Human Parathyroid Hormone (1-34) Restores Ovariectomy-Induced Cortical And Cancellous Bone Loss Within Axial And Appendicular Skeletal Sites Of C57BL/6J Mice

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Introduction: Post menopausal osteoporosis is a major health problem in elderly woman as it increases osteoporotic fractures. Parathyroid hormone is an anabolic agent now used to treat osteoporosis in humans and has been shown to stimulate bone formation and reverse the bone loss associated with estrogen deficiency. However, the tissue and cellular responsivity to PTH in the skeleton is still not well understood. In this study we have used high resolution microcomputed X-ray tomography (\(\mu\)CT) to assess the efficacy of intermittent PTH treatment on skeletal sites in C57BL/6J mice.

Materials and Methods: C57BL/6J mice were ovariectomized (OVX) or sham operated and after 7 weeks treated with human parathyroid hormone (hPTH(1-34)) or vehicle intramuscularly for 4 weeks. After cessation of treatment, the left femur, tibia and fourth lumbar vertebrae were removed and cleaned of soft tissue and analysed by \(\mu\)CT using the SkyScan-1072 high-resolution desk-top micro-CT system (Skyscan N.V., Aartselaar, Belgium.) The left proximal tibia, distal femur and vertebral body were imaged using an X ray tube voltage of 80kV and current 120\(\mu\)A, with a 1 mm aluminium filter. The scanning angular rotation was 180 degrees and the angular increment 0.68 degrees. Datasets were reconstructed using the cone beam reconstruction software (NRecon version 1.4.4) based on the Feldkamp algorithm and segmented in to binary images using adaptive local thresholding. Bone volume analysis was performed using the CT analyser software (Skyscan). Trabecular bone distal to the proximal tibial growth plate or proximal to the distal femoral growth plate was selected for analysis within a conforming volume of interest (cortical bone excluded) extending 80\(\mu\)CT sections from the growth plate with a thickness of 600\(\mu\)m. Trabecular vertebral bone was calculated within 400\(\mu\)CT sections. Morphometric parameters including trabecular number (Tb.N), trabecular separation (Tb.Sp), and trabecular volume (BV) were calculated using the mean intercept length method. Trabecular thickness (Tb.Th) was calculated according to the method of Hildebrand and Ruegsegger [1]. In the mid-diaphysis cortical volume was assessed by similar methods in a region 1.2mm from the growth plate and extending 400\(\mu\)CT slices distally. Three dimensional images were generated using Cone-Beam Reconstruction and 3D Realistic Visualisation software.

Results: \(\mu\)CT analysis indicated that 11 weeks of estrogen deficiency induced a 33% loss of trabecular bone in the proximal tibia, 24% bone loss in the distal femur, and 26% loss in the vertebral body. This decrease was accompanied by a substantial disruption of the cancellous architecture characterised by fewer trabeculae and a deteriorated trabecular network. OVX mice also exhibited reduced cortical bone volume in the femoral and tibial diaphysis. Four weeks of hPTH (1-34) treatment was able to compensate significantly for OVX induced bone loss in the tibia and vertebrae but not in the femur, with a greater increase in the proximal tibia (33%) than in the vertebral body (15%). hPTH (1-34) fully compensated for OVX-induced trabeculae thinning by significantly increasing trabecular thickness in the tibia and femur to values above that of sham operated mice. hPTH (1-34) treatment did not appear to significantly affect trabeculae number or spacing in these bone tissues. In addition, hPTH (1-34) markedly enhanced cortical bone volume in the femoral and tibial diaphysis to that above sham and OVX vehicle treated mice.

Discussion: OVX induces severe cancellous bone loss in the tibia of C57BL/6J mice and to a lesser extent induces bone loss in the femur and vertebral body. Intermittent PTH treatment appears to significantly restore OVX-induced bone loss in both axial and appendicular bones in C57BL/6J mice by significantly enhancing cortical bone volume and trabecular thickness.