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12. MINERAL CONTENT ANALYSIS OF TENSION/COMPRESSION SKELETAL SYSTEMS: INDICATIONS OF POTENTIAL STRAIN-SPECIFIC DIFFERENCES J.G. Skedros*, D. Ota*, and R.D. Bloebaum* (Intro. by H. Winet) Bone and Joint Research Laboratory, V.A. Medical Center, Salt Lake City, UT 84184

Strain magnitude and strain mode (e.g., tension vs. compression) are considered to be important in establishing thresholds for bone remodeling/modeling processes. The present study examines the idea that bone structural/material organization can reflect the capacity of bone cell populations to recognize regional nonuniformities in strain-related loading conditions. Two simple skeletal tension/compression systems were studied to see if spatial differences in strain mode and/or magnitudes can be recognized as adaptations in bone organization. Calcanei from two large quadrupeds were studied. Justification for the use of this model is based on in vivo data showing the unambiguous presence of prevailing compression in the cranial cortex and tension in the caudal cortex. Sections (0.5 cm thick) were obtained from the middle third of skeletally mature horse (n=14) and elk (n=9) calcanei. The compression (cranial) cortex was cut into three pieces corresponding to subperiosteal (SP), middle (MD), and endosteal (EN) regions. The narrower tension (caudal) cortex was cut into two pieces corresponding to SP and EN regions. Specimens were defatted in chloroform, dried to constant weight, and ashed at 550C. Mineral content (ash fraction) was expressed by dividing ash weight by dry weight. Results showed that in both horse and elk calcanei the compression cortex had a significantly higher mineral content than the tension cortex ($p < 0.001$, all regions averaged) [elk: 69.9 (1.5 = S.D.) vs. 65.5 (1.7); horse: 65.5 (0.9) vs. 61.5 (1.0)]. In elk calcanei there were no significant differences between the compression cortical regions (SP, MD, and EN) in all possible comparisons ($p > 0.05$). Similarly, there were no significant differences between the endosteal and subperiosteal regions of the tension cortex of both the elk and horse calcanei ($p > 0.05$). In contrast, the mineral content of the subperiosteal region of the horse compression cortex was significantly less than the other two regions ($p < 0.001$) [64.8 (1.1) SP, 66.3(0.8) MD, 66.2 (0.8) EN]. Mineral content differences shown between tension and compression cortices may be adaptive responses to the well-documented disparity in the mechanical properties of bone loaded in tension versus compression. Increased mineral content in the compression cortex may enhance mechanical properties in this more highly stressed region. If these mineral content differences reflect adaptation to prevailing strain differences, it is suggested that this may represent both local (e.g., within one cortex) and global (e.g., between cortical regions) processing of strain information.

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VERTEBRAL BODY TOUGHNESS AND RESILIENCE IN RATS AFTER PARATHYROID HORMONE (PTH) TREATMENT M.P. Akhter, H. Maeda, D.B. Kimmel, I.Y. Tang, and R.R. Recker Creighton University; Omaha, NE.

Systemic treatments that enhance bone formation and increase bone mass should also improve bone's performance as a structure *in vivo*. While increased breaking strength is desirable, increased resilience [ability to resist permanent deformation during loading] is also crucial. Our aim is to evaluate breaking strength, toughness, and resilience of vertebral bodies from rats treated with human PTH (1-84).

6mos old female Sprague-Dawley rats were used. In one experiment, hPTH (50μg/kg/d) or vehicle (V) was given to intact rats for 29d. In a second, hPTH (50μg/kg/d) was given to osteopenic, ovariectomized rats for 28d. Fourth lumbar vertebrae were collected. Bodies were isolated and prepared with parallel end surfaces for compression testing in stroke control (MTS 810 servo-hydraulic) at 4mm/min. The load-deformation curve was recorded and analyzed to reveal ultimate load (UL), yield load (YL), toughness (T) and resilience (R). Resilience is the energy absorbed before permanent deformation. Toughness is the energy absorbed before failure.

Group	UL N	YL N	T N-mm	R N-mm
Intact	154±58	138±63	27±11	16±11
Intact+hPTH	219±60[a]	186±43[b]	52±27[a]	29±20[b]
OVX+Veh	124±48	91±36	19±14	7±3
OVX+hPTH	242±62[c]	198±64[c]	58±38[c]	27±28[c]
greater than Intact [a](P<.05) [b](.1>P>.05); greater than OVX+Veh [c](P<.05) [d](.1>P>.05)				

In intact rats given hPTH, ultimate and yield load improved 40%, while toughness and resilience doubled. In OVX, osteopenic rats given hPTH, ultimate and yield load doubled, while toughness and resilience rose 3-4X fold.

