Multiparametric MR imaging reveals early cartilage degeneration at 2 and 8 weeks after ACL transection in a rabbit model

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Abstract

In this study, the rabbit model with anterior cruciate ligament transection (ACLT) was used to investigate early degenerative changes in cartilage using multiparametric quantitative magnetic resonance imaging (qMRI). ACLT was surgically induced in the knees of skeletally mature New Zealand White rabbits (n = 14). ACL transected and contralateral knee compartments—medial femur, lateral femur, medial tibia, and lateral tibia—were harvested 2 (n = 8) and 8 weeks (n = 6) post-surgery. Twelve age-matched nonoperated rabbits served as control. qMRI was conducted at 9.4 T and included relaxation times T₁, T₂, continuous-wave T₁ρ (CWT₁ρ), adiabatic T₁ρ (AdT₁ρ), adiabatic T₂ρ (AdT₂ρ), and relaxation along a fictitious field (TRAFF). For reference, quantitative histology and biomechanical measurements were carried out. Posttraumatic changes were primarily noted in the superficial half of the cartilage. Prolonged T₁, T₂, CWT₁ρ, and AdT₁ρ were observed in the lateral femur 2 and 8 weeks post-ACLT, compared with the corresponding control and contralateral groups (P < .05). Collagen orientation was significantly altered in the lateral femur at 2 weeks post-ACLT compared with the corresponding control group. In the medial femur, all the studied relaxation time parameters, except TRAFF, were increased 8 weeks post-ACLT, as compared with the corresponding contralateral and control groups (P < .05). Similarly, significant proteoglycan loss was observed in the medial femur at 8 weeks following surgery (P < .05). Multiparametric MRI demonstrated early degenerative changes primarily in the superficial cartilage with T₁, T₂, CWT₁ρ, and AdT₁ρ sensitive to cartilage changes at 2 weeks after surgery.

KEYWORDS

ACLT, cartilage, MRI, osteoarthritis, rabbit model
1 | INTRODUCTION

Osteoarthritis (OA), characterized by cartilage loss, often follows traumatic injury to the articular cartilage and anterior cruciate ligament (ACL).\(^1,2\) ACL rupture might change loading conditions of the knee joint, leading to degenerative changes in metabolism and structure of articular cartilage.\(^4\) These alterations are biomarkers of early OA in the joint.\(^6\) Where proteoglycan (PG) content is decreased and collagen network becomes fibrillated.\(^6\) Since available ex vivo human joint tissue samples generally represent advanced OA, a rabbit model with ACL transection (ACLT) has been successfully used to investigate early events of OA progression in cartilage.\(^5,7,9\) Hence, detection of these compositional changes, typically occurring in the early stage of disease, is crucial for the diagnosis and treatment of OA.\(^10\)

Magnetic resonance imaging (MRI) relaxometry measurements facilitate noninvasive, quantitative, and objective probes for the microscopic composition of cartilage, particularly essential in early and preradiographic stages of OA.\(^11,12\) Among others, these measures include longitudinal and transverse relaxation times (T\(_1\) and T\(_2\)), continuous-wave (CW) rotating-frame of reference (RFR) longitudinal relaxation time (CWT\(_{1p}\)), adiabatic RFR longitudinal and transverse relaxation times (AdT\(_{1p}\), and AdT\(_{2p}\)), and relaxation along a fictitious field (T\(_{RAFF}\)). T\(_1\) relaxation time has been reported to be a biomarker for macromolecular content and interstitial water content,\(^13\) while T\(_2\) has been linked to tissue hydration and collagen fiber organization in articular cartilage.\(^14,15\) CWT\(_{1p}\) has been shown to be sensitive to the low-frequency interactions between water molecules and large slow tumbling macromolecules, such as PG and collagen, which constitute the majority of the cartilage matrix.\(^16\) T\(_{1p}\) dispersion, which is characterized by the dependence of T\(_{1p}\) values on the strength of a spin-locking radio frequency (RF) field,\(^17\) is another MRI technique that can be used for tissue characterization. In addition to the continuous wave spin-locking technique, T\(_{1p}\) can also be measured with adiabatic hyperbolic secant RF pulses (AdT\(_{1p}\) and AdT\(_{2p}\)).\(^18\) A study using AdT\(_{1p}\) and AdT\(_{2p}\) reported superior sensitivity of these parameters, compared with the conventional ones, towards detecting early degenerative changes in cartilage.\(^5\) T\(_{RAFF}\) exploits relaxation during nonadiabatic RF swept pulses, creating measurable contrast with low RF deposition in the tissues, thereby making it suitable in clinical settings.\(^19\)

