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Evaluation of Subchondral Bone Marrow Lipids of Acute Anterior Cruciate Ligament (ACL)-Injured Patients at 3 T

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Abstract

Rationale and Objectives—The objectives of this study were to investigate the changes in compartment-specific subchondral bone marrow lipids of femoral–tibial bone in acute anterior cruciate ligament (ACL)-injured patients compared to that of healthy volunteers and patients with osteoarthritis (OA) (Kellgren–Lawrence [KL] grade 2–3).

Materials and Methods—A total of 55 subjects were recruited in the study and subdivided into three subgroups: 17 healthy controls (4 females, 13 males; mean age = 41 ± 16 , age range 24–78 years), 17 patients with acute ACL injury (3 females, 14 males; mean age = 30 ± 11 , age range 18–61 years), and 21 patients with KL2–3 OA (12 females, 9 males; mean age = 65 ± 12 , age range 44–89 years). Routine clinical proton density–weighted fast spin echo images in sagittal (without fat saturation), axial, and coronal (fat saturation) planes were acquired on a 3 T clinical scanner for cartilage morphology using Whole-Organ Magnetic Resonance Imaging Score grading. A voxel of $10 \times 10 \times 10 \text{ mm}^3$ was positioned in the medial and lateral compartments of the tibia and femur for proton magnetic resonance spectroscopy measurements using the single voxel stimulated echo acquisition mode pulse sequence. All proton magnetic resonance data were processed with Java-based magnetic resonance user interface. Wilcoxon rank sum test and mixed model two-way analysis of variance were performed to determine significant differences between different compartments and examine the effect of ACL injury, OA grade and compartment, and their interactions.

Results—The index of unsaturation in lateral tibial compartment in ACL-injured patients was significantly higher ($P < .05$) than all compartments except lateral femoral in patients with KL2–3

OA. Significantly lower values ($P < .05$) were also identified in saturated lipids at 2.03 ppm in all compartments in ACL-injured patients than those of all compartments in patients with KL2–3 OA.

Conclusions—The preliminary results suggest that the indices of unsaturation in the lateral tibial compartment and the peaks of saturated lipids at 1.3 and 2.03 ppm in medial tibial compartment may be clinically useful to characterize subchondral bone marrow among healthy controls, acute ACL-injured patients, and patients with OA.

Keywords

Proton MR spectroscopy; anterior cruciate ligament injury; osteoarthritis; bone marrow lipids; lipid metabolism

Single voxel spectroscopy is a simple commonly used technique for in vivo examination of metabolites within a small volume of tissue. This technique provides a powerful noninvasive and nondestructive chemical assessment tool for studying vertebral body bone marrow (1,2), evaluating the metabolites and biochemical profiles in gliomas and other human brain tumors (3), and investigating the lipid metabolism of human skeletal muscles (4–8).

Anterior cruciate ligament (ACL) injury is associated with increased risk for the development of posttraumatic knee osteoarthritis (OA) 10–20 years after the injury (9,10). Before the onset of structural changes, the cartilage tissue is subject to molecular modifications within the cartilage matrix (10). OA is the main cause of mobility-related disability in elderly persons (2). Although cartilage loss is the leading pathologic feature of OA, abnormal bone has been documented as another possible etiology (9,11–13). Felson et al. (11) have revealed that bone marrow edema-like (BMEL) lesions are a potential risk factor for structural deterioration in knee OA, and BMEL lesions strongly correlate with the presence of pain in patients with OA. On the other hand, significantly elevated water and unsaturated lipids, and decreased saturated lipids are seen in BMEL lesions that are subjacent to areas of cartilage degeneration in OA (1). Other literature (12–14) has indicated that some weight-bearing joints such as the knee and hip suffering from OA resulted from increased joint mechanical loading, and some possible risk factors such as obesity and knee pain because of previous injury may play an important role in the process of OA (13).

