Introduction

Fatty acid triglycerides (FATs) are ubiquitous in nature and serve as energy stores or precursors for lipid-based structures (e.g., cell membranes), hormones, and various intracellular signaling molecules. FATs in bone marrow are of interest as bone-marrow susceptibility effects have been exploited as a means to obtain information on trabecular bone density and structure. These methods paradoxically assume either the static dephasing regime to apply where diffusion is negligible, or that diffusion is significant enough so that the internal fields due to susceptibility are well sampled. Data on FAT diffusion would provide insight into the validity of the basic assumptions of these different methods. To the best of our knowledge, accurate values of FAT diffusion coefficients have not been reported. Using pulsed-gradient spin-echo (PGSE) NMR, we report FAT apparent diffusion coefficients (ADC) by means of a custom-built 50T/m z-gradient/RF coil set in order to probe the slow diffusion of FATs found in intact bone yellow marrow.

Method

Bone marrow was collected from lamb shanks, where marrow is entirely adipocytic, i.e., yellow marrow, and frozen for storage. Experiments were performed with a custom-built 50 T/m z-gradient with a solenoidal RF coil (4-turn, 3 mm i.d.) set interfaced to a 9.4 T spectrometer/micro-imaging system (Bruker DMX 400 with Micro2.5 gradients and BAFPA40 amplifiers). Samples were placed in a 3 mm NMR tube and experiments were conducted with a PGSE sequence at ambient temperature (19°C), where Δ and δ are the diffusion time and diffusion gradient duration, respectively. The gradient input voltage was measured and b-values were calculated assuming a rectangular pulse shape. In order to improve RF and gradient homogeneity, sinc pulses were used to select a 1 mm slice in the center of the RF coil leading to an excited volume of 4.5 μl. The NMR signal was manually shimmed. To test our technique, we measured the ADC of water (1.9 ± 0.3 cm²/s at 19°C, data not shown), which matched literature values.

First, the ADC of FAT in intact marrow was measured using the following parameters: TR=2.5 s, SW=7kHz, 1024 points, TE/Δδ=15.7/8/1 ms, NEX=4, and 64 b-values (bmax ~ 1.3×10⁸ s/cm²). The second experiment used a solution of marrow (12.5% w/v in CDCl₃): TR=2.5 s, SW=7kHz, 4096 points, TE/Δδ=15.7/8/1 ms, NEX=4, and 64 b-values (bmax ~ 7.8×10⁸ s/cm²). After Fourier transformation, the ADC was calculated from a linear fit of the logarithm of the amplitudes of each of the spectral peaks (averaging over 5 peak points if necessary) in the magnitude spectrum versus b-value. ADC calculations were based on the first 40 b-values as not all spectral components had the same SNR.

Results and Discussion

Fig. 1 shows bone marrow spectra obtained with our gradient/RF coil set and with a standard Bruker spectrometer. Fig 2 shows spectral peak attenuation versus b-value for various spectral components for intact marrow and 12.5% w/v marrow diluted in CDCl₃. Table 1 lists the calculated ADCs from linear fit of slopes in Fig. 2 (R²≈0.99). There is clearly more dispersion in the ADCs calculated among different sites in the FAT molecule. A t-test between pairs of regression slopes indicated that the ADCs measured in the intact marrow were significantly different from each other (all p < 0.005) whereas the ADCs measured in the marrow solution were not significantly different (all p > 0.05 except one p = 0.04). Diffusion minimizes intermolecular associations and enhances the rate of diffusion by two orders of magnitude.

Although the current gradient coil design did not allow for temperature control, we believe that the order of magnitude of FAT ADCs would not change significantly at the more physiologically relevant temperature of 37°C. It is noted that the diffusion coefficient of water changes by a factor of 1.5 as the temperature is raised from 20 to 40 °C. The intact marrow FAT ADCs reported here would therefore suggest that FAT diffusion is slow enough to fulfill the static dephasing regime. Further, the slow FAT ADC suggests that the applicability of methods that rely on diffusive sampling of internal fields would be severely limited.

Fig. 2 Spectral peak attenuation versus b-value for (a) intact marrow and (b) 12.5% w/v marrow in CDCl₃. Peaks were normalized to Mid-chain peak.

Conclusion

Diffusion behavior of FAT in lamb shank marrow was investigated using a home-built 50T/m z-gradient/RF coil set. The ADC of FAT in intact bone yellow marrow was found to be three orders of magnitude slower than that of water suggesting that the static dephasing regime is fulfilled and there is no significant diffusive sampling of internal fields at diffusion times of tens of milliseconds.


Is bone marrow diffusion too slow or too fast for susceptibility-based methods to assess trabecular bone architecture?

H. H. Ong¹, A. C. Wright¹, S. L. Wehrli², C. E. Jones², and F. W. Wehrli¹

¹Laboratory for Structural NMR Imaging, Department of Radiology, University of Pennsylvania School of Medicine, Philadelphia, PA, United States, ²NMR Core Facility, Children’s Hospital of Philadelphia, Philadelphia, PA, United States