Previous studies of rabbit ACLT models have reported signs of cartilage degeneration 3 to 4 weeks after the surgery.\(^7,9\) In some studies, contralateral knee joints were used as a control group.\(^4,20\) Even though, contralateral joints have been found to be affected by the ACL transection in the ipsilateral knee.\(^4,20\) Therefore, a separate group of animals, without operation to any knee, can better represent control group measurements and values.\(^5\)

The aim of this study was to investigate the potential of quantitative MRI techniques (T\(_1\), T\(_2\), CWT\(_{1p}\), AdT\(_{1p}\), AdT\(_{2p}\), and T\(_{RAFF}\)) to capture OA degenerative changes as early as 2 and 8 weeks after ACLT in a rabbit model, which represent very early time points after the operation. The lateral and medial femoral, and tibial plateaus of transected, contralateral, and nontransected control knees were selected for analysis. For reference, MRI methods were correlated with mechanical properties of articular cartilage and quantitative histology, namely, digital densitometry (DD), reflective of PG content, and polarized light microscopy (PLM) of the collagen fibril network. As ACL injury has been reported to cause joint instability and development of posttraumatic OA,\(^1,4\) we hypothesized that (a) quantitative MRI parameters can detect tissue changes in cartilage as early as 2 weeks following ACLT; (b) quantitative MRI relaxation parameters are prolonged as a result of tissue alterations post-ACLT; and (c) using multiple quantitative MRI relaxation parameters can provide complementary information about cartilage structure and properties.

2 | MATERIALS AND METHODS

2.1 | Sample preparation and handling

Unilateral ACLT was surgically performed in the knees of skeletally mature female New Zealand White rabbits (n = 14, Oryctolagus, cuculus, age 12 months at the time of surgery, 4.8 ± 0.1 kg) under general anesthesia. The rabbits recovered postoperatively on a heating pad covered with a blanket until they were mobile and returned to their cages (76 × 64 × 41 cm), where they were allowed to move freely. The animals were sacrificed 2 weeks (n = 8) (ACLT-2w) and 8 weeks (n = 6) (ACLT-8w) postsurgery. An additional 12 age-matched (12.5 months (n = 8) and 14 months (n = 4)) nonoperated rabbits served as a control group. For controls aged 12.5 months (CTRL-2w) the knee specimens were retrieved from a single leg per animal, while in controls aged 14 months (CTRL-8w), knee specimens were harvested from both legs.

All the rabbits were anesthetized with isoflurane and euthanized with an intracardiac injection of pentobarbital sodium (200 mg/kg; Euthanyl, Bimeda-MTC Animal Health Inc, Cambridge, ON). All procedures were conducted according to the guidelines of the Canadian Council on Animal care and were approved by the Animal Ethics committee at the University of Calgary. Osteochondral samples for MRI were prepared by dissecting the four compartments of the knee, namely, digital densitometry (DD), reflective of PG content, and polarized light microscopy (PLM) of the collagen fibril network. As ACL injury has been reported to cause joint instability and development of posttraumatic OA,\(^1,4\) we hypothesized that (a) quantitative MRI parameters can detect tissue changes in cartilage as early as 2 weeks following ACLT; (b) quantitative MRI relaxation parameters are prolonged as a result of tissue alterations post-ACLT; and (c) using multiple quantitative MRI relaxation parameters can provide complementary information about cartilage structure and properties.

2.2 | Magnetic resonance imaging

MRI was conducted at room temperature on a 9.4 T MRI scanner (Oxford Instruments Plc, Witney, UK) in combination with a 19-mm quadrature volume RF transceiver (RAPID Biomedical GmbH, Rimpar, Germany) and Varian Vnmr 3.1A console (Varian Inc, Palo Alto, CA). Prior to MRI, samples were mounted in the same orientation as for biomechanical...
TABLE 1  Sequence parameters for MRI protocols

| MRI contrasts | Preparation parameters | Pulse power | Acquisition time (min:s) |
|---------------|------------------------|-------------|-------------------------|
| T₁            | TI = 200, 500, 800, 1100, 1400, and 3000 ms | yB₁ = 500, 600, 800, 1000, and 2000 Hz | 11:43 |
| T₂            | TE = 8.7, 12.6, 18.2, 26.4, 38.2, 55.3, and 80 ms | γB₁,max = 2.5 kHz | 18:83 |
| CWT₁p        | Spin-lock embedded between two AHP pulses of +90 and −90, TSL = 0, 4, 8, 16, 32, 64, and 128 ms | yB₁ = 500, 600, 800, 1000, and 2000 Hz | 73:6 |
| AdT₁p        | Trains of 0, 4, 8, 12, 24, and 32 HS1-AFP pulses, pulse duration 4.5 ms | γB₁,max = 2.5 kHz | 16:17 |
| AdT₂p        | Trains of 0, 4, 8, 12, and 24 HS1-AFP pulses between AHP pulses, pulse duration 4.5 ms | γB₁,max = 2.5 kHz | 13:52 |
| RAFF         | Trains of 0, 2, 4, 8, and 16 RAFF pulses, pulse duration 9 ms | γB₁,max = 625 Hz | 26:83 |

