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PLATELET DERIVED GROWTH FACTOR (PDGF) STIMULATION OF IN VIVO BONE FORMATION IS INITIATED BY SITE-SPECIFIC STROMAL AND CELLULAR INTERACTIONS. D. Lacey§, W. Kenney*§, J. Chu*¶, W. Benson*§, D. Hill*§, P. Ruegg*¶, and W.E. Hufferd. §AMGEN, Inc., Thousand Oaks, CA 91320 and ¶University of Colorado Health Sciences Center, Denver, CO 80262.

The recent observation showing that PDGF BB enhances bone formation in vivo was only partially predicted by in vitro experiments indicating PDGF BB to be a mitogen, but not a differentiation factor, for osteoblasts. To address both this apparent discrepancy and ascertain potential mechanism(s) for this in vivo effect, femurs and tibiae from 200 gm female Lewis rats injected (IV) with PDGF BB (0.1, 1, or 5 mg/kg/day, up to 7 days) were analyzed by histology, immunohistochemistry, in situ hybridization, and morphometry. While PDGF BB dose-dependently induced new bone formation in this model, the process involved non-osteoblast cells and was geographically specific. By 3 days, enlarged endosteal osteoblasts, alkaline phosphatase positive marrow stromal cells, reticulin fibrils, and cells expressing osteopontin mRNA appeared at or near the diaphyseal endocortex. Additionally, TGF β1 mRNA-expressing megakaryocytes were present in the altered marrow stroma. By day 5, these changes had extended into the subcortical metaphysis and now the zone of altered marrow stroma included osteoid, enlarged, type I collagen and osteocalcin mRNA-expressing osteoblasts and bridging trabeculae, which mineralized by day 7. Histomorphometry confirmed these findings and showed no effects on bone formation or mineralization in primary or secondary trabeculae or periosteum. Interestingly, Factor VIII positive (endothelial, megakaryocyte, and platelet marker) debris and entrapped hematopoietic cells were present in the matrix, but not in the cortical or metaphyseal trabecular bone. As the bone forming process progressed in the marrow, blood platelet levels fell in the 1 and 5 mg/kg groups to 50 and 20% of control by 7 days. We conclude that PDGF BB induces bone formation through a process initiated by a site-specific stimulation of marrow stromal/osteoblastic cells, which potentially involves interactions of these cells or their stroma with megakaryocytes, platelets, and/or endothelial cells. These findings provide a morphologic basis for the discrepancy seen between the in vitro and in vivo effects of PDGF BB and provide a rationale for developing biologically relevant, albeit more complex, models of in vitro bone formation.

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THE ARTIODACTYL CALCANEUS AS A MODEL FOR EXAMINING MECHANISMS GOVERNING REGIONAL DIFFERENCES IN REMODELING ACTIVITIES IN CORTICAL BONE. J.G. Skedros, F. Chow*, and M.J. Patzakis*
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The artiodactyl (e.g., sheep) calcaneus has been introduced as a potential model for examining the veracity of stated mechanisms and predictions of Frost's Mechanostat Theory of mechanically induced bone adaptation [Skedros et al., 1994 Anatomical Record, vol. 239]. Since this bone is primarily subject to loading in the sagittal plane, it receives habitual cranial-caudal bending. In comparison to the caudal ("tension") cortex, significantly greater mineral content and osteon population densities have been measured in the cranial ("compression") cortex of deer, sheep, elk, and horse calcanei. It has been hypothesized that these differences reflect increased remodeling activity in the caudal cortex, which occurs when strains fall below a minimum effective strain (MES) remodeling threshold. If this interpretation is correct, then remodeling in the cranial cortex should be relatively quiescent and the caudal cortex should have hallmarks of increased activity, including increased population densities of resorption spaces (RSPD) and newly forming secondary osteons (nOPD). Additionally, since high strains that produce microdamage would not be expected in this natural model, there should be no regional disparities in the prevalence of microdamage. To test these hypotheses 12mm thick sections were cut from the mid-to-proximal shafts of one calcaneus from each of 11 mature domestic sheep, 11 mature wild male mule deer, and 11 mature wild male North American elk. All sections were bulk stained in 1% basic fuchsin. Using on a low-speed diamond blade saw, three 100micron slices were cut from each bulk section (33 sections/species) and were examined in the light microscope for RSPD, nOPD, and in vivo microcracks (Mcrs) (present before staining). Results are reported as no. /cm² in the cranial (Cr), caudal (Cd), medial (M), and lateral (L) cortices:

	Sheep				Deer				Elk			
	Cr	Cd	M	L	Cr	Cd	M	L	Cr	Cd	M	L
RSPD	3.6	13.4	0	0.5	0.7	10.3	0.3	0.3	1.3	21.0	1.2	0.2
nOPD	2.4	9.1	0	1.5	0	14.6	0.3	0	1.3	14.1	0.2	0.4
Mcrs	0	0	0	0	0	2	0	0	0	0.6	0	0

These data support the hypothesis that remodeling is occurring more rapidly in the caudal cortex than in other regions ($p<0.001$). This may result from stress shielding by the plantar ligament, which reduces ambient strains. However, the increased Mcr prevalence in the caudal cortex of the deer is not consistent with expectations in a low strain environment. Since the sheep and elk calcanei do not exhibit similar regional Mcr disparities, the general increased remodeling in the caudal cortices of the these bones can not be attributed to this form of microdamage – consistent with predictions of the Mechanostat Theory.

