Published in final edited form as:

NMR Biomed. 2012 April ; 25(4): 545–555. doi:10.1002/nbm.1770.

Assessment of Subchondral Bone Marrow Lipids in Healthy Controls and Mild Osteoarthritis Patients at 3T

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Abstract

The compartment-specific lipid changes in femoral-tibial bone of healthy controls and mild osteoarthritis (OA) patients were quantified at 3.0T. Healthy volunteers (Kellgren Lawrence [KL] grade = 0; n = 15, 4 females, 11 males, mean age 39 ± 16 years, age range = 24–78 years) and mild OA patients (KL = 1, 2; n = 26, 12 females, 14 males, mean age 61 ± 14 years, age range = 27–80 years) were scanned on a 3T scanner. Clinical proton density (PD)-weighted fast spin echo (FSE) images in sagittal (without fat-saturation), axial, and coronal (fat-saturation) planes were acquired for cartilage Whole-Organ MR Imaging Score (WORMS) grading. A voxel of $10 \times 10 \times 10$ mm³ was positioned in the medial and lateral compartments of the tibia (MT, LT) and femur (MF, LF) for proton MR Spectroscopy (MRS) measurements using the single voxel stimulated echo acquisition mode (STEAM) pulse sequence. All MRS data were processed with Java-based Magnetic Resonance User Interface (JMRUI). Wilcoxon rank sum test and mixed model two-way analysis of variance (ANOVA) were performed to determine significant differences between different compartments as well as examine the effect of OA grade and compartment, and their interactions. Generally, the MF compartment index of unsaturation was increased in healthy subjects compared to OA subjects (whether graded by KL or WORMS score). Differences between MF at KL0 and all other compartments at KL1 except LF approached statistical significance ($P < 0.05$). Differences in saturated lipids signals could be observed predominantly in the 2.03 ppm frequency shift. Healthy controls in MF compartment had the lowest saturated lipid signals, and mild OA patients with KL2 and WORMS5–6 in MF compartment had the highest saturated lipid signals compared to other compartments at 2.03 ppm ($P < 0.05$).

Keywords

proton MR spectroscopy; knee; osteoarthritis; index of unsaturation; saturated lipids; bone marrow lipids; lipid metabolism; T₂*

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Key findings presented in the paper:

Our results suggest that the indices of unsaturation in the MF compartment, the peaks of saturated lipids at 2.03 ppm, and the median T₂* values of the femoral-tibial bone marrow at 1.3 ppm and 5.31 ppm in LF, MT, and MF compartments may be clinically potential biomarkers for assessment of OA degeneration and progression.

INTRODUCTION

Proton MR Spectroscopy (^1H -MRS) can provide a non-invasive method to quantify biochemical or metabolic changes in various diseases. Single voxel spectroscopy (SVS) is a simple commonly used technique for in vivo examination of metabolites within a small volume of tissue. MR spectroscopic imaging (MRSI) allows the examination of the spatial distribution of metabolic changes within tissues (1). Both techniques provide a powerful noninvasive and nondestructive chemical assessment tool for studying vertebral body bone marrow (2), evaluating the metabolites and biochemical profiles in gliomas and other human brain tumors (3–5), and investigating the lipid metabolism of human skeletal muscles (6–12).

Osteoarthritis (OA) is the main cause of mobility-related disability in elderly persons (2, 3). While cartilage loss is the leading pathologic feature of OA, abnormal bone has been documented as another possible etiology (13, 14). Previous work (13, 14) has revealed that bone marrow edema-like (BMEL) lesions are a potential risk factor for structural deterioration in knee OA, and bone marrow edema-like lesions strongly correlate with the presence of pain in patients with OA. Another study (2) has described increased bone marrow fat in subjects with osteoporosis; specifically, saturated lipids increase preferentially compared to unsaturated lipids. On the other hand, significantly elevated water and unsaturated lipids, and decreased saturated lipids are seen in bone marrow edema-like lesions that are subjacent to areas of cartilage degeneration in OA (1).

Although previous studies have used the MRSI method to measure the major components of the signal within bone marrow edema-like lesions, including water and lipids (1, 2), to the best of our knowledge, there have been no quantitative assessments of subchondral bone marrow lipids alone in different compartments of femoral-tibial bone in healthy controls and mild OA patients at 3T. Therefore, the objective of the present study was to quantify the compartment-specific lipid changes in femoral-tibial bone in healthy controls and mild OA patients at 3.0T.

EXPERIMENTAL METHODS

Water Phantom for Normalization

Characteristic spectra from subchondral bone marrow regions (LF, LT, MF, MT) of the knee in healthy and mild OA subjects consisted of known lipid peaks between 0.5 and 2.3 ppm and at 5.3 ppm with no water resonance at 4.7 ppm. Since in vivo metabolites are subject to variations between individuals and variations over time within the same individual, a fixed and stable external signal was used for reliable measurement of changes in all lipid components. The external signal consisted of the water peak from the Siemens water phantom (plastic bottle 1000 ml, 31-11-361-K2140, per 1000g H_2O distilled, 1.25g $\text{NiSO}_4\text{X}6\text{H}_2\text{O}$, 5g NaCl). The water signal was used for normalization of in-vivo lipid peaks.

Human Subjects

Forty-one subjects ($n = 25$ males and $n = 16$ females, ranging in age from 24 to 80 years, mean $\pm$ SD = 53 ± 18 years) (Table 1) in total were recruited. The subjects were classified as being healthy or as having mild OA based on radiographs [Kellgren-Lawrence (KL) grading scale 0, 1, and 2; $n = 15$ as KL0, $n = 12$ as KL1, $n = 14$ as KL2] (15). The subjects' body height and weight were obtained in order to calculate the body mass index (BMI). Subjects with a BMI of greater than 24.9 were classified as overweight, and subjects with a BMI of greater than 29.9 were classified as obese (16). The mean BMI of the subjects included in this study was 25.6 ± 3 . The BMI was within the normal range in 24 subjects (59%); 10

subjects were overweight (24%) and 7 subjects were obese (17%). All the subjects provided written informed consent to participate in the study, which was approved by the local institutional review board (IRB).

