SU164

52. Ontogenetic Development of the Ovine Calcaneus: A Model for Examining the Relative Contributions of Genetic, Epigenetic, and Extra-genetic Stimuli. J. G. Skedros, K. J. Hunt, C. L. Sybrowsky*. Dept of Orthop Surg, U of UT, Salt Lake, UT, USA.

Bone morphology may be largely constrained by genetic and epigenetic processes (e.g., via tissue interactions) that govern development along a given growth trajectory. We hypothesize that: 1) structural variations (e.g., trabecular orientation, cross-sectional shape), which dominate in early ontogeny, are primarily genetically/epigenetically derived, and 2) material (e.g., collagen fiber orientation (CFO)) and/or structural variations that appear later in development are primarily environmentally, or "extra-genetically" derived (e.g., as a response to strain transduction or microdamage formation). Because of its highly nonuniform morphologic organization, the ovine calcaneus appears to be a good model for examining mechanisms that mediate the modeling and remodeling processes that produce spatio-temporal developmental variations. Calcanei were obtained from 24 sheep (fetal - adult). In vivo strain data demonstrate that this bone receives relatively simple bending, with prevalent compression in the cranial and tension in the caudal cortex. Geometric measurements included: 1) cranial, caudal, medial, and lateral cortical thickness at 60% 'length', 2) maximum (Imax) and minimum (Imin) 2nd moments of inertia, and 3) cortical area (CA) and total area (TA). Lateral roentgenograms were analyzed for arched trabecular patterns, which may reflect tension/compression stress trajectories. Predominant CFO was determined using circularly polarized light in thin ultra-milled sections. Results showed that although CA/TA and Imax/Imin ratios increased linearly with bone length ($p<0.001$), significant cranial vs. caudal CFO and cortical thickness differences occurred only in the subadult and adult groups. Arched trabecular patterns were identified in fetal bones. The relatively early appearance of preferred trabecular orientation most likely represents programmed development. The 'delayed' temporal development of cross-sectional asymmetry and regional CFO anisotropy probably reflect influences of strain transduction. Mounting evidence suggests that the role of mechanotransduction, especially in the developing skeleton, is to provide the necessary threshold values required for implementation or maintenance of growth patterns that are mediated by modeling processes and guided principally by positional information. In contrast, we speculate that adaptations that are mediated by remodeling activities (e.g., non-uniform CFO patterns) may be more strongly influenced by strain transduction or byproducts of functional loading (e.g., regional or strain-mode-related differences and/or characteristics of microdamage).

SU165

Interactions between Estrogen and Mechanical Strain Effects on Osteoblasts Are not Influenced by Estrogen Receptor Type. T. Thomas[1], F. Lima*[1], M. Lafage-Proust[1], P. van der Saag[2], C. Alexandre[1], L. Vico[1]. [1]Inserm 9901, University Hospital of St-Etienne, Saint-Etienne, France, [2]Netherlands Institute for Developmental Biology, Utrecht, Netherlands.

Estrogens (E) and mechanical strain exert direct effects on osteoblast activity, with good evidence of interactions between their respective effects. Osteoblasts express both forms of estrogen receptors (ER) ERa and ERb, and previous studies have suggested a specific role for each receptor. Therefore, our working hypothesis was that the interactions between E and mechanical strain on osteoblast activity vary depending on which ER is preferentially activated. Using the human osteoblastic cells, U2Os, stably transfected either with ERa or ERb, we evaluated the effects of cyclical cell loading on a F-3000 Flexercell Strain Unit (1.5% elongation, 10 min/day), in presence of estradiol (E2) 10-8 M or not. The U2Os cell lines, either ERa or ERb transfected or the original U2Os cell line which does not express ER, were characterized by low alkaline phosphatase (AP) activity. They expressed all osteoblastic markers but osteocalcin because of a specific gene mutation. In both U2Os-ERa and U2Os-ERb cell lines, mechanical strain induced similar increases in AP activity and gene expression as measured by quantitative RT-PCR (Light Cycler, Roche), and a decrease in type I collagen gene expression. No change in proliferation rate was observed. Strain and E2 had a synergistic effect on AP activity as compared to each stimulus alone. Neither proliferation nor differentiation of the original U2Os cell line, was altered by strain or E2. In summary, the differences observed between the U2Os and the U2Os-ERa or -ERb cell lines are consistent with previous studies suggesting that ER play a critical role in mechanotransduction. However, these data do not support the hypothesis of differing roles for ERa and ERb in these defined experimental conditions. Understanding the mechanisms mediating interactions between estrogens and mechanical strain at the cellular level still requires further investigations.

