# Proton NMR Study of Transverse Relaxation of Rabbit and Rat Cortical Bone

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## Introduction

Bone is a composite material consisting of a collagenous matrix that is mineralized with nonstoichiometric calcium apatite. In addition, it contains water part of which is bound to collagen while a larger fraction occupies the spaces of the haversian and lacunocanalicular system. Proton NMR has previously been used to study the effect of demineralization in hypophosphatemic osteomalacia[1] and has potential applications to humans[2]. However, the signal is comprised of protons in the collagen matrix, water bound to collagen fibrils, and bulk water in the pore spaces of the Haversian canals and lacuno-canalicular system. A quantitative assessment of cortical bone pore water, and thus porosity, requires an understanding of the various contributions to the overall signal and NMR properties of the different proton populations. For example, NMR relaxometry may provide insight into bone architecture due to its sensitivity to water diffusion in the Haversian system[3,4]. Towards that goal, in this work, we quantified the $T_2^*$ and $T_2$ components of rabbit and rat tibia cortical bone specimens from bi-exponential fits of FIDs and CPMG signal decays.

## Materials and Methods

Rectangular sections of cortical bone ($10\times4\times1 mm^3$), with the marrow removed, were harvested from the tibia mid-shafts of healthy 20 week-old New Zealand white rabbits (N=5). Whole tibia mid-shafts were also harvested and demarrowed from healthy 16 week-old Sprague-Dawley rats (N=4). Upon removal from storage in saline (to prevent leaching of minerals), surface water was removed by brief padding with tissue paper. The specimens were then placed in a 5mm NMR tube in a vertical-bore 9.4T spectrometer (DMX-400, Bruker Instr.). All experiments were performed at ambient temperature of 20°C. First, a CPMG sequence was run (16 scans, $\tau_{90}/\tau_{180}$=4.6/9.2$\mu s$, TR=2$s$, TE=0.2-20$ms$ with an inter-echo spacing of 0.2$ms$, dwell time=5$\mu s$). After Fourier transformation and phase correction of the acquired signals, the spectral peak maximum was recorded as a function of TE. Second, an FID was acquired (16 scans, $\tau_{90}$=4.6$\mu s$, TR=3$s$, time=5$\mu s$). Both the normalized CPMG signal decays and FIDs (only the first 1$ms$ of the FID was used due to signal decay) were fitted to a bi-exponential of the form $M_1\exp(-t/T_a) + M_2\exp(-t/T_b)$ in the least-squares fashion (Levenberg-Marquardt algorithm in Matlab), where t is either TE or FID time axis. $M_1$ and $M_2$ are the magnitudes of the two components, and $T_a$ and $T_b$ represent either $T_2^*$ or $T_2$.

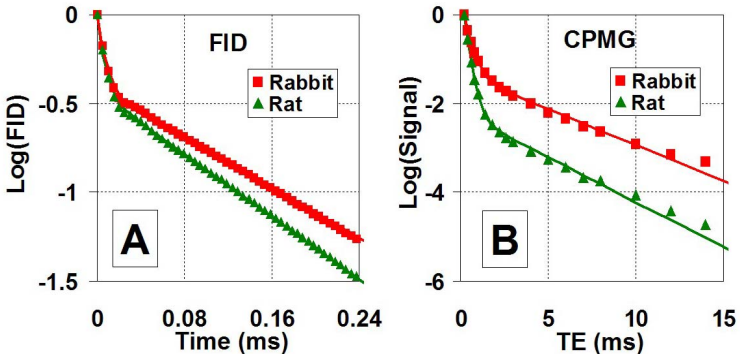


**Figure 1**. Sample rabbit and rat logarithmic plots of normalized (A) FIDs and (B) CPMG signal decay curves. Squares and triangles are the data points. Solid lines bi-exponential fits.