Imaging Hardware

All experiments were performed using a 3.0T clinical scanner (MAGNETOM Trio, Siemens Medical Solutions, Erlangen, Germany). An 18-cm inner-diameter 8 channel transmit/receive knee RF coil (In Vivo Corp., Gainesville, FL) was employed for all the MR imaging and spectroscopy measurements.

MR Imaging and Spectroscopy

The morphology of the cartilage was assessed by acquiring clinical sagittal proton density (PD)-weighted without fat saturation images and axial and coronal PD-weighted fast spin echo (FSE) fat-saturation images for cartilage Whole-Organ MR Imaging Score (WORMS) grading.

The parameters for the sagittal PD-weighted without fat saturation sequence were: TR/TE = 3000ms/19ms, slice thickness = 2.5 mm, NEX = 1, FOV = 100 mm, matrix = 512×512, in-plane spatial resolution = 0.19 mm × 0.19 mm, pixel bandwidth = 255 Hz/Pixel, echo train length = 5.

The parameters for the axial PD-weighted FSE fat-saturation sequence were: TR/TE = 3240ms/19ms, slice thickness = 2.5 mm, NEX = 1, FOV = 100 mm, matrix = 512×512, in-plane spatial resolution = 0.19 mm × 0.19 mm, pixel bandwidth = 255 Hz/pixel, echo train length = 5.

The parameters for the coronal PD-weighted FSE fat-saturation sequence were: TR/TE = 3240ms/19ms, slice thickness = 2.5 mm, NEX = 1, FOV = 100 mm, matrix = 512×512, in-plane spatial resolution = 0.19 mm × 0.19 mm, pixel bandwidth = 255 Hz/pixel, echo train length = 5.

For the spectroscopy measurements of the external reference water phantom, the MRS data were obtained from a 1 cm³ voxel in the four different spatial positions (top right, bottom right, top left, bottom left) (Fig. 1 (a)) using the stimulated echo acquisition mode (STEAM) pulse sequence with TE = 20 ms, TR = 5000 ms, 1024 data points, spectral bandwidth = 2000 Hz, number of averages = 15. The acquisition time was 2 minutes 14 seconds. The measurements were repeated four times with four types of voxel position shifts being the same between each measurement: Anterior-Posterior (AP)/Left-Right (LR)/Feet-Head (FH) = 66 mm/41 mm/4 mm for top right voxel position; 66 mm/45 mm/58 mm for bottom right; 66 mm/15 mm/25 mm for top left, and 77 mm/11 mm/58 mm for bottom left, respectively.

For all spectroscopy measurements of subchondral bone marrow lipids in the four different compartments of femoral-tibial bone (LF, LT, MF, MT), after localizer images were acquired, a voxel of 10×10×10 mm³ was positioned in the corresponding compartment of femoral-tibial bone. Fig. 1 (b) shows representative single voxel locations in different compartments of femoral-tibial bone (LF, LT, MF, MT) from an OA subject. All MRS data were obtained from a 1 cm³ voxel using the stimulated echo acquisition mode (STEAM) pulse sequence with TE = 20 ms, TR = 2000 ms, 1024 data points, spectral bandwidth = 2000 Hz and without water suppression, number of averages = 50. The acquisition time was 2 minutes 36 seconds. To evaluate the intra-and inter-subject reproducibility of MRS measurement, four subjects (OA patients) were scanned two times with voxel position shifts between two measurements (Fig. 1(b)). The coefficient of variation (CV) is computed and

reported for each subject separately. The root mean square (RMS) value of the CV across the subjects is reported as well.

Data Analysis and Spectral Fitting

The clinical radiologic evaluation of the cartilage was performed using 2D-sagittal PD-weighted FSE without fat saturation as well as axial and coronal PD-weighted FSE with fat saturation images. Clinical evaluation of the knee joint was performed by an experienced musculoskeletal radiologist (by __). The radiologist was blinded to subjects' specific information and KL scores. Cartilage scoring was performed using the WORMS grading as defined in (17): 0 = normal thickness and signal; 1 = normal thickness but increased signal; 2.0 = partial thickness focal defect <1 cm in greatest width; 2.5 = full thickness focal defect <1 cm in greatest width; 3 = multiple areas of partial thickness (grade 2.0) defects intermixed with areas of normal thickness, or a grade 2.0 defect wider than 1 cm but <75% of the region; 4 = diffuse ($\geq 75\%$ of the region) partial thickness loss; 5 = multiple areas of full thickness loss (grade 2.5) or a grade 2.5 lesion wider than 1 cm but <75% of the region; 6 = diffuse ($\geq 75\%$ of the region) full-thickness loss. Subjects with cartilage changes graded as 0 to 1 were assigned as normal, 2 to 4 as early degeneration, and 5 to 6 as advanced degeneration. Representative images of cartilage ranging from normal to severe degeneration are shown in Fig. 2.

All MRS data were exported and processed on an offline computer using the Java-based Magnetic Resonance User Interface (JMRUI) (18) software using AMARES (Advanced Method for Accurate, Robust and Efficient Spectral) (19) time domain fitting procedure. The water resonance at 4.7 ppm was absent from all subchondral regions of bone marrow in the knee joint. Observed resonances include the olefinic ($-\text{CH}=\text{CH}-$) peak at 5.31 ppm, the methylene peaks at 1.3 ppm ($-(\text{CH}_2)_n-$) and 2.03 ppm ($-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$) and the methyl ($-\text{CH}_3$) peak at 0.9 ppm, which were fitted with Lorentzian lines. Peak integrals of these resonances were corrected for coil loading with the transmitter reference amplitude (20) from individual measurements, and then used to normalize the methyl, methylene, and olefinic lipid signals to the corrected peak integral value of the external reference water phantom. Lateral Femur (LF) was normalized to the top right voxel of the phantom, LT to the bottom right, MF to the top left, and MT to the bottom left voxel of the phantom, respectively using the following equation different from that in (1, 2):

$$\frac{I(0.9\text{ppm})}{I(\text{phantom})}, \frac{I(1.3\text{ppm})}{I(\text{phantom})}, \frac{I(2.03\text{ppm})}{I(\text{phantom})}, \frac{I(5.31\text{ppm})}{I(\text{phantom})} \quad (1)$$

The normalized peak integrals were also used in the calculation of the index of unsaturation (2):

$$U = \frac{I(5.31\text{ppm})}{I(5.31\text{ppm}) + I(1.3\text{ppm}) + I(2.03\text{ppm}) + I(0.9\text{ppm})} \quad (2)$$

A Wilcoxon rank sum test was used to determine the significance of differences in the index of unsaturation, saturated lipid signals, and T_2^* values (measured via line widths) of the voxel of interest (VOI) among the four different compartments (LF, LT, MF, MT) of femoral-tibial bone between healthy controls and mild OA patients. For each comparison, a P value of less than or equal to 0.05 was considered to indicate a significant difference.