SU166

Mechanically Related Changes in Gene Expression Using Real Time RT-PCR. M. E. Squire[1], L. R. Donahue*[2], M. Hadjiargyrou[1], C. Rubin[1], S. Judex[1]. [1]Biomedical Engineering, SUNY Stony Brook, Stony Brook, NY, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA.

Low level mechanical stimuli (<5 microstrain) can be anabolic to bone if induced at high frequencies (30-90 Hz). Conversely, removal of the normal mechanical environment can cause loss of bone tissue. We have recently demonstrated in BALB/cByJ mice that 10 minutes per day of whole body vibration at 45 Hz significantly increased trabecular bone formation rates in the proximal tibia by 34% while hind limb suspension (disuse) decreased bone formation rates by 48%. The identification of key molecular events behind this adaptive process may ultimately lead to the discovery of delivery targets and novel therapeutics for osteoporosis. Using reverse transcription polymerase chain reaction (RT-PCR), we previously characterized the expression of several candidate genes in response to both our anabolic and catabolic stimulus. Here, we tested whether real-time RT-PCR increases the sensitivity of detecting subtle changes in gene expression. Sixteen-week old female BALB/cByJ mice were randomly assigned to control, disuse (tail suspension), and mechanical stimulation groups (n=2 each). Mechanically stimulated mice were placed on a vibrating platform (0.25g, 45 Hz) for 10 min/d, 5 d/wk. After 4 days, RNA was extracted from the whole left tibiae (including bone marrow and cartilage). Real time RT-PCR quantified the expression of the genes coding for collagen type I (α1) and beta-2 microglobulin (beta-2). Mechanical stimulation up-regulated the relative expression (normalized to the housekeeping gene beta-2) of collagen type I by 30% +/- 4.4% (mean +/- SE) while disuse down-regulated the transcriptional levels by 40% +/- 4.8%. These results support our previous findings that collagen type I mRNA levels are rapidly reduced by mechanical unloading. The data also indicate that the expression of the most abundant protein in bone is increased after only 4 days of mechanical vibration, a result that we were unable to observe previously with conventional RT-PCR. Intriguingly, the relative changes in the expression of collagen type I as induced by low level mechanical vibration and disuse accurately reflected the relative changes in trabecular bone formation rates. In summary, this study indicates the superior sensitivity of real time RT-PCR in quantifying small changes in molecular expression levels of bone during the mechanically adaptive process. This sensitivity is currently being utilized for detecting subtle changes in the transcriptional level of genes critical for bone formation, resorption and maintenance.

SU167

Mechanical Properties of the Extracellular Matrix Regulate Fluid Shear Induced Metabolism of Prostaglandins in Osteoblasts. S. M. Ponik, J. Pavalko. Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN, USA.