Although previous studies have used the magnetic resonance spectroscopy (MRS) imaging method to measure the major components of bone marrow signals including water and lipids (1,2,15), to the best of our knowledge, there have been no quantitative assessments of bone marrow lipids alone in different femoral–tibial bone compartments in acute ACL-injured patients. Therefore, we hypothesized in this study that the compartment-specific subchondral lipid variations may be possible precursor of articular degeneration in acute ACL-injured patients, and the MRS of bone marrow signals may be helpful to characterize subchondral bone marrow among healthy controls, acute ACL-injured patients, and patients with OA.

MATERIALS AND METHODS

Water Phantom for Normalization

Characteristic spectra from subchondral bone marrow regions (lateral femoral [LF], lateral tibial [LT], medial femoral [MF], and medial tibial [MT]) of the knee in healthy, acute ACLinjured, and OA subjects (KL2 or KL3) 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. Because in vivo metabolites are subject to variations between individuals and variations over time within the same individual, a fixed and stable external reference signal (water phantom) was used for reliable measurement of changes in all lipid components. The phantom replacement technique is best suited for assessing sub-chondral bone marrow lipids because there is no water signal to be used as an internal reference, and practical considerations rule out the use of an external reference vial placed in the coil along the patient knee. The phantom replacement technique and coil loading correction using the transmitter amplitude of a 90° pulse or the reference transmitter amplitude are techniques that have been validated by others in the field (16,17) and have been commonly used for metabolite quantification with in vivo MRS. The external reference signal was used for normalization of in vivo lipid peaks (15).

Human Subjects

A total of 55 volunteers ($n = 36$ males and $n = 19$ females, ranging in age from 18 to 89 years, mean $\pm$ standard deviation = 47 ± 20 years) (Table 1) were recruited. The volunteers were classified as being healthy or as having minimal-moderate OA based on radiographs (Kellgren–Lawrence [KL] grading scale 0, 2, and 3) (18). For the healthy subcohort, the inclusion criteria were as follows: absence of clinical symptoms (and based on radiographs) in the knee joint until the time of the magnetic resonance imaging (MRI) examination and age between 18 and 50 years. For the subcohort of patients with acute ACL tear, the inclusion criteria were as follows: either partial or complete disruption of ACL fibers with ligament discontinuity within 1–2 weeks of injury and age between 18 and 40 years. The patterns of ACL tears include anterior medial, posterior lateral, or full (both bundles) tears. For the OA subcohort, the inclusion criteria were the presence of symptoms (frequent knee pain including lateral, medial, patellofemoral, or a combination) and radiographic evidence of OA required to be in the same knee.

Anteroposterior radiographs of the knee in the standing position in all recruited volunteers were obtained to determine the KL grade. The radiographs were read by an experienced (15 years of experience) musculoskeletal radiologist (J.T.B.) who assigned a KL grade to each knee. The volunteers were then further divided into three groups as healthy controls, patients with acute ACL injury, and OA with minimal-moderate radiographic changes, respectively (Table 1). The volunteers' body height and weight were obtained to calculate the body mass index (BMI). Volunteers with a BMI >24.9 kg/m² were classified as overweight, and volunteers with a BMI >29.9 kg/m² were classified as obese (19). All the recruited volunteers provided written informed consent to participate in the study, which was approved by the local institutional review board.

Imaging Hardware

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

MRI and MRS

Themorphology 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 Magnetic Resonance Imaging Score (WORMS) grading (20).

Likewise, for the spectroscopy measurements of the external reference water phantom (phantom replacement method) and subchondral bone marrow lipids in the four different compartments of femoral–tibial bone (LF, LT, MF, and MT), the parameters for MRI and MRS measurements were the same as those in Ref. (15).

