SU073

41. **Ontogeny of Cancellous Bone Anisotropy in a Natural "Trajectorial" Structure: Genetics or Epigenetics?** J. G. Skedros,[1] J. H. Brady.*[2] [1]Orthopaedics, Univ. Utah, Salt Lake City, UT, USA, [2]Univ. Utah, Salt Lake City, USA.

Since the late 1800s, preferred orientations of cancellous bone trabeculae have been interpreted as adaptations for principal tension and compression stress trajectories produced by habitual bending. During Wolff's formulation of the trajectorial theory of cancellous bone architecture (Wolff's "law" in a strict sense), he often used the human proximal femur as an example of a trajectorial structure. Many other bones exhibiting arched trabeculae have been used in this context (e.g., calcaneus, proximal tibia, metatarsals). Such patterns are commonly used to infer local loading history in extant and extinct animals. However, recent authors who have reviewed the historical use of the trajectorial hypothesis suggest that principal tension and compression strains may not be important in the formation of these distinctive trabecular anisotropies. To examine this possibility, we studied lateral radiographs of an ontogenetic series of sheep and deer calcanei (in vivo "tension/compression" bones; n=15 each), ranging from fetus to adult, for the presence of arched trabeculae (as seen clearly in mature bones). Their presence or absence was also considered in the context of predominant collagen fiber orientation (CFO) in mid-diaphyseal transverse sections (100micron) of the cranial ("compression" = C) and caudal ("tension" = T) cortices of the deer bones. We hypothesized that: 1) fetal bones lack obvious arched trabecular patterns, and 2) the temporal appearance of this anisotropy correlates with strain-mode-specific (T/C) CFO differences. All fetal bones showed the presence of obvious arched trabeculae. However, fetuses and fawns lacked cranial-caudal CFO differences. Since predominant CFO is sensitive in detecting a T/C distribution, it seems unlikely that the trabecular core (relatively lower strains) exhibits strain-mode-specific adaptation when the contiguous/adjacent cortices (higher strains) do not. The arched trabecular tracts may be strongly influenced by the orientation of the epiphyseal growth plate -- the caudal tract and growth plate have the same orientation. The cranial tract's orientation may then represent the programmed deposition of bony trabeculae that tend to maintain a consistent orientation with respect to the plate, and hence form quasi-orthogonal "intersections" with the caudal tract. Arched trabeculae in proximal femora, which strongly resemble those in the calcanei, may follow the same construction rules. This interpretation suggests that a genetically driven developmental program may be relatively more important than epigenetic mechanical stimuli in the initial construction of trabecular patterns in mammalian limb bone epiphyses.

SU074

Role of Bone Formation in Fracture Healing. T. Beil, J. M. Rueger, M. Amling. Trauma Surgery, Hamburg University, Hamburg, Germany.

Fractures are the major clinical problem associated with almost any bone disease. Although our means of therapeutic intervention have been significantly improved through the last decades, the biology of fracture healing remains poorly understood. Given the recently discovered hypothalamic control of bone formation, and thereby its accessibility to external modulation, we reasoned that bone formation would be a possible target for therapeutic intervention to enhance fracture healing in general and improve skeletal repair in cases of delayed- or non-union. Therefore, the aim of this study was to elucidate the specific role of bone formation on fracture repair. Here we compared the process of fracture healing in three mouse models with genetically determined differences in bone formation rate. More specifically, we took advantage of the fact that the ob/ob and the db/db mice, that are both deficient in leptin signaling, have a genetically determined two to three fold increase in bone formation rate in comparison to wildtype mice. To make genetic mouse models accessible to such studies, we modified the tibial fracture model, first described by Einhorn, and developed a standardized closed fracture model of the femur. This model has the advantage of improved diaphyseal fracture reproduceability (no fibula) and better accessibility to subsequent biomechanical testing. Already radiological analysis and callus morphology demonstrated significant differences in fracture repair dependant on bone formation rate. Furthermore, we will study the qualitative and quantitative micromorphology using histology and histomorphometry. The results of these experiments will be presented at the meeting. To our knowledge this is the first report of a significant influence of bone formation on fracture healing. Therefore these data suggest a possible new approach to enhance fracture repair by targeted modulation of bone formation.