Mixed model two-way analysis of variance (ANOVA) was also performed to examine the effect of OA grade and compartment on each study endpoint. A separate analysis was

conducted for each endpoint. In each case, the dependent variable consisted of the endpoint values obtained from each subject within each of the four compartments (LF, LT, MF, and MT). The model included OA grade (represented as KL or WORMS in separate analyses) and compartment as fixed classification factors as well as the term representing their interaction. A significant interaction would imply that differences among compartments vary as a function of OA grade; in this case, the comparisons among compartments were stratified by OA grade. To adjust for multiple comparisons, post hoc pair-wise comparisons among compartments were conducted only if the composite test of the main effect for compartment was found to be significant at the Bonferroni corrected level of $0.05/9 = 0.0056$ (there were nine study endpoints: the index of unsaturation; saturated lipids at 0.9 ppm, 1.3 ppm, and 2.03 ppm; unsaturated lipids at 5.31 ppm; T_2^* at 0.9 ppm, 1.3 ppm, 2.03 ppm, and 5.31 ppm) and the pair-wise comparisons were subjected to a Tukey multiple comparison correction. To account for statistical dependencies among the observations derived for the same subject, an anonymous subject identifier was incorporated into the model as a random classification factor. As a result, observations were treated as correlated when acquired from the same subject and were considered independent when obtained from different subjects. All reported P values are two-sided. Statistical significance was defined as $P < 0.05$. SAS 9.3 (SAS Institute, Cary, NC) was used for all computations.

RESULTS

13 subjects were clinically evaluated as WORMS0–1, 11 as WORMS2–4, and 17 as WORMS5–6, respectively.

All the indices of unsaturation, saturated lipid signals, and T_2^* values of the voxel of interest (VOI) among the four different compartments (LF, LT, MF, MT) of femoral-tibial bone in healthy controls and mild OA patients were quantified and categorized based on both conventional radiographs (KL) and standard clinical MRI (WORMS), respectively.

The CV varied between 0.7% and 5.1% for the model water phantom across all four spatial voxel regions (top right, bottom right, top left, and bottom left) (Fig. 1 (a)). The CVs and the RMS-CVs for the intra- and inter-subject MRS reproducibility of measurement in the four compartments (LF, LT, MF, and MT) are reported in Table 2 and shown in Fig. 1 (c), respectively.

The mixed model two-way analysis of variance (ANOVA) showed that there was no significant interaction between compartment and either KL score ($P > 0.19$) or WORMS ($P > 0.056$) in terms of their effect on any study endpoint. Irrespective of whether OA grade was represented as KL score or WORMS, the main effect of compartment (with 3 degrees of freedom) was significant at the Bonferroni-corrected level of 0.0056 for all endpoints except the index of unsaturation. As a result, pairwise comparisons between compartments were conducted for each endpoint except the index of unsaturation and the comparisons were adjusted for KL score and WORMS rather than stratified according to these factors (Table 3).

Fig. 3 shows box and whisker plots of bone marrow indices of unsaturation within the four compartments (LF, LT, MF, MT) based on KL grade (Fig. 3 (a)) and WORMS (Fig. 3 (b)) in healthy controls and mild OA subjects, respectively. The box and whisker plot shows the six statistics (minimum, first quartiles, mean (horizontal dotted line), median (horizontal thick line), third quartiles, and maximum). The corresponding values of median bone marrow indices of unsaturation among the four compartments (LF, LT, MF, MT) were 10.1 ± 1.4 , 9.4 ± 1.4 , 10.0 ± 0.9 , and 9.6 ± 1.2 for KL0, 9.0 ± 1.5 , 8.6 ± 1.5 , 8.9 ± 1.4 , and 8.6 ± 1.5 for KL1, 10.5 ± 2.1 , 8.2 ± 2.1 , 9.5 ± 3.5 , and 9.6 ± 4.3 for KL2, 10.2 ± 1.3 , 9.6 ± 1.5 ,

10.0 $\pm$ 1.0, and 9.7 $\pm$ 1.2 for WORMS0–1, 10.3 $\pm$ 3.4, 8.7 $\pm$ 1.6, 9.5 $\pm$ 1.8, and 9.6 $\pm$ 3 for WORMS2–4, 9.4 $\pm$ 3.1, 8.3 $\pm$ 1.8, 9.1 $\pm$ 3.1, and 8.9 $\pm$ 3.3, respectively. When categorized based on KL scores, an obvious trend was seen in that the indices of unsaturation decreased with increasing KL grade of cartilage mainly in LT compartment. Healthy controls had the highest index of unsaturation among them (Fig. 3(a)). There were significant differences ($P < 0.05$) in index of unsaturation between MF (KL0) and all other compartments (KL1) except LF (KL1) (Fig. 3(a)).

