bone strength at all investigated sites. It also showed that zinc acted on the bone strength in a dose-dependent manner except for the distal metaphyseal, where there was no significant difference between the group fed with the normal zinc diet and the group fed with a hypo-zinc diet. However, at all three skeletal sites there was a significant difference between the group fed with hypo-zinc compared with the other two groups.

We conclude that dietary zinc supplementation in growing rats induced an increase of bone strength in both the femoral neck and the femoral diaphysis. Zinc also improved the rates of growth in the rats. The weight, length, and diameter of the femora were all higher in the rats given zinc supplementation. These results further support the view that zinc has a potent anabolic effect on bone metabolism.

P42 S
OSTEON SIZE IS RELATED TO MECHANICAL STRAIN: A HUMAN RETRIEVAL STUDY
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Introduction - Recently, evidence was found that remodeling is regulated by deformation of the bone matrix [1]. Osteocytes thereby play the role of mechanosensors, which control osteoclast and osteoblast activity at the surface. This theory [1] also predicts, that the size of osteons depends on the amount of matrix deformation: under large strains, osteocytes around the cutting cone should inhibit osteoclasts earlier, leading to a narrower resorption tunnel. This hypothesis was tested with data of osteons, where strongly different loading conditions occur in the bone tissue between the threads.

Materials and Methods - In order to determine the deformation of the bone tissue around the screw, a Finite Element analysis was performed, which showed that the largest volumetric strains occur on the compression side above the thread, and only small strains appear in the bone below the threads.

Five HA-coated titanium dental screws were retrieved from a 50 years old patient. The implants had functioned well for 2.5 years, but had to be removed for psychological problems. Histological sections were made in the longitudinal plane, and the section from the mid-portion of each retrieval was analysed. Bone was analysed in two areas within each screw thread, according to the calculated strain pattern. For each area, the bone density, the number of osteons and the size of the osteons were determined.

Results - See Table 1.

Discussion - It was hypothesised that the size of osteons is determined by the amount of deformation. It was found, that the bone density around the implants was associated with the volumetric straining of the bone tissue. Moreover, the osteons in the more strongly deformed areas were much smaller than in less deformed areas. These findings support the hypothesis, that the size of an osteon is determined (at least) by mechanical loading.


Parameter
B/VYT (%) 92.6 (±4.7) 87.0 (±4.4) 0.088
# osteons/screw 6.0 (±2.0) 4.0 (±1.0) n.s.
osteon diameter (micron) 84.6 (±11.2) 129.9 (±20.4) 0.002

Table 1: Summary of the histological parameters. Values are means from 5 screws. Standard deviations are between brackets.

P43 W
BMU-COUPING IS REGULATED BY LOCAL PATTERNS OF FLUID FLOW
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Simulation - It is known that BMU-coupling is regulated by deformation of the bone matrix [1]. Evidence also accumulates, that mechanical loading by osteocytes is related to extra- and intracellular fluid flow. The purpose of this study is to relate the theory of extracellular fluid flow to BMU coupling. To that end, we determined the pattern of fluid flow around a tunnelling osteon under axial loading.

Methods - The problem is approached with Biot’s theory of poroelectromics, and calculated with the finite element method. The tunnelling osteon was modelled axisymmetrically as a cylindrical gap with a spherical end. The bone matrix was described as an isotropic medium with fully saturated lacuno-canalicular porosity. The load applied to the model was that of a person walking at 4 km/h. The maximum deformation of the bone matrix was 1500 microstrains.

Results - On loading, a typical flow pattern appears around the cutting cone: along the tube flow flows into the bone matrix, but at the tip fluid flows into the bone matrix. This is due to a local area of volumetric expansion in front of the cutting cone. Inside the bone matrix, an outflow pattern exists along the cylindrical wall, which dips out at a depth of some 0.1 mm. In front of the cutting cone, however, the inflow at the surface changes to an outflow at a depth of some 10 micron. So, just below the surface of the cutting cone, the fluid flow is close to zero. At unloading, the fluid flow pattern is more or less reversed.

Discussion - The pattern of bone fluid flow around a tunnelling osteon was determined for a walking cycle, using Biot’s theory of poroelectromics and a finite element model. The main finding was that the fluid flow pattern is different near the cutting cone as compared to the closing cone, which suggests that the osteocytes within the bone matrix sense different patterns of fluid flow near sites of osteoclastic and osteoblastic activity. This is compatible with the hypothesis that local patterns of bone fluid flow regulate BMU-coupling.


