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 metaphysis, where there was no significant difference between the group fed with the normal-zinc diet and the group fed with a hyper-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 alimentary 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 remodelling 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 retrievals of dental screws, 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 (a.o.) by mechanical loading.

References - [1] JBMR 2000: 301-307

Parameter	A	B	p
BV/TV (%)	92.6 (±4.7)	87.0 (±5.4)	0.008
# osteons/screw	6.0 (±2.0)	4.0 (±1.0)	n.s.
osteon diameter (microm)	84.6 (±11.2)	152.9 (±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-COUPLING IS REGULATED BY LOCAL PATTERNS OF FLUID FLOW

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Introduction - There is evidence that BMU-coupling is regulated by deformation of the bone matrix [1]. Evidence also accumulates, that mechanosensing by osteocytes is related to extra-cellular 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 poroelasticity, 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 material 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 microstrain.

Results - On loading, a typical flow pattern appears around the cutting cone: along the the closing cone, fluid is pressed out of 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 damps 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 microm. 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 poroelasticity 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.

References - [1] JBMR 2000: 301-307

P44 T

DENSIFICATION BY INFILLING MARROW SPACE IN RESPONSE TO EXERCISE IN THOROUGHBRED HORSE DISTAL CANNON BONE

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We examined changes induced by training in equine third metacarpal bones (McIII). Distal parts of McIIIs were obtained from controlled training experiments in which 2 year old Thoroughbreds were subjected to strongly contrasting exercise routines. McIIIs were sliced longitudinally in para-sagittal or 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 electron imaging. Entire bone slices were analysed using automated scanning of sub-fields. For each sub-field, anatomical region, and whole slice, 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 in 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 extremities. The bone was of mixed woven and lamellar nature and, where relatively thick, formed in relation to numerous fine blood vessels which it incorporated to form canals. It was deposited upon prior lamellar bone surfaces without the intervention of prior resorption and without the formation of a hypermineralised cement line. In the immediate subchondral bone zone, the open canal or marrow space was much less in the exercised groups, whilst extensive 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 the histology showing repetitive turnover in small domains. The greater amount of bone formed in response to training exercise was of net lower density, reflecting increased material compliance.

P45 S

45. COLLAGEN FIBER ORIENTATION: A CHARACTERISTIC OF STRAIN-MODE-RELATED REGIONAL ADAPTATION IN CORTICAL 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 consistent 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. Undecalcified mid-diaphyseal transverse segments from mature bones (n=7 each) were embedded in methacrylate: sheep, deer, and horse calcanei, sheep and horse radii, horse third metacarpals (MCIIIs), and sheep tibiae. The horse MCIIIs and sheep tibiae served as 'controls' since they experience comparatively complex loading. In the experimental bones, tension and compression strains of unequal magnitudes prevail on opposite (cranial/caudal) cortices; this disparity is diminished along the neutral axis (medial/lateral cortices). Ultramilled (100±5 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 cranial, caudal, medial, and lateral cortices. Additional quantitative analyses included fractional area of secondary bone, secondary osteon population density, mean area of secondary osteons, cortical thickness, cross-sectional second moments 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 tibiae and the cranial-caudal cortices of horse MCIIIs. The consistent regional strain-mode-related material heterogeneity may reflect adaptations for specific biomechanically important

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 chromatin 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 analyse 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 in 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 least 24 hours. A maximal response was obtained with 30 ng/ml of BMP-2 (2.2 fold). Kinetic analysis indicated that BMP-2 increased the maximal rate (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 Pi 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 is 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-Pharmacia Biotech). Primary cultures were established at 5×10^5 cells/cm^2. 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^{-7}M), a potent inducer of osteogenesis in HMC. Just prior to confluence, first passage cultures were established at 10^4 cells/cm^2. All cultures were treated with ascorbate phosphate at 100 microg/ ml. Selected cultures were treated with BMP-2 at 100 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 dex-treated 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 inhibi BMP. 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 irrespective of primary culture condition and AP level in first passage. We co that human marrow stromal cells possess a latent osteogenic response to which can be induced by dex treatment.

