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16. EXAMINATION OF TRABECULAR BONE FOR MATERIAL ADAPTATION TO HABITUAL DIFFERENCES IN PHYSIOLOGIC STRAIN MODES. J.G. Skedros Univ. of Southern California Dept. of Orthopaedics, Los Angeles and Veterans Affairs Medical Center, Salt Lake City, Utah

Although it is well known that trabecular bone can accommodate prevailing physiologic loading conditions by adapting its architecture (structural organization), it is not clear if this tissue also has the capacity to adapt its material organization. It is hypothesized that material adaptation, if present in normal trabecular bone, would be most conspicuous between regions that receive a consistent strain distribution which is characterized by distinct regional differences in strain mode (i.e., tension in one region vs. compression in the other). That such material adaptation might exist is supported by studies showing trabecular bone to have yield and ultimate strength and strain that are each 30% lower for tensile vs. compressive loading [Keaveny et al., 1994 J. Biomechanics vol. 27(9)]. To test this hypothesis the artiodactyl and perissodactyl calcaneus models were selected since they receive cranial-caudal bending confined to the sagittal plane and have corresponding cranial ("compression") and caudal ("tension") trabecular tracts. Specimens included 5mm thick transverse sections from the mid-to-proximal shaft of one calcaneus from each of 13 skeletally mature standard breed horses and 13 wild North American elk (Cervus elaphus). Specimens of trabecular bone obtained from the cranial ("compression") and caudal ("tension") trabecular tracts, and cortical bone from the corresponding adjacent cortices and from the medial and lateral cortices, were defatted in chloroform, dried to constant weight, and ashed at 550C for 24 hours. Ash fraction (mineral content) was determined by dividing the weight of the bone ash by the weight of the dried bone. Results show that the mineral contents of the opposing trabecular tracts are nearly equivalent (horse: 2.7% greater, cr 64.6(0.8) vs. cd 62.9(1.1) $p<0.001$; elk 0.7% greater, cr 66.3(0.8) vs. cd 65.8(0.7) $p=0.29$). The medial and lateral cortices, which fall along a theoretical neutral axis, also exhibit small mineral content differences (horse: medial 65.8 vs. lateral 64.2 $p<0.001$; elk: medial 68.4 vs. lateral 66.8 $p<0.01$). In contrast, the corresponding cranial and caudal cortices exhibit relatively large mineral content differences (horse: 8.5% greater, cr 65.5(0.8) vs. cd 60.5(0.7) $p<0.001$; elk: 6.9% greater, cr 69.4(1.1) vs. cd 64.9(1.4) $p<0.001$). Trabecular struts in the opposing tracts do not adapt their material organization in the manner that is demonstrated by their neighboring cranial and caudal cortices. This difference most likely reflects the capacity of compact bone to exhibit adaptation through extensive osteon remodeling (which lowers mineral content and increases porosity). In addition to equivalent mineral content between tracts, the presence of quasi-orthogonal cross struts observed between trabeculae within each cranial and caudal tract suggest that each region may be equally adapted for tension and compression.

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EFFECTS OF INTERLEUKINS ON CYTOKINE PRODUCTION BY MARROW CULTURED FROM POSTMENOPAUSAL WOMEN. D. Cheleuitte*, S. Mizuno*, H. Reichel*, J. Glowacki, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115

We examined the kinetics of basal and stimulated cytokine production by marrow from postmenopausal women. Mononuclear cells were isolated from bone marrow discarded during total hip replacement. Cells were seeded in 24-well plates (10^6/ml/2 cm^2) in basal phenol red-free αMEM, 10% fetal bovine serum, antibiotics ± IL-1 or IL-6 as stimuli. Secreted cytokines [IL-1, IL-6, IL-6 soluble receptor (IL-6 sR), IL-11, and granulocyte/macrophage-colony stimulating factor (GM-CSF)] were measured with specific ELISAs. Basal secretion of IL-6, IL-6 sR, and IL-11, but not GM-CSF, increased with time in culture. There was no evidence of basal IL-1 production by human marrow. In previous work using an IL-6 bioassay, we found that premenopausal women and postmenopausal women on estrogen replacement therapy (ERT) had lower basal production of IL-6 than untreated perimenopausal and postmenopausal women. In a time-course study, we found that, although IL-6 secretion was much lower in cultures from a woman receiving ERT, it increased with time. Addition of IL-1 (20 U/ml) significantly stimulated both IL-6 and IL-11 secretion. A more detailed dose-response curve (IL-1 from 20-250 U/ml) showed significant increases in IL-6, IL-11, and GM-CSF production and a trend for increased secretion of IL-6 sR. Notwithstanding wide variations, as great as 640-fold, in the magnitude of IL-6 and GM-CSF secretion in response to interleukins by marrow from different subjects (N=9), these data clearly show that IL-1 stimulated IL-6, GM-CSF, and IL-11; and that IL-6 (50 ng/ml) stimulated secretion of GM-CSF, IL-6 sR, and IL-11, but not IL-1. The kinetic and dose-response data imply that IL-6 may be mediating some of the effects of IL-1 on marrow cells. Results with marrow from women on ERT showed blunting of IL-1's stimulation of cytokines and suggest that marrow cultures may be useful to reveal both systemic and local mechanisms of cytokine regulation in bone and marrow.