Abbreviations: AdT₁p, adiabatic T₁p; AdT₂p, adiabatic T₂p; AFP, adiabatic full-passage; AHP, adiabatic half-passage; CWT₁p, continuous-wave T₁p; RAFF, relaxation along a fictitious field; TI, time to inversion; TSL, spin-lock time.

FIGURE 1  Representative relaxation time maps of T₁, T₂, continuous-wave (CW) T₁p (500 Hz), CWT₁p (600 Hz), CWT₂p (800 Hz), CWT₁p (1000 Hz), CWT₂p (2000 Hz), adiabatic T₁p (AdT₁p), adiabatic T₂p (AdT₂p), and RAFF in cartilage from the lateral femoral condyles of the rabbits. Optical density (OD) images acquired by digital densitometry (DD), and polarized light microscopy (PLM) represent reference measurements (bottom). Increased relaxation times in the superficial cartilage of post-ACLT models can be seen with T₁, T₂, continuous-wave (CW) T₁p (1000 Hz), continuous-wave (CW) T₂p (2000 Hz), adiabatic T₁p (AdT₁p), adiabatic T₂p (AdT₂p), and RAFF. In line with the prolonged relaxation times, DD reveals small fissesures as early as 2 weeks post-ACLT. The scale bar indicates scale in the relaxation time maps [Color figure can be viewed at wileyonlinelibrary.com]

Testing on a custom-made sample holder, with cartilage weight-bearing surface perpendicular to B₀ to minimize the magic angle effect at the site of mechanical testing. The holder could bear two specimens simultaneously. To provide ¹H signal-free background, samples fixed on the holder were immersed in perfluoroether (Galden, Solvay, TX) inside a Teflon tube. For MRI experiments, a magnetization preparation block was modified, as presented in Table 1, to provide the following measurement techniques: T₁, T₂, CWT₁p, AdT₁p, AdT₂p, and RAFF. Experiments were repeated for spin-lock frequencies (SLF) (γB₁ = 500, 600, 800, 1000, and 2000 Hz) to assess CWT₁p dispersion. The preparation block was followed by a single-slice fast spin echo (FSE) readout (TR = 5 s, TE = 4.8 ms, slice thickness = 1 mm, matrix size = 256 × 128, FOV = 18 × 18 mm, resolution along the cartilage depth (frequency encoding direction) = 70.3 μm per pixel). The slice was positioned centrally, covering the biomechanical testing sites of both lateral and medial femoral condyles, and lateral and medial tibiae. Relaxation time maps were calculated with Aedes software (http://aedes.uef.fi/) and in-house written plugins using mono-exponential two-parametric fitting on a pixel-by-pixel basis for T₁, T₂, CWT₁p, AdT₁p, AdT₂p, and RAFF, and three-parametric mono-exponential fitting with steady state for RAFF. The mean values and 95% confidence intervals of each relaxation parameter were determined in a single 2-D slice for 2-mm wide regions of interest (ROIs) drawn manually (AWK) in the load-bearing areas: highest point of the lateral and medial femur and the center of the lateral and medial tibia. The slice and the ROIs covered the tissue marks which corresponded to the biomechanical testing site and histology. In addition to full-thickness of cartilage, the mean values for each parameter were determined for two equally spaced layers, superficial 50% and deep 50%, similar to the procedures typically used in clinical settings.22

2.3 | Reference measurements

Reference measurements were performed as reported in earlier studies. Briefly, full-thickness profile analysis of cartilage tissue was
conducted using DD of Safranin-O dye and PLM to measure PG content and orientation angle of collagen fibrils, respectively. For DD measurement, 3-μm thick sections of each sample were stained with Safranin-O dye, which binds stoichiometrically to the negative charge of the PG macromolecules. The optical density (absorbance) of the cationic dye, which is directly proportional to the PG content, was assessed. For PLM measurement, 5-μm thick unstained sections of each sample with enzymatic PG removal were used to determine the orientation angle of the collagen fibrils (0° = parallel to cartilage surface).