Data Analysis and Spectral Fitting

The clinical evaluation of cartilage and ACL injury was performed using two-dimensional–sagittal PD-weighted FSE without fat saturation and axial and coronal PD-weighted FSE with fat saturation by an experienced musculoskeletal radiologist (J.T.B., 15 years of experience). The radiologist was blinded to volunteers' specific information, KL scores, and MRS data. Cartilage scoring was performed using the WORMS grading as defined in Ref. (20): 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 (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; and 6 = diffuse (75% of the region) full-thickness loss.

All MRS data were exported and processed on an offline computer using the Java-based magnetic resonance user interface (21) software using the advanced method for accurate, robust, and efficient spectral (22) time domain fitting procedure. Stimulated echo acquisition mode sequence, which allows for a shorter echo time and is less sensitive to J-modulation, has been commonly used for fat quantification (23). Quantification of overlapping peaks has been successfully done on MR spectra with similar resolution as ours (24). Overlapping peak quantification is especially problematic in the frequency domain using simple processing techniques such as numerical integration of the peaks, although 2D sequences such as Localized Correlation Spectroscopy (L-COSY) have been used to possibly resolve the overlapping resonances (25). The MRS data were analyzed in the time domain with the advanced method for accurate, robust, and efficient spectral, which has been proven to be more accurate than other available techniques for fitting multiplets and overlapping peaks (22).

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 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 (15,16). Lateral femur was normalized to the top right voxel of the phantom, lateral tibia to the bottom right, medial femur to the top left, and medial tibia to the bottom left voxel of the phantom, respectively, using the following equation different from that in Refs. (1,2) in response to the water phantom replacement method (15,16) applied in the present work:

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

The normalized peak integrals were also used in the calculation of the index of unsaturation (2,15):

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

All the indices of unsaturation, saturated and unsaturated lipid signals of the voxel of interest (VOI) among the four different compartments (LF, LT, MF, and MT) of femoral–tibial bone in healthy controls, ACL-injured patients, and patients with KL2–3 OA were quantified and categorized based on conventional radiographs (KL).

A Wilcoxon rank sum test was used to determine the significance of differences in the index of unsaturation and saturated lipid signals of the VOI among the four different compartments (LF, LT, MF, and MT) of femoral–tibial bone of healthy controls, acute ACL-injured patients, and patients with KL2–3 OA. For each comparison, a *P* value of 0.05 was considered to indicate a statistically significant difference.

Analysis of variance (ANOVA) was performed to compare volunteer groups (healthy controls, ACL-injured patients, and patients with KL2–3 OA) in terms of each study endpoint. A separate analysis was conducted for each endpoint within each of the four compartments (LF, LT, MF, and MT). In each case, the endpoint was the dependent variable and the model included subject group as the classification factor. Analysis of covariance (ANCOVA) was performed to make these same comparisons when adjusted for age, gender, and BMI. A Kolmogorov–Smirnov test was applied to the residuals of each model to assess the underlying assumption of normality. In all cases, rejection of the hypothesis of normality led to a reanalysis of the relevant data following a normalizing transformation. To adjust for multiple comparisons, post hoc pairwise comparisons among volunteer groups were conducted only if the composite test of the main effect for group was found to be significant at the Bonferroni-corrected level of $0.05/9 = 0.0056$, where the divisor corresponds to the

number of study endpoints, and the pairwise comparisons for any endpoint were subjected to a Tukey multiple comparison correction. All reported P values are two-sided. Statistical significance was defined as $P < .05$. SAS 9.3 (SAS Institute, Cary, NC) was used for all computations.

RESULTS

Twenty-four subjects were clinically evaluated as WORMS0–1, 16 as WORMS2–4, and 15 as WORMS5–6, respectively. When graded based on KL scoring, 34 subjects were graded as KL0, 16 as KL2, and 5 as KL3, respectively. All the subjects were then divided into three groups as healthy controls ($n = 17$, KL = 0), patients with acute ACL injury ($n = 17$, KL = 0), and patients with minimal-moderate (KL2–3) OA ($n = 21$, KL = 2 or KL = 3), respectively (Table 1). The mean BMI of the subjects included in this study was 25.4 ± 3.4 kg/m². The BMI was within the normal range in 31 subjects (56%); 16 subjects were overweight (29%) and 8 subjects were obese (15%).

