31P NMR Relaxation of Cortical Bone Mineral Investigated by Partial Demineralization and Deuterium Exchange

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Introduction: Phosphorus, whose only isotope, 31P, has spin I=1/2, is a major component of bone mineral [1]. It has been shown that solid-state 1H and 31P MRI have the potential to quantify bone matrix and mineral mass per unit volume of bone tissue, from which true bone mineral density can be computed [2-4]. Imaging of bone phosphorus is made difficult by its extremely short T1 and long T2 relaxation times. In order to better understand the mechanisms responsible for these unfavorable relaxation times and assess their dependence on bone mineralization, we have measured the effects of deuterium exchange and partial demineralization (a model for osteomalacia) on T1 and T2 relaxation times of bone mineral phosphorus.

Methods:

Specimens: Five cylindrical pieces of cortical bone (10mm length, 4mm diameter) were cut from the tibial midshaft of lamb shanks obtained from a butcher. Five samples of cortical bone powder, weighing 300 mg each, were cryogenically ground from the same tibias. All data were acquired at room temperature.

Hardware: 3T and 7T: Siemens whole-body MRI (3T TIM Trio, 7T Magnetom) and custom-made transmit/receive solenoidal RF coil with dual-conductor windings; 9.4T: Bruker Avance III vertical-bore NMR and vendor-supplied saddle-coil/gradient probe.

T1 measurements: Due to the difficulty in generating short 180° RF pulses, saturation recovery rather than inversion recovery was used (Fig. 1). During the SAT preparation, generation of stimulated echoes was not a concern because the duration of QSTOP ≥ 5 ms >> T2*. All receiver dead times were ≤350μs. The recovery time tSAT was incremented by powers of two from 16 ms to 512 s (1024 s for D3O-exchanged at 7T). The entire set of blocks was averaged two (D2O exchange) or four (partial demineralization) times.

T2* measurements: The acquisition at the longest saturation recovery time was used for measurement of T2*. Partial Demineralization: Relaxation measurements were performed on five samples of bone powder slurry in saline. The saline was removed by centrifugation and saved. To partially demineralize the samples, they were left in 1.2 mL of 1% EDTA solution at room temperature for six days; the liquids were changed on day three. The EDTA solutions were removed and each sample was rinsed with water. 1.2 mL of saline was then added and relaxation times were measured. All EDTA solutions, rinse liquids, and saline in contact with each sample were concentrated to 0.5 mL by lyophilization, and scanned with a calibrated methylene diphosphonate (MDP) capillary using high-resolution 31P NMR spectroscopy to measure the amount of phosphorus removed from the bones. This six-day cycle was performed three times. Finally, the powders were liquefied in 1.2M HCl and scanned using high-resolution 31P NMR spectroscopy to measure the amount of phosphorus remaining in the bones.

Deuterium Exchange: Relaxation measurements were performed on five bone samples at 3T and 7T. The bones were then blotted dry and immersed in 3 mL (>25-fold volume excess) of 99.9% D2O-saline at 4°C for 72h and measurements were repeated.

Data Processing: Data were Fourier transformed and phased. A Lorentzian function was fitted to the single 31P peak in each real-component solid-state spectrum (mean R2=0.94). T1 was calculated by fitting an exponential function to the peak amplitudes at each tSAT (mean R2=0.99). T2* was calculated from the fitted Lorentzian line width (FWHM) of the spectrum acquired after the longest saturation recovery time. If the internuclear distance vectors are unchanged by deuterium exchange, then R1(1H,31P) = (8/3)(ν2π/γ1Hγ31P)R1(1H,1H)P, where ν2 = 42.58 MHz/T and ν2H = 6.54 MHz/T. This relationship was used to measure the fraction of the longitudinal relaxation rate R1 = 1/T1, which is due to 1H-31P heteronuclear dipolar interaction.

Results: At each stage of partial demineralization, T1 decreased significantly, but T2* was unchanged, as shown in Figure 2. Replacement of exchangeable hydrogen atoms with deuterons caused dramatic increases in T1, but only very small increases in T2*, as shown in Table 1. The percentage of longitudinal relaxation rate due to 31P heteronuclear dipolar interaction is 78.6 ± 2.0% at 3T and 74.3 ± 2.1% at 7T.

Discussion and Conclusions: The observation that the majority of longitudinal relaxation rate of cortical bone 31P is due to heteronuclear dipolar interaction with nearby hydrogen nuclei explains the decrease in T1 after partial demineralization; because demineralization occurs under constant total volume [5], a loss of mineral causes an increase in bone water, which increases the number of 1H nuclei available to interact with 31P nuclei. The result is more rapid longitudinal relaxation in demineralized bone. Because of this, it is not feasible to assume a single T1 value for bone mineral in vivo, as this value will likely vary significantly with bone health.


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