs were monitored weekly using CT imaging for four weeks following surgery. In the OVX model, it has been well documented that removal of the ovaries initiates bone degradation due to hormone deprivation that results in reproducible bone loss,

characterized by site-dependent decreases in overall bone mass as well as diminished trabecular structure and cortical expansion [19, 20]. Volume fractions of increased Hounsfield unit (HU) value ($\text{PRM}_{\text{HU}+}$) or decreased HU value ($\text{PRM}_{\text{HU}-}$) as well as total bone volume (BV) and bone mineral density (BMD) were assessed temporally as clinically relevant measures of bone loss. After the fourth week of imaging, animals were sacrificed and tibiae were harvested for μCT *ex vivo* imaging. We found that $\text{PRM}_{\text{HU}-}$ was able to detect bone mineral changes in the OVX model as early as two weeks post-surgery while providing detailed spatial information on the extent and location of bone loss, while standard *in vivo* measurements of BMD changes based on statistical summary techniques were not detectable until 3 weeks post-surgery. Assessment of these clinically relevant measures of bone loss suggest that PRM may provide additional sensitivity as well as spatial information over standard approaches that can be used by clinicians for the early diagnosis of bone weakening and osteoporosis.

Methods

Animal Model

Twelve female Sprague Dawley rats, 16 weeks old, were obtained from Charles River Labs and housed randomly in cages (2 per cage) fed with standard rat chow and tap water. Rats were randomly divided into ovariectomized (OVX, $n=8$) and sham-operated control ($n=4$) groups. When the rats were 17 weeks old, bilateral ovariectomy operation from a dorsal approach was performed on the OVX group, while surgery with no ovary removal was performed on the Sham animals. Ovariectomy was performed using standard protocols [21]. Briefly, animals were given 0.3mg/kg buprenorphine pre-operatively and anesthesia was achieved using 5% isoflurane in 1 liter/min oxygen until unconscious. The eyes were lubricated and all animals received a bolus dose of 5ml warmed Lactated Ringers subcutaneously. Rats were then maintained on a warming table during surgery. The site of incision was shaved and then prepped using warm chlorhexidine and saline. Skin incisions were made from the second to fifth lumbar vertebrae on each side, about 2cm in length, using a scalpel blade. The retro-peritoneal incisions were made ventral to the rector spinae muscles just caudal to the last rib. The ovaries were exteriorized by gentle retraction, then a 5-0 Vicryl suture was placed around the cranial portion of the uterus and uterine vessels followed by removal of the ovary, oviduct and a small portion of the uterus. Skin and peritoneum incisions were closed with 5-0 Vicryl sutures, and then rats were recovered under heat lamp until ambulatory. A second dose of buprenorphine was administered 8 hours post-surgery and incisions were observed daily until fully healed. The animal experiments described in this study complied with all relevant federal and institutional policies.

μ Computed Tomography (μCT)

In vivo imaging was performed on a Siemens Inveon system with the following acquisition parameters: 80kVp, 500 μA , 300ms exposure time, 501 projections over 360 degrees, 49.2mm field of view (FOV, 96.1 μm isotropic voxel size). Imaging was performed one day prior to surgery and days 6, 13, 20, and 27 post-surgery, capturing both tibiae as well as the distal femora of each rat. Right tibiae and femora were excised on day 28 post-surgery and stored in PBS-soaked gauze at -20°C until *ex vivo* μCT imaging was performed.

Ex vivo μCT imaging was performed on a General Electric eXplore Locus SP system with the following parameters: 80 kVp, 80uA, 1600ms exposure time, 400 projections, 0.5 degrees per projection, 4 frames averaged per projection, 18 μm isotropic voxel size. For imaging, the sample was submerged in water, and X-rays were pre-filtered using 0.02"

aluminum. Each image captured the proximal tibia, from the tibial head to about 20mm distally.