Table 2 lists the mean $\pm$ standard deviation of each study endpoint within each subject group. Each P value is from the composite test of group differences as determined by ANOVA or ANCOVA. Table 3 displays the Tukey-corrected P values from ANOVA and ANCOVA to pairwise compare subject groups in terms of each endpoint without and with adjustment for age, gender, and BMI, respectively. Results are provided only for endpoints showing a significant composite test for group differences from both ANOVA and ANCOVA.

Figure 1 shows representative single voxel locations in different compartments of femoral–tibial bone (LF, LT, MF, and MT) from an acute ACL-injured subject with Figure 1a (LF), Figure 1b (LT), Figure 1c (MF), and Figure 1d (MT), respectively.

Assessment of Index of Unsaturation

Figure 2 shows the box and whisker plots comparing bone marrow index of unsaturation among the four compartments (LF, LT, MF, and MT) of healthy controls, ACL-injured patients, and patients with KL2–3 OA. 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).

As shown in Figure 2, the index of unsaturation was significantly higher ($P < .05$) predominantly in LT compartment in ACL-injured patients than that of all compartments except LF in patients with KL2–3 OA.

Assessment of Saturated and Unsaturated Lipids

Box and whisker plots comparing saturated lipids at 0.9 ppm (Fig. 3a), 1.3 ppm (Fig. 3b), 2.03 ppm (Fig. 3c), and unsaturated lipids at 5.31 ppm (Fig. 3d) among the four compartments (LF, LT, MF, and MT) of healthy controls, ACL-injured patients, and patients with KL2–3 OA are shown in Figure 3. The horizontal dashed lines on the boxes show the corresponding mean values.

As shown in Figure 3a, the values of saturated lipids at 0.9 ppm predominantly in the MT compartment in healthy controls were significantly higher ($P < .05$) than those of all compartments except LT in ACL-injured patients.

In Figure 3b, significantly higher values ($P < .05$) were identified at 1.3 ppm saturated lipids in the MT compartment in healthy controls than those of ACL-injured patients. There were also significantly lower differences ($P < .05$) at 1.3 ppm saturated lipids in the LF compartment in ACL-injured patients than in healthy controls. Significantly higher differences ($P < .05$) were identified at 1.3 ppm saturated lipids in the MT compartment in patients with KL2–3OA than in healthy controls as well. Similarly, there were significantly higher differences ($P < .05$) at 1.3 ppm saturated lipids in the MT compartment in patients with KL2–3 OA than in ACL-injured patients.

As shown in Figure 3c, significantly higher differences ($P < .05$) were identified at 2.03 ppm saturated lipids in the LF compartment in healthy controls than in ACL-injured patients. There were significantly higher differences ($P < .05$) at 2.03 ppm saturated lipids in the MF compartment in healthy controls than in ACL-injured patients. Significantly higher differences ($P < .05$) were also identified at 2.03 ppm saturated lipids in the MT compartment in healthy controls than in ACL-injured patients. There were significantly lower differences ($P < .05$) at 2.03 ppm saturated lipids in the LT compartment in ACL-injured patients than in healthy controls. There were also significantly higher differences ($P < .05$) at 2.03 ppm saturated lipids in the MF compartment in patients with KL2–3 OA than in healthy controls. There were significantly lower differences ($P < .05$) at 2.03 ppm saturated lipids in the LT compartment in healthy controls than in patients with KL2–3 OA. Significantly lower differences ($P < .05$) were also identified at 2.03 ppm saturated lipids between all compartments in ACL-injured patients than all compartments in patients with KL2–3 OA.