Conclusion: In rats, PTH not only improves vertebral body breaking strength, but also improves its ability to resist permanent deformation. The improvements in toughness and resilience may be greater than those in breaking strength.

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PROTEOLYTIC INACTIVATION OF MATRIX METALLOPROTEINASE 3 IS INDUCED BY BONE ACIDIC GLYCOPROTEIN-75. Y. Chen, K. Suzuki*, H. Nagase*, and J.P. Gorski, Univ. of Missouri-KC, Kansas City, MO, and, Univ. of Kansas Med. Ctr., Kansas City, KS 64110.

Recent work has shown that bone acidic glycoprotein-75 (BAG-75) is an inactivator of matrix metalloproteinases 1 and 3 (MMP-3). To identify and characterize presumptive BAG-75/MMP-3 complexes, microtiter wells were coated with a range of BAG-75 input concentrations (2×10^{-9} to 2×10^{-7} M). Bound BAG-75 was then incubated for 24 h at 37°C with 6×10^{-8} M MMP-3; osteopontin (OP) coated wells and plastic alone served as negative controls. Unbound MMP-3 was removed and residual proteolytic activity assayed with ^{3}H-transferrin; in other lanes, ^{125}I-MMP-3 was used to follow binding directly. Above background binding was observed only in wells with BAG-75, reaching a maximum at 9×10^{-8} M input. Proteolytic activity in unbound supernatant fractions followed in an inverse manner the binding curve for MMP-3; however, activity recovered was only 15% of that expected based upon radioactivity. Immunoblotting analyses of supernatant fractions demonstrated a loss of MMP-3 bands at Mr=45 and 28 kDa which corresponded with estimates of proteolytic activity. Autoradiography provided additional support for a degradative mechanism; macromolecular radioactivity was barely detectable in supernatant fractions from wells shown to have bound MMP-3. In contrast, studies with TIMP/^{125}I-MMP-3 complex revealed only background binding to BAG-75 and showed no changes in MMP-3 in supernatant fractions. Recoveries of MMP-3 proteolytic activity and antigenicity from OP-coated or plastic wells alone were similar and as expected. The results indicate an autolytic mechanism of inactivation of MMP-3 induced by immobilized BAG-75.

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BONE MORPHOGENETIC PROTEIN (BMP)2 EFFECTS ON THE EXPRESSION OF GENES ENCODING ALKALINE PHOSPHATASE TYPE I PROCOLLAGEN, OSTEOCALCIN AND OSTEOPONTIN IN RAT OSTEOBLASTIC OSTEOSARCOMA CELLS. Y.Tsuchimoto*, M. Shigide*, J. Wozney* and M. Noda, (Intr. by Y. Imai) Dept. Molecular Pharmacology, Medical Research Institute, Tokyo Medical & Dental University, Chiyoda-Ku, Tokyo, Japan 101 *Genetics Institute, Cambridge, MA

BMP was identified first in bone matrix as an activty which induces ectopic bone formation in vivo. In vitro studies also indicated that BMPs enhance expression of phenotypes in osteoblastic cells or their precursor cells. To elucudate further the mode of action of BMP, on the expression of genes encoding osteoblastic phenotype-related proteins in relatively mature type osteoblast-like cells, we examined BMP effects on the expression and promoter activities of these genes. We first examined the effects of human recombinant BMP2 on the osteoblastic phenotypic expression in rat osteosarcoma ROS 17/2.8 cells. These cells form bone when implanted in animals, express most of the phenotypic genes including high levels of alkaline phosphatase (AP) osteocalcin (OC) and osteopontin (OP) and, thererore, are considered to be a model of relatively mature osteoblasts. Treatment with BMP2 at 100ng/ml enhanced AP activity more than three fold. Concominantly, AP mRNA level was also enhanced about four fold. Funthermore, the abundance of type I procollagen, osteocalcin and osteopontin mRNA was enhanced by about 70%, 60% and 30% more in BMP2 treated cells than control respectively. These BMP2 effects were observed within 24hrs and lasted at least up to 48hrs. The concentration of serum in the media did affect the basal steady state level of some mRNAs while BMP2 effects on these mRNAs were similarly observed regardless of the serum concentration. The growth of these cells were not affected significantly by the treatment with 100ng/ml hrBMP2 in the presence of either 0.5 or 5% serum, although the mean values were about 10-20% smaller in BMP2 treated cells. The BMP2 effects on the activity of the fragments of the promoter region of alkaline phosphatase gene were examined by transfecting chloramphenicol acetyltransferase constructs into the cells. Although the basal promoter activities were observed, BMP2 effects on these particular promoter fragments were not significant. In summury, BMP2 enhances expression of genes related to osteoblastic phenotypes in ROS17/2.8 cells.