

# LABORATORY FOR THE STUDY OF SKELETAL DISORDERS AND REHABILITATION, SUMMARY OF CURRENT RESEARCH

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Our group has devoted to the application of Nuclear Magnetic Resonance (NMR) spectroscopy and Nuclear Magnetic Resonance Imaging (MRI) to basic science and clinical problems of bone mineral and matrix, and to the diseases associated with bone. NMR spectroscopy provides an analysis of the ionic composition and structure of the mineral crystals and matrix, i.e., the properties of bone as a material or substance, while MRI yields the spatial properties of bone as a tissue based on the spatial variation of the NMR signals of bone.

## SOLID STATE MAGNETIC RESONANCE SPECTROSCOPY STUDY OF BONE MINERAL

### HYDROXYL ION IN BONE MINERAL CRYSTALS

The chemistry of calcium phosphate salts, in particular the apatite minerals, is highly complex. Many of the biological and mechanical functions of bone mineral are controlled by the composition and crystal structure of the calcium phosphate bone mineral crystal. Issues relating to bone mineral chemistry and bone resorption carry important implications for public health.

It has long been established that bone mineral crystals are compositionally and structurally similar to the synthetic mineral calcium hydroxyapatite,  $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$ . However, in contrast to typical synthetic hydroxyapatite, bone mineral is nanocrystalline, structurally disordered, and compositionally nonstoichiometric. It exhibits a high specific surface containing labile chemical groups, and a remarkable ability to support ionic vacancies and substitutions.

A decades-old controversy concerning the relationship between bone mineral and hydroxyapatite is whether bone mineral crystals contain hydroxyl (OH<sup>-</sup>) ions. Although the OH<sup>-</sup> content of hydroxyapatite itself is readily measurable with wet chemical and instrumental techniques, such measurements on the biological apatite crystals of bone suffer interference from

the organic matrix, which contains variable amounts of water and complex mixture of proteins (primarily collagen I) and other biopolymers as well as small molecules. Both the matrix and the water interfere with wet chemical and infrared analysis for OH<sup>-</sup> in the crystalline phase. Hence, most studies of the OH<sup>-</sup> content of bone mineral require removal of the water by drying and removal of the organic matrix ("deproteinization") by chemical pretreatment to yield "anorganic" bone. Such chemical treatment always raises the question of whether the mineral has been chemically altered.

We have demonstrated that a two dimensional solid state <sup>1</sup>H to <sup>31</sup>P NMR spectroscopy technique (HetCor) eliminated the need for specimen pretreatment of any kind other than cryogenic grinding, and detected that the bone crystal OH<sup>-</sup> of human cortical bone of about 20% of the amount expected in stoichiometric hydroxyapatite (1).

In the two dimensional HetCor method, proton magnetization was first excited. Magnetization of phosphorus nuclei was obtained by the so-called cross polarization from the excited proton, and its (<sup>31</sup>P) intensity was recorded by the spectrometer. A series of such <sup>31</sup>P spectra with various proton magnetization evolution times are collected. A two-dimensional Fourier Transform applied on the series of <sup>31</sup>P data yielded a two dimensional NMR spectrum, with one axis of <sup>31</sup>P and the other axis of <sup>1</sup>H. In the proton side, only those resonances arising from protons located within atomic distances (much less than 1 nm) of phosphorus nuclei were shown. By this method, only OH<sup>-</sup> and the surface-absorbed water of bone mineral crystals were detected while the interfering matrix signals were suppressing. But due to the nature of two-dimensional spectroscopy, the measurement time is generally very long. To obtain a 2D spectrum for one cross polarization time constant at reasonable signal to noise ratio would require 24 hours. Usually, to determine the amount of OH<sup>-</sup> in the crystal, four spectra at various cross polarization contact time are needed.

A new NMR spectroscopy method to speed up the measurement is currently developed in our lab. Instead of exciting the proton magnetization in the first place, the <sup>31</sup>P magnetization was excited first and transferred to proton by cross polarization. The resulted <sup>1</sup>H magnetization is observed directly. This is a one dimensional <sup>31</sup>P to <sup>1</sup>H cross polarization method. With the same principle of cross polarization in the 2D method, the spectrum obtained in this new method shows only those resonances arising from protons very close to phosphorus nuclei. Since it is one dimensional, the measurement time is much

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shorter. For a spectrum with similar signal to noise ratio, the one dimensional method needs only 4 hours.

