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PERSPECTIVES

Is Bone Mineral Crystal Size a Significant Contributor to "Bone Quality"?

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It has long been established that bone mineral is an analogue of the naturally occurring calcium phosphate mineral hydroxyapatite. Geologic apatite crystals may be several feet long, whereas mineral crystals in bone range from 10-40 nm in their largest dimension. Recent studies by Eppell *et al*. (1,2) used atomic force microscopy to measure the precise size of these small crystals, confirming the size measured in bulk specimens by physicochemical techniques. In healthy bone, mineral crystals align with their long axes roughly parallel to the collagen fibril axis (3), providing stiffness and strength to the more compliant and weaker collagen matrix. Not all crystals in a given bone are of the same size or orientation. In fact, there is a relatively non-Gaussian distribution of mineral crystals in a given osteon or trabecula (4). The maximal size achieved by crystals is most likely determined by the spacing of the collagen fibrils. There is controversy, however, as to whether a greater proportion of smaller crystals, larger crystals, or a broad distribution of crystal sizes is preferable. This is a crucial question because the therapies currently used for osteoporosis affect bone mineral crystal size and distribution.

The structural strength of bone is determined by bone quantity (*i.e.*, mass, density, and size), geometric form, and bone quality (5), an elusive term that refers to internal architecture and material properties. Architecture and material properties are influenced by many factors, such as bone turnover, mechanical environment, and disease. Furthermore, material properties are usually thought to depend on many factors, such as tissue composition, amount of secondary mineralization, collagen crosslinking, and the presence of microdamage. Because mineral composition, particle size, and distribution are not always the same, the properties of the crystals should also be considered as one of the factors contributing to material properties, when discussing the ability of bone to resist fracture.

Evidence supporting the theory that crystal size affects mechanical strength is mainly anecdotal, because few investigators have measured crystal size distribution in bones that have also been mechanically tested. Nonetheless, there are examples of comparisons between groups of experimental animals that have been analyzed for crystal size and tested mechanically, which provide some initial

validation of the theory. Animals with increased crystal size and perfection relative to age- and sex-matched controls include ovariectomized monkeys (6), osteonectin-null mice (7), and osteopontin-null mice (8). Ovariectomized monkeys and osteonectin-null mice have reduced mechanical properties. Because of increased bone size, osteopontin-null mice should have increased bone structural strength; however, this has not yet been reported in the literature. Animals with decreased crystal size and perfection include ia/ia osteopetrotic rats (9); two models of osteogenesis imperfecta in mice (10-12), in which the mice have weak bones; and osteocalcin-null mice (13), which have increased whole bone strength, most likely because of increased cortical diameter (14). Magnesium-deficient rat bones also have larger crystals than do magnesium-replete controls and show a significant decrease in maximum three-point bend strength in the absence of significant changes in bone diameter or bone mineral content (15). In contrast, *Hyp* mice have very ductile bones (16) and decreased mineral content, but no significant variation in crystal size. Similarly, chicks deficient in vitamin B-6 have a different maximum fracture load and offset yield, which is attributable to differences in bone diameter and collagen properties, without showing any difference in mineral crystal size (17), and ovariectomized sheep show decreased compressive strain relative to controls, without detectable changes in bone mineral crystal size (18).

There are a few examples of investigations in which the same bones were used to measure bone mechanical properties and crystal size and perfection, allowing direct correlations to be made. The classic study of Chatterji *et al.* (19) found that in human femoral cortical bone, average bone mineral particle size distribution shifted to higher values, with increasing age after the fourth decade, whereas in an early study, these same samples showed intrinsic tensile strength of bones decreasing after the fourth decade (20). In a study based on ultrasound and physicochemical measures in rats given growth hormone, mineral crystal width and cortical porosity were correlated with Poisson's ratios. In these animals, measures of bone quality (*i.e.*, density and crystal size) varied inversely with measures of bone quantity (*i.e.,* cortical area and moments of inertia) after growth hormone treatments (21). The compressive strength of vertebrae from rats given fluoridated water varied inversely with the width of the crystals (22,23). Because apatite crystal width tends to decrease as length of apatite crystals increases, these data suggest that crystal length may be positively correlated with mechanical strength. However, where crystal size distribution is sharpened, as in alendronate-treated minipig bones (24), an increase in mechanical strength was reported.

From these disparate data, it could be argued that crystal size has neither a positive nor negative effect on bone mechanical properties. However, in the studies mentioned above, the animals were examined at different stages of development. Some studies used x-ray diffraction of ground bone to evaluate crystal size, some used electron microscopy of selected material, and others used vibrational spectroscopic imaging with 1-20 μm spatial resolution. These methods also varied in sensitivity and spatial resolution. Different loading modes were used to assess mechanical properties, and different properties were reported. Furthermore, in many instances, bone geometry, a major determinant of bone structural strength, was not consistently considered. Thus, additional studies will be required to test the hypothesis that bone mineral crystal size is a significant contributor to bone mechanical properties.

Distribution of crystal size may be much more important than average crystal size. In humans and primates with osteoporosis, the broad distribution seen in age-matched controls disappears (6), whereas in PTH-treated ovariectomized monkeys, whose whole bone strength improves with treatment, the distribution of crystal size is broadened (25). Other studies in osteoporotic patients show a wide range of changes in crystal size and perfection, perhaps because fracture callus was included in the material analyzed, or where lack of differences were detected, the relative sensitivity of the method used for analysis.

To resolve the controversy, mechanical testing and determination of crystal size and distribution should be performed on the same samples. Spectroscopic studies might include both Fourier transform infrared microspectroscopic analysis and Raman imaging, as well as atomic force microscopy. Mechanical testing should include structural measures of bone stiffness and failure load in well-defined loading modes, accurate determinations of geometry and architecture, and assessments of material properties. Then, direct correlations between crystal size and size distribution could be made with structural or material mechanical attributes.

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