Fluid shear stress (FSS) resulting from mechanical loading of bone in vivo is a potent stimulus for bone cell metabolism. In this study we tested the hypothesis that FSS-induced cell signaling may be regulated by changing the physical state of the extracellular matrix (ECM) or by modulating expression of integrin adhesion molecules, which link the actin cytoskeleton to the ECM. MC3T3-E1 osteoblasts were cultured on fibronectin or collagen that was adsorbed directly onto untreated glass slides or onto poly-L-lysine coated slides. Cells were allowed to rearrange the matrix for 16 hours and then were subjected to 90 minutes of laminar fluid shear. Following FSS, cells were incubated for 30-minutes during which released prostaglandins were allowed to accumulate in 1 ml of cell culture media. Prostaglandin-E_2 (PGE_2) released into the media was analyzed by enzymeimmunoassay. Integrin expression in cells grown on the different matrix proteins was evaluated by immunoprecipitation of biotin labeled cell surface proteins. Cells grown on either untreated glass or glass coated with fibronectin or collagen expressed both the α5 and αv integrin subunit at the cell surface. However, in response to FSS osteoblasts cultured on fibronectin and collagen released more PGE_2 then cells cultured on untreated glass. The α2 integrin subunit was expressed at the cell surface only in cells cultured on collagen, however, the FSS induced PGE_2 release was not different between cells grown on fibronectin and collagen. This suggests that cells grown on fibronectin or collagen are more responsive to FSS than cells grown on uncoated glass. To further investigate the role of mechanical properties of the ECM in FSS-induced mechanotransduction, glass slides were first coated with poly-lysine prior to adsorption of fibronectin or collagen to immobilize the matrix and decrease the ability of osteoblasts to rearrange the ECM. Interestingly, FSS-induced PGE_2 release from osteoblasts cultured on poly-L-lysine-immobilized fibronectin or collagen was significantly decreased compared to cells cultured on fibronectin or collagen adsorbed to untreated glass. These data suggest that limiting the ability of osteoblasts to reorganize the ECM by immobilizing the ECM proteins decreases the cell's ability to respond to FSS.

SU168

Do Bones Have Memory? Glutamate Receptor Expression in Bone. K. Black*, B. L. Theriault*, G. I. Anderson. Pharmacology, Dalhousie, Halifax NS, Canada.

Learning and memory involve both ionotropic and metabotropic glutamate receptors. Glutamate receptors have been identified in the bones of rat, rabbit, and mouse. Mechanical stimulation elicits changes in glutamate receptor expression. We postulate that glutamate receptors mediate mechanical stimulation to increase bone density in a manner analogous to their role in mediating CNS synaptic plasticity. Estrogen modulates glutamate receptor expression in the CNS: we suggest a similar role in postmenopausal osteoporosis. A mouse osteoclast model is being used to characterize the sensitivity of glutamate receptor expression to mechanical stimulation and estrogen levels. In some cultures, glutamate receptor expression has been seen to decrease or disappear during prolonged culture. To validate the use of our osteoclast culture model, we have verified the expression of the glutamate receptor subtypes and examined their sensitivity to mechanical strain. Bone marrow cells were flushed from the femora and tibiae of 7-week old female CD1 mice using minimal essential medium, 8 x 106 cells/35mm well were seeded onto collagen-I coated flexible silastic membranes and cultured for 7 days. Cells were mechanically stimulated (4 Hz, 900 cycles) on days 4, 5, and 6 of culture. The presence of glutamate receptor subunits in the cultured cells was analyzed using immunohistochemistry with mouse hippocampal slices as a positive control. After 7 days of culture, sufficient mature multinucleated osteoclasts differentiated from hematopoeitic progenitor cells to permit analysis. The expression of the following glutamate receptor subunits was confirmed in unstimulated multinucleated osteoclasts: NMDAR1, 2A, 2B, 2C; AMPAR1, 2/3, 4; mGluR1a and 5, but not mGluR2/3. The highest expression levels were observed for NMDAR2B and GluR4, suggesting that they play an enhanced role in the transduction of mechanical strain stimuli. Mechanical stimulation did not appear to alter the expression of NMDAR2B. AMPAR2 or AMPAR4, but caused decreases in NMDAR1, NMDAR2A, NMDAR2C. AMPAR1, mGluR1a, and mGluR5 expression. Additionally, we observed marked differences in distribution of the different receptor subtypes: NMDA receptor subtypes 1, 2A, and were mostly expressed in the periphery where podosomes form, further supporting the role mechanotransduction. We postulate that glutamate receptor subunit expression changes with mechanical stimulation and modulates osteoclast responsiveness to osteoblast glutamate signalling. We are currently tracking the mRNA levels of these immunohistochemically observed changes in glutamate receptor subtype expression induced by mechanical stimulation and the effects of varying estrogen levels in this model.