P44 T
DENSIFICATION BY INFILLING MARROW SPACE IN RESPONSE TO EXERCISE IN THOROUGHBRED HORSE DISTAL CANON BONE
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We examined changes induced by training in equine third metacarpal bones (MeIII). Distal parts of MeIIIs were obtained from controlled training experiments in which 2 year old Thoroughbreds were subjected to strongly contrasting exercise routines. MeIIIs were sliced longitudinally in parasagittal and central and dorsal- and palmar-oblique medio-lateral planes, embedded in PMMA, the blocks micromilled and carbon coated and the bone mineralisation density studied at the cubic micron scale using quantitative digital backscattered electrons imaging. Entire bone slices were analysed using automated skeletal diagnostics. For each sample, bone volume fraction (BVF) was calculated with mean and median BSE grey levels: volume fractions of 16 density phases were estimated. The fraction of the organ volume occupied by any form of bone tissue is the distal extremities was increased in the exercised group. Most of this extra bone was deposited within the former marrow space in the central regions of the condyles. The bone was of mixed woven and lamellar nature and, where relatively thick, formed in relation to numerous fine blood vessels which incorporated to form canals. It was deposited upon prior laminar bone surfaces without the intervention of prior resorption and without the formation of a hypermineralised cement line. The immediate subchondral bone zone, the open canal or marrow space was much less in the exercised groups, whilst extrinsic spaces, representing resorption episodes, were more easily seen in the control group. The more loaded immediately subchondral zones, e.g. the palmar regions in the condyles, had the highest BVF and a lower mean level of mineralisation, with well-defined mid-diaphyseal transverse segments from mature bones (n=7 each) were embedded in methacrylate; sheep, deer, and horse calcanes, sheep and horse radii, horse third metacarpals (MCIIIs), and sheep ilial. The horse MCIIIs and sheep ilia were subjected to 'controlled' exercise since they experienced comparatively low loads. In the experimental bones, tension and compression strains of unequal magnitudes prevail on opposite (crural/caudal) cortices; this disparity is diminished along the neutral axis (medial/lateral cortices). Ultrathin (10x5 micron) specimens were viewed under circularly polarized light. Variations in predominant collagen fiber orientation (CFO) were expressed as relative differences in the amount of transmitted light in the crural, caudal, medial, and lateral cortices. Additional quantitative analyses included fractional area of secondary bone, secondary osteon population density, mean area and secondary osteons, cortical thickness, cross-sectional second moment of inertia along the major and minor axes, and polar moments of inertia. Regional mineral content (ash%) was determined in adjacent segments. Results showed that only CFO exhibited a consistent relationship with loading history - in experimental bones, compression cortices had a significantly more oblique-to-transverse collagen than tension cortices (p=0.017; range of differences: 15% (sheep radii) to 69% (horse radii)). As anticipated, there were no significant CFO differences between the cranial-caudal and medial-lateral cortices of sheep tibio and the cranial-caudal cortices of horse MCIII. The consistent regional strain-mode-related material heterogeneity may reflect adaptations for specific biomechanically important

45. COLLAGEN FIBER ORIENTATION: A CHARACTERISTIC OF STRAIN-MODE-RELATED REGIONAL ADAPTATION IN BONE
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Skeletal morphologists often examine the structural and material organization of skeletal tissues as a means to interpret loading history. However, it is unclear if even a simple loading history can be reliably inferred from specific structural and material characteristics. Various limb bones were examined to determine if such characteristics exhibit consistencies adaptations to customary strain distributions. Each 'experimental' bone was subject to an in vivo history of bending in a consistent direction. Notable adaptations were expected because mechanical properties of cortical bone markedly differ in tension, compression, and shear strain modes. Unicortical mid-diaphyseal transverse segments from mature bones (n=7 each) were embedded in methacrylate; sheep, deer, and horse calcanes, sheep and horse radii, horse third metacarpals (MCIIIs), and sheep ilial. The horse MCIIIs and sheep ileals were subjected to 'controlled' exercise since they experienced comparatively low loads. In the experimental bones, tension and compression strains of unequal magnitudes prevail on opposite (crural/caudal) cortices; this disparity is diminished along the neutral axis (medial/lateral cortices). Ultrathin (10x5 micron) specimens were viewed under circularly polarized light. Variations in predominant collagen fiber orientation (CFO) were expressed as relative differences in the amount of transmitted light in the crural, caudal, medial, and lateral cortices. Additional quantitative analyses included fractional area of secondary bone, secondary osteon population density, mean area and secondary osteons, cortical thickness, cross-sectional second moment of inertia along the major and minor axes, and polar moments of inertia. Regional mineral content (ash%) was determined in adjacent segments. Results showed that only CFO exhibited a consistent relationship with loading history - in experimental bones, compression cortices had a significantly more oblique-to-transverse collagen than tension cortices (p=0.017; range of differences: 15% (sheep radii) to 69% (horse radii)). As anticipated, there were no significant CFO differences between the cranial-caudal and medial-lateral cortices of sheep tibio and the cranial-caudal cortices of horse MCIII. The consistent regional strain-mode-related material heterogeneity may reflect adaptations for specific biomechanically important

S110
features of local strain history. In contrast to adaptations that affect global (i.e., whole bone) stiffness/strength requirements, these adaptations may enhance fatigue resistance and fracture toughness for local loading conditions.