SU075

Osteocyte-Bone Lining Cells System Responds to Cyclic Loading in a Dose-Dependent Manner. A. Rubinacci,*[1] M. Covini,*[2] C. Bisogni,*[2] I. Villa,*[1] M. Galli,*[2] C. Palumbo,*[3] M. Ferretti,*[3] A. Ardizzoni,*[3] G. Marotti.*[3] [1]Bone Metabolic Unit, Scientific Institute H San Raffaele, Milano, Italy, [2]Dept of Bioengineering, Politecnico, Milano, Italy, [3]Dept of Morphological Sciences, University of Modena, Modena, Italy.

The mutual interaction between osteocyte-bone lining cells system (OBLCS) and the surrounding bone extracellular fluid (BECF) which fills the continuous network of lacuno-canalicular microcavities plays a role in mechanotransduction. Since BECF could be modified by the application of a load via streaming potential and stretch activated cation channels mechanisms and since OBLCS is able to respond to axial loading by increasing ion exchanges at the BECF/ECF (systemic extracellular fluid) interface, it is likely that the response of OBLCS to mechanical strain could vary depending on the characteristics (amplitude and frequency) of the load applied.To verify this hypothesis, metatarsal bones of weanling mice were subjected ex vivo, immersed in ECF medium, to axial cyclic loading for two minutes by respectively varying the loading parameters, amplitude and frequency. The electric (ionic) currents at the bone-medium interface were monitored by a voltage-sensitive two-dimensional vibrating probe system before and after loading. By varying the load from 0.7 g to 12 g without changing neither the loading frequency (1Hz) nor the time (2'), the increment in current density was dependent upon the applied loads reaching a plateau at 8 g. Post load current density decreased following different time dependent exponential decays to different asyntotic values depending upon the applied load. By varying the loading frequency from static load to 2 Hz with constant load (5 g) and time, the increment in current density was dependent on the applied frequencies reaching a plateau at 1.5 Hz. Post load current density decreased following different time dependent exponential decays to different asyntotic values depending upon the applied frequency. Static load did not induce any change in the current density. The post load increment in current density was associated to the total energy transferred to bone during the entire loading cycle, as defined by the loading parameters. Post load current density in the dead bones was significantly lower than in the living ones, linearly decayed to background level and did not show any relationship with the applied load. This study showed that OBLCS responds to cyclic loading depending on the applied loading parameters in a dose dependent manner. The higher is the load related perturbation of BECF the faster is the restoration of the preload conditions by OBLCS. Static load did not elicit any detectable OBLCS response.

SU076

BMD Changes up to 3 Years Following Treatment with Zoladex or CMF in Pre-/Perimenopausal Women with Early Breast Cancer Participating in the ZEBRA Study. I. Fogelman, G. M. Blake. Guy's, King's and St Thomas' Hospital Medical School, London, United Kingdom.

