ment, but showed an increased tooth movement as the activation duration proceeded from 1 week to 3 weeks. The periodontal ligament space surrounding the tooth was also measured by micro CT coronal sections of the distobuccal root of the treated molar. In the WT treated group the periodontal ligament space ratio of the tension side (distal periodontal ligament) to the pressure side (mesial periodontal ligament) was significantly greater compared to the KO group ($p<0.05$). Histological examinations of both WT and OPN deficient alveolar complex with treatment showed a reverse pattern in bone remodeling compared to the control. On the pressure side, there was clear presentation of TRAP positive osteoclasts, while in the tension side alkaline phosphatase positive new bone forming cells were observed. However there was no significant difference in TRAP and Alkaline phosphatase positive cells between the WT, OPN deficient group. This indicates OPN deficiency could be backed up by unknown compensating mechanisms for surrounding bone remodeling in the experimental tooth movement in mice

SU059

Osteopontin Deficiency Suppresses New Bone Formation in the Sutures Under Tensile Mechanical Stress in vivo. M. Morinobu[1], M. Ishijima[1], S. R. Rittling[2], K. Tsuji[1], H. Yamamoto*[3], A. Nifuji[1], D. T. Denhardt[2], M. Noda[1]. [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Rutgers University, Piscataway, NJ, USA, [3]Ehime University, Matsuyama, Japan.

Mechanisms underlying mechanical stress-dependent control of bone remodeling are largely unknown. Mechanical stress enhances osteopontin (OPN) expression in osteoblasts, and OPN deficiency prevents unloading-induced reduction in bone formation (Journal of Exp. Med. 193:399, 2001). Thus, OPN could act as a transducer for mechanical stress. However, the function of OPN in bone formation under mechanical stress is not known. Therefore, we examined the role of OPN in bone formation under tensile mechanical stress in vivo in mice. Sagittal sutures of mice were subjected to expansion by orthodontic springs and bone formation was examined. One week expansion of the sutures resulted in bone formation in the edges of the parietal bones. RT-PCR analysis indicated increase in the levels of OPN mRNA expression in the cells in the wild type calvariae subjected to expansion. In addition, type I collagen mRNA was also expressed in the calvariae under the mechanical stimuli. Immunohistchemical analysis revealed highly abundant expression of OPN protein in the matrix of newly formed bone in the mechanically expanded sutures and on the inner edge of the parietal bone where stress was applied. Osteoblasts forming bone under tensile stress also exhibited high levels of OPN protein expression in the operated mice. In sham-operated mice (without expansion), OPN positive osteoblasts were also observed on the inner edges of parietal bone, however accumulation of OPN protein on the bone matrix was significantly less than expansion group. After the application of mechanical stress, OPN was expressed at low levels in fibroblastic cells in the fibrous interzone. On the primary matrix front, highly OPN positive-oval shaped transchondral cells were observed. Bone formation was clearly observed in the expanded suture area four weeks later in wild type mice. In contrast, reduction in such bone formation in the expanded suture area was observed in the OPN knockout mice. These observations revealed that OPN is required for bone formation under the tensile mechanical stress.

SU060

Skeletal Phenotype of Transgenic Mice Expressing the Beta-1 Integrin Cytoplasmic Tail In Osteoblasts. R. K. Globus[1], M. C. H. van der Meulen[2], C. Damsky*[3], J. B. Kim*[3], D. Amblard*[1], Y. Nishimura*[4], E. Almeida*[1], U. T. Iwaniec[5], T. J. Wronski[5]. [1]NASA Ames Research Center, Moffett Field, CA, USA, [2]Cornell University, Ithica, NY, USA, [3]UCSF, San Francisco, CA, USA, [4]NASA Ames Research Center, Moffett Field, CA, USA, [5]University of Florida, Gainesville, FL, USA.

To define the physiologic role of β1 integrin in bone formation and mechanical loading, transgenic mice were generated by expressing the cytoplasmic tail and transmembrane domain of β1 integrin under the control of the osteocalcin promoter. In cultured cells, this truncated fragment of β1 can act as a dominant negative. Previously, the matrix of calvariae was shown to be abnormal in transgenic (TG) compared to wildtype (WT) mice. In this study, we analyzed appendicular bone in TG and WT, male and female mice at 14, 35, 63, 90 and 365 days old (n=8-12/gp). To assess β1 integrin function in mechanical loading, a pilot study using hindlimb unloading by tail suspension was performed. 35d old TG and WT females were hindlimb unloaded for 4 wks (n=3-5). Body mass, bone mineral content, histomorphometric (distal femur) and biomechanical parameters were analyzed. Statistical significance ($P<.05$) was defined by ANOVA using the Tukey-Kramer post-hoc test. We confirmed transgene expression by immunoprecipitating then immunoblotting bone lysates using an antibody against the β1 tail. Body masses of TG mice at 63, 90 and 365d old were greater (16-25%) than WT. Some TG female mice at 365d appeared obese; mean abdominal fat mass was 415% greater in TG than WT mice. Tibiae were longer (5-7%) in TG than WT mice at 63 and 90d. Tibial mineral mass of 35d males was 7% lower in TG than WT mice, but at 63d was 21% higher. The % osteoblast surface in 35d TG mice was 20% higher than WT, and at 63d was 17% lower, while % osteoclast surface did not differ. In 365d mice, cancellous bone volume (125%) and endocortical mineral apposition rate (40%) were greater in TG than WT males but not females. In WT mice, hindlimb unloading caused a reduction in mineral mass of tibiae (-20%) and lumbar vertebrae (-22%) relative to normally loaded controls. Surprisingly, hindlimb unloading also caused a relative reduction (-13%) in humerus mass. The effects of hindlimb unloading on tibia and humerus mass were less obvious in TG than in WT mice. Since hindlimb unloading caused skeletal changes in both loaded and unloaded bones, systemic changes may contribute to bone responses observed using this animal model. In conclusion, transgene expression resulted in marked metabolic changes during growth and in the aged female. Our results demonstrate that expression of the β1 integrin cytoplasmic tail *in vivo* causes gender- and age-specific changes in select morphometric parameters, bone length, and bone mass.