***In Vivo* Image Analysis**

PRM analysis was performed using Matlab (Natick, MA) algorithms developed in-house. *In vivo* CT images were converted to Hounsfield units using a 0 HU phantom on each time point. All post-OVX image time points were registered to baseline images using mutual information as an objective function and simplex as an optimizer [24]. Registration was automatic and assumed rigid-body geometry, meaning rotation and translation only. Bone volumes of interest (VOI) were contoured on the baseline image using an automatic segmentation algorithm, selecting the tibia from the tibia/fibula junction to the proximal tibial head. Images were analyzed for bone volume fraction relative to total bone volume (BV/TV) and bone mineral density using a threshold of 600HU for selecting mineralized bone tissue. Parametric response maps of quantitative CT as expressed in Hounsfield units (PRM_{HU}) were generated over the same region by first calculating the difference between the Hounsfield units ($\Delta HU = HU_{\text{post-surgery}} - HU_{\text{pre-surgery}}$) for each voxel within the bone pre- and post-surgery. Individual voxels were classified based on the extent of change observed in ΔHU . Voxels yielding a ΔHU greater than a predetermined threshold were designated red, decreased by more than the threshold were designated blue, and otherwise designated green (indicating no significant change from pre-surgery). Volume fractions of the total bone were calculated for the three classifications: PRM_{HU+} (red voxels denoting increased HU), PRM_{HU-} (blue voxels denoting decreased HU), and PRM_{HU0} (green voxels denoting unchanged HU). The threshold that designates a significant change in HU within a voxel was empirically calculated from one random subject imaged twice on the same day, separated by an interval of one hour. Following registration and conversion to HU of the two images from the same animal, a linear least squares analysis was performed and the 95% confidence interval was determined for use as the PRM threshold, which was set as $\pm 391 \text{ HU}$.

***Ex Vivo* Image Analysis**

Ex vivo images were analyzed at week 4 post-surgery to verify that significant changes in bone microenvironment had occurred following ovariectomy in this well-established model of osteoporosis relative to sham animals. Images were analyzed using MicroView (GEHC). Trabecular VOI were drawn by hand and extrapolated between slices over a 3mm-long region near the proximal tibia (shown in figure 4). Measures of mean trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), total bone volume (BV), bone volume fraction (BV/TV), mean bone mineral density (BMD), and structure model index (SMI) were analyzed. Cortical bone VOI were automatically delineated over the bottom four slices from the trabecular VOI. Measures of mean cortical thickness, cross-sectional area, and inner and outer perimeters were analyzed.

Data and Statistical Analysis

Data is presented as the mean $\pm$ the standard error of the mean (SEM). Differences in outcome measures between groups at each time point was determined by a two-tailed, unpaired student's t-test with $p < 0.05$ denoting significance.

Results

Comparison of PRM_{HU} and standard whole-bone analyses

To assess the effectiveness of the PRM method at identifying bone loss we analyzed weekly μCT images using PRM compared to mean BV/TV and BMD between groups. Analysis

was constrained to tibial bone from proximal tibial plateau distally to tibia/fibula junction segmented on the baseline image. The results in Figure 1 show that BV/TV and BMD were significantly different between groups by week 3. In the OVX group, BV/TV decreased by $3.1 \pm 0.6\%$ at the end of the study. BMD decreased by $4.2 \pm 1.0\%$ on week 3 but saw no further change the following week (week 4).

The PRM method, with spatial sensitivity, revealed trabecular bone loss as well as cortical expansion in the OVX group. Figure 2 shows PRM analysis with a representative axial slice through the CT image (I–ii) and the scatter plot with pre-surgery HU on the x-axis and post-surgery HU at a specific time on the y-axis for the entire VOI (iii) over the study time period. The representative slice shown near the proximal tibial plateau was chosen to include changes in both trabecular and cortical bone. Trabecular degradation is apparent in the OVX animal, $\text{PRM}_{\text{HU-}}$, seen as blue in the PRM overlay and scatterplot. Also in the OVX group, $\text{PRM}_{\text{HU+}}$ (red voxels) indicates a shift in the cortical bone outward, reflecting cortical expansion. These two changes in bone structure are typical of this osteoporosis model [19]. In contrast to the OVX animal, the sham animal had very little change in PRM metrics. The few red and blue pixels observed were the result of natural bone growth and reflected modeling changes associated with skeletal growth.

