

NMR Measurement of Bone Quality

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The problem of increased risk of skeletal fractures due to bone mass loss in aging or disease is a major clinical problem with associated estimated health care costs of nearly \$17 billion in the US [1]. It has been estimated that 40%-46% of all women over the age of 50 and 13%-22% of all men over the age of 50 will suffer a fracture as a result of bone loss [2]. With the number of persons aged 60 years or older projected to almost triple by the year 2050 [3], the aging of the general population will lead to a significant increase in the at-risk population for fractures. As such, the number of world-wide fractures will likely increase from 1.26 million, as estimated in 1990, to 2.6 million by 2025 [4]. Notwithstanding the economic burden, non-vertebral fractures are a significant cause of morbidity and mortality in the aging population [5-7]. Thus, concerted efforts are needed to not only identify those at risk of bone fractures, but also to identify treatment strategies that can maintain the health of the skeleton with age.

Age-related increase in the incidence of skeletal fractures results from interactions among a variety of factors including impaired balance and reflexes, reduced bone mineral density, changes in bone geometry, porosity, and architecture, physicochemical properties of bone's mineral and organic phases, and accumulation of damage in bone tissue [8-12]. The latter five factors are collectively referred to as "*bone quality*." It is becoming increasingly evident that bone mass alone cannot account for variation in observed fracture risk and that more accurate fracture risk prediction will only be possible through incorporation of measures of bone quality [13]. To improve our ability to predict fracture risk by including measures of bone quality, we must first understand the mechanisms through which various measures of bone quality act to control bone mechanical properties and, ultimately, bone strength. This is not currently well understood.

To understand the full range of determinants of fracture risk, bone must be analyzed at each of the hierarchical levels of its organization. Bone has a complex hierarchical structure that resembles a composite at various length scales ranging from nanometers to nearly meters [14, 15]. The basic building blocks in a bone are nanometer-sized platelet-shaped crystals of carbonate apatite or hydroxyapatite (HA), which are arranged in parallel layers with a collagen matrix to form a mineralized collagen fibril [14]. At the next hierarchical level the mineralized collagen fibrils are organized into various structures with different property characteristics. In lamellar bone, the mineralized collagen fibrils are ordered into arrays in which the fibril axes and the crystals are aligned into a three-dimensional structure resembling a nanometer-sized composite exhibiting anisotropic elastic and fracture properties. Well-organized lamellae of different thicknesses and fibril orientations form the structure in an osteon, including a Haversian canal and numerous lacunae located at lamellae interfaces. In contrast, disorganized fibril arrangements and orientations constitute the structure in interstitial bone tissue. At the next hierarchical scale, bone structure is comprised of a microstructure of osteons and interstitial lamellae that are separated by cement lines. At the macroscopic or continuum scale, bone can be considered a porous solid whose elastic and fracture properties depend on bone mass, porosity, and pore size distribution. Bone microstructure evolves as damaged bone is remodeled to form damage-free bone tissues.

Bone tissue is 25% water. Water is not only present in the microscopic pores as free mobile water (~18% by volume), it also exists within the extracellular matrix of bone tissue as tightly or loosely bound water (~7% by volume). Previous studies have suggested that the removal of water from the extracellular matrix, in addition to that removed from the void spaces within bone tissue, affects the mechanical properties of bone. The objective of this investigation was to

determine the porosity, mobile, and bound water distribution, and, for the first time, attempt to determine the loosely and tightly bound water in cortical bone *in vitro* using a non-destructive low-field NMR technique and to characterize how changes in bound water within bone tissue are related to bone mechanical properties.

We found no age-related differences in either bone porosity or the ratio of bound to mobile water between young and old baboon bone. However, we did find a significant difference in loosely bound water between the young and old bone. Furthermore, it appears that this loosely bound water may play an important role in bone mechanical properties, particularly in the tensile yield and post yield behavior of bone tissue. In our previous study, we found that bound water plays an important role in human bone mechanical properties [16]. Since the ratio of bound water to mobile water is much higher in this sample of baboon bone than in human bone, it is reasonable to assume that the loosely bound water within the bone matrix plays an important function in bone mechanical properties. However, a more detailed characterization of the bound water (such as the fraction of loosely bound or tightly bound water) is needed to assess the mechanical integrity of bone tissue.

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