As shown in Figure 3d, there were significantly higher differences ($P < .05$) in unsaturated lipids at 5.31 ppm predominantly in the MT compartment in healthy controls than in ACL-injured patients, in patients with KL2–3 OA than in healthy controls, and in patients with KL2–3 OA than in ACL-injured patients, respectively.

DISCUSSION

Only a handful of reports have been published addressing the use of MRI in determining the association of ACL tear and associated cartilage damage (10,26). In this study, for the first time the proton MRS method was used to quantify the compartment-specific lipid changes in femoral–tibial subchondral bone in ACL-injured patients compared to healthy controls and patients with KL2–3 OA at 3.0 T with the hypothesis that the compartment-specific subchondral lipid changes may be clinical indicators in characterizing subchondral bone marrow among healthy controls, acute ACL-injured patients, and patients with OA. The BMEL lesions and knee pain have been demonstrated to strongly correlate with the deterioration and progression of knee OA (11). Previous work has implied that significantly elevated water and unsaturated lipids, and decreased saturated lipids are seen in BMEL lesions that are subjacent to areas of cartilage degeneration in OA (1). However, the cause of

bone marrow lesions and their relationship to cartilage degeneration remain unclear. The investigation of bone marrow lesions in patients with OA 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 (2,15,27).

In general, there was a distinct increase in the indices of unsaturation of femoral–tibial bone marrow lipids mainly in ACL-injured patients compared to patients with KL2–3 OA. This occurred predominantly within three compartments (LT, MF, and MT). However, no significant differences were seen in the LF between these two groups (Fig. 2). On the other hand, no significant differences were identified in the indices of unsaturation of femoral–tibial bone marrow lipids between healthy controls and acute ACL-injured patients, and healthy controls and patients with KL2–3 OA. These results suggested that the indices of unsaturation of femoral–tibial bone marrow lipids may elevate more markedly especially in acute ACL-injured patients compared to both patients with KL2–3 OA and healthy controls.

There was also an evident increase in the peak of saturated lipids at 0.9 ppm of femoral–tibial bone marrow lipids mainly in healthy controls compared to ACL-injured patients. This occurred predominantly within three compartments (LF, MF, and MT). However, no significant differences were seen in the LT compartment between these two groups (Fig. 3a). The decreased peak of saturated lipids at 0.9 ppm of femoral–tibial bone marrow lipids in acute ACL-injured patients may correspond to the previous findings that decreased saturated lipids are seen in BMEL lesions adjacent to the location of cartilage degeneration in OA (1). The significantly decreased peak of saturated lipids at 1.3 ppm was also found in both LF and MT compartments in acute ACL-injured patients than in healthy controls. Of note, there was a distinct increase in the peak of saturated lipids at 1.3 ppm in the MT compartment in patients with KL2–3 OA compared to both healthy controls and ACL-injured patients (Fig. 3b). This may imply that the peak of saturated lipids at 1.3 ppm in the MT compartment possibly decreases drastically in patients with acute ACL injury. The peak of saturated lipids at 2.03 ppm was generally lower in all four compartments in ACL-injured patients than in healthy controls. There was also a distinct increase in the peak of saturated lipids at 2.03 ppm in both LT and MF compartments in patients with KL2–3 OA compared to healthy controls. Specifically, the peak of saturated lipids at 2.03 ppm was higher in all compartments in patients with KL2–3 OA than those in ACL-injured patients (Fig. 3c). This result suggests that the peaks of saturated lipids at 2.03 ppm among the four compartments of femoral–tibial bone may represent an early indicator to discriminate between ACL-injured and osteoarthritic patients. Additionally, the peak of unsaturated lipids at 5.31 ppm was higher in the MT compartment in patients with KL2–3 OA than those in both healthy controls and ACL-injured patients (Fig. 3d). This result is partly in response to previous findings (1) with an exception that no significantly elevated peak of unsaturated lipids at 5.31 ppm was found in acute ACL-injured patients compared to patients with KL2–3 OA.

