HRMAS NMR spectroscopic analysis and quantification of marrow adiposity in the B6 mouse model after exposure to rosiglitazone treatment

Keerthi Shet[1], Hikari Yoshihara[1], John Kurhanewicz[1], Ann Schwartz[2], Cliff J Rosen[3], and Xiaojuan Li[1]

[1]Radiology, University of California, San Francisco, San Francisco, CA, United States, [2]Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, United States, [3]Jackson Laboratory, Bar Harbor, Maine

Introduction

Bone marrow adiposity is related to both bone quantity and bone quality [1]. It has been observed in clinical proton magnetic resonance (1H-MRS) studies that increased bone marrow adiposity is associated with decreased bone mineral density (BMD) in patients with osteoporosis or osteopenia [2-4]. However, only very limited studies in the literature have investigated whether the composition of marrow adipose tissue (MAT) changes as bone density declines, and these studies have provided mixed results [3, 5]. A recent study showed preliminary evidence that suggested that less unsaturated and more saturated fatty acids in MAT, as measured by in vivo MRS, predicted fractures in postmenopausal women with or without type-2 diabetes [6]. Thus, the composition of MAT together with the quantification of the saturated and unsaturated components during the aging process and exposure to drug regimens is crucial in developing an understanding of the paracrine effects of marrow adiposity of skeletal remodeling. The mechanism that triggers an increase in marrow fat and reduces bone mineral density in rodents (and humans) can be externally activated by the anti-diabetic drug rosiglitazone. In particular, in C57BL/6J (B6) the drug induced bone loss is linked to increase in marrow adiposity and these changes are magnified as the B6 mice age [7, 8]. However, it is not known if the changes in adiposity are also associated with changes in MAT composition and if these changes vary with age. The objective of this study is to use high resolution magic angle spinning spectroscopy at 11.7T to study the effects of aging and rosiglitazone exposure in the B6 model and to further explore the relationship of bone marrow fat with skeletal metabolism. Our long term goal is to develop non-invasive MR based quantitative imaging techniques for evaluating bone marrow adiposity that will be capable of identifying novel imaging markers for determining bone quality and fracture risk.

Materials and methods

Specimen preparation: Marrow samples were extracted from the central distal femur B6 mice and flash-frozen at -80˚C in 200 µL eppendorf tubes storage until further biochemical analysis. Marrow specimens were extracted from 12 week old mice for the control vs. rosiglitazone treated study. HRMAS NMR spectroscopy protocol: 10-15uL of the marrow specimen was pipetted out into a 30µL zirconium rotor and scanned in a 500 MHz spectrometer while being spun at a rate of 2.25 kHz. 5µL of TSP standard was added to provide the locking signal and to obtain a chemical reference at 0 ppm. Spectral data was acquired following a single 90˚ RF pulse acquisition which was preceded by a water pre-saturation pulse to attenuate the water signal at 4.8 ppm. 124 signal averages were acquired with a total acquisition time of 12 minutes and a spectral bandwidth of 20 kHz. Spectral Data processing: The resulting HRMAS spectra was processed using ACD Labs 1D NMR processor (ver. 9.0). The 1D FID's were zero-filled to 131K points, apodized with an exponential function, Fourier transformed, phase corrected, baseline corrected and referenced to TSP at 0ppm. Quantitation was achieved using the peak fitting/ integration routines in ACD Labs processor. Quantification technique: The triglyceride composition was calculated using the method described in the literature [8]. The resulting fraction of fatty acids that are doubly unsatured (FDU), mono unsaturated (FMU), unsaturated (FU=FDU+FMU), and saturated (FS) was determined in each case. To account for the inter-sample/experimental setup variations, all measurements were normalized to the 0.9 ppm resonance which was assigned a fixed integral value of 100 units in case of all samples.

Results

Figure 1 shows sample NMR spectra of marrow specimen harvested from a B6 mice. The resonances (0.9, 2.03, 2.25, 2.77, 5.31 ppm) used in calculating the triglyceride composition as described in [8] and the corresponding fatty acid chain are also indicated. The difference in fatty acids composition of MAT of control (N=3) vs. rosiglitazone treated group (N=3) is provided in Figure 2. An increase in FDU (p=0.035) and decrease in FMU (p=0.002) with rosiglitazone treatment was observed and this change was statistically significant ($p < 0.05$). A trend of decreased FU (p=0.08) and increased FS (p=0.085) was also observed in the treated mice group.

Conclusion and Discussion

Our study showed the feasibility of using HRMAS NMR to quantify MAT composition with high-resolution spectra. Previous studies observed significantly increased MAT and decreased bone mass and strength with rosiglitazone treatment, through activation of peroxisome proliferator-activated receptor-gamma. These changes are magnified as B6 mice age. Our results suggested that there are significant changes in MAT composition along with the rosiglitazone-induce MAT increase. In particular, the decreased unsaturated lipids and increased saturated lipids may be correlated with decreased bone strength with rosiglitazone treatment. Thus, consistent with the observation in vivo that decreased MAT unsaturation level is associated with fracture risk [6], the fatty acid unsaturation/saturation level of MAT can be a potential imaging marker for bone quality and fracture. In this ongoing study, we will perform measurement in mice at different ages with and without treatment, and correlate MAT composition with bone mass and strength measures.

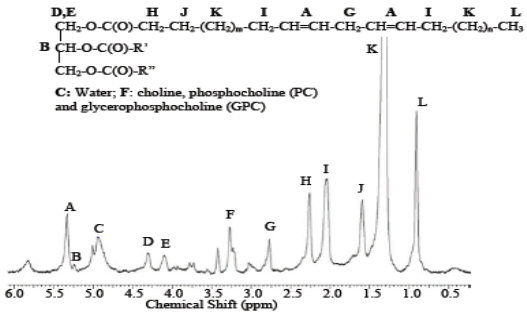

Figure 1. Representative spectra of a bone marrow sample from B6 mice

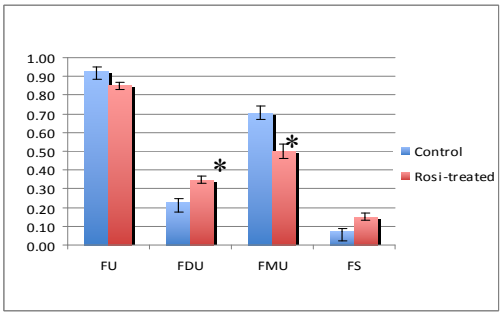

Figure 2. Comparison of fatty acid composition in control and treated mice

References:

1. Rosen, C.J., et al.,Crit. Rev Eukaryot Gene Expr, 2009. 19(2):p.109-24.
2. Griffith, J., et al., Radiology, 2005. 236(3):P.945-51.
3. Yeung, D., et al., J Magn. Reson Imag, 2005. 22(2):p.279-85.
4. Blake, G., et al., Bone, 2008
5. Griffith, J.F., et al., Bone, 2009. 44(6): p. 1092-6.
6. Patsch, J., et al., Talk #FR0049 , ASBMR (2011), San Diego, CA.
7. Lazarenko, O.P., et al., Endocrinology, 2007. 148(6):p.2669-80.
8. Ren, J., et al., J Lipid Res. 2008. 49: p. 2055-62.