Box and whisker plots indicate the levels of saturated lipids at 0.9 ppm, 1.3 ppm, 2.03 ppm, and unsaturated lipids at 5.31 ppm (LF, LT, MF, MT) based on KL grade and WORMS in healthy controls and mild OA subjects are shown in Fig. 4 and Fig. 5, respectively. The corresponding saturated lipids at 0.9 ppm, 1.3 ppm, 2.03 ppm, and unsaturated lipids at 5.31 ppm (with the peak of the external reference water phantom used for normalization) varied between 0.06 $\pm$ 0.03 and 0.1 $\pm$ 0.09 for KL0, 0.05 $\pm$ 0.02 and 0.1 $\pm$ 0.06 for KL1, 0.06 $\pm$ 0.03 and 0.1 $\pm$ 0.07 for KL2 at 0.9 ppm; 0.43 $\pm$ 0.07 and 0.62 $\pm$ 0.12 for KL0, 0.51 $\pm$ 0.06 and 0.64 $\pm$ 0.17 for KL1, 0.5 $\pm$ 0.08 and 0.54 $\pm$ 0.2 for KL2 at 1.3 ppm; 0.06 $\pm$ 0.02 and 0.08 $\pm$ 0.03 for KL0, 0.08 $\pm$ 0.02 and 0.11 $\pm$ 0.03 for KL1, 0.09 $\pm$ 0.02 and 0.1 $\pm$ 0.04 for KL2 at 2.03 ppm; 0.06 $\pm$ 0.01 and 0.09 $\pm$ 0.02 for KL0, 0.06 $\pm$ 0.01 and 0.08 $\pm$ 0.03 for KL1, 0.07 $\pm$ 0.03 and 0.08 $\pm$ 0.03 for KL2 at 5.31 ppm; 0.06 $\pm$ 0.03 and 0.11 $\pm$ 0.09 for WORMS0–1, 0.06 $\pm$ 0.04 and 0.09 $\pm$ 0.06 for WORMS2–4, 0.05 $\pm$ 0.02 and 0.1 $\pm$ 0.07 for WORMS5–6 at 0.9 ppm; 0.45 $\pm$ 0.07 and 0.58 $\pm$ 0.09 for WORMS0–1, 0.45 $\pm$ 0.07 and 0.63 $\pm$ 0.17 for WORMS2–4, 0.51 $\pm$ 0.14 and 0.596 $\pm$ 0.14 for WORMS5–6 at 1.3 ppm; 0.07 $\pm$ 0.01 and 0.07 $\pm$ 0.02 for WORMS0–1, 0.08 $\pm$ 0.02 and 0.11 $\pm$ 0.02 for WORMS2–4, 0.09 $\pm$ 0.02 and 0.11 $\pm$ 0.04 for WORMS5–6 at 2.03 ppm; 0.06 $\pm$ 0.01 and 0.08 $\pm$ 0.01 for WORMS0–1, 0.06 $\pm$ 0.02 and 0.09 $\pm$ 0.03 for WORMS2–4, and 0.08 $\pm$ 0.03 and 0.07 $\pm$ 0.03 at 5.31 ppm, respectively (Fig. 4, Fig. 5). There were significant differences ($P < 0.05$) in saturated lipids at 1.3 ppm between MT (KL0) and all compartments (KL1 and KL2) (Fig. 4(b)). There were significant differences ($P < 0.05$) in saturated lipids at 2.03 ppm between MF (KL0) and all compartments (KL2) (Fig. 4(c)). On the other hand, there were significant differences ($P < 0.05$) in saturated lipids at 1.3 ppm between MT (WORMS0–1) and all compartments (WORMS5–6), MT (WORMS2–4) and all other compartments (WORMS5–6) except MF (WORMS5–6) (Fig. 5(b)). There were significant differences ($P < 0.05$) in saturated lipids at 2.03 ppm between MF (WORMS0–1) and all compartments (WORMS5–6), MT (WORMS5–6) and all other compartments (WORMS0–1) except MT (WORMS0–1) (Fig. 5(c)).

Box and whisker plots of median T_2^* values at 0.9 ppm, 1.3 ppm, 2.03 ppm, and 5.31 ppm within the four compartments (LF, LT, MF, MT) based on KL grade and WORMS in healthy controls and mild OA subjects are shown in Fig. 6 and Fig. 7, respectively. The corresponding T_2^* values of bone marrow VOIs at 0.9 ppm, 1.3 ppm, 2.03 ppm, and 5.31 ppm among the four compartments (LF, LT, MF, MT) based on KL grade and WORMS varied between 7.3 $\pm$ 2.2 ms and 9.9 $\pm$ 3.0 ms for KL0, 9.0 $\pm$ 3.3 ms and 11.3 $\pm$ 2.4 ms for KL1, 8.3 $\pm$ 3.3 ms and 11.9 $\pm$ 3.9 ms for KL2 at 0.9 ppm; 5.4 $\pm$ 0.7 ms and 8.2 $\pm$ 1.8 ms for KL0, 7.1 $\pm$ 1.3 ms and 9.2 $\pm$ 1.4 ms for KL1, 6.9 $\pm$ 1.6 ms and 9.7 $\pm$ 2.9 ms for KL2 at 1.3 ppm; 6.4 $\pm$ 0.6 ms and 6.9 $\pm$ 0.5 ms for KL0, 6.7 $\pm$ 0.7 ms and 6.9 $\pm$ 0.4 ms for KL1, 6.3 $\pm$ 0.7 ms and 6.9 $\pm$ 1.3 ms for KL2 at 2.03 ppm; 4.2 $\pm$ 0.6 ms and 6.1 $\pm$ 1.7 ms for KL0, 6.3 $\pm$ 2.0 ms and 7.6 $\pm$ 1.4 ms for KL1, 5.9 $\pm$ 2.8 ms and 8.3 $\pm$ 2.6 ms for KL2 at 5.31 ppm; 7.3 $\pm$ 2.7 ms and 9.4 $\pm$ 2.9 ms for WORMS0–1, 8.1 $\pm$ 2.2 ms and 10.6 $\pm$ 2.9 ms for WORMS2–4, 8.8 $\pm$ 3.3 ms and 11.8 $\pm$ 3.7 ms for WORMS5–6 at 0.9 ppm; 5.4 $\pm$ 0.6 ms and 8.3 $\pm$ 1.8 ms for WORMS0–1, 6.6 $\pm$ 1.9 ms and 8.8 $\pm$ 1.9 ms for WORMS2–4, 7.1 $\pm$ 1.3 ms and 9.7 $\pm$ 2.4 ms for WORMS5–6 at 1.3 ppm; 6.4 $\pm$ 0.7 ms and 7.0 $\pm$ 0.5 ms for WORMS0–1, 6.4 $\pm$ 0.7 ms and 6.7 $\pm$ 0.5 ms for WORMS2–4, 6.6 $\pm$ 0.7 ms and 7.0 $\pm$ 1.1 ms for WORMS5–6 at 2.03 ppm; 4.1 $\pm$ 0.7 ms and 6.1 $\pm$ 1.8 ms for WORMS0–1, 5.5 $\pm$ 2.7 ms and 7.1 $\pm$ 2.2 ms for

