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Increased calcium content and inhomogeneity of mineralization render bone toughness in osteoporosis: Mineralization, morphology and biomechanics of human single trabeculae

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ABSTRACT

The differentiation and degree of the effects of mineral content and/or morphology on bone quality remain, to a large extent, unanswered due to several microarchitectural particularities in spatial measuring fields (e.g., force transfer, trajectories, microcalli). Therefore, as the smallest basic component of cancellous bone, we focused on single trabeculae to investigate the effects of mineralization and structure, both independently and in superposition. Transiliac Bordier bone cores and T12 vertebrae were obtained from 20 females at autopsy for specimen preparation, enabling radiographical analyses, histomorphometry, Bone Mineral Density Distribution (BMDD) analyses, and trabecular singularization to be performed. Evaluated contact X-rays and histomorphometric limits from cases with osteoporotic vertebral fractures generated two subdivisions, osteoporotic ($n=12$, Ø 78 years) and non-osteoporotic ($n=8$, Ø 49 years) cases, based on fracture appearance and bone volume (BV/TV). Measurements of trabecular number (Tb.N.), trabecular separation (Tb.Sp.), trabecular thickness (Tb.Th.), trabecular bone pattern factor (TBPf) and eroded surface (ES/BS) were carried out to provide detailed structural properties of the investigated groups. The mechanical properties of 400 rod-like single vertebral trabeculae, assessed by three-point bending, were matched with mineral properties as quantified by BMDD analyses of cross-sectioned rod-like and plate-like trabeculae, both in superposition and independently. Non-osteoporotic iliac crests and vertebrae displayed linear dependency on structure parameters, whereas osteoporotic compartments proved to be non-correlated with bone structure. Independent of trabecular thickness, osteoporotic rod-like trabeculae showed decreases in Young's modulus, fracture load, yield strength, ultimate stress, work to failure and bending stiffness, along with significantly increased mean calcium content and calcium width. Non-osteoporotic trabeculae showed biomechanically beneficial properties due to a homogeneous mineralization configuration, whereas osteoporotic trabeculae predominantly demonstrated various mineralized bone packets, eroded surfaces, highly mineralized cement lines and microcracks. The Young's moduli of single trabeculae exhibited significantly negative linear correlations with trabecular thickness. Because of increased, but inhomogeneously distributed, calcium content, osteoporotic trabeculae may be subject to shear stresses that render bone fragile beyond structure impairment due to cracks and lacunae.

Introduction

Bone fragility and fracture risks due to osteoporosis are related to the quality of bone tissue. Bone structure and the degree of bone mineralization are the factors that primarily affect bone quality, although the bone turnover status also plays a substantial role [1–4]. Currently, non-invasive dual energy X-ray absorptiometry (DXA) measurements are considered as the standard technique for the diagnosis of osteoporosis. DXA scans provide information regarding a patient's bone mineral density (BMD) in the lumbar spine and/or the proximal femur [5,6]. However, these three-dimensional bone corpora are converted into two-dimensional reproductions that reflect the mass of calcium crystals per volume by the X-ray absorption in the unit of area (g/cm^2) by the DXA technique

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[1,2,3,7]. Thus, the degree of bone structure and/or bone mineralization effects on human bone quality is non-differentiable by DXA. Therefore, losses of bone mass may be unrecognized due to the occurrence of concurrently high bone mineral density in one case, while decreases of bone mineral density may be unrecognized due to an occurrence of concurrently high bone mass in another [8]. Although these situations may indicate pathological changes of bone tissue accompanied by elevated fracture risk, the appropriated BMD value, or T-score, may vary without pathological findings. In contrast, fractured and deformed vertebrae [7], bone islands [9], and micro-callus formations within the vertebrae may lead to high DXA-BMD and should therefore not be used for DXA assessment. According to this, the differentiation and degree of mineral content and/or morphology effects on bone toughness remain, to a large extent, unanswered due to several microarchitectural aspects contained in spatial measuring fields [10]. In particular, the design of the bone, orientation of trabecular architecture, stress trajectories and local strains may have a considerable effect on bone strength [11–14]. Therefore, we hypothesized that combined measurements of the bone mineral density distribution (BMDD) and three-point bending of single trabeculae, the smallest basic component of cancellous bone, may help to gain insights into bone mineralization effects on bone strength beyond structural properties due to their minimization. The evaluations of single trabeculae from non-osteoporotic cases, as well as from osteoporotic donors, are used to show how the mineralization profile affects the material properties of trabecular networks.