The mean volume fractions, $\text{PRM}_{\text{HU+}}$ and $\text{PRM}_{\text{HU-}}$, from both groups were monitored over the study time period (Figure 3). The $\text{PRM}_{\text{HU+}}$ results showed a temporary increase on week 2 over control values. This significant difference was lost after week two indicating a transient remodeling effect on OVX animals. The PRM map shows that the majority of $\text{PRM}_{\text{HU+}}$ is along the bone's outer edge, indicating that this increase is due mainly to cortical expansion. The subsequent loss of significance between groups is likely normal bone growth in the sham group catching up with the remodeling effect in the OVX group. The $\text{PRM}_{\text{HU-}}$ plot (Figure 3B) reflects progressive bone loss which is characteristic of this animal model, with significantly higher $\text{PRM}_{\text{HU-}}$ values observed in OVX than sham animals at all time-points after week 1 post-surgery. As shown in Figure 2, $\text{PRM}_{\text{HU-}}$ voxels are primarily found in the cancellous bone space and indicate loss in trabecular bone mass. The increase is nearly significant even at the week 1 imaging time point ($p=0.083$). By the end of the study, at 4 weeks post-surgery, OVX and sham groups resulted in bone loss as measured by $\text{PRM}_{\text{HU-}}$ of $16.0\% (\pm 2.3)$ and $2.5\% (\pm 0.8)$, respectively ($p<0.001$).

Ex vivo μCT measurements of tibial trabecular and cortical bone

To verify that OVX animals had undergone extensive bone loss following surgery relative to sham, we performed *ex vivo* μCT after 4 weeks on all animals in the study. Images were acquired with $18\mu\text{m}$ resolution allowing quantification of trabecular structures. Figure 4 illustrates the process of analysis for both trabecula and cortex, with resulting measurements. Figure 4A indicates the location of the trabecular analysis slab (left), region for maximum intensity projection (MIP) in B (middle), and slab for cortical analysis (right) as a yellow box. Figure 4B shows representative MIP images for OVX and sham animals, with a clearly lower trabecular bone mass in the OVX animal. Figure 4C shows representative isosurfaces for the two groups, taken from the yellow region indicated in B. Figure 4D shows an isosurface of the cortical bone from a representative animal, which was used for cortical analysis. Resulting measurements are listed in Figure 4E, and group means are shown in Tables 1 and 2 for trabecular bone and cortical bone, respectively. Significant differences were seen between groups in all trabecular measurements, indicating degradation of trabecular structure. Structural model index (SMI) measurements quantify the extent of rod- or disc-like shaping of the trabecular lattice, with higher values indicating more rod-like and lower indicating more disc-like shaping. Cortical measurements of average thickness, inner, and outer perimeters also showed significant differences between groups. Larger perimeters and decreased cortical thickness in the OVX group indicate significant cortical

expansion, which is consistent with this model. No significant change in cross-sectional area indicates that remodeling occurred without significant loss of total cortical bone.

Discussion

The goal of this study was to evaluate voxel-based PRM analysis of bone mineral changes using *in vivo* μ CT and compare these results to those determined using conventional measures of bone mineral density and bone volume. Toward this end we used a well-documented model of osteoporosis in rats in which removal of the ovaries initiates bone degradation due to hormone deprivation. This animal model has been shown to result in highly-reproducible bone loss, characterized by site-dependent decreases in overall bone mass as well as diminished trabecular structure and cortical expansion [19, 20]. Clinical osteoporosis is characterized by decreases in either bone mineral density (BMD) or bone mineral content (BMC) of over 2.5 standard deviations below the young adult reference mean (-2.5 T-score), which leads to increased fragility and consequently a greater risk of SREs [19]. It is reported that the earliest time of statistically detectable cancellous bone loss is approximately 14 days post-OVX in this animal model [19, 25]. In this study, PRM showed a near-significant change in PRM_{HU} by one week post-surgery, which became significant 2 weeks post-OVX, well before any significant difference in BMD was detected. In addition to being an early biomarker of bone remodeling, PRM also provided locally-resolved spatial information revealing bone degradation and growth which was not attainable by BMD measurements.