WORMS2–4, and 6.4 ± 2.1 ms and 8.3 ± 2.0 ms for WORMS5–6 at 5.31 ppm, respectively (Fig. 6, Fig. 7). There were significant differences ($P < 0.05$) in mean T_2^* values at 0.9 ppm between MT (KL1) and all other compartments (KL0) except MT (KL0) (Fig. 6(a)). There were significant differences ($P < 0.05$) in mean T_2^* values at 1.3 ppm between MF (KL0) and all other compartments (KL1) except LF (KL1), MT (KL1) and all other compartments (KL0) except MT (KL0), LF (KL0) and all compartments (KL2), MF (KL0) and all other compartments (KL2) except LF (KL2), LT (KL2) and all other compartments (KL0) except MT (KL0), MT (KL2) and all other compartments (KL0) except MT (KL0) (Fig. 6(b)). There were significant differences ($P < 0.05$) in median T_2^* values at 5.31 ppm between MF (KL0) and all other compartments (KL1) except LF (KL1), MF (KL0) and all other compartments (KL2) except LF (KL2), LT (KL2) and all compartments (KL0) (Fig. 6(d)). On the other hand, there were significant differences ($P < 0.05$) in median T_2^* values at 0.9 ppm between MT (WORMS5–6) and all other compartments (WORMS0–1) except MT (WORMS0–1) (Fig. 7(a)). There were significant differences ($P < 0.05$) in median T_2^* values at 1.3 ppm between MF (WORMS0–1) and all other compartments (WORMS5–6) except LF (WORMS5–6), MT (WORMS5–6) and all other compartments (WORMS0–1) except MT (WORMS0–1) (Fig. 7(b)). There were significant differences ($P < 0.05$) in median T_2^* values at 5.31 ppm between MF (WORMS0–1) and all other compartments (WORMS5–6) except LF (WORMS5–6), LT (WORMS5–6) and all compartments (WORMS0–1), MT (WORMS5–6) and all other compartments (WORMS0–1) except LT (WORMS0–1) (Fig. 7(d)).

DISCUSSION

It has been demonstrated that bone marrow edema-like lesions and knee pain strongly correlate with the deterioration and progression of knee OA (13, 14). However, the cause of bone marrow lesions and their relationship to cartilage degeneration remain poorly understood. The investigation of bone marrow lesions in OA patients may provide further insight into disease pathogenesis and the risk for OA progression. ^1H -MRS is a useful technique for exploring the chemical composition of bone marrow in vivo and allows the evaluation of changes in the composition of bone marrow lipids (2, 21–24).

To the best of our knowledge, this is the first study to quantify the compartment-specific lipid changes in femoral-tibial subchondral bone in healthy controls and mild OA patients at 3.0T. In general, there was a slight trend for the indices of unsaturation of femoral-tibial bone marrow lipids to decrease with increasing WORMS score. This occurred predominantly within three compartments (LT, MF, and MT). However, more significant differences were seen mainly in the LF, LT, and MF compartments (Fig. 3(b)). In contrast, when KL scores were used to grade disease severity, significant differences were seen only between the MF compartment in healthy controls (KL0) and all other compartments except LF in mild OA patients (KL1) (Fig. 3(a)).

The peaks of saturated lipids at 1.3 ppm and 2.03 ppm (with the signal peak of the external reference water phantom used to normalize among the four femoral-tibial compartments) increased with increases in both KL grade and WORMS score. This was especially evident for the peaks of saturated lipids at 2.03 ppm. Compared to peaks of saturated lipids at 0.9 ppm and unsaturated lipids at 5.31 ppm, significant differences were seen predominantly in the peaks of saturated lipids at 2.03 ppm mainly within three compartments (LF, LT, and MF) of femoral-tibial subchondral bone (Fig. 4 and Fig. 5). This indicates that the peaks of saturated lipids at 2.03 ppm among the three compartments of femoral-tibial bone may represent an early marker to discriminate healthy and osteoarthritic knees.

Similarly, the median T_2^* values of the VOIs of the femoral-tibial subchondral bone marrow among the four compartments (LF, LT, MF, MT) in general increased with KL grade and WOMBS score. This was especially evident at 0.9 ppm, 1.3 ppm, and 5.31 ppm based on both KL grade and WOMBS (Fig. 6(a, b, d) and Fig. 7(a, b, d)). Significant differences were also seen predominantly in the median T_2^* values of the VOIs of the femoral-tibial bone marrow at 1.3 ppm and 5.31 ppm among the four compartments of femoral-tibial bone especially in LF, LT, and MF compartments based on both KL grade and WOMBS compared to those at 0.9 ppm and 2.03 ppm among the four compartments of femoral-tibial bone. This suggests that the median T_2^* values of the femoral-tibial bone marrow at 1.3 ppm and 5.31 ppm in the LF, LT, and MF compartments may represent potential reference measures by which to monitor OA degeneration and progression.

MRS offers a non-invasive approach to explore biochemical changes within bone marrow, especially alterations in water and lipid content (1). Previous work has reported that bone marrow edema is related to the progression of knee osteoarthritis, and it remains unclear whether it is causal or an epiphenomenon (25). Bone marrow edema pattern in OA knees represents histologically noncharacteristic abnormalities including bone marrow necrosis, bone marrow fibrosis, and trabeculae abnormalities and it may be a multi-faceted process of progressive deterioration (26). Others have also demonstrated preferentially increased saturated lipids in comparison with unsaturated lipids within the marrow of subjects with osteoporosis (2). The indices of unsaturation of femoral-tibial bone marrow lipids tended to decrease with increasing cartilage WOMBS score (Fig.3 (b)). Similarly, the peaks of saturated lipids at 1.3 ppm and 2.03 ppm (Fig. 4, Fig. 5) and the median T_2^* values (Fig. 6, Fig. 7) of the VOIs of the femoral-tibial subchondral bone marrow among the four compartments (LF, LT, MF, MT) roughly increased with KL grade and WOMBS score. All these findings in our current work appear to reveal that the indices of unsaturation, the peaks of saturated lipids, and the median T_2^* values of the femoral-tibial bone marrow may be potential metrics for assessing OA degeneration and progression in advance.