P46 W

ZINC STAINING OF MATRIX METALLOPROTEINASES AND ENDONUCLEASES IN GROWTH CARTILAGE
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A new method for zinc histochemistry was applied to stain zinc atoms from matrix metalloproteinases and endonucleases to localize their distribution in epiphyseal plate rat cartilage. Though these zinc ions are firmly bound and essentially they are not available, drastic ammonium sulfide exposure rendered them reactive for staining. Matrix metalloproteinases were detected in chondrocytes and in extracellular matrix along the longitudinal septa before matrix calcification. A second localization was found at the resorptive limit of calcified matrix adjacent to the zone of vascular invasion. Zinc of endonucleases involved in apoptosis was stained within the nuclei in the last rows of hypertrophic chondrocytes precisely where chondrin was condensed.

P47 T

BONE MORPHOGENETIC PROTEIN 2 STIMULATES INORGANIC PHOSPHATE TRANSPORT AND MINERALIZATION IN OSTEOBLAST-LIKE CELLS
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Bone morphogenetic proteins (BMPs) play an important role in the development of bone and cartilage. BMP-2 is produced by osteogenic cells including osteoblasts and stimulates the differentiation of preosteoblasts and the activity of osteogenic cells. Inorganic phosphate (Pi) is an important element for the calcification of the bone matrix. Recent studies in cultured MC3T3-E1 cells suggest a specific role of the Pi transport system PiT-1 in initial events of matrix mineralization. The aim of the present study was to analyze whether BMP-2 regulates the expression and activity of PiT-1 and investigate the possible role of this transporter in the BMP-2-induced matrix mineralization. BMP-2-time- and dose-dependently stimulated Na-dependent Pi transport at day-6 is MC3T3-E1 cells. An effect of BMP-2 on Pi transport was detected after 3 hours. It was maximal after 6 hours and remained expressed at 24 hours. A maximal response was obtained with 36 ng/ml of BMP-2 (22 fold). Kinetic analysis was indicated that BMP-2 increased the maximal rate (Vm) and Vmax of the transport system but did not affect the apparent affinity for Pi. Pretreatment of the cells with either actinomycin D (2.5 microg/ml) or cycloheximide (5 microM) completely abolished the stimulation of Pi transport induced by BMP-2. Northern blotting analysis showed an increased expression of mRNA encoding PiT-1 after 2 hours BMP-2 exposure. In parallel with the stimulation of Pi transport, BMP-2 enhanced both ALP activity and the formation of mineralized bone nodules in differentiating cells.

In conclusion, the results of this study indicate that BMP-2 stimulates the expression and activity of the transporter PiT-1 in osteoblast-like cells via a RNA and protein synthesis dependent process. This effect is associated with enhanced expression of bone matrix mineralization suggesting a possible role of this Pi transport system in bone matrix calcification.

P48 S

BMP RESPONSIVENESS IN HUMAN MARROW STROMAL CELLS
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INTRODUCTION: Cultured bone marrow stromal cells from various species have been shown to possess an inducible osteogenic phenotype. Interestingly, the potency of individual inducers is species-dependent. BMP has a relative potent osteogenic effect on rat and mouse stromal cells yet it usually a poor inducer of osteogenesis in cultured human stromal cells. We have been examining why the BMP effect is poor and variable in human cells.

METHODS: Human stromal cells (HMC) were isolated from marrow aspirated from femora during total hip arthroplasty. The marrow was washed to remove fat and the mononuclear cells concentrated on Ficoll-Paque (Amersham-Pharcmaics Biotech). Primary cultures were established at 2 x 103 cells/cm2. Media in primary cultures were initially changed on day 3 and, generally, every second day thereafter. Half of the primary cultures from individual samples were treated with dexamethasone (dex; 10-3 M), a potent inducer of osteogenesis in HMC. Just prior to confluence, first passage cultures were established at 105 cells/cm2. All cultures were treated with ascorbic acid phosphate at 100 microg/ml. Selected cultures were treated with BMP-2 at 1.00 ng/ml. Cultures were harvested at day 6 for alkaline phosphatase (AP) assay and total RNA isolation.