The clinical significance of all these findings is that they might be used to stratify subjects who are at risk for posttraumatic knee osteoarthritis initiation and progression. Our present work is still a preliminary study to evaluate the compartment-specific lipid metabolism changes in femoral–tibial bone among healthy controls, ACL-injured patients, and patients

with KL2–3 OA. Previous studies (12–15,28) have implied that OA may be a systematic disorder affecting the entire musculoskeletal system and involve altered lipid metabolism. In these studies (12–14), obesity and joint pain are indicated as possible risk factors of articular degeneration because of increased knee joint mechanical loading and previous joint injury. Other studies (14,15) have suggested that OA may be a multifactorial disorder in which disturbances of lipid homeostasis may be involved. Further, some recent publications (12–14) have indicated that the adipose tissue-derived hormones such as leptin, adiponectin, and resistin, may have a marked effect on the initiation and progression of OA. Increased evidence has suggested that leptin played a key role in the regulation of body weight by way of decreasing food intake and stimulating energy consumption (13). Other literature (12,13) has shown that resistin made plenty of biological effects on arthritis, and resistin could be detected locally in the inflamed joints with rheumatoid arthritis and OA and elevated in rheumatoid arthritis. Given all this evidence, studies on different adipocytokines have revealed that they may have played proinflammatory and catabolic/anabolic role during the pathophysiology of OA, and OA is a multifaceted disease and the exact mechanism of adipocytokines in obesity-induced OA remains unclear, and much work is needed to clarify their roles in OA. Notably, one risk factor of OA initiation lies in the knee misalignment, which may affect the load to the subchondral bone, and thus the changes determined in this work (29). Altogether, these studies indicate that OA pathogenesis may be a complicated process, and more work remains warranted to further explore 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 ($n = 17$ for KL0 as healthy controls, $n = 17$ for KL0 as ACL-injured patients, and $n = 21$ for KL2–3 as patients with minimal-moderate OA) were studied. The age mismatch among healthy controls, ACL-injured patients, and patients with KL2–3 OA (41 ± 16 vs. 30 ± 11 vs. 65 ± 12 years) is also a deficiency of the present study, which possibly affects the significance of the comparison results. Other limitations of this study are the subjectivity of: (1) the KL and WOMBS scales as semiquantitative, integer-based assessments of OA, and (2) the manually chosen VOIs within the four compartments of femoral–tibial bone (LF, LT, MF, and MT). It should be noted, however, that the KL and WOMBS systems are the current reference standards used to grade OA severity. Future studies involving larger sample sizes would help verify the results in the present study. Moreover, in the future, we hope to correlate the subchondral bone marrow lipid content with the magnitude of the bone marrow edema pattern and with the presence of any trabecular microfracture, potentially by using highresolution bone microarchitecture imaging techniques as in previous work (30).

CONCLUSIONS

In this study, for the first time we have quantified the compartment-specific subchondral lipid changes in femoral–tibial bone among healthy controls, ACL-injured patients, and patients with KL2–3 OA at 3.0 T using single voxel MRS technique. Our preliminary results suggest that the indices of unsaturation in the LT compartment and the peaks of saturated lipids at 1.3 and 2.03 ppm in the MT compartment of the femoral–tibial bone marrow at 1.3, 2.03, and 5.31 ppm in LF and LT compartments may be clinically beneficial to characterize

bone marrow lesions among healthy controls, acute ACL-injured patients, and patients with OA.

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Figure 1.

Representative images of the voxel positions and lipid peaks obtained from a patient with acute anterior cruciate ligament injury: **(a)** lateral femoral bone, **(b)** lateral tibial bone, **(c)** medial femoral bone, and **(d)** medial tibial bone, respectively.

Figure 2.