Historically, the most utilized method of tracking bone mineral changes *in vivo* has been dual-energy x-ray absorptiometry (DXA). This method, although highly accurate in its measurements, is limited in its ability to provide spatial information. The attractiveness of DXA in most cases is its measurement accuracy as well as its minimal exposure to ionizing x-rays. Doses from quantitative CT imaging can be hundreds of times higher than DXA, but provide 3D voxel sizes able to resolve bone micro-structures. Despite the widespread usage of BMD, recent studies have indicated that measurement of BMD alone is insufficient for evaluation of bone strength [26–29]. Recent studies have explored the use of high resolution CT imaging to model bone mechanical characteristics in order to predict fracture risk through finite element analysis (FEA) [26, 30–32]. Until recently, mechanical properties of bone were tested *ex vivo*, requiring the use of a large number of animals to provide sufficient data points. Several recent studies have shown great accuracy in predicting fractures in femoral and vertebral cases using FEA [30, 33]. However very powerful computing hardware is necessary for this technique, especially when using nonlinear modeling strategies and the constraints and limitations in application are not yet fully understood. Using the spatially resolved bone changes obtained from PRM analysis, evaluation of increased fracture risk may be predictable without such rigorous computational modeling making this technique more clinically relevant. PRM has the capability of monitoring global bone mass changes as well as identification of focal bone loss which would otherwise be attenuated in global bone measurements that use summary statistical methods (e.g. mean value over a region of interest). For this reason PRM may provide a better foundation for determining correctional orthopedic strategies.

The tradeoff between resolution and imaging time (and therefore radiation dose) is well known. For the purposes of this study and its future clinical implications, image resolution was sacrificed in order to prioritize low radiation doses, below known tissue response limits [22, 23]. Due to the lower resolution of our *in vivo* measurements, quantification of such trabecular parameters as thickness, spacing, and SMI were not feasible. The voxel-wise comparison of HU in PRM, however, was able to detect loss in trabecular bone structure as a decrease in HU rather than the loss in binary threshold bone volume that is typically

monitored in higher resolution *ex vivo* images. PRM maps are also able to spatially resolve these changes in 3D space, allowing for focal changes in bone mineral density to be identified and quantified.

Previous studies have reported results from using a longitudinal voxel-wise comparison approach, similar to PRM, to subjectively show spatial changes in bone mass *in vivo* [34–36]. Even though these studies performed voxel-wise comparison, it was used only for qualitative presentation and not quantified statistically. Schulte et al. [8] used registered high resolution μ CT to extrapolate measures of bone formation and resorption rates in the trabecula of mouse vertebrae. The study focused primarily on bone histomorphometry and the processes of bone adaptation, and would result in high doses of ionizing radiation (IR) to achieve the level of resolution necessary, therefore the approach was not designed for clinical application. Since PRM compares quantitative Hounsfield units and not binary bone images it is able to detect bone structural changes without requiring excessively small resolution (and thus high IR doses). The experiments presented here are the first to use PRM to quantify voxel-based changes in bone mass *in vivo*.

Although PRM was demonstrated here on a model of osteoporosis, PRM analysis may have wider clinical applications. Bisphosphonates, used clinically for several years, inhibit the resorption of bone by osteoclasts [37]. Interestingly, the degree of fracture risk reduction following bisphosphonate therapy is not well explained for by changes in bone mass alone. Following 1 year of Risedronate therapy in 2087 individuals, Watts et al. [38] found that fracture risk reduction was not dependent on change in BMD, indicating that other factors such as remodeling of bone geometry, etc. must play significant roles. PRM analysis may provide a sensitive biomarker of bone response to these therapies, leading to prediction of overall outcome by direct observation of local sites of anabolic or anti-catabolic effect. Another application of PRM is in the assessment of bone response to metastatic cancer [35] where diseases characterized by low bone mass and deterioration of microarchitecture often lead to bone fragility [5, 19, 39]. Skeletal metastases develop in approximately 70–80% of patients with breast or prostate primary cancer, with over 250,000 and 100,000 deaths worldwide each year from breast and prostate cancer, respectively [40]. Cancer that has metastasized to the bone may present osteolytic, osteoblastic, or a mix of these characteristics. Patients presenting with these diseases have a high risk of incurring fractures, resulting in patient pain and increased morbidity. Local changes in bone mass due to metastatic disease can significantly impact the mechanical integrity of the skeleton, leading to focal sites of high fracture susceptibility [41]. PRM analysis may provide a unique and sensitive measure in differentiating the osteoblastic and osteolytic sites which would be highly valuable in strategizing corrective therapy based on local fragility. Recent studies have uncovered a close interaction between bone and cancer metastases through molecular signaling [42], in which growth of the cancer is highly dependent on the remodeling of the surrounding bone. PRM_{HU} analysis may also prove clinically useful in the identification of initial forming micro-metastases in the bone but may also help in assessing treatment efficacy for targeted therapies that disrupt the molecular signaling between bone and cancer.