The large (n=1640), multicentre, randomized ZEBRA (Zoladex Early Breast Cancer Research Association) study has previously reported that Zoladex (3.6 mg every 28 days for 2 years) is as effective as cyclophosphamide/ methotrexate/5-fluorouracil (CMF; 6 x 28-day cycles) in pre-/perimenopausal patients with estrogen receptor positive early breast cancer. In a protocolled sub-study, bone mineral density (BMD) of the lumbar spine (L2–L4) and neck of femur were assessed by dual-energy X-ray absorptiometry at baseline then annually for up to 5 years. Patients with a baseline and at least one post-baseline measurement at the same site in a protocolled time window were included in the analysis. In total, 96 selected patients from eight centers (Zoladex, n=53; CMF, n=43) were included in the analysis of data to 3 years follow-up. Demographic characteristics and baseline BMD data were well balanced. Mean percentage BMD losses for Zoladex and CMF were 8.2 vs. 4.5 (p=0.00008) at 1 year and 10.5 vs. 6.5 (p=0.0005) at 2 years for lumbar spine, and 4.5 vs. 4.4 (p=0.70) at 1 year and 6.4 vs. 4.5 (p=0.04) at 2 years for neck of femur. After 3 years (i.e. 1 year after cessation of Zoladex) partial recovery of BMD was observed in the Zoladex group, whereas losses persisted in the CMF group overall (lumbar spine: 6.2 with Zoladex [n=29] vs. 7.2 with CMF [n=26], p=0.26; neck of femur: 3.1 with Zoladex [n=30] vs. 4.6 with CMF [n=26], p=0.48). As a result, no significant differences in BMD were observed between the two groups at 3 years. All Zoladex patients in the BMD sub-study became amenorrhoeic while receiving treatment compared with 63% of the CMF group at 48 weeks and 69% at 2 years. Menses returned in the majority of Zoladex patients after cessation of therapy, whereas amenorrhoea was permanent in most CMF patients. In the CMF group, based on amenorrhoea status at 48 weeks, mean percentage BMD losses at the lumbar spine were greater for amenorrhoeic than non-amenorrhoeic patients (2 years: 9.7 [n=18] vs. 2.0 [n=10]). In summary, ovarian suppression resulting in amenorrhoea was closely related to BMD loss in both groups, with the partial recovery of BMD in the Zoladex group associated with return of ovarian function in the majority of patients. Longer term follow-up, including analysis of data to 5 years follow-up, is planned to determine the degree of potential recovery of BMD with Zoladex and whether there is continuing progressive bone loss with CMF.

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SU077

Carboxyl Terminal Trimer of Collagen type I (C3) Induces Directional Migration and Metalloproteinase-2 Activation in Tumor Cells. D. Palmieri,* S. Poggi,* V. Ulivi,* P. Manduca. DOBIG, University of Genova, Genova, Italy.

We have previously shown that the agent secreted in the conditioned medium (CM) from mature osteoblasts and inducing specifically directional migration in endothelial cells, is the carboxyl trimer of procollagen type I (C3, Palmieri et al. 2000 J.Biol.Chem. 275, 32658). Endothelial cells directional migration is dependent on G0/i proteins activity, integrin b1 and b3 and MMP-2 and –9 functionality. Also tumor cells (breast and prostatic carcinoma and melanoma) are chemoattracted by osteoblast CM (Giunciuglio et al. 1995, Cancer letters 97 , 69 and Festuccia et al. 1999, Oncol.Res.11,17). We here show that purified C3 is responsible for the chemoactivity of CM on tumor cells. Chemotaxis is induced with purified C3 and is inhibited by antibodies against C3 chains a1 and a2, by PTX and by antibodies against MMP-2, –9 and uPA.The directional migration induced by C3 in Boyden chambers is concomitant to the induction of MMP-2 and to the activation of MMP-2 in two lines of breast carcinoma cells (BCC) and one of human melanoma. Secreted, free uPA and tPA are constitutively produced by BCC and are not quantitatively changed in migrating cells. The induction of MMP-2 and –14 and the activation of MMP-2 occurs increasingly in time upon exposure of not migratory (adhering to the culture dish) BCC to C3. In these conditions no significant changes occur in the free uPA and tPA levels. C3 is not a mitogen for BCC.C3 is identified by these experiments as the agent produced by mature osteoblasts capable to induce directional migration of both endothelial and carcinoma cells. It is the only agent presently known that induces migration targeting specifically both cell types. C3 production by Collagen type I producing stroma might therefore play a role in the promotion of tumor progression, by affecting tumor cells pericellular proteolysis and their spatial convergence with endothelial cells.