



Graduate Studies

Department of Physiology
MICHIGAN STATE UNIVERSITY

Mechanisms Regulating Bone Health and Density

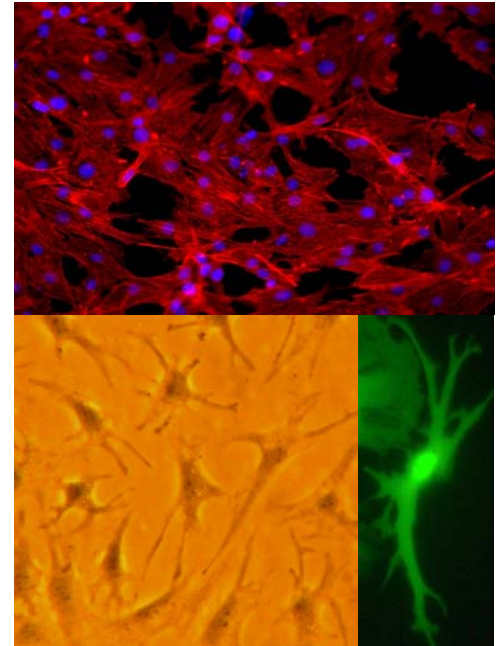
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Over 34 million Americans have decreased bone mass and an additional 10 million are classified as osteoporotic (severe bone loss). Aging, disuse and disease contribute to decreased bone density and its associated increase in fracture risk. In the elderly, a bone fracture is strongly associated with depression and morbidity. Most therapies prevent bone resorption, while few are able to enhance bone formation. By taking an integrative approach to examine bone adaptation to **disuse** and **diabetes**, my lab is working toward identifying mechanisms regulating bone formation. We are also developing **therapeutics** to target our identified mechanisms/pathways to increase bone formation.

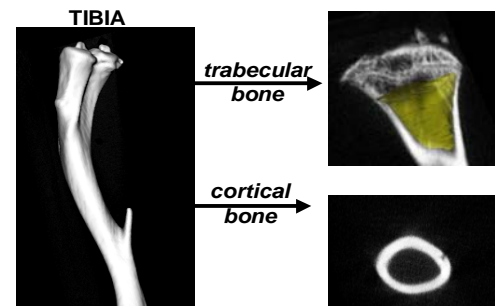
Disuse and Hypoxic Regulation of Bone Mass: Mechanosensing allows bones to adaptively strengthen at areas where increased force is sensed, and reduce bone volume at areas where strong bone is no longer needed. We hypothesize that bone loss associated leads to decreased bone formation. We've demonstrated that hypoxia is a key regulator of bone cell maturation and suppresses runx2 expression. Runx2 is a key transcription factor required for stem cell selection to be a bone cell and bone cell differentiation and bone formation. In addition, hypoxia stimulates PPAR γ expression, a transcription factor that suppresses bone cell maturation and stimulates fat cell number/adipogenesis. Selection of adipogenesis over osteoblastogenesis is thought to contribute to bone loss associated with a variety of conditions associated with osteoporosis. We suspect that decreased oxygen delivery contributes to this outcome. We've identified a highly conserved proximal region of the runx2 promoter that is sufficient for hypoxic response and are currently working on identifying the factors involved by utilizing promoter mutations, supershift analyses, footprinting, and chromatin immunoprecipitation (to look for corepressor or coactivator modulation). In addition, we know that hypoxia inducible factors (HIF transcription factors) are involved in runx2-suppression and are using animal models of bone hypoxia and transgenic animals to test the role of HIFs in bone mineral density regulation.

Mechanisms of Diabetes Associated Bone Loss: Insulin dependent diabetes mellitus (IDDM, type I diabetes) is a chronic disease stemming from little or no insulin production, and elevated blood glucose levels and osmolarity. IDDM is associated with complications including the less well-known bone loss in both men and women, which makes them susceptible to osteoporosis and fractures. We hypothesize that several factors underlie suppressed osteoblast differentiation and bone loss in type I diabetics including osteoblast osmoadaptation, induction of PPAR γ expression, and modified cellular metabolism (perhaps involving hypoxia). We've demonstrated that indeed, osteoblasts respond to osmotic stress through the immediate activation of p38 and protein kinase C signaling pathways that lead to altered transcription factor activities, gene expression and differentiation. We've also characterized the bone phenotype of a streptozotocin-induced IDDM mouse model and found a decrease in bone volume and an increase in PPAR γ mRNA levels. Our studies suggest that adipogenesis is favored over osteogenesis under diabetic conditions and we are currently examining the mechanism for this response.

Development of Potential Therapeutics: Hip and knee joint implants are used in more than 1,500,000 operations each year. In collaboration with faculty from Chemistry and Engineering, my lab is applying our basic knowledge about anabolic pathways to enhance bone fracture and implant healing. We are examining surface responses, manipulation of surface structures and effects of growth factors on the success of implant integration. In addition, we are testing therapies for bone loss under conditions of disuse.



Our *in vitro* bone models. Osteoblasts (top picture) undergo three stages of maturation (proliferation, matrix maturation, and matrix mineralization) to produce bone nodules in culture dishes that are similar to real bone. Osteocytes (bottom figures) are the cells embedded within the bone mineral. Both cells are used in my lab to examine mechanisms regulating bone formation under conditions of hypoxia and disease. The green cell represents a cell that we have introduced a gene that makes it produce fluorescent proteins.



Bone effects *in vivo*. To test our hypotheses *in vivo* we are using mouse models of diabetes (streptozotocin injection) and disuse (hindlimb suspension). Effects on bone mineral density are determined histologically and by micro-computed tomography (as shown above). In addition, gene expression and protein levels are determined by RNA analyses and immunohistochemistry, respectively.