In conclusion, PRM proved highly sensitive in the detection and spatial localization of bone mass changes resulting from osteoporosis. Spatial identification of focal sites of bone loss over time will provide many new opportunities for clinical application. The voxel-wise analysis of registered serial data is a highly flexible tool, and can be applied to a variety of bone disease pathologies to provide for detection of spatially varying changes in CT skeletal images over time.

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References

1. Fast Facts. National Osteoporosis Foundation; Aug 29. 2011 <<http://www.nof.org/node/40>>
2. Krestan CR, Nemec U, Nemec S. Imaging of insufficiency fractures. *Semin Musculoskelet Radiol*. 2011; 15:198–207. [PubMed: 21644194]
3. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. *J Bone Miner Res*. 2007; 22:465–75. [PubMed: 17144789]
4. Ammann P, Rizzoli R, Slosman D, Bonjour JP. Sequential and precise in vivo measurement of bone mineral density in rats using dual-energy x-ray absorptiometry. *J Bone Miner Res*. 1992; 7:311–6. [PubMed: 1585832]
5. Honig S. Osteoporosis - new treatments and updates. *Bull NYU Hosp Jt Dis*. 2010; 68:166–70. [PubMed: 20969546]
6. Jacobson JA, Jamadar DA, Hayes CW. Dual X-ray absorptiometry: recognizing image artifacts and pathology. *AJR Am J Roentgenol*. 2000; 174:1699–705. [PubMed: 10845510]
7. Lorente-Ramos R, Azpeitia-Arman J, Munoz-Hernandez A, Garcia-Gomez JM, Diez-Martinez P, Grande-Barez M. Dual-energy x-ray absorptiometry in the diagnosis of osteoporosis: a practical guide. *AJR Am J Roentgenol*. 2011; 196:897–904. [PubMed: 21427343]
8. Schulte FA, Lambers FM, Kuhn G, Muller R. In vivo micro-computed tomography allows direct three-dimensional quantification of both bone formation and bone resorption parameters using time-lapsed imaging. *Bone*. 2011; 48:433–42. [PubMed: 20950723]
9. Moffat BA, Chenevert TL, Lawrence TS, Meyer CR, Johnson TD, Dong Q, Tsien C, Mukherji S, Quint DJ, Gebarski SS, Robertson PL, Junck LR, Rehemtulla A, Ross BD. Functional diffusion map: a noninvasive MRI biomarker for early stratification of clinical brain tumor response. *Proc Natl Acad Sci U S A*. 2005; 102:5524–9. [PubMed: 15805192]
10. Galban CJ, Chenevert TL, Meyer CR, Tsien C, Lawrence TS, Hamstra DA, Junck L, Sundgren PC, Johnson TD, Galban S, Sebolt-Leopold JS, Rehemtulla A, Ross BD. Prospective analysis of parametric response map-derived MRI biomarkers: identification of early and distinct glioma response patterns not predicted by standard radiographic assessment. *Clin Cancer Res*. 2011; 17:4751–60. [PubMed: 21527563]
11. Galban CJ, Chenevert TL, Meyer CR, Tsien C, Lawrence TS, Hamstra DA, Junck L, Sundgren PC, Johnson TD, Ross DJ, Rehemtulla A, Ross BD. The parametric response map is an imaging biomarker for early cancer treatment outcome. *Nat Med*. 2009; 15:572–6. [PubMed: 19377487]
12. Galban CJ, Mukherji SK, Chenevert TL, Meyer CR, Hamstra DA, Bland PH, Johnson TD, Moffat BA, Rehemtulla A, Eisbruch A, Ross BD. A feasibility study of parametric response map analysis of diffusion-weighted magnetic resonance imaging scans of head and neck cancer patients for providing early detection of therapeutic efficacy. *Transl Oncol*. 2009; 2:184–90. [PubMed: 19701503]
13. Hamstra DA, Galban CJ, Meyer CR, Johnson TD, Sundgren PC, Tsien C, Lawrence TS, Junck L, Ross DJ, Rehemtulla A, Ross BD, Chenevert TL. Functional diffusion map as an early imaging biomarker for high-grade glioma: correlation with conventional radiologic response and overall survival. *J Clin Oncol*. 2008; 26:3387–94. [PubMed: 18541899]
14. Lee KC, Bradley DA, Hussain M, Meyer CR, Chenevert TL, Jacobson JA, Johnson TD, Galban CJ, Rehemtulla A, Pienta KJ, Ross BD. A feasibility study evaluating the functional diffusion map as a predictive imaging biomarker for detection of treatment response in a patient with metastatic prostate cancer to the bone. *Neoplasia*. 2007; 9:1003–11. [PubMed: 18084607]
15. Lee KC, Sud S, Meyer CR, Moffat BA, Chenevert TL, Rehemtulla A, Pienta KJ, Ross BD. An imaging biomarker of early treatment response in prostate cancer that has metastasized to the bone. *Cancer Res*. 2007; 67:3524–8. [PubMed: 17440058]

