The effect of freezing on measurements of trabecular bone structure based on NMR spectroscopy

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Introduction: Nuclear magnetic resonance (NMR) measurements provide an effective tool for non-invasive evaluation of trabecular bone structure and bone marrow composition [1]. Trabecular bone consists of series of interconnecting plates and rods and is filled with bone marrow. Since the calcified tissue provides only a negligible NMR signal at conventional pulse sequences, the NMR signal is obtained from the water and fat components of the bone marrow. Sensitivity of T_2 and $T_{1\rho}$ relaxation to different relaxation mechanisms, including dipolar interaction with slowly tumbling large molecules, chemical and molecular exchange as well as diffusion in local magnetic field gradients, varies. Hence, relaxation times of fat and water in bone marrow may be used as a magnetic resonance marker of bone structure and molecular changes in the bone marrow. Bone samples from patients are often frozen before studied ex vivo. However, freezing can have an effect on the histological morphology of bone [2]. Further, the bone marrow cells are affected by deep-freezing [3], which can cause changes at the molecular level. The aim of the present study was to investigate the effect of freezing on trabecular bone, as assessed by the structural changes seen in the bone marrow by NMR.

Materials and methods:

Fresh bovine tibias (n=10, age=18 months) were obtained from the local slaughterhouse. Cylindrical trabecular bone samples (∅=10 mm, L=20 mm) were isolated from the proximal tibia near the central marrow cavity. Fresh samples were measured within 24 hours of slaughter. Thereafter, they were stored in the freezer (T = -20°C) for 7 days. Finally, they were thawed 24 hours before the second measurement at room temperature and stored overnight at +4°C.

NMR measurements were performed using a 4.7 T/31 cm horizontal magnet, Varian^{UNITY} INOVA console and an in-house-built surface coil (transmit/receive mode). The trabecular bone samples were placed in the center of the coil. A 3x3x3 mm^3 voxel was localized into the middle of the sample. Seven spin-lock times (19.5-79.5 ms) were used at ten different B1 field strengths (0.018-0.18 mT) to determine $T_{1\rho}$ [3]. Separate experiments were done to obtain on-resonance $T_{1\rho}$ for both fat and water. The T_2 relaxation time was measured using single-voxel LASER pulse sequence for localization and a double spin-echo preparation block (TEs between 23 and 143 ms) [4]. Carr-Purcell-T_2 (CP) (τ_{CP} = 4 ms) was determined either using four 180° pulses (4P) (τ_{CP} = 12 ms) and the same pulse sequence with 0° adiabatic pulses inserted symmetrically around 180° pulses (DP) (TEs between 23 and 117 ms, τ_{CP} = 12 ms). The fat and water signals were calculated as the area under the respective peaks [4].

As a reference, micro computed tomography (μCT) (SkyScan 1172, Artselaar, Belgium) was performed to determine the structural parameters of trabecular bone. An isotropic voxel size of 13μm was used. A cylindrical ROI was binarized and the structural parameters, i.e. bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N) and structure model index (SMI) were calculated. NMR parameters before and after freezing were statistically compared with the Wilcoxon Signed rank test. Linear correlations between the μCT structural parameters and the NMR parameters were studied both before and after freezing using the Pearson's correlation test.

Results: After freezing $R_{1\rho}$ values for water, calculated at higher B_1 fields (at ON-resonance), were different to those measured before freezing (Fig 1B). Differences were found (Fig 1D) in the R_2 values for water, whereas no difference was observed for fat (Fig 1C). Before freezing, several significant correlations ($p<0.05$) were observed, e.g. between Tb.N and $R_{1\rho}$ (at all B^1 fields) for water (R = 0.761 – 0.861); between BS/BV and $R_{1\rho}$ (at all B^1 fields) for fat (R = 0.776 – 0.836). However, after the freeze-thaw cycle, only non-significant associations were found between same variables.

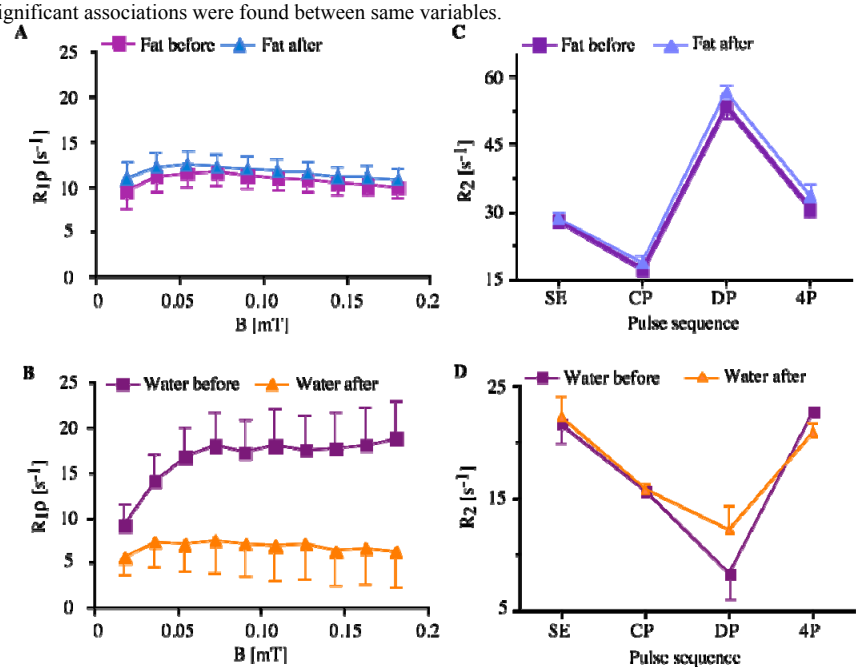

Fig 1.: The relationship between the relaxation rate ($R_2=1/T_{1\rho}$) for fat (A) and water (B) components of the bone marrow at ON-resonance, before and after freezing. The dependence of R_2 for fat (C) and water (D) on the pulse sequence (SE: Spin-echo, CP: Carr-Purcell, DP: Dummy pulse, 4P: Empty pulse) before and after freezing.

Conclusion: Different relaxation times of water and fat correlate significantly with the structural parameters of fresh trabecular bone. This may relate to diffusion of molecules by the local field gradients at the near interface of calcified bone and bone marrow. As the calcified bone may be insensitive to freezing, there is a potential effect of freezing on the structure of bone marrow. This indicates that no frozen bone samples should be used when addressing the dynamic behavior of molecules in bone marrow by NMR.

References:

1. Yeung DK Chem Phys Lipids; 151(2):103-9, 2008; **2.** Andrade MG et al., Cell Tiss Bank 9:279-287, 2008 **3.** van Putten LM, Ann N Y Acad Sci;114:695-700, 1964, **4.** Lammentausta et al., Phys Med Biol 53:543-55, 2008