## Results and Discussion

Figure 1 shows a logarithmic plot of typical FIDs and CPMG signal decays for a rat and rabbit bone specimen. Both datasets fit well to a bi-exponentially decaying signal (all $R^2$ >0.95). Table 1 shows average short and long $T_2^*$ and $T_2$ values with their relative magnitudes for each species.

**Table 1. Average values of bi-exponential fit parameters**

| | FID | | | | CPMG | | | |
|---|---|---|---|---|---|---|---|---|
| **Species** | **Short $T_2^*$ Magnitude** | **Short $T_2^*$ ($\mu s$)** | **Long $T_2^*$ Magnitude** | **Long $T_2^*$ ($\mu s$)** | **Short $T_2$ Magnitude** | **Short $T_2$ ($\mu s$)** | **Long $T_2$ Magnitude** | **Long $T_2$ ($ms$)** |
| **Rabbit** | 0.34±0.002 | 7.9±0.3 | 0.66±0.002 | 307±23 | 0.72±0.01 | 422±20 | 0.28±0.01 | 5.5±0.5 |
| **Rat** | 0.35±0.002 | 7.1±0.2 | 0.65±0.002 | 240±10 | 0.87±0.05 | 319±13 | 0.13±0.05 | 5.3±0.9 |

The short and long $T_2^*$ components are most likely the protons in collagen and bone water, respectively, since the short $T_2^*$ component persists while the long $T_2^*$ component disappears after soaking the bone specimen in $D_2O$ (data not shown) as has been previously observed in hydrated collagen[5]. Our results indicate that the majority of the FID signal (65%) is from water. The spectral peak from the CPMG experiments is also most likely water as the CPMG echo does not exhibit the short $T_2^*$ component (data not shown). The short and long $T_2$ components of the CPMG signal decay may originate from water in the lacunae and Haversian canals, respectively. It is well known that diffusion in porous systems results in multi-exponential relaxation[3]. Our data are in qualitative agreement with those by Ni et al in human cortical bone[4]. As $T_2$ scales with the square of the pore size, the short $T_2$ component should logically be assigned to water in the lacunae, which are an order of magnitude smaller than the Haversian canals. Furthermore, the CPMG signal decay in both species is dominated by the short $T_2$ component, which is similar in value to the long $T_2^*$ of the FID. This suggests that the majority of spin-spin dephasing experienced by bone water is irreversible. A well known relaxation mechanism in porous media and possible mechanism for this irreversible dephasing is translational self-diffusion in the internal gradients caused by the susceptibility difference at the bone-bone water interface due to the greater diamagnetism of bone compared with water[6,7].

The major difference in relaxation behavior between rat and rabbit specimens was the increased fraction of the short $T_2$ component for rat, which may suggest a decreased fraction of Haversian canals in rat bone. It has been reported that rabbit tibiae have less mineral content then that of rat[8,9]. Assuming equal bone mineralization, lower mineral content would indicate greater porosity, most likely due to a greater density of Haversian canals in rabbit bone.

## Conclusion

Characterization of transverse relaxation properties of bone proton signal provides insight into the micro-architecture of cortical bone. The data presented here provide evidence of inter-species differences between rabbit and rat cortical bone.

**References:** 1. Anumula S, et al, *Bone*, In Press (2007). 2.Bydder GM, et al, *NMR Biomed*, **19**:765 (2006). 3.Brownstein KR and Tarr CE, *Phys Rev A*, **19**:2446 (1979). 4. Ni Q, et al, *Meas Sci Technol*, **15**:58 (2004). 5. Edzes H, et al, *Nature*, **256**:521 (1977). 6. Hopkins J, et al, *MRM*, 37:494 (1997). 7. Huerlimann MD, *JMR*, **131**:232 (1998). 8. Ayers RA, et al, *Biomed Sci Instr*, **32**:251 (1996). 9. Biltz RM, et al, *J Bone Jnt Sur*, **51**:456 (1969). **Acknowledgements:** NIH R01 AR50068