16. Moffat BA, Chenevert TL, Meyer CR, McKeever PE, Hall DE, Hoff BA, Johnson TD, Rehemtulla A, Ross BD. The functional diffusion map: an imaging biomarker for the early prediction of cancer treatment outcome. *Neoplasia*. 2006; 8:259–67. [PubMed: 16756718]
17. Reischauer C, Froehlich JM, Koh DM, Graf N, Padevit C, John H, Binkert CA, Boesiger P, Gutzeit A. Bone metastases from prostate cancer: assessing treatment response by using diffusion-weighted imaging and functional diffusion maps--initial observations. *Radiology*. 2010; 257:523–31. [PubMed: 20829534]
18. Tsien C, Galban CJ, Chenevert TL, Johnson TD, Hamstra DA, Sundgren PC, Junck L, Meyer CR, Rehemtulla A, Lawrence T, Ross BD. Parametric response map as an imaging biomarker to distinguish progression from pseudoprogession in high-grade glioma. *J Clin Oncol*. 2010; 28:2293–9. [PubMed: 20368564]
19. Jee WS, Yao W. Overview: animal models of osteopenia and osteoporosis. *J Musculoskelet Neuronal Interact*. 2001; 1:193–207. [PubMed: 15758493]
20. Turner RT, Wakley GK, Hannon KS. Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats. *J Orthop Res*. 1990; 8:612–7. [PubMed: 2355301]
21. Olson ME, Bruce J. Ovariectomy, ovariectomy and orchidectomy in rodents and rabbits. *Can Vet J*. 1986; 27:523–7. [PubMed: 17422731]
22. Brouwers JE, van Rietbergen B, Huiskes R. 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*. 2007; 25:1325–32. [PubMed: 17568420]
23. Laperre K, Depypere M, van Gastel N, Torrekens S, Moermans K, Bogaerts R, Maes F, Carmeliet G. Development of micro-CT protocols for in vivo follow-up of mouse bone architecture without major radiation side effects. *Bone*. 2011; 49:613–22. [PubMed: 21763477]
24. Meyer CR, Boes JL, Kim B, Bland PH, Zasadny KR, Kison PV, Koral K, Frey KA, Wahl RL. Demonstration of accuracy and clinical versatility of mutual information for automatic multimodality image fusion using affine and thin-plate spline warped geometric deformations. *Med Image Anal*. 1997; 1:195–206. [PubMed: 9873906]
25. Wronski TJ, Cintron M, Dann LM. Temporal relationship between bone loss and increased bone turnover in ovariectomized rats. *Calcif Tissue Int*. 1988; 43:179–83. [PubMed: 3141020]
26. Griffith JF, Engelke K, Genant HK. Looking beyond bone mineral density : Imaging assessment of bone quality. *Ann N Y Acad Sci*. 2010; 1192:45–56. [PubMed: 20392217]
27. Sornay-Rendu E, Munoz F, Duboeuf F, Delmas PD. Rate of forearm bone loss is associated with an increased risk of fracture independently of bone mass in postmenopausal women: the OFELY study. *J Bone Miner Res*. 2005; 20:1929–35. [PubMed: 16234965]
28. Schuit SC, van der Klift M, Weel AE, de Laet CE, Burger H, Seeman E, Hofman A, Uitterlinden AG, van Leeuwen JP, Pols HA. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. *Bone*. 2004; 34:195–202. [PubMed: 14751578]
29. Cummings SR, Karpf DB, Harris F, Genant HK, Ensrud K, LaCroix AZ, Black DM. Improvement in spine bone density and reduction in risk of vertebral fractures during treatment with antiresorptive drugs. *Am J Med*. 2002; 112:281–9. [PubMed: 11893367]
30. Imai K. Vertebral fracture risk and alendronate effects on osteoporosis assessed by a computed tomography-based nonlinear finite element method. *J Bone Miner Metab*. 2011
31. Genant HK, Engelke K, Prevrhal S. Advanced CT bone imaging in osteoporosis. *Rheumatology (Oxford)*. 2008; 47(Suppl 4):iv9–16. [PubMed: 18556648]
32. Kalpakcioglu BB, Engelke K, Genant HK. Advanced imaging assessment of bone fragility in glucocorticoid-induced osteoporosis. *Bone*. 2011; 48:1221–31. [PubMed: 21320651]
33. Keaveny TM. Biomechanical computed tomography-noninvasive bone strength analysis using clinical computed tomography scans. *Ann N Y Acad Sci*. 2010; 1192:57–65. [PubMed: 20392218]
34. Brouwers JE, van Rietbergen B, Huiskes R, Ito K. Effects of PTH treatment on tibial bone of ovariectomized rats assessed by in vivo micro-CT. *Osteoporos Int*. 2009; 20:1823–35. [PubMed: 19262974]