Box and whisker plots comparing median bone marrow index of unsaturation among the four compartments (LF, LT, MF, and MT) of healthy controls, ACL-injured patients, and patients with KL2–3 OA. 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). There were statistically significant differences ($P < .05$) in the index of unsaturation between LT in ACL-injured patients and all compartments except LF in patients with KL2–3 OA. ACL, anterior cruciate ligament; KL2–3, Kellgren–Lawrence grade 2–3; LF, lateral femoral; LT, lateral tibial; MF, medial femoral; MT, medial tibial; OA, osteoarthritis.

Figure 3.

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, and MT) of healthy controls, ACL-injured patients, and patients with KL2–3 OA. The *horizontal dashed lines* on the boxes show the corresponding mean values. ACL, anterior cruciate ligament; KL2–3, Kellgren–Lawrence grade 2–3; LF, lateral femoral; LT, lateral tibial; MF, medial femoral; MT, medial tibial; OA, osteoarthritis.

TABLE 1

Characteristics of Human Subjects (Healthy, ACL-injured, and KL2–3 OA)

Subject Group and Characteristic	Healthy Controls	ACL-injured Patients	Patients with OA (KL2–3)
All subjects			
No. of subjects	17	17	21
Age (years) *	41 ± 16	30 ± 11	65 ± 12
BMI (kg/m ²)	25.0 ± 2.7	26.1 ± 4.2	25.0 ± 3.4
Total WORMS [†]	2.6 ± 6.9	1.7 ± 1.4	13.5 ± 11.5
Female subjects			
No. of subjects	4	3	12
Age (years)	38 ± 15	33 ± 8	63 ± 13
Age range (years)	24–59	24–38	44–89
Male subjects			
No. of subjects	13	14	9
Age (years)	41 ± 17	29 ± 12	68 ± 11
Age range (years)	25–78	18–61	46–80

ACL, anterior cruciate ligament; BMI, body mass index; KL2–3, Kellgren–Lawrence grade 2–3; OA, osteoarthritis; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

* There were significant differences ($P < .05$) in subjects' age between healthy controls and ACL-injured patients, healthy controls and patients with KL2–3 OA, and ACL-injured patients and patients with KL2–3 OA.

[†] There were significant differences ($P < .05$) in total WORMS between healthy controls and patients with KL2–3 OA, and ACL-injured patients and patients with KL2–3 OA.