Biomechanical properties of the cartilage were determined using indentation testing with a plane-ended indenter (diameter = 1 mm) on the apex of femoral condyles and central point of the tibial plateaus. The indentation points were marked on the specimen for registering the mechanical testing location to MRI. The cartilage-on-bone specimens were first glued to the measurement chamber at their bottom. Then, a step-wise stress-relaxation test (5% strain of the remaining cartilage thickness and three steps with 15 min relaxation time after each step), followed by sinusoidal dynamic testing (frequency = 1 Hz and amplitude = 2% of the remaining cartilage thickness) were performed to define the equilibrium ($E_{eq}$) and dynamic ($E_{dyn}$) elastic moduli, respectively.

All samples were macroscopically assessed and Osteoarthritis Research Society International (OARSI) grading system was utilized to evaluate abnormalities in cartilage structure. The structure of cartilage was assessed with scores ranging between 0 to 11, 0 indicating normal, ≥2 indicating fissures, ≥4 indicating erosion; and ≥8 indicating full-depth erosion.

![Boxplots of the measured T1, T2, adiabatic T1p (AdT1p), adiabatic T2p (AdT2p), and relaxation along a fictitious field (TRAFF) relaxation times in full-thickness, superficial, and deep ROIs of the lateral femoral condyle. ACLT-2w, CL-2w, and CTRL-2w represent surgical, intact contralateral and control groups at 2-week and ACLT-8w, CL-8w, and CTRL-8w represent the groups at 8-week time points, respectively. The whiskers and the boxes indicate full and 25 to 75 percentile ranges, respectively. Straight horizontal lines on the boxes represent median (dark) and mean (red) values. The black solid diamonds are outliers. Brackets indicate significant differences (**P < .01, *P < .05) between the animal groups. ROIs, regions of interest [Color figure can be viewed at wileyonlinelibrary.com]
All statistical analyses were conducted using SPSS software (Version 24, IBM SPSS Statistics, New York). Distributions of MRI relaxation times, biomechanics, DD, and PLM were compared between 2-week control (CTRL-2w), 2-week contralateral (CL-2w), 2-week ACLT (ACLT-2w), 8-week control (CTRL-8w), 8-week contralateral (CL-8w), and 8-week ACLT (ACLT-8w) groups. The comparisons were made using a linear mixed model, which takes into account the dependence between samples and animals. Pair-wise group comparisons were carried out using Dunn-Bonferroni corrections. Differences were considered to be statistically significant at $P$ values of less than .05. The relationships between MRI and reference parameters were studied using Spearman’s rank correlation analysis of CTRL, CL, and ACLT pooled data. Mean values from full-thickness ROIs were used for correlating MRI parameters with the biomechanical properties. Superficial and deep ROIs (50% of tissue thickness each) were used to correlate MRI relaxation times with the average values comprising 50% of the cartilage depth for DD and PLM.

### RESULTS

#### 3.1 MRI parameters

One sample in the 2-week ACLT group was affected by susceptibility artifact and thus was eliminated from the analysis along with the rest of the samples from the same knee joint. Analyzing MRI relaxation parameters in full-thickness cartilage, different parameters demonstrated variable responses to the injured ACLT group as compared with the CTRL and CL groups (Figure 1). $T_1$ and CWT$_1$ (γ$B_1$ = 1 and 2 kHz) relaxation times were longer in both the 2- and 8-week post-ACLT transected lateral femur compared with the corresponding CTRL and CL-8w groups (Figures 2 and 3). $T_2$ and AdT$_1$ values were each prolonged in 2- and 8-week post-ACLT compared to corresponding CTRL groups, respectively (Figure 2). In the medial femur, all relaxation times except $T_{RAFF}$ were increased at 8-week following ACLT compared with their corresponding CTRL group values (Figures 4 and 5). Moreover, $T_1$, $T_2$, CWT$_1$ (γ$B_1$ = 1 and 2 kHz) and...
AdT2ρ were also increased in ACLT-8w compared with CL-8w (Figures 4 and 5). No significant differences were found with TRAFF in any compartments.