Furthermore, as is shown in Fig. 3(a, b), there were significant differences in the index of unsaturation mostly in the MF compartment in healthy controls and all other compartments in OA patients based on both KL grade and WOMBS score. In saturated lipids, statistically significant differences were also identified mostly between MT at 1.3 ppm, LF and MF at 2.03 ppm in healthy controls and all other compartments in OA patients based on both KL grade and WOMBS score (Fig. 4(b, c) and Fig. 5(b, c)). Similarly, there were also mostly significant differences in T_2^* values between LF, MF at 1.3 ppm and 5.31 ppm in healthy controls and all other compartments in OA patients based on both KL grade and WOMBS score (Fig. 6(b, d) and Fig. 7(b, d)). These results raise the possibility that the femoral-tibial compartments of MF, MT, and LF experience relatively higher weight-bearing and have a greater possibility of developing OA. This corresponds well with the results reported in the literature (16) as these femoral-tibial compartments have relatively more significant differences in index of unsaturation, saturated lipids, and T_2^* values between healthy controls and OA patients. The clinical importance of these findings is that they might be utilized to identify subjects who are at risk for OA progression. Our current work is still a preliminary study to evaluate the compartment-specific lipid metabolism changes in femoral-tibial bone of healthy controls and mild OA patients. Previous studies (27, 28) have hypothesized that OA may be a systematic disorder affecting the entire musculoskeletal system and involving altered lipid metabolism. Other studies (29, 30, 31) have suggested that OA may be a multi-factorial disorder in which disturbances of lipid homeostasis may be involved. Altogether, these investigations indicate that OA pathogenesis may be a complicated process and more work remains to be done concerning the potential reason and mechanism of lipid changes in OA.

There are limitations in this study. A relatively small number of subjects in each group ($n = 15$ for KL0, $n = 12$ for KL1, $n = 14$ for KL2) were studied. The age mismatch between healthy controls and mild OA subjects (39 ± 16 yrs vs. 61 ± 14 yrs) is also a deficiency of the current study. Future work will address this issue. Other limitations of this study are the subjectivity of: 1) the KL and WORMS scales as semi-quantitative, integer-based assessments of OA and 2) the manually-chosen VOIs within the four compartments of femoral-tibial bone (LF, LT, MF, MT). It should be noted, however, that the KL and WORMS systems are the current reference standards utilized to grade OA severity. Future studies involving larger sample sizes would help verify the results in the current study.

CONCLUSIONS

In this study, for the first time we have quantified the compartment-specific lipid changes in femoral-tibial bone of healthy controls and mild OA patients at 3.0T employing MR spectroscopy. Our results suggest that the indices of unsaturation in the MF compartment, the peaks of saturated lipids at 2.03 ppm, and the median T_2^* values of the femoral-tibial bone marrow at 1.3 ppm and 5.31 ppm in LF, MT, and MF compartments may potentially serve as biomarkers for the assessment of OA degeneration and progression.

Abbreviations

WORMS	Whole-Organ MR Imaging Score
OA	Osteoarthritis
KL	Kellgren Lawrence
LF	lateral femoral
LT	lateral tibial
MF	medial femoral
MT	medial tibial
VOI	voxel of interest
BMEL	Bone Marrow Edema-like Lesion
BMI	Body Mass Index
CV	Coefficient of Variation
RMS-CV	Root Mean Square Coefficient of Variation

Acknowledgments

The authors would like to acknowledge support by research grants RO1 AR053133 and RO1 AR056260 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH). Ding Xia, M.Sc, from Center for Biomedical Imaging, Department of Radiology, New York University Langone Medical Center, is thanked for technical support.

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Fig. 1.

First two rows of Fig.1 (a) show the representative images of the voxel positions in the external reference water phantom and the corresponding spectra with the voxel position being top left, top right, bottom left, and bottom right, respectively. The third row of Fig. 1 (a) shows the corresponding coefficients of variation (CVs) of measurement with each voxel position kept the same between four times of repeated MRS measurements. Fig.1 (b) shows the representative images of the voxel position shift of the in-vivo MRS measurement and the corresponding spectra in LF, LT, MF, and MT compartment, respectively. Four knee OA patients were scanned two times with different voxel position shift between two measurements. For subject 1 in LF compartment, Anterior-Posterior (AP)/Left-Right (LR)/Feet-Head (FH) = 81 mm/102 mm/10 mm for measurement 1, 68 mm/102 mm/10 mm for measurement 2, in LT, 75 mm/99 mm/44 mm for measurement 1, 64 mm/102 mm/43 mm for measurement 2, in MF, 76 mm/63 mm/10 mm for measurement 1, 61 mm/63 mm/11 mm for measurement 2, in MT, the lipid peaks were unable to be differentiated, so ignored (also see Table. 2); for subject 2 in LF, 75 mm/115 mm/19 mm for measurement 1, 80 mm/113 mm/20 mm for measurement 2, in LT, 79 mm/111 mm/53 mm for measurement 1, 61 mm/109 mm/53 mm for measurement 2, in MF, 66 mm/73 mm/25 mm for measurement 1, 82 mm/75 mm/26 mm for measurement 2, in MT, /61 mm/81 mm/55 mm for measurement 1, 73 mm/83 mm/56 mm for measurement 2; for subject 3 in LF, 80 mm/73 mm/10 mm for measurement 1, 68 mm/71 mm/10 mm for measurement 2, in LT, 64 mm/71 mm/43 mm for

measurement 1, 75 mm/73 mm/43 mm for measurement 2, in MF, 85 mm/ 29 mm/13 mm for measurement 1, 73 mm/27 mm/13 mm for measurement 2, in MT, 78 mm/28 mm/46 mm for measurement 1, 65 mm/26 mm/46 mm for measurement 2; for subject 4 in LF, 68 mm/109 mm/25 mm for measurement 1, 68 mm/109 mm/29 mm for measurement 2, in LT, 67 mm/111 mm/62 mm for measurement 1, 67 mm/111 mm/59 mm for measurement 2, in MF, 85 mm/61 mm/42 mm for measurement 1, 61 mm/61 mm/45 mm for measurement 2, and in MT, 79 mm/64 mm/66 mm for measurement 1, 80 mm/62 mm/65 mm for measurement 2, respectively. Fig. 1(c) shows the corresponding CVs of measurement at 0.9 ppm, 1.3 ppm, 2.03 ppm, and 5.31 ppm, respectively in each compartment with different voxel positions between two times of repeated MRS measurements.