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THE OVARIECTOMY-INDUCED UPREGULATION OF OSTEOCLAST AND OSTEOBLAST PROGENITORS IN THE MURINE BONE MARROW SUBSIDES WITH TIME. M. Munshi, R.L. Jilka, and S.C. Manolagas, Center for Osteoporosis & Metabolic Bone Diseases, and GRECC, VAMC, Univ. of Arkansas For Med. Sci., Little Rock, AR 72205.

The increased rate of bone remodeling caused by loss of ovarian function in the mouse is associated with an upregulation of osteoclast and osteoblast formation in the bone marrow, as evidenced by the demonstrated upregulation of colony forming units for the osteoclast progenitor granulocyte/macrophages (CFU-GM), as well as the osteoblast progenitor CFU-fibroblast (CFU-F). Here, we have determined the time course of the CFU-GM and CFU-F changes following ovariectomy. In these experiments, Swiss Webster mice (60 days old) were ovariectomized or sham-operated. At 3 days, or at 1, 2, 4, 8,16 or 28 weeks after the operation, triplicate ex-vivo cultures of marrow cells were established from bone marrow aspirates from individual animals (n=4 to 5 animals per group), and the number of CFU-GM and CFU-F were determined. For the CFU-GM assay, $3x10^4$ cells were cultured in 35 mm tissue culture dishes containing methylcellulose semisolid medium supplemented with stem cell factor and granulocyte/macrophage colony stimulating factor. The cycling status of CFU-GM was determined by treating marrow cells with ^{3}H-thymidine (to kill replicating cells) prior to establishment of the culture. After 7 days, plates were scored for the presence of colonies (>50 cells) containing both granulocytes and macrophages. For the CFU-F assay, cells were cultured for 10 days in αMEM with 15% FCS, 50 µg/ml ascorbic acid and 10 mM β-glycerophosphate. Cells were fixed and stained for alkaline phosphatase, counterstained with hematoxylin, and scored for the presence of colonies exhibiting fibroblastic characteristics. The total number of CFU-GM as well as the number of cycling CFU-GM was unchanged at 3 days but increased at 1 week following ovariectomy, compared to sham-operated controls, and remained elevated for as long as 4 weeks following ovariectomy. However CFU-GM and cycling CFU-GM in cultures of bone marrow cells obtained from mice 8 weeks following ovariectomy and beyond were identical to those in the controls. The number of CFU-F also increased following ovariectomy, but this change did not become apparent until 2 weeks following the operation. The CFU-F number remained elevated until 8 weeks and began to decline thereafter. At 16 weeks, CFU-F levels were identical to that observed in sham-operated animals. These results demonstrate that the osteoclastogenic and osteoblastogenic response of the bone marrow caused by the acute loss of ovarian function in the mouse is transient and subsides within 4 to 8 weeks following the operation, as do the serum markers of bone remodeling.

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THE EFFECTS OF AGE, BODY WEIGHT AND GENETIC VARIATION ON BONE DENSITY OF THE SPINE AND RADIUS IN BABOONS (*Papio hamadryas*) MEASURED USING DUAL ENERGRY X-RAY ABSORPTIOMETRY. J. Rogers[1], M.C. Mahaney[1], N.J. Whittam[1*], W. J. Hodgson[1*], M.B. Rocha[1*], P. Parry[2*], and C.M. Kammerer[1] [1]Southwest Foundation for Biomedical Research, San Antonio, TX 78228, [2]Sequana Therapeutics, Inc. La Jolla, CA 92037

Previous studies indicate baboons (*Papio hamadryas*) are an appropriate nonhuman primate model for studies of bone mass and bone loss. We used DEXA to measure bone mineral density (BMD) in 442 pedigreed baboons. Five skeletal sites were studied: three lumbar vertebrae, the ultradistal radius and the point 33% proximal on the radial diaphysis. The animals were fed a standard monkey chow diet providing all essential nutrients including 1% calcium and >2000 IU vitamin D3/day. The study population included 174 males and 268 females, from 5.5 to 30 years old. There were no manipulations (surgical or pharmacological) to alter bone formation or bone loss. Maximum likelihood methods were used to generate preliminary estimates of the effects of covariates (age, sex, weight) and additive genetic variation on the five DEXA measures of bone density. No significant sex difference is detected in BMD, but there are highly significant positive effects of age and negative effects of age^2 on spinal density. Body weight has a significant positive correlation with BMD in all five skeletal sites. Genetic variation accounts for 23-39% of BMD variation among vertebrae, 47% of variation in radial diaphysis density and 46% of ultradistal radius density. A DNA polymorphism in the vitamin D receptor locus showed no association with BMD at any measurement site, even after accounting for covariates. We conclude this species is a valuable animal model for studies of the genetic basis of variation in peak bone density and for studies of age-related bone loss.