SU061

Demonstration of MAP Kinase Activation in Response to Mechanical Stimulation in a Novel Rat Model of Fracture Healing. N. J. Hubbard*, S. K. Volkman*, S. A. Goldstein, M. R. Moalli. Orthopaedic Research Laboratories, University of Michigan, Ann Arbor, MI, USA.

The MAP Kinase family of threonine and serine kinases is activated in response to diverse extracellular stimuli, and various in vitro studies have demonstrated their responsiveness to mechanical strain. However, the signal transduction mechanisms which cells use to respond to the local strain environment during fracture healing are currently poorly characterized. We have developed the first in vivo model that enables the study of how mechanical stimulation influences the cellular and molecular processes that regulate fracture healing under normal, aged and osteopenic conditions. Using this model we examined the hypothesis that the stage of fracture healing, as well as the local strain environment determine the activation profile of MAPK. Mechanical load-induced MAPK activation was evaluated during the reparative and remodeling stages of fracture healing, which occur at either 8 days or 5 weeks post-operatively in this model, as determined by histological analysis. This study utilized young adult Fischer 344 female rats that received bilateral, 0.6mm, mid-diaphyseal femoral osteotomies. The osteotomies were stabilized with custom four pin external fixators that enabled normal cage activity post-operatively. At either 8 days or 5 weeks post-operatively, a uni-axial compressive load was applied to one fracture site, while the other fracture site was designated as the unloaded control. Due to the predominantly fibroblastic component of the 8 day reparative tissue, the mechanical stimulus was a displacement-controlled force, corresponding to a 15% strain of the fracture tissue. In contrast, due to increased consolidation in the 5 week tissue the mechanical stimulus was a load-controlled force, also corresponding to a 15% strain. Both experimental groups were loaded for 900 cycles at 1Hz. Specimens were harvested immediately after loading, snap frozen and prepared for immunoblot analysis using MAPK and phospho-specific MAPK antibodies. In the 8 day specimens MAPK activation was increased in loaded as compared to the unloaded tissue. Interestingly, MAPK activation was decreased in the loaded as compared to the unloaded tissue of the 5 week specimens. It appears that the viscoelastic nature of the undifferentiated, 8 day repair tissue may be dictating MAPK activation in response to mechanical stimulation. Immunolocalization studies are currently underway to identify the mechanoresponsive cellular phenotypes.

SU062

53. Use of Predominant Collagen Fiber Orientation for Interpreting Cortical Bone Loading History: Bending vs. Torsion. J. G. Skedros. Dept of Orthop Surg, U of UT, Salt Lake, UT, USA.

Habitual bending appears to be highly conserved in functional loading of appendicular long bones in terrestrial animals. In fact, empirical strain data demonstrate that bending produces the majority (>70%) of longitudinal strains occurring during controlled in vivo activity. Non-uniform patterns of predominant collagen fiber orientation (CFO) in cortical bone are highly correlated with the regional prevalence of tension and compression strain modes. Predominant CFO therefore appears to be highly sensitive and specific for interpreting loading history in a limb diaphysis when strain data are not available. Some bone regions, however, experience habitual torsion. While notable examples include the human femoral neck, and mid diaphyses of the sheep tibia, pigeon humerus, and turkey ulna, only the non-human bones have been verified with in vivo strain measurements. Due to the widely shifting neutral axis that occurs during torsional loading, non-uniform CFO patterns would not be expected in these regions. To test this hypothesis, transverse segments from mid diaphyses of 9 skeletally mature chicken femora were embedded in polymethyl methacrylate and examined for tissue adaptations. In vivo strain gauge studies have demonstrated that this region experiences prevalent torsion (Carrano and Biewener, 1999. J Morph). Undecalcified sections were milled to 100+/-5 microns and viewed under circularly polarized light. CFO is expressed as the mean graylevel in each microscopic field (approx. 2.3mm2) in the mid-cortical region of the cranial (Cr), caudal (Cd), medial (M), and lateral (L) cortices. Graylevels were quantified from pixel histograms obtained from digitized images. Embedded transverse sections from mid shafts of calcanei from a skeletally mature sheep and mule deer, and a horse radius, were used as "control" bones since strain gauge analyses have shown that they have a habitual tension/compression distribution. Results showed no statistically significant CFO differences between any of the chicken femora cortical regions, consistent with the hypothesis of the apparent absence of non-uniform regional CFO patterns in torsional loading. These results are consistent with recently reported findings in mid diaphyses of torsionally loaded mature sheep tibiae and turkey ulnae. It is suggested that the uniform CFO in the chicken femora actually represents adaptation for shear strains produced by torsion. CFO is also uniform in the neutral axes of the control bones where shear stains are prevalent. These observations suggest that predominant CFO is reliable for interpreting strain-mode-specific (tension, compression, shear) adaptations in bending and torsion.