Fig. 2.

Representative cartilage images from normal to severe degeneration based on WORMS grading. Arrowheads mark the location of cartilage lesions. WORMS = 0 for all regions, a) coronal proton density (PD)-weighted FSE fat-saturated, b) lateral and c) central sagittal PD-weighted without fat saturation; WORMS = 1 for lateral patellar facet, d) axial PD-weighted FSE fat-saturated; WORMS = 2 for medial posterior tibial plateau, e) sagittal PD-weighted without fat saturation; WORMS = 2.5 for medial femoral condyle, f) coronal PD-weighted FSE fat-saturated, g) sagittal PD-weighted without fat saturation; WORMS = 4 for medial femoral central condyle, h) coronal PD-weighted FSE fat-saturated, i) sagittal PD-weighted without fat saturation; WORMS = 5 for lateral patellar facet, j) axial PD-weighted FSE fat-saturated; WORMS = 5 for medial tibial anterior plateau, k) coronal PD-weighted FSE fat-saturated; WORMS = 6 for central tibial plateau, l) coronal PD-weighted FSE fat-saturated, m) sagittal PD-weighted without fat saturation; WORMS = 6 for lateral tibial plateau, n) sagittal PD-weighted without fat saturation.

Fig. 3.

Box and whisker plots comparing median bone marrow index of unsaturation among the four compartments (LF, LT, MF, MT) based on KL grade (a) and WORMS (b) in healthy controls and mild OA subjects. The horizontal dashed lines on the boxes show the corresponding mean values. The box and whisker plot shows the five statistics (minimum, first quartiles, median, third quartiles, and maximum). Fig. 3(a), there were significant differences ($P < 0.05$) in index of unsaturation between MF (KL0) and all other compartments (KL1) except LF (KL1). Fig. 3(b), significant differences ($P < 0.05$) existed in index of unsaturation between LF (WORMS0–1) and all other compartments (WORMS5–6) except LF (WORMS5–6), MF (WORMS0–1) and all other compartments

(WORMS5–6) except LF (WORMS5–6), LT (WORMS5–6) and all other compartments
(WORMS0–1) except LT (WORMS0–1).

Fig. 4.

Box and whisker plots comparing median saturated lipids at 0.9 ppm (a), 1.3 ppm (b), 2.03 ppm (c), and (d) median unsaturated lipids at 5.31 ppm among the four compartments (LF, LT, MF, MT) based on KL score in healthy controls and mild OA subjects. The horizontal dashed lines on the boxes show the corresponding mean values. Fig. 4(b), there were significant differences ($P < 0.05$) in saturated lipids at 1.3 ppm between MT (KL0) and all compartments (KL1), LF (KL1) and all other compartments (KL0) except LF (KL0), LT (KL1) and all other compartments (KL0) except LF (KL0), MT (KL0) and all compartments (KL2). Fig. 4(c), there were significant differences ($P < 0.05$) in saturated lipids at 2.03 ppm between LF (KL0) and all other compartments (KL1) except MF (KL1), MF (KL0) and all compartments (KL1), LT (KL1) and all compartments (KL0), LF (KL0) and all other compartments (KL2) except MT (KL2), MF (KL0) and all compartments (KL2), LT (KL2) and all other compartments (KL0) except LT (KL0). Fig. 4(d), there were significant differences ($P < 0.05$) in unsaturated lipids at 5.31 ppm between MT (KL0) and all other compartments (KL1) except MT (KL1), LF (KL0) and all other compartments (KL2) except LF (KL2).

Fig. 5.

Box and whisker plots comparing median saturated lipids at 0.9 ppm (a), 1.3 ppm (b), 2.03 ppm (c), and median unsaturated lipids at 5.31 ppm (d) among the four compartments (LF, LT, MF, MT) based on WORMS in healthy controls and mild OA subjects. The horizontal dashed lines on the boxes show the corresponding mean values. Fig. 5(b), there were significant differences ($P < 0.05$) in saturated lipids at 1.3 ppm between MT (WORMS0-1) and all compartments (WORMS5-6), LT (WORMS5-6) and all other compartments (WORMS0-1) except LF (WORMS0-1), MT (WORMS2-4) and all other compartments (WORMS5-6) except MF (WORMS5-6). Fig. 5(c), there were significant differences ($P < 0.05$) in saturated lipids at 2.03 ppm between LF (WORMS2-4) and all compartments (WORMS0-1), LT (WORMS2-4) and all compartments (WORMS0-1), LF (WORMS0-1) and all compartments (WORMS5-6), LT (WORMS0-1) and all other compartments (WORMS5-6) except MF (WORMS5-6), MF (WORMS0-1) and all compartments (WORMS5-6), LF (WORMS5-6) and all other compartments (WORMS0-1) except MT (WORMS0-1), LT (WORMS5-6) and all compartments (WORMS0-1), MT (WORMS5-6) and all other compartments (WORMS0-1) except MT (WORMS0-1).

Fig. 6.

Box and whisker plots comparing median T_2^* values at 0.9 ppm (a), 1.3 ppm (b), 2.03 ppm (c), and 5.31 ppm (d) among the four compartments (LF, LT, MF, MT) based on KL score in healthy controls and mild OA subjects. The horizontal dashed lines on the boxes show the corresponding mean values. Fig. 6(a), there were significant differences ($P < 0.05$) in mean T_2^* values at 0.9 ppm between MT (KL1) and all other compartments (KL0) except MT (KL0). Fig. 6(b), there were significant differences ($P < 0.05$) in mean T_2^* values at 1.3 ppm between LF (KL0) and all compartments (KL1), MF (KL0) and all other compartments (KL1) except LF (KL1), LT (KL1) and all other compartments (KL0) except MT (KL0), MT (KL1) and all other compartments (KL0) except MT (KL0), LF (KL0) and all compartments (KL2), MF (KL0) and all other compartments (KL2) except LF (KL2), LT (KL2) and all other compartments (KL0) except MT (KL0), MT (KL2) and all other compartments (KL0) except MT (KL0). Fig. 6(d), there were significant differences ($P < 0.05$) in median T_2^* values at 5.31 ppm between LF (KL0) and all compartments (KL1), MF (KL0) and all other compartments (KL1) except LF (KL1), LT (KL1) and all compartments (KL0), MT (KL1) and all compartments (KL0), LF (KL0) and all other compartments (KL2) except LF (KL2), MF (KL0) and all other compartments (KL2) except LF (KL2), LT (KL2) and all compartments (KL0).