In the layer-wise analyses of the MRI parameters, statistically significant differences were most common in the superficial half of the cartilage (Figures 2–5). Evaluating the superficial half, T1 and CWT1p (γB1 = 1 and 2 kHz) were increased in the lateral femur at both, the 2- and 8-week post-ACLT time points compared with the corresponding values in the CTRL and CL group specimens (Figures 2 and 3). T2 was elongated in ACLT-2w compared with CL-2w values (Figure 2). The superficial cartilage in the medial femur was affected at 8-week post-ACLT, as increased values of T1, T2, CWT1p (γB1 = 1 and 2 kHz), AdT1p, and AdT2p were observed in ACLT-8w compared with CTRL-8w (Figures 4 and 5). T1, T2, CWT1p (γB1 = 1 and 2 kHz), and AdT1p were also increased in ACLT-8w compared with the CL-8w values. Assessing the relaxation parameters in the deep half of the articular cartilage, T1 and CWT1p (γB1 = 1 and 2 kHz) values were significantly increased in the lateral femur of ACLT-2w group animals compared with those found in CTRL group animals (Figures 2 and 3). Moreover, in deep cartilage of lateral femur, CWT1p (γB1 = 1 and 2 kHz) was significantly different in ACLT-2w group animals compared with CWT1p values of CL-2w and ACLT-8w group animals (Figure 3).

Evaluating CWT1p with varying spin-lock fields, the number of statistically significant differences between injured ACLT knees and CTRL group knees increased with higher SLF (Figures 3 and 5).
Prolonged CWT$_{1p}$ values were mainly observed in ACLT-2w animals in the lateral femur and ACLT-8w animals in the medial femur at SLFs ($\gamma B_1 = 1$ and 2 kHz). The boxplots of MRI parameters for tibia are accessible in the Supplementary Information.

### 3.2 | Correlation of MRI parameters with histology

For the pooled data of all five experimental groups, all MR imaging parameters were inversely correlated with optical density, reflective of PG content, in the superficial cartilage of the femoral condyles (Table 2). For full-thickness cartilage, weak to moderate correlations between MRI parameters and optical density were found for $T_2$, CWT$_{1p}$, AdT$_{1p}$, and AdT$_{2p}$ (Table 2). The correlation between CWT$_{1p}$ and optical density became stronger with increasing the spin-lock field. $T_2$ was the only parameter weakly correlated to collagen fiber orientation in the deep tibial cartilage (Table 2).

### 3.3 | Correlation of MRI parameters with biomechanical properties

For the pooled data, the equilibrium modulus was moderately negatively correlated with $T_1$, $T_2$, CWT$_{1p}$ ($\gamma B_1 \geq 1000$ Hz), AdT$_{1p}$ and AdT$_{2p}$ in femoral condyle cartilage (Table 3). In the tibial cartilage, weak to moderate negative correlation was found between elastic modulus and all MRI parameters (Table 3). The strongest correlations were observed with $T_1$ and AdT$_{1p}$. For dynamic modulus, weak to moderate negative correlation was found with $T_1$, CWT$_{1p}$, AdT$_{1p}$, AdT$_{2p}$, and $T_{RAFF}$ in tibial cartilage (Table 3).

### 3.4 | Reference analysis

The presence of intact cartilage was reported for all the samples following macroscopic evaluation of the specimens. For cartilage...
TABLE 2  Pooled data Pearson correlation coefficients (r) with P-values between MRI parameters and optical density (DD, reflective of proteoglycan content) and collagen orientation angle (PLM)

| MRI parameters  | Femoral condyles (n = 86) | Tibias (n = 86) |
|-----------------|---------------------------|-----------------|
|                 | Full-thickness DD          | Superficial DD  | Deep DD |
|                 | Full-thickness DD          | Superficial DD  | Deep DD |
| $T_1$           | $r = -.206$               | $r = -.416$     | $r = .130$ |
|                 | $P = .057$                | $P < .001$      | $P = .231$ |
| $T_2$           | $r = -.444$               | $r = -.458$     | $r = -.345$ |
|                 | $P < .001$                | $P < .001$      | $P = .001$ |
| CWT$_{1\text{p}}$ (500 Hz) | $r = -.390$               | $r = -.353$     | $r = -.269$ |
|                 | $P < .001$                | $P = .001$      | $P = .012$ |
| CWT$_{1\text{p}}$ (600 Hz) | $r = -.373$               | $r = -.363$     | $r = -.270$ |
|                 | $P < .001$                | $P = .001$      | $P = .012$ |
| CWT$_{1\text{p}}$ (800 Hz) | $r = -.420$               | $r = -.428$     | $r = -.283$ |
|                 | $P < .001$                | $P < .001$      | $P = .008$ |
| CWT$_{1\text{p}}$ (1000 Hz) | $r = -.361$               | $r = -.402$     | $r = -.173$ |
|                 | $P = .001$                | $P < .001$      | $P = .110$ |
| CWT$_{1\text{p}}$ (2000 Hz) | $r = -.406$               | $r = -.483$     | $r = -.162$ |
|                 | $P < .001$                | $P < .001$      | $P = .135$ |
| AdT$_{1\text{p}}$ | $r = .263$                | $r = -.458$     | $r = .137$ |
|                 | $P = .014$                | $P < .001$      | $P = .208$ |
| AdT$_{2\text{p}}$ | $r = -.364$               | $r = -.385$     | $r = -.179$ |
|                 | $P = .001$                | $P < .001$      | $P = .099$ |
| $T_{\text{RAFF}}$ | $r = -.116$               | $r = -.179$     | $r = -.044$ |
|                 | $P = .287$                | $P < .001$      | $P = .688$ |