Materials and methods

Transiliac Bordier bone cores as well as spinal columns were obtained from 20 females at autopsy (Fig. 1A). All specimens were taken during outright autopsy at the Department of Legal Medicine, University Medical Center Hamburg-Eppendorf, Germany. The circumstances leading to death were motor vehicle or train accidents, assaults, suicides and other unnatural or unexpected causes. Individuals who suffered from cancer, renal diseases, primary hyperparathyroidism, Paget's disease or showed any other signs or symptoms of bone diseases apart from postmenopausal osteoporosis were excluded from the study due to full autopsy. None of the cases received medication that interfered with bone metabolism or affected the bone tissue. During the autopsies, radiographical analyses of the spinal columns, as well as of the transiliac Bordier bone cores, was performed routinely (Figs. 1B, C). In order to assess histomorphometric structure parameters of the thoracic vertebrae (T12) and iliac crest bone samples, cross-sections of the samples were prepared to undecalcified histologic specimens (Figs. 1D, E),

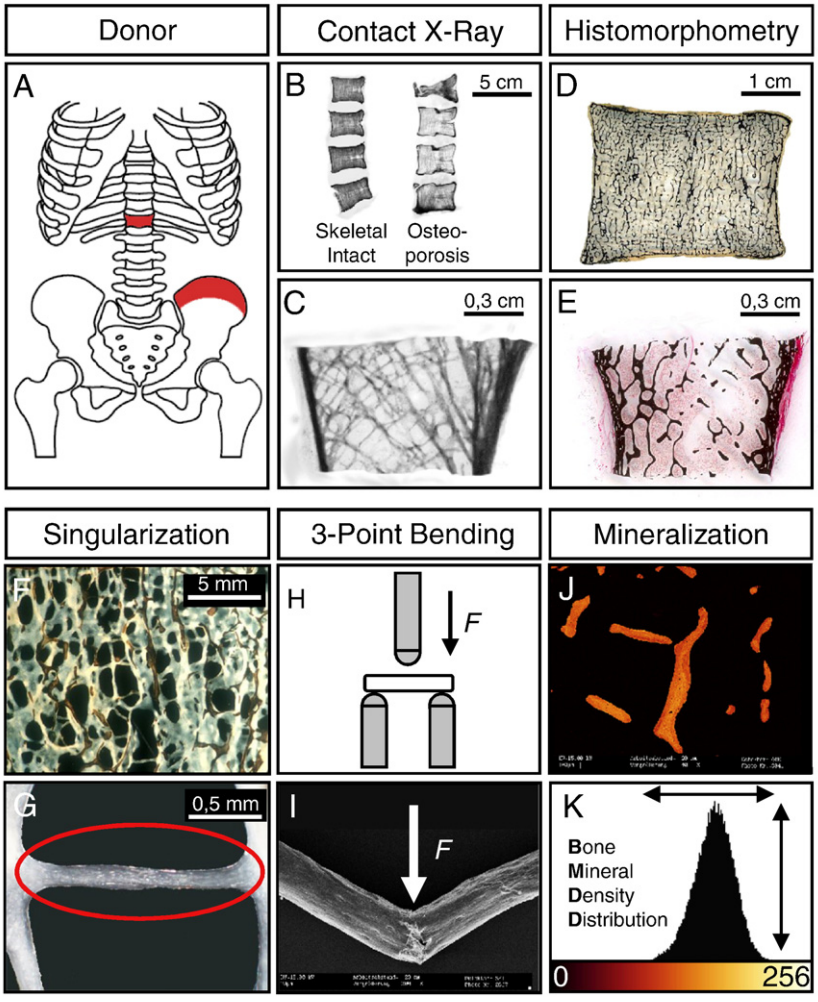

Fig. 1. Operation chart.(A) Sampling location of the T12 vertebrae and iliac crests. (B) Contact X-rays of a skeletal intact spinal column (left) and an osteoporotic spinal column with fractured vertebrae (right) carried out during outright autopsy. (C) Contact X-ray of a transiliac Bordier bone core performed during autopsy. (D) T12 vertebrae processed to a surface-stained block specimen. (E) von Kossa-stained transiliac Bordier bone sample. (F) Surface-stained block specimen under darkfield illumination showing separable trabeculae. (G) Trabeculae under darkfield illumination applicable for singularization and materials testing. (H) Schematically mounted single trabeculae in terms of three-point bending. (I) Scanning electron microscope image of one fractured trabeculae due to three-point bending (200x). (J) Backscattered electron image to evaluate the bone mineral density distribution (40x). (K) Gray value histogram of backscattered signal intensities reflecting the bone mineralization profile.