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

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Introduction: Phosphorus, whose only isotope, ^{31}P, has spin I=½, is a major component of bone mineral [1]. It has been shown that solid-state ^{1}H and ^{31}P 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 T_2^* and long T_1 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 T_1 and T_2^* 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 fresh 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.

T_1 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 $G_{SPOIL} \geq 3$ ms >> T_2^*. All receiver dead times were ≤30μs. The recovery time τ_{SR} was incremented by powers of two from 16 ms to 512 s (1024 s for D_2O-exchanged at 7T). The entire set of blocks was averaged two (D_2O exchange) or four (partial demineralization) times.

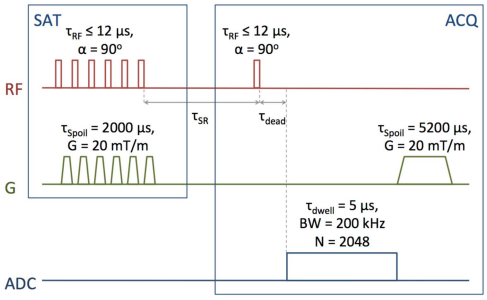

Figure 1: Saturation recovery pulse sequence

T_2^* measurements: The acquisition at the longest saturation recovery time was used for measurement of T_2^*.

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 ^{31}P 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 ^{31}P 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% D_2O-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 ^{31}P peak in each real-component solid-state spectrum (mean R^2=0.94). T_1 was calculated by fitting an exponential function to the peak amplitudes at each t_{SR} (mean R^2=0.99). T_2^* 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 $R_{1,2H\text{-}31P} = (8/3)(\gamma_{2H}^2/\gamma_{1H}^2)R_{1,1H\text{-}31P} = 0.0629R_{1,1H\text{-}31P}$, where γ_{1H} = 42.58 MHz/T and γ_{2H} = 6.54 MHz/T. This relationship was used to measure the fraction of the longitudinal relaxation rate $R_1 = 1/T_1$ which is due to ^{1}H-^{31}P heteronuclear dipolar interaction.

B_0	Condition	T_1 (s)	T_2^* (μs)
3T	Unmodified	25.9 ± 1.4	189 ± 2.2
	D_2O-Exchanged	99.0 ± 10.5	203 ± 2.1
7T	Unmodified	66.0 ± 0.8	119 ± 0.4
	D_2O-Exchanged	218 ± 15	121 ± 1.0

Table 1: Relaxation times before and after deuterium exchange

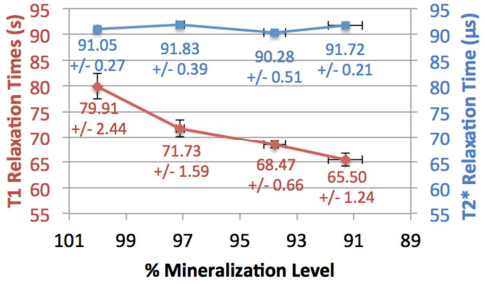

Figure 2: ^{31}P relaxation times at several stages of demineralization

Results: At each stage of partial demineralization, T_1 decreased significantly, but T_2^* was unchanged, as shown in Figure 2. Replacement of exchangeable hydrogen atoms with deuterons caused dramatic increases in T_1, but only very small increases in T_2^*, as shown in Table 1. The percentage of longitudinal relaxation rate due to ^{1}H-^{31}P 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 ^{31}P is due to heteronuclear dipolar interaction with nearby hydrogen nuclei explains the decrease in T_1 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 ^{1}H nuclei available to interact with ^{31}P nuclei. The result is more rapid longitudinal relaxation in demineralized bone. Because of this, it is not feasible to assume a single T_1 value for bone mineral in vivo, as this value will likely vary significantly with bone health.

References: [1] Bringhurst FR In: Fauci AS, et al. Harrison's Principles of Internal Medicine. 17ed: McGraw-Hill Professional; 2008. [2] Wu Y, et al, PNAS 1999;1574-78. [3] Anumula S, et al, MRM 2006; 56:946-56. [4] Robson MD, et al, MRM 2004;51:888-92. [5] Robinson RA, et al., J Bone Joint Surg Am 1957;39-A(1):167-188.

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