P49 W

DISTRIBUTIONS OF MRNAS FOR BMP-2 AND BMP RECEPT IN OSTEOARTHRITIC CARTILAGE

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[Purpose] Osteophytes are neoplastic tissues made up of osseou cartilaginous components with fibrous mesenchymal layers. The tissues originate from the margin of osteoarthritic joints, especially at the sy chondral junctions. The aim of this study is to examine the involvement of E signaling in the process of cellular differentiation during osteophyte format the human.

[Materials and Methods] In situ hybridization (ISH) utilizing digogi labeled cRNA probes for human bone morphogenetic protein (BMP)-2 and receptor IB, and immunohistochemistry (IH) with a monoclonal antibody a human BMP-2/4. To determine the phenotypes of cells, ISH using cRNA prob human collagens types I, II and III (Col I, II, III) were also performed. Osteo 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 adjacent to ap osteophytes, and in chondrocytes (positive for Col II) located in neo-plastic h cartilage. BMP-2 was also localized in chondrocytes in fibrocartilaginous (p for Col II and Col III) and in mesenchymal cells undergoing intramembr ossification forming osteophyte. In mesenchymal cells although BMP-2 synthesized by in mesenchymal cells surrounding the osteophyte, mRN BMPRIB were predominantly localized in chondrocytes themselves. Neither 2 mRNA and protein was detected in cells in non-osteophytic cartilage. results is summarized in a Table.

[Discussion] These results suggest that mesenchaymal cells contribute to i and promote formation of osteophyte via synthesis of BMP-2. Co-localizati BMP-2 and BMP receptor type IB in cells in mesenchymal layers in mesenc cells located in the periphery of osteophyte suggest that BMP-2 may play rc osteophyte fromation in osteoarthritic cartilage.

P50 T

ADENOVIRUS MEDIATED BMP-2 GENE THERAPY ENHANC BONE FORMATION IN A MURINE METAPHYSEAL BONE DEFECT

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We have investigated the capacity of bone morphogenetic protein (BMP enhance bone healing in a metaphyseal bone defect model in the mouse. Fo purpose a recombinant adenovirus (RAdBMP-2) harboring the complete c sequence of the human BMP-2 under the control of cytomegalovirus IE pro was constructed. RAdBMP-2 viruses were injected into the defect site in the metaphysis of the femur immediately after surgery. Control defects were inj with recombinant adenoviruses harboring the LacZ gene. The healing proces followed at 7, 14, 21 and 42 days using histology, peripheral quantitative com tomography (pQCT), biomechanical testing and molecular biologic anal Histologically, a characteristic effect of BMP-2 was enhanced osteogenesis v the medullary cavity and periosteal chondrogenesis adjacent to the d particularly during the first week of healing. At two weeks, pQCT analysis rev increased bone mineral content (BMC) in the defect area injected with RAdBI when compared with the controls. Similarly, an increasing trend was seen i bending stiffness of the healing femur at two weeks after RAdBMP-2 inje Analysis of the chondrogenic and osteogenic activity in the defect area by Nor analyses revealed that the mRNA levels for cartilage and bone componen defects injected with RAdBMP-2 remained essentially unchanged, indicati balanced increase in chondrogenesis and osteogenesis. The production of h BMP-2 in defect area was demonstrated by a reverse transcription-polym chain reaction (RT-PCR) assay. The highest levels of recombinant BMP-2 seen already at one week of healing. In summary, the data demonstrate the cap of transient overproduction of BMP-2 to induce both chondrogenesis osteogenesis. As the defect model was developed in the mouse, it can now be t for the biological activity of other bone inducing factors both in normal mice a transgenic mice harboring various type of gene modifications using adeno mediated gene transfer.