

properties determination and a rigorous method verification by modeling a bone that was actually tested.

Methods: A 10x10x10 mm cube of trabecular bone was taken from a vertebral body of a whale, the specimen was tested in three directions, in each a strain of 0.3% was applied at the platens and the axial stress and transverse strains were assessed. Each experiment was repeated four times. The morphology of the cube was digitised in a serial sectioning procedure, and numerically reconstructed in a voxel matrix. The voxels that represented bone were transformed in a FE Model. At each face of the cube a thin slice (max. 0.6mm) was eliminated from the model to ensure that a representative part of the trabeculae in the model were load carrying. The resulting model consisted of 511,109 three dimensional brick elements of 80x80x80 micron. The elastic modulus of the elements was initially set at 1,000 Mpa, the poisson ratio was set to 0.3. The three experimental compression tests were simulated assuming a frictionless contact between specimen and platen. A special purpose FE code was used to solve the resulting FE problem. Because the apparent elastic modulus is a linear function of the local tissue modulus, the latter could be estimated by fitting the experimental to the analytical results.

Results: The experimental results show similar apparent moduli in the x and y direction and a lower modulus in the z direction. The poisson ratio for the cube as a whole can be calculated from the transverse strains and is approximately 0.3 for the z direction and approximately half that value for the x and y directions. The best fit between experimental results and FE results was found for a tissue modulus of $E=5758$ Mpa ($sd=710$). The apparent transverse strains and moduli, determined by the FE model after scaling the results for the tissue modulus show good agreement with the experimental results.

Discussion: The apparent anisotropic material properties found experimentally were reasonably well reproduced by the FE model with isotropic material properties. Differences between the experimental results and the simulation results can be explained in part by artefacts in the bone specimen boundary area that were not modelled in the FE model. It was concluded that the methods used in this study provide an accurate tool for the quantification of relations between parameters at the tissue and at the apparent level.

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COMPARATIVE ASSESSMENT OF CORTICAL BONE BY 2 METHODS: DEXA (NON-INVASIVE TECHNIQUE) v SEM (INVASIVE TECHNIQUE).

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Introduction: Bone is essential to better understand structure disorders and bone remodeling, to confirm a diagnosis, to provide a predictive risk of fracture or to monitor the effect of therapy on the skeletal mass. In the last three decades, numerous techniques have been developed and improved in order to perform non-invasive measurement of the mineral content of both axial and appendicular skeleton. Recently, techniques which have been adapted to follow changes that occur in bone presented the potential for predicting mechanical properties. The goal of this original study is to assess the precision of DEXA (dual-energy X-ray absorptiometer) measurements by comparing this non-invasive technique to an invasive method (scanning electron microscope or SEM) which is taken as a reference.

Materials and methods: The mid-diaphysis of 7 pairs of tibias and femurs were obtained from dogs of different ages, sexes, weights and breeds. Slices 2 cm thickness were cut using a low speed saw. For each sample, bone mineral content (BMC) and bone mineral density or BMD (g/cm^3) were determined by using a DEXA (Lunar radiation, Madison, WI). Four and 6 sectors (circumferential analysis) were defined for femurs and tibias, respectively. Each specimen was placed in a plastic dish and covered by 12 cm of water to simulate soft tissues. BMD were defined for all regions of interest. Furthermore, each sample was polished with diamond paste, dehydrated stepwise in ethanol

(60-100%) and covered with a thin carbon film. Then, observation was performed using a SEM (JEOL JSM840). The chemical composition was defined by net intensity ratio (Ca/IP) and mineral content $[(Ca + IP)/(Ca + IP)] \times \%S$. A synthetic hydroxyapatite cube was employed as a control object (c) in the aim to calibrate the microscope and quantify chemical parameters.

Results: First, sectorial analyses were performed for both devices in order to study the BMC and chemical composition variations for femurs and tibias. From DEXA measurements a slight dominance of BMD was observed along the lateral-medial (LM) axis for both two bones and also in the postero-lateral (PL) and postero-medial (PM) sectors for tibia. From SEM, a mineral variation was also observed for both tibia and femur but in different sectors. We noted also important standard deviation (SD). Second, correlation (between the 2 techniques was investigated; the threshold for good correlation was set to $r^2 > 0.70$ $p < 0.05$) results are obtained for tibias.

Discussion: The study demonstrated that for global analysis (mean values) no variation was detected in BMD. However, on a sample per sample basis, variations consistent with SEM measurements could be observed. A poor correlation was found between results from DEXA and SEM. The non-zero intercept for regression line indicates that DEXA never estimates the BMC of femur and tibia. This may be explained by the fact that SEM measures a smaller volume compared to DEXA and only considers the mineral elements whereas DEXA is based on photon absorption through the thickness of the material and takes other elements such as marrow into account. This study suggests that DEXA only gives qualitative data and information for both BMC and BMD. Moreover, the major conclusion from this work was the lack of correlation between the two techniques.