|                 | PLM                       | PLM                  | PLM |
|                 | PLM                       | PLM                  | PLM |
| $T_1$           | $r = .075$                | $r = .116$           | $r =.003$ |
|                 | $P = .494$                | $P = .290$           | $P = .981$ |
| $T_2$           | $r = -.084$               | $r = -.018$          | $r = -.048$ |
|                 | $P = .447$                | $P = .872$           | $P = .663$ |
| CWT$_{1\text{p}}$ (500 Hz) | $r = -.087$               | $r = -.091$          | $r = -.187$ |
|                 | $P = .430$                | $P = .406$           | $P = .087$ |
| CWT$_{1\text{p}}$ (600 Hz) | $r = -.100$               | $r = -.028$          | $r = -.179$ |
|                 | $P = .361$                | $P = .801$           | $P = .100$ |
| CWT$_{1\text{p}}$ (800 Hz) | $r = -.114$               | $r = -.057$          | $r = -.198$ |
|                 | $P = .300$                | $P = .606$           | $P = .069$ |
| CWT$_{1\text{p}}$ (1000 Hz) | $r = -.033$               | $r = -.031$          | $r = -.129$ |
|                 | $P = .761$                | $P = .780$           | $P = .240$ |
| CWT$_{1\text{p}}$ (2000 Hz) | $r = -.033$               | $r = -.031$          | $r = -.171$ |
|                 | $P = .762$                | $P = .777$           | $P = .117$ |
| AdT$_{1\text{p}}$ | $r = .046$                | $r = .051$           | $r = -.052$ |
|                 | $P = .676$                | $P = .642$           | $P = .636$ |
| AdT$_{2\text{p}}$ | $r = -.152$               | $r = -.057$          | $r = -.093$ |
|                 | $P = .165$                | $P = .603$           | $P = .397$ |
| $T_{\text{RAFF}}$ | $r = .007$                | $r = .089$           | $r = -.119$ |
|                 | $P = .951$                | $P = .418$           | $P = .278$ |

Note: Statistically significant values are indicated in bold.
Abbreviations: AdT$_{1\text{p}}$, adiabatic T$_{1\text{p}}$; AdT$_{2\text{p}}$, adiabatic T$_{2\text{p}}$; CWT$_{1\text{p}}$, continuous-wave T$_{1\text{p}}$; DD, digital densitometry; MRI, magnetic resonance imaging; PLM, polarized light microscopy; RAFF, relaxation along a fictitious field.
structure, statistically significantly higher OARSI scores were noted for ACLT-8w (score = 3.5 [2.4, 4.6]) compared with corresponding CTRL-8w (score = 1.1 [0.1, 2.1]) and CL-8w (score = 0.8 [−0.2, 1.9]) in the medial femoral condyles.

Statistical differences in DD, reflective of PG content, were found in the medial tibio-femoral cartilage (Figures 6 and 7). In the medial femur, a significantly reduced PG content was observed in both superficial and deep cartilage of ACLT-8w, as compared with CTRL-8w and CL-8w (Figure 6). In the medial tibia, CL-2w was found to have a higher PG content compared with CTRL-2w and ACLT-2w (Figure 7). Statistically significantly altered collagen fibril orientation, as determined by PLM, was observed in the superficial half of ACLT-2w compared with CTRL-2w (Figure 6). Moreover, in the deep half of the medial femur, decreased PG content was observed in ACLT-8w compared with CTRL-8w and CL-8w (Figure 6). No statistically significant difference was found between the biomechanical properties of the groups. The mean values and 95% confidence of intervals of biomechanics and OARSI are accessible in the Supplementary Information.