RESULTS: Baseline AP activity in first passage cultures derived from excised primaries was higher than activity in cultures derived from non-dex primaries. In most first passage cultures derived from non-dex primaries, BMP-2 had no significant effect on AP activity; some isolates showed modest inhibition. In first passage cultures derived from dex-treated primaries, I significantly increased AP activity (p<0.01). However, the magnitude of this varied widely. Interestingly, BMP-2-induced noggin mRNA in first passage c of primary culture condition and AP level in first passage. We co that human marrow stromal cells possess a latent osteogenic response which can be induced by dext treatment.

P49 W

DISTRIBUTIONS OF MRNAS FOR BMP-2 AND BMP RECEPTORS IN OSTEORHITHIC CARTILAGE
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[Purpose] Osteocytes are neoplastic tissues made up of ossae cartilaginous components with fibrous mesenchymal layers. The tissues originate from the margin of osteorhithic joints, especially at the chondral junctions. The aim of this study is examine the involvement of signaling in the process of cellular differentiation during osteocyte formation in the human.

[Materials and Methods] In situ hybridization (ISH) utilizing digoxigenin labeled cRNA probes for human bone morphogenetic protein (BMP) and receptor I, and receptor II, and immunohistochemistry (IH) with monoclonal antibody to a human BMP-2/4. To determine the phenotypes of cells, ISH using cRNA probes for collagen types I, II, and III (Col I, II, III) were also performed. Osteoblasts obtained at the surgery, with consent, from 8 specimens from 6 individuals used in this study.

[Results] BMP-2 mRNA and protein were distributed in mesenchymal positive for Col I and III, negative for Col II overlying or adjacently to the osteocytes, and in chondrocytes (positive for Col II) located in non-osteohcartilage. BMP-2 was also localized in chondrocytes in fibrocartilage (positive for Col II and III) and in mesenchymal cells undergoing intracellular ossification forming osteocyte. In mesenchymal cells although BMP-2 is synthesized by in mesenchymal cells surrounding the osteocyte, mRNA BMP/RIDB were predominantly localized in chondrocytes themselves. Neither BMP-2 mRNA and protein was detected in cells in non-osseous cartilage. results is summarized in a Table.

[Discussion] These results suggest that mesenchymal cells contribute to and promote formation of osteocyte via synthesis of BMP-2. Co-localization of BMP-2 and BMP receptor type IIB cells in mesenchymal layers in mesenchymal cells located in the periphery of osteocyte suggest that BMP-2 may play an osteocyte formation in osteorhithic cartilage.

P50 T

ADVERSEXUS MEDICATED BMP-2 GENE THERAPY ENHANCE BONE FORMATION IN A MURINE METAPHYSAL BONE DEFECT
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We have investigated the capacity of bone morphogenetic protein (BMP) enhance bone healing in a metaphysal bone defect model in the mouse. Po purpose a recombinant adenovirus (RAd/BMP-2) expressing the complete sequence of the human BMP-2 under the control of cytosine virus IE pro was constructed. RAd/BMP-2 viruses were injected into the defect site in the metaphysis of the femur immediately after surgery. Control defects were infected with recombinant adenovirus harboring the lacZ gene. The healing process was followed at 7, 14, 21 and 42 days using histology, peripheral quantitative computed tomography (pQCT), biomechanical testing and microstructural analysis. Histologically, a characteristic effect of BMP-2 was enhanced osteogenesis in the medullary cavity and periscaphoid chondrogenesis adjacent to the 0 particularly during the first week of healing. At all times pQCT analysis reveal increased bone mineral content (BMC) in the defect area injected with RAd/BMP when compared with the controls. Similarly, an increasing trend was seen healing of the bone defects at two weeks after RAd/BMP-2 injection. Analysis of the chondrogenic and osteogenic activity in the defect area by Nor analyses reveal that the mRNA levels for collagen and bone components defects identified with RAd/BMP-2 remained essentially unchanged, indicating an increased in chondrogenesis and osteogenesis. The production of BMP-2 in defect area demonstrated by a reverse transcription-polymer chain reaction (RT-PCR) assay. The highest levels of expression of BMP-2 seen control 0 week of healing. In summary, the data demonstrate injection of a BMP-2 to enhance both chondrogenesis and osteogenesis. As the defect model was developed in the mouse, it may not be applicable to the biological activity of bone inducing factors both in normal mice a transgenic mice harboring various type of gene modifications using adenovirus mediated gene transfer.