This new method has been applied to synthetic hydroxyapatite and 27 week-old chicken bone samples. The spectrum of hydroxyapatite shows only the OH<sup>-</sup> peak, while the spectrum of chicken bone sample shows the peaks of OH<sup>-</sup> and the surface-absorbed water of bone crystals. A systematic study using this new method of various biologic and synthetic samples is currently being conducted in our lab.

## MAGNETIC RESONANCE IMAGING

### BONE MATRIX DENSITY MEASUREMENT OF RAT FEMUR BONE SPECIMENS BY SOLID STATE PROTON MRI

Progress in new and improved methods for characterization of bone tissue occurred at a rapid pace in the past decade, largely driven by the increasing importance of osteoporosis as a major public health factor in the elderly. An NIH consensus development panel concluded in 2001 that in the United States alone, 10 million people already have osteoporosis, and 18 million more have low bone mass.

One of the crucial parameters for bone qualities is the degree of bone mineralization (DM), conventionally defined as the weight of bone mineral in unit volume of bone matrix. A closely related definition of DM is the ratio of bone mineral density (BMD) divided by bone matrix density. Knowledge of this parameter will enable us to detect the compositional changes in bone substance, and is decisively valuable to distinguish osteoporosis (low BMD but normal DM) from other metabolic bone diseases such as osteomalacia (low BMD and low DM). This data will also significantly help in evaluation of the effectiveness of treatment for heritable and metabolic diseases of the skeleton. The clinical significance of the diagnosis of mineralization defect in patients with low BMD is obvious, since appropriate measures can be undertaken to correct this abnormality, whereas available antiosteoporotic treatments would not have any effect or might even be harmful. Indeed, defective mineralization has been commonly discovered in patients who underwent bone biopsies to find an explanation for antiosteoporotic treatment failure.

In our previous studies, we have demonstrated that the bone mineral density and bone matrix density of relatively large porcine and bovine bone specimens (a few cm of diameter) can be obtained, respectively, by solid state <sup>31</sup>P MRI and water and

fat suppression proton MRI (WASPI), and DM can be mapped by the ratio of these two measurements (2).

Because the anatomy of the rat skeleton has many similarities with the human skeleton, and also because the study on rats can be conducted relatively cheap in relatively short-term, rats are the most commonly used animal models to investigate the pathogenesis and treatment of skeletal disorders as they occur in humans. A special CT (micro-CT or  $\mu$ CT), which utilizes a microfocus x-ray tube as a source, an image intensifier as a 2D detector and a cone-beam algorithm as reconstruction methods, has gained popularity in bone and mineral research on small rat bone specimens (< 18 mm) due to its high spatial resolution. But as an X-ray based method,  $\mu$ CT measurement of bone only reflects the mineral content of bone, i.e., the BMD, since the X-ray beam is mostly attenuated by calcium, the constituent with the highest atomic number and concentrated in bone mineral, to an intermediate extent by phosphorus, also mostly concentrated in bone mineral, and much less by oxygen, carbon and hydrogen in bone matrix. The measurement of  $\mu$ CT does not determine the solid matrix content (mostly collagen) of bone.

We are currently exploring the feasibility of WASPI to image the solid matrix content of rat bone specimens, with the challenges of small sample size and the demanded resolutions.

In order to obtain better signal to noise ratio of the small size rat bone specimens, a specially designed double tuned <sup>31</sup>P/<sup>1</sup>H RF probe of inside diameter ~ 1.5 cm were utilized in this study. The split-solenoid type coil provided greater axial homogeneity that allows quantifiable measurements of spin density as can be seen.

New method of WASPI has also developed to improve the suppression of water and fat signals from the bone specimens.

The MRI experiments were carried out with Bruker 4.7 T system equipped with 400 mT/m gradient. The <sup>1</sup>H Larmor frequency was 400 MHz.

<sup>1</sup>H WASPI images of rat femur (< 10 mm) solid matrix have been obtained. It is the first time that bone matrix only image of rat femur was obtained by MRI, as far as we know, described in the literature. The resolution of 0.7 mm can be achieved by 35 min of scan time. These results will be presented in the NIH-ASBMR Scientific Meeting, Bone Quality: What is it and Can We measure It, Bethesda, Maryland, 2005.

## References

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