### DISCUSSION

ACL transection in rabbits provides a well-established experimental model for studying early cartilage degeneration.\(^7,9,28\) Rabbit knee anatomy mimics that of human knees,\(^7\) and OA degenerative changes caused by post-ACL transection injuries resemble, but progress much faster, than those observed in human OA.\(^29\) Previous studies in the rabbit ACLT model reported posttraumatic OA changes at 3 to 12 weeks post-ACLT.\(^7,9,28\) Posttraumatic OA changes have been reported with T2, CWT1ρ (SLF = 1 and 2 kHz), AdT1ρ, and AdT2ρ, in ACL transected rabbits 4 weeks after ACLT surgery.\(^7\) To complement the understanding on the qMRI features of the disease progression after ACLT in the rabbit model, this study included time points before and after those in the previous study.\(^9\) This study confirmed that changes in T1, T2, CWT1ρ, and AdT1ρ are detected as early as 2 weeks post-ACLT in the lateral femur. In contrast to previous MRI studies where ACL transected joints were compared with the corresponding contralateral knees,\(^7,9,28\) post-ACLT findings in the current study are compared with both the contralateral and nontransected control knees. In accordance with findings of quantitative histology, T1, T2, CWT1ρ (SLF = 1 and 2 kHz), AdT1ρ, and AdT2ρ relaxation times were able to detect the degenerative changes in the medial cartilage 8-week post-ACLT. Moreover, the MR relaxation times were correlated moderately to biomechanical properties and articular cartilage PG content.

Consistent with prolonged values in MR imaging parameters, the histological investigation revealed advanced degeneration (fissures and erosion) of cartilage structure in the 8-week post-ACLT group. Quantitative histology revealed early changes in collagen orientation of the lateral femoral condyles 2 weeks after surgery and decreased PG content in the medial compartment 8-week post-ACLT. The increased PG content observed in the contralateral joints (statistically significant in the medial tibia) may be an adaptive response to the altered mechanical stress caused by the ACL transection in the corresponding opposite knee.\(^30\) ACLT alters loading in the rabbit knee and causes instability of the knee joint. It changes rotations and translations of the tibia relative to the femur, which increases contact forces on the lateral side of the knee joint during hopping.\(^31,32\) This may explain the significant changes observed with 2-week post-ACLT in MRI parameters of the lateral femoral condyles in the present study. On the other hand, decreased PG content and prolonged MRI parameters, 8-week post-ACLT in the medial femur suggest reduced mechanical stress compared with the lateral side.

A multiparametric MRI study provides a more comprehensive assessment of articular cartilage structural changes in a postranssection model of OA than previous single parameter imaging or mechanical studies. The quantitative MRI parameters studied, T1, T2, CWT1ρ, and AdT1ρ, relaxation times, were sensitive to alterations of articular cartilage structure and integrity at both 2 and 8 weeks post-ACLT. Onset of early OA, ACLT-2w, in the lateral femur was followed by a more pronounced, later stage OA, ACLT-8w, in the medial femur, showing a characteristic, time-dependent progression of OA.

| MRI parameters | Femoral condyles (n = 86) | Tibias (n = 86) |
|---------------|-----------------|----------------|
|               | Eeq  | Edyn |       | Eeq  | Edyn |
| T1            | r = −.350 | P = .001 | r = −.135 | P = .217 | r = −.573 | P < .001 | r = −.542 | P < .001 |
| T2            | r = −.367 | P = .001 | r = −.197 | P = .069 | r = −.325 | P < .001 | r = −.210 | P < .001 |
| CWT1ρ (500 Hz) | r = −.039 | P = .723 | r = −.202 | P = .062 | r = −.333 | P < .001 | r = −.238 | P < .001 |
| CWT1ρ (600 Hz) | r = −.070 | P = .525 | r = −.118 | P = .280 | r = −.260 | P = .016 | r = −.180 | P = .098 |
| CWT1ρ (800 Hz) | r = −.154 | P = .157 | r = −.171 | P = .116 | r = −.438 | P < .001 | r = −.295 | P < .001 |
| CWT1ρ (1000 Hz) | r = −.308 | P = .004 | r = −.124 | P = .256 | r = −.462 | P < .001 | r = −.335 | P < .001 |
| CWT1ρ (2000 Hz) | r = −.368 | P < .001 | r = −.082 | P = .455 | r = −.513 | P < .001 | r = −.401 | P < .001 |
| AdT1ρ         | r = −.452 | P < .001 | r = −.153 | P = .159 | r = −.559 | P < .001 | r = −.576 | P < .001 |
| AdT2ρ         | r = −.373 | P < .001 | r = −.125 | P = .251 | r = −.380 | P < .001 | r = −.253 | P < .001 |
| TRAFF         | r = −.116 | P = .288 | r = −.056 | P = .606 | r = −.301 | P < .005 | r = −.353 | P < .001 |