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18. POTENTIAL EVIDENCE OF FATIGUE-RELATED MATERIAL ADAPTATION BETWEEN LIMB BONES OF A CURSORIAL MAMMAL. J.G. Skedros, T.R. Parry*, P. Durand*, and R.D. Bloebaum*. Univ. Southern California Dept. of Orthopaedics, Los Angeles and VA Medical Center, Salt Lake City, UT

Based on data showing an increased incidence of fracture in distal limb bones of race horses that had not fallen, J. Currey hypothesized that different long bones in the limb of a cursorial mammal will exhibit different material organizations in accordance with regional differences in fatigue-related functional requirements. Referring to experimental studies showing that primary bone is more fatigue resistant than secondary osteon bone, he suggested that the amount of secondary osteon (remodeled) bone would exhibit a progressive increase from the distal bones (near the hoof) to the axial skeleton. In turn, no differences in fatigue microdamage would be expected to occur between different limb bones. To test these hypotheses, 11 fresh skeletons of wild mature male mule deer (Odocoileus hemionus hemionus) were collected and 3 adjacent transverse sections were cut from mid-shafts of the left proximal phalanx of the medial digit, principal metacarpal, radius, and humerus, and middle third of the 6th rib. Sections include: 1) one 100micron slice from each bone of 3 animals (15 sections) for determining secondary osteon population density (OPD) (#/mm²), 2) 3mm for mineral content analysis (55 sections), and 3) 10 to 16mm for microcrack (Mcr) analysis (55 sections). Mineral content (ash weight/dry weight) of each bone is expressed as the mean of the transcortical pieces obtained from the cranial, caudal, medial, and lateral regions – which were defatted in chloroform and ashed at 550C for 24 hrs. The third sections were bulk stained in 1% basic fuchsin and 3 thin slices from each section (165 slices) were examined in the light microscope for the presence of in vivo Mcrs. Results [mean(s.d.)]:

	OPD (#/mm²)	# Mcr (all sections)	Ash (%)	p value (Ash data)
Rib	10.35(1.67)	13	69.81(0.32)	} p<0.00001
Humerus	4.24(0.88)	0	75.37(1.19)	
Radius	6.81(2.49)	1	73.15(1.19)	} p=0.07
Metacrpl.	1.08(0.65)	0	72.37(1.69)	} p<0.00001
Phalanx	4.38(0.92)	0	69.35(1.50)	

Although the hypothesized pattern of between-bone differences in OPD (highest in the rib to lowest in the phalanx) is not supported by these data, the absence of between-bone differences in microcrack prevalence supports the hypothesis that the limb bones are adapted for fatigue requirements. Presence of progressive, and relatively large (up to 8%), mineral content differences may, in addition to other unrecognized structural/material features, represent adaptations unique to each bone. Increased Mcrs in ribs may reflect their increased loading cycles and a lower biologic cost of microdamage.

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HYPERMINERALIZED PERIPHERAL LAMELLAE IN PRIMARY OSTEONS OF DEER ANTLER: POTENTIAL FUNCTIONAL ANALOGUES OF CEMENT LINES IN MAMMALIAN SECONDARY BONE. J.G. Skedros, P. Durand*, and R.D. Bloebaum* VA Medical Center, Salt Lake City, UT and University of Southern California Dept. of Orthopaedics, Los Angeles, CA

Traditionally considered to be highly mineralized lamellae at the periphery of secondary osteons (Haversian systems), cement lines (arrest lines) are ascribed substantial functional importance in fracture mechanics, energy absorption, viscous damping and elastic function, and fatigue processes in compact bone. Cement lines enhance fatigue life and fracture toughness by attenuating the propagation of microcracks. Since antlers of wild deer sustain considerable impact and torsional loads during the mating season, their fracture toughness greatly exceeds that of human limb bone. This property has been ascribed to the relatively lower mineral content of the antler compacta. However, the attenuation of microcrack propagation at the microscopic level would seem paramount in this tissue. We hypothesize that deer antler will exhibit interfaces that are functionally analogous to cement lines even though antlers do not undergo secondary osteon remodeling. Eleven antlers of similar size, each with two tines, were obtained from 11 male Rocky Mountain mule deer. The periosteal covering ("velvet") had been shed, indicating that the antlers were mature. One 5cm transverse section was cut from one tine approximately 4cm distal to the bifurcation. The sections were embedded in one block of polymethyl methacrylate, ground, polished, and prepared for imaging in the backscattered electron (BSE) mode of a SEM (JEOL 6100) using 0.75nA probe current and a 30keV beam. Image contrast and brightness were computer controlled and image graylevels (atomic number contrasts) were calibrated using 98% pure magnesium and magnesium oxide wires according to our published protocol. One calibrated, digitized BSE image (1500X) was obtained of the peripheral lamella and adjacent bone of each of three primary osteons in each specimen (3 osteons/bone). Relative differences in the mineralization of peripheral lamellae and adjacent interstitial and intraosteon bone were quantified, and are expressed as corresponding differences in the mean graylevels. Results show that the peripheral lamellae of primary osteons are invariably more highly mineralized than the immediately adjacent osteon ($p<0.0001$) and interstitial ($p<0.0001$)bone: lamellae 154.8(17.7); interstitial bone 129.1(20.4); intraosteon bone 122.9(21.1). Mineralization of interstitial and osteon bone are not significantly different ($p>0.05$). Hypermineralized lamellae were also observed to be extensively distributed within the non-osteon primary bone. Similar to cement lines, the hypermineralized lamellae may have an important micromechanical role as an interface and region of modulus mismatch.