CALLUS GROWTH; THE EFFECT OF ANTIPROSTAGLANDINS ON THE RATE OF HEALING AND PRODUCTION OF CALLUS IN THE UNDISPLACED TIBIAL FRACTURE.

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The effect of diclofenac on callus production in humans is investigated. In a prospective randomised trial patients were allocated one of two analgesia regimens following fracture: oral diclofenac 50mg at eight hourly intervals as required (Group 1), or oral dihydrocodeine 60mg at six hourly intervals (Group 2). Fracture healing was studied in 15 consecutive footballers with undisplaced tibial fractures. We measured the Callus Index from lateral radiographs taken at regular intervals.

Serial measurement of Callus Index from the lateral radiograph was a useful indicator of healing. This technique allowed an accurate predictor of healing when compared with time to removal of all support, clinical assessment of healing, callus bridging, time to full weight bearing and return to work.

No significant difference was detected between the two groups for any of the summary measures used ($p=0.164$). Our study indicates that the use of diclofenac in the early stages of fracture healing has no detectable effect on the inhibition of callus formation in patients with transverse undisplaced tibial fractures. The probability that diclofenac could cause an atrophic non union is small. A much larger study would be required to prove an effect of reduction of callus growth.

CHANGES IN HUMAN BONE HYDROXYAPATITE DURING INDIVIDUAL AGEING

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The mineral component of bone is thought to undergo significant changes in structure and composition during the course of development, but these modifications are little known as regard the microstructural level and the close

relationship between bone crystals, chiefly hydroxyapatite (HA), and the organic components of bone tissue. The aim of this study was to investigate the changes in the HA crystals of human bone occurring during life, relating their crystallinity degree, internal energy level, and mechanical properties.

Material and method: Cortical bone biopsy specimens were carried out from the femoral and tibial mid-shaft during the course of surgical procedures in 61 males of different age (min=5ys; max=79ys) affected with traumatic fractures of the inferior limbs. Each bone sample was divided into 3 fragments which underwent histological, differential thermal analysis (DTA), X-ray diffraction (XRD) and chemical analysis.

Samples were carefully sectioned in accordance with frontal and transversal plane, cleaned of periosteum, adherent soft tissue and marrow, further fractionated and powdered in order to allow chemical, DTA and XRD analysis. The crystallinity index (CI), the crystal size (D value), and the Ca/P ratio were calculated.

Results: Histology of the specimens almost always showed normal cortical bone; features showing the presence of osteoporosis were observed in very few specimens taken from elderly patients. XRD analysis showed a low degree of crystallinity in cortical bone specimens taken from young subjects (mean age = 11.6ys) as revealed by the mean values of (0.51) and D (162.8). The values of the crystallinity parameters increased in adult subjects with a mean age of 40.8 ys (CI=0.61; D=195.4), and reached the highest level, i.e. the highest degree of crystalline perfection, in subjects aged about 73.8 ys (CI=0.67; D=205.3). DTA showed that the level of internal energy of each bone specimen decreased with increasing individual age and that it was inversely proportional to the degree of crystallinity of bone mineral.

Chemical analysis showed that the average of the Ca/P ratio was 1.49 in the group of young subjects, 1.63 in the group of adults and 1.64 in the group of the elderly subjects.

Discussion: Our results clearly show that, in physiological conditions, the Ca/P molar ratio of bone mineral proportionally increases with age. In the same time, the degree of crystallinity of bone mineral, expressed by the D values, increases with individual age, i.e. with the age of mineral. Even if the number of cases analysed is too small to draw any conclusion, nevertheless, we may identify three phases in the development of bone crystals and their degree of crystallinity: a) an initial phase when bone mineral is scarcely crystallised and rapidly increases its crystalline order and its Ca/P ratio up to 30 years of age, b) a middle phase during which bone little increases its Ca/P ratio, but much improves its crystalline perfection up to 50 years, and c) a late phase during which nevertheless the Ca/P ratio maintains an almost constant value, bone mineral increases its degree of crystallinity and the HA crystals size. It may be that bone mineral continues to undergo structural changes even after full or nearly-full mineralisation is achieved, and bone mineral increases its crystalline perfection after mineralisation is virtually complete. Such an increasing degree of crystal perfection would be expected to influence the mechanical properties of bone.

EFFECTS OF BISPHOSPHONATE IN A RAT MODEL OF CORTICAL AND TRABECULAR OSTEOPOROSIS.

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Postmenopausal osteoporosis is characterised by increased bone turnover where bone resorption exceeds new bone formation. Bisphosphonates are potent inhibitors of osteoclast activity and have been used in several diseases with increased bone metabolism. We have found that treating oophorectomized rats on a low Ca diet with the bisphosphonate, clodronate, gives an increased femoral stiffness and maximum stress in the femur. Also the mechanical parameters of the femoral neck increased in the clodronate treated group. Since the femoral geometry and ashing show a significant reduction in femoral growth in the treated group, the increased strength suggests an increase in the material property of the bone in the clodronate-treated group.