35. Johnson LC, Johnson RW, Munoz SA, Mundy GR, Peterson TE, Sterling JA. Longitudinal live animal micro-CT allows for quantitative analysis of tumor-induced bone destruction. *Bone*. 2011; 48:141–51. [PubMed: 20685406]
36. Waarsing JH, Day JS, van der Linden JC, Ederveen AG, Spanjers C, De Clerck N, Sasov A, Verhaar JA, Weinans H. Detecting and tracking local changes in the tibiae of individual rats: a novel method to analyse longitudinal in vivo micro-CT data. *Bone*. 2004; 34:163–9. [PubMed: 14751574]
37. Green JR, Guenther A. The backbone of progress--preclinical studies and innovations with zoledronic acid. *Crit Rev Oncol Hematol*. 2011; 77 (Suppl 1):S3–S12. [PubMed: 21353178]
38. Watts NB, Geusens P, Barton IP, Felsenberg D. Relationship between changes in BMD and nonvertebral fracture incidence associated with risedronate: reduction in risk of nonvertebral fracture is not related to change in BMD. *J Bone Miner Res*. 2005; 20:2097–104. [PubMed: 16294263]
39. Krug R, Burghardt AJ, Majumdar S, Link TM. High-resolution imaging techniques for the assessment of osteoporosis. *Radiol Clin North Am*. 2010; 48:601–21. [PubMed: 20609895]
40. Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. *Cancer Treat Rev*. 2001; 27:165–76. [PubMed: 11417967]
41. Coleman RE, Rubens RD. The clinical course of bone metastases from breast cancer. *Br J Cancer*. 1987; 55:61–6. [PubMed: 3814476]
42. Onishi T, Hayashi N, Theriault RL, Hortobagyi GN, Ueno NT. Future directions of bone-targeted therapy for metastatic breast cancer. *Nat Rev Clin Oncol*. 2010; 7:641–51. [PubMed: 20808302]

Figure 1.

