ANALYSIS OF FAT, LEAN AND BONE TISSUES IN OVARIECTOMIZED RATS BY MAGNETIC RESONANCE AND X-RAY TECHNIQUES

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Introduction:
The most prevalent non-invasive technique used to quantify bone at axial and appendicular sites is dual energy x-ray absorptiometry (DXA), which measures bone mineral density (BMD, g/cm2) and bone mineral content (BMC, g). BMD has been shown to correlate with fracture incidence and proven to be highly useful in evaluating progression of osteoporosis. However, because DXA BMD is a two dimensional parameter, its value and correlation is projection dependent. As a result, QCT appears to be superior to DXA because true volumetric analyses can be conducted in three dimensions. QCT have been shown to be capable of microstructural analyses of trabecular bone in 3D with resolution approaching that of histomorphometry. However, unlike single-energy QCT, both DXA and MRI can measure soft tissues, such as fat or water (lean tissue).

The effects of ovariectomy and estrogen treatment on fat, lean (H2O), and bone tissues were evaluated longitudinally and after euthanasia by magnetic resonance imaging (MRI) and spectroscopy (MRS), dual energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT). The aim of this study was to investigate the utility of MRI in quantitating longitudinal regional changes in the fat and water composition of tissues in the bone environment in vivo in the aged ovariectomized rat model of postmenopausal osteoporosis. These data showed that MRI and DXA are complementary techniques with MRI capable of high resolution compositional analyses within bones, while DXA is better suited to whole body analyses.

Methods:
Two sets of 6 month old, pathogen-free, virgin, Sprague-Dawley rats (Charles River, Canada, Harlan, Indianapolis) were either sham-ovariectomized (SHAM, group 1) or ovariectomized (OVX, group 2) and maintained on a calcium and phosphorus defined diet (Teklad TD 89222 with 0.5% Ca and 0.4% P). Group 3 was ovariectomized and treated with 17-β estradiol (E2) by oral gavage at a dose of 100 µg/kg/day in vehicle (20% hydroxypropyl-b-cyclodextrin) from day 4 post-surgery until 56 days post-surgery. High resolution (150 micron isotropic) 3D MRI of tibial fat distribution and MRS of three tibial locations were performed at days -10, -2 pre-surgery and +1, +21, +35, +56 days post-surgery, using a 3.5 cm diameter radio-frequency volume coil at 9.4 Tesla. Additionally, preserved left tibias (from animals sacrificed at Day 56) were imaged ex vivo by MRI at 11.7 Tesla at 6 transverse 1mm thick slices (90 microns in plane resolution) producing both water and fat images. DXA (Eclipse, Norland, Ft. Atkinson, WI) analyses of whole body fat, lean, and bone content were also conducted at baseline, +7, +21, +35, and +56 days post-surgery. QCT was conducted on serial cross-sections of an overlapping region of the proximal tibia after euthanasia, using voxel dimensions of 148×148×1200 µm (960A pQCT, Norland). Results:

Ovariectomy was confirmed to induce a significant increase in body weight compared to baseline and Sham in 4 out of 5 sets of rats, while E2 prevented the ovariectomy induced gain in body weight. MRI analysis in vivo showed an ovariectomy induced increase in subcutaneous fat, which was inhibited by E2. No significant differences in total body lean tissue (H2O) were observed between Sham, OVX, and E2. DXA analysis of whole body BMC showed a transient reduction for OVX at 7 days post-surgery, but no difference between Sham and OVX by 56 days. DXA analysis of the tibia and femur was transient reduction for OVX at 7 days post-surgery, but no difference between Sham, OVX, and E2. DXA analysis of whole body BMC showed a significant increase in subcutaneous fat, which was inhibited by E2. These data showed that ovariectomy stimulates an increase in body weight that is primarily due to an increase in body fat which includes an increase in subcutaneous fat as well as accumulation of fat in the marrow. E2 prevented this increase in body weight and accumulation of fat.

Previously, subcutaneous administration of 0.1 mg/kg E2 was shown to fully prevent bone loss due to ovariectomy in the proximal tibia (1,2,3). In this study, both MRI and QCT showed that oral administration of 0.1 mg/kg E2 was insufficient to completely prevent bone loss due to ovariectomy. These data suggest that E2 is less efficacious when administered orally as compared to subcutaneous administration and that fat is more sensitive to systemic estrogen levels in vivo than bone.

References:

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MRI of tibial water content clearly distinguished between Sham and OVX groups, the OVX group showing approximately double the marrow water content compared to Sham, with E2 showing an intermediate value. Ex vivo fat content alone did not show a difference seen between groups, however the ex vivo MRI fat/water ratios did correlate very strongly with in vivo MRS fat/water ratios, confirming the large animal-to-animal variations in tibial fat content observed in vivo. Spatial distribution analysis showed that the fat content is higher in the proximal tibia, relative to distal tibia.

Regional analysis of the proximal tibia was also attempted by DXA, but DXA had limited ability to discriminate between Sham and OVX, as compared to QCT. Serial QCT analysis of the identical tibia showed significant differences in BMD and BMC between Sham and OVX for the epiphysis and metaphysis. However, BMD and BMC for E2 were typically not different from OVX, suggesting that oral administration of 17-β estradiol is less efficacious than subcutaneous administration (1,2,3).

Discussion:
We have applied MRI techniques on the ovariectomized rat model of osteoporosis. MRI was shown to be the only technique capable of quantitating changes in fat, water and composition within bones at high spatial resolution, as a result of changing levels of circulating estrogen. That is, while DXA was useful in measuring whole body BMC, water (lean) and fat content, only MRI had sufficient resolution for regional tissue analyses. Therefore, MRI, and DXA are complementary techniques useful in the quantitation of longitudinal changes in fat, water, and bone. The cumulative data showed that ovariectomy stimulates an increase in body weight that is primarily due to an increase in body fat which includes an increase in subcutaneous fat as well as accumulation of fat in the marrow. E2 prevented this increase in body weight and accumulation of fat.

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