TABLE 2

The mean $\pm$ SD of Each Study Endpoint within Each Subject Group

Compartment	Measure	Healthy	ACL-injured	OA KL2–3	P Values	
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	ANOVA	ANCOVA
LF	Sat 0.9 ppm	0.099 $\pm$ 0.083	0.086 $\pm$ 0.030	0.092 $\pm$ 0.057	.7994	.8414
	Sat 1.3 ppm	0.617 $\pm$ 0.116	0.518 $\pm$ 0.074	0.589 $\pm$ 0.192	.0116	.0539
	Sat 2.03 ppm	0.071 $\pm$ 0.022	0.054 $\pm$ 0.016	0.095 $\pm$ 0.031	<.0001	.0659
	Unsat 5.31 ppm	0.088 $\pm$ 0.017	0.075 $\pm$ 0.031	0.088 $\pm$ 0.028	.2820	.3844
	UI*	0.101 $\pm$ 0.013	0.101 $\pm$ 0.036	0.105 $\pm$ 0.035	.9027	.9796
LT	Sat 0.9 ppm	0.076 $\pm$ 0.058	0.071 $\pm$ 0.034	0.071 $\pm$ 0.049	.9495	.6127
	Sat 1.3 ppm	0.508 $\pm$ 0.112	0.453 $\pm$ 0.083	0.554 $\pm$ 0.121	.0192	.0961
	Sat 2.03 ppm	0.077 $\pm$ 0.027	0.061 $\pm$ 0.018	0.104 $\pm$ 0.037	<.0001	.3376
	Unsat 5.31 ppm	0.068 $\pm$ 0.019	0.078 $\pm$ 0.027	0.073 $\pm$ 0.025	.4864	.5132
	UI*	0.093 $\pm$ 0.013	0.117 $\pm$ 0.037	0.090 $\pm$ 0.024	.0322	.0757
MF	Sat 0.9 ppm	0.083 $\pm$ 0.042	0.072 $\pm$ 0.012	0.085 $\pm$ 0.056	.5922	.2533
	Sat 1.3 ppm	0.504 $\pm$ 0.071	0.480 $\pm$ 0.037	0.525 $\pm$ 0.116	.2935	.1708
	Sat 2.03 ppm	0.066 $\pm$ 0.016	0.053 $\pm$ 0.011	0.089 $\pm$ 0.036	<.0001	.0004
	Unsat 5.31 ppm	0.073 $\pm$ 0.011	0.071 $\pm$ 0.011	0.074 $\pm$ 0.019	.8677	.3500
	UI*	0.100 $\pm$ 0.009	0.106 $\pm$ 0.019	0.097 $\pm$ 0.028	.3645	.2651
MT	Sat 0.9 ppm	0.080 $\pm$ 0.044	0.046 $\pm$ 0.024	0.063 $\pm$ 0.030	.0146	.0317
	Sat 1.3 ppm	0.420 $\pm$ 0.091	0.350 $\pm$ 0.068	0.495 $\pm$ 0.091	<.0001	.0004
	Sat 2.03 ppm	0.073 $\pm$ 0.021	0.049 $\pm$ 0.014	0.089 $\pm$ 0.025	<.0001	.0021
	Unsat 5.31 ppm	0.058 $\pm$ 0.014	0.057 $\pm$ 0.022	0.068 $\pm$ 0.026	.2895	.3001
	UI*	0.095 $\pm$ 0.011	0.112 $\pm$ 0.038	0.095 $\pm$ 0.035	.2387	.2744

ACL, anterior cruciate ligament; ANOVA, analysis of variance; ANCOVA, analysis of covariance; KL2–3, Kellgren–Lawrence grade 2–3; LF, lateral femoral; LT, lateral tibial; MF, medial femoral; MT, medial tibial; OA, osteoarthritis; Sat, saturated; SD, standard deviation; Unsat, unsaturated; UI, index of unsaturation.

Each *P* value is from the composite test of group differences as determined by ANOVA or ANCOVA. *P* values are shown in bold italic font when significant at the Bonferroni-corrected level of 0.0056.

* *P* values were derived using data subjected to a normalizing transformation; the mean and SD values for these measures were derived using data on the original scale of measurement.

TABLE 3

Tukey-corrected P Values from ANOVA and ANCOVA to Pairwise Compare Subject Groups in Terms of Each Endpoint without and with Adjustment for Age, Gender, and BMI, Respectively

Compartment	Measure	Healthy versus ACL-injured		Healthy versus OA KL2–3		ACL-injured versus OA KL2–3	
		ANOVA	ANCOVA	ANOVA	ANCOVA	ANOVA	ANCOVA
MF	Sat 2.03 ppm *	.0115	.0133	.0207	.0235	.0005	.0004
MT	Sat 1.3 ppm	.0167	.0388	.0444	.0372	.0004	<.0001
	Sat 2.03 ppm	.0060	.0010	.4323	.0812	.0070	<.0001

ACL, anterior cruciate ligament; ANOVA, analysis of variance; ANCOVA, analysis of covariance; KL2–3, Kellgren–Lawrence grade 2–3; MF, medial femoral; MT, medial tibial; OA, osteoarthritis; Sat, saturated.

Results are provided only for endpoints showing a significant composite test for group differences from both ANOVA and ANCOVA.

* *P* values were derived using data subjected to a normalizing transformation. *P* values are shown in bold italic font when significant at the 5% level. There was no one measure in the LF compartment that showed a significant result in Table 2 for both ANOVA and ANCOVA (Sat 2.03 ppm showed a significant composite test for ANOVA but not for ANCOVA). Therefore, results for the subcompartment LF are correctly excluded in this table.