Plots of relative change in (A) bone volume fraction, BV/TV, and (B) bone mineral density, BMD, over the study time period. Quantitative values from registered images were determined from a volume-of-interest over the proximal tibial plateau distally to the tibia/fibula junction on baseline images. Differences between groups were not seen in either BV/TV or BMD until week 3 post-OVX, with decreases of $4.4 \pm 1.0\%$ ($p=0.002$) and $3.4 \pm 1.1\%$ ($p<0.001$), respectively, in the OVX group at the end of the study. Data is presented as group mean $\pm$ SEM. Significant difference between groups was assessed at $p<0.05$ and indicated by *.

Figure 2.

Representative PRM images from (A) an OVX animal and (B) a sham animal, displayed as an axial slice over time (from left to right: weeks 0 to 4, respectively). The position of the slice shown is indicated by the yellow box on the surface rendering to the left of the PRM results. Presented for each representative animal are (i) grayscale images, (ii) PRM overlays, and (ii) PRM scatterplots of all individual voxels of the analyzed tibia with x-axes pertaining to pre-surgery HU and y-axes pertaining to post-surgery HU at the respective time point (column).

Figure 3.

Bar plots showing the volume fraction of (A) increased HU, PRM_{HU+}, and (B) decreased HU, PRM_{HU-}. The OVX group showed a significant increase in PRM_{HU+} on week 2 which disappeared at later time points and a progressive increase in PRM_{HU-} until the end of the study. For week 1 PRM_{HU-}, group differences were nearly significant, with $p=0.08$. Data is presented as a group mean $\pm$ SEM. Significant difference between groups was assessed at $p<0.05$ and indicated by *.

Figure 4.

Representative images of *ex vivo* images of proximal tibiae four weeks post-surgery. (A) Surface renderings show regions used for *ex vivo* analysis (yellow boxes) of trabecula (left) and cortex (right). (B) Maximum intensity projections from a middle slab (200 μ m thick) show a clear difference between OVX and Sham animals. (C) Surface renderings of OVX (Left) and Sham (Right) trabecular bone (location indicated by yellow box in B) show a significant drop in trabecular structure following OVX surgery. (D) Surface rendering of region used for cortical analysis, excluding the trabecular region. (E) Parameters obtained from trabecular (left) and cortical (right) analyses.

Table 1

Trabecular Bone Analysis

Group	Tb.Th (µm)	Tb.Sp (µm)	Volume (mm ³)	BV/TV	BMD (mg/mm ³)	SMI
OVX	50.8 (1.94)	173 (16.5)	8.6 (0.62)	0.29 (0.021)	363 (16.7)	1.57 (0.14)
Sham	73 (4.03)	65.3 (10.87)	14.2 (0.88)	0.6 (0.045)	564 (25)	-2.16 (0.858)
p-value	0.0058	0.0003	0.0019	0.0026	0.0007	0.0210

Note: Data are shown as mean with standard error in parentheses, for trabecular thickness and spacing (Tb.Th and Tb.Sp, respectively), bone volume (BV), bone volume to total volume ratio (BV/TV), bone mineral density (BMD), and structural model index (SMI).

Table 2

Cortical Bone Analysis

Group	Thickness (mm)	Area (mm ²)	Mineral (mg)	Inner Perimeter (mm)	Outer Perimeter (mm)
OVX	0.45 (0.014)	4.51 (0.164)	0.1 (0.002)	11.1 (0.28)	17.2 (0.2)
Sham	0.53 (0.013)	4.86 (0.067)	0.102 (0.0026)	9.7 (0.34)	15.3 (0.51)
p-value	0.0023	0.0815	0.5329	0.0166	0.0278

Note: Data are shown as mean with standard error in parentheses.