Fig. 7.

Box and whisker plots comparing median T_2^* values at 0.9 ppm (a), 1.3 ppm (b), 2.03 ppm (c), and 5.31 ppm (d) among the four compartments (LF, LT, MF, MT) based on WORMS in healthy controls and mild OA subjects. The horizontal dashed lines on the boxes show the corresponding mean values. Fig. 7(a), there were significant differences ($P < 0.05$) in median T_2^* values at 0.9 ppm between LT (WORMS5-6) and all compartments (WORMS0-1), MT (WORMS5-6) and all other compartments (WORMS0-1) except MT (WORMS0-1). Fig. 7(b), there were significant differences ($P < 0.05$) in median T_2^* values at 1.3 ppm between LF (WORMS0-1) and all other compartments (WORMS2-4) except LF (WORMS2-4), LF (WORMS0-1) and all compartments (WORMS5-6), MF (WORMS0-1) and all other compartments (WORMS5-6) except LF (WORMS5-6), LT (WORMS5-6) and all other compartments (WORMS0-1) except MT (WORMS0-1), MT (WORMS5-6) and all other compartments (WORMS0-1) except MT (WORMS0-1). Fig. 7(d), there were significant differences ($P < 0.05$) in median T_2^* values at 5.31 ppm between LF (WORMS0-1) and all other compartments (WORMS2-4) except LF (WORMS2-4), LF (WORMS0-1) and all compartments (WORMS5-6), MF (WORMS0-1) and all other compartments (WORMS5-6) except LF (WORMS5-6), LT (WORMS5-6) and all compartments (WORMS0-1), MT (WORMS5-6) and all other compartments (WORMS0-1) except LT (WORMS0-1), LF (WORMS2-4) and all other compartments (WORMS5-6).

except LF (WORMS5–6), LT (WORMS5–6) and all other compartments (WORMS2–4)
except LT (WORMS2–4).

Table 1

Characteristics of Human Subjects (healthy and mild OA)

Subject Group and Characteristic	Healthy controls	Mild OA
All subjects		
No. of subjects	15	26
Age (yrs) *	39 ± 16	61 ± 14
BMI (kg/m ²)	25.1 ± 2.8	25.9 ± 3.8
Total WORMS **	2.7 ± 7.9	19.8 ± 16.6
Female subjects		
No. of subjects	4	12
Age (yrs)	37.8 ± 15.3	56.9 ± 15.2
Age range (yrs)	24 – 59	27 – 78
Male subjects		
No. of subjects	11	14
Age (yrs)	39.5 ± 17.2	64.3 ± 12.8
Age range (yrs)	25 – 78	46 – 80

WORMS = Whole-Organ MR Imaging Score.

BMI = Body Mass Index.

* There were significant differences ($P < 0.05$) in subjects' age between healthy controls and mild OA patients.** There were significant differences ($P < 0.05$) in Total WORMS between healthy controls and mild OA patients.

Table 2

The intra- (CV) and inter-subject (RMS-CV) MRS Reproducibility of Measurement in the four Compartments (LF, LT, MF, and MT)

CV (%)		LF	LT	MF	MT
0.9 ppm	Subject 1	9.7	7.2	8.7	NA*
	Subject 2	10.5	4.1	21.7	12.0
	Subject 3	8.6	19.7	2.9	4.4
	Subject 4	3.2	9.6	4.2	15.7
1.3 ppm	Subject 1	9.1	0.8	7.3	NA
	Subject 2	6.2	0.1	2.3	3.7
	Subject 3	12.2	0.9	11.2	0.4
	Subject 4	7.7	1.4	0.7	1.0
2.03 ppm	Subject 1	1.0	23.9	20.5	NA
	Subject 2	8.5	7.5	6.6	11.4
	Subject 3	4.0	16.6	12.2	4.4
	Subject 4	2.2	3.8	1.8	12.8
5.31 ppm	Subject 1	9.4	3.3	15.3	NA
	Subject 2	13.3	6.5	6.1	10.3
	Subject 3	7.0	9.0	13.9	7.0
	Subject 4	3.3	4.5	0.2	7.7
RMS-CV (%)	0.9 ppm	8.5	11.7	12.0	11.7
	1.3 ppm	9.1	0.9	6.8	2.2
	2.03 ppm	4.9	15.2	12.4	10.2
	5.31 ppm	9.0	6.2	10.8	8.4

NA - data not available (The lipid peaks were unable to be differentiated).

Tukey-corrected P values from the mixed model analysis to compare compartments adjusting for KL score. The comparisons among compartments adjusted for WORMS are perfectly consistent with the results tabulated here in the sense that the exact same set of comparisons were found to be significant after Tukey-correction.

Table 3

Comparison Items	LF vs. LT	LF vs. MF	LF vs. MT	LT vs. MF	LT vs. MT	MF vs. MT
Unsaturation Index	< 0.05	0.7489	0.4711	0.2728	0.5247	0.9698
Saturated Lipids at 0.9 ppm	0.0826	0.3303	< 0.05	0.8956	0.4657	0.1399
Saturated Lipids at 1.3 ppm	0.4114	< 0.05	< 0.0001	0.2744	< 0.05	0.4918
Saturated Lipids at 2.03 ppm	0.1121	0.7074	0.9127	< 0.05	< 0.05	0.9759
Unsaturated Lipids at 5.31 ppm	< 0.05	< 0.05	< 0.0001	0.9883	0.1226	0.2328
T ₂ * at 0.9 ppm	< 0.05	0.9929	< 0.05	< 0.05	0.9884	< 0.05
T ₂ * at 1.3 ppm	< 0.0001	0.0545	< 0.0001	< 0.0001	0.9990	< 0.0001
T ₂ * at 2.03 ppm	0.9845	< 0.05	0.5352	< 0.05	0.6875	0.9342
T ₂ * at 5.31 ppm	< 0.0001	0.0941	< 0.0001	< 0.0001	0.5459	< 0.05