Note: Statistically significant values are indicated in bold. Abbreviations: AdT1ρ, adiabatic T1ρ; AdT2ρ, adiabatic T2ρ; CWT1ρ, continuous-wave T1ρ; MRI, magnetic resonance imaging; RAFF, relaxation along a fictitious field.
in rabbits.\(^8\) Primary changes at both 2- and 8-weeks after ACLT were noted mainly in the superficial half of the cartilage of the femoral condyles, consistent with the findings of quantitative histology and previous multiparametric studies.\(^9,33\) Moreover, the aforementioned relaxation times were increased two weeks postsurgery in the deep cartilage of the lateral femur. These early signs in the deep cartilage could be indicative of structural changes in the collagen matrix or of cartilage-bone cross-talk.\(^34,35\) \(\text{AdT}_2\) differentiated between changes in the early and the late stage of the experimental period, ACLT-8w. \(\text{T}_{\text{RAFF}}\) did not differ between the groups, consistent with previous study.\(^9\)

Though \(\text{T}_1\) without contrast is less frequently used in characterizing cartilage structure, the relaxation time has been linked to PG and water content, and biomechanical properties of cartilage.\(^36,37\) In the present study, out of the MRI parameters studied, \(\text{T}_1\) had the strongest correlation with equilibrium modulus. Concerning group discrimination, \(\text{T}_1\) differentiated the injured ACLT-8w group not only from the intact controls but also from the corresponding contralateral knee joint samples. \(\text{T}_1\) relaxation times also discriminated between ACLT-2w and the control group specimens.

Earlier studies demonstrated a strong correlation of \(\text{T}_2\) relaxation time with collagen network integrity.\(^14,15,38\) Surprisingly, the weak association between \(\text{T}_2\) and PLM, reflective of collagen fiber orientation, was observed in the current study. Differences in cartilage structure and mechanical properties between rabbits and other animal models could partially explain the weak correlation between \(\text{T}_2\) and PLM in this study. Furthermore, averaging \(\text{T}_2\) over cartilage thickness (superficial 50% and deep 50%) may mask its association with PLM by small sample-to-sample variations. Similarly, Rautiainen et al\(^7\) reported no association between \(\text{T}_2\) and collagen anisotropy. However, in the current study, \(\text{T}_2\) was significantly correlated to PG content and biomechanical properties. Moreover, \(\text{T}_2\) could differentiate injured 2- and 8-week post-ACLT group cartilage from the contralateral and intact control knee joint cartilages.

\(\text{CWT}_{1\rho}\) dispersion, or frequency dependence of \(\text{T}_1\) relaxation in the rotating-frame,\(^17\) has been linked to alterations in PG content in the cartilage matrix.\(^14\) In this study, the correlation of \(\text{CWT}_{1\rho}\) with PG content increased with the spin-lock frequency. \(\text{CWT}_{1\rho}\) at spin-lock field (\(\gamma B_1 = 1 \text{kHz}\)) was the only MRI parameter to detect OA progression from 2 to 8 weeks, discriminating ACLT-8w from ACLT-2w. Moreover, \(\text{CWT}_{1\rho}\) at higher spin-lock fields (\(\gamma B_1 = 1\) and 2 kHz) could differentiate between the 2- and 8-week injured femurs and the corresponding contralateral and control femurs. The reason behind this finding could be associated with a reduction of the relative contribution of dipolar interactions with increasing spin-lock frequencies.\(^39\) Akella et al\(^7\) found that the difference between \(\text{CWT}_{1\rho}\) values at the magic angle, and other angles, disappeared when the spin-lock frequency was equal to or higher than 2 kHz at 4.7 T. Therefore, increasing the spin-lock frequency enables \(\text{CWT}_{1\rho}\) to be less
dependent on the laminar appearance of cartilage (the “magic angle effect” in cartilage), and more sensitive to changes in PG content. However, using higher spin-lock frequency increases RF deposition in the tissues, which is restricted not only by the limitations of the hardware but critically also by the regulations on specific absorption rate (SAR) in clinical imaging. To maintain SAR within acceptable limits, most previous clinical studies using CWT1\(\rho\) have been conducted at 500 Hz.\(^{40,41}\) In our study, T1\(\rho\) at 500 Hz spin-lock amplitude was found to be one of the least sensitive parameters. Thus, the potential of CWT1\(\rho\) to detect cartilage degeneration appears to not only depend on the spin-locking field, but also on the other details of the experimental setup, such as the degenerative phenotype, the imaging field strength, and so forth.

In AdT1\(\rho\) and AdT2\(\rho\), spin-lock frequency is swept, creating a wide range of effective frequencies sensitive to an extended range of molecular processes.\(^{18}\) Some recent studies showed an association between elevated AdT1\(\rho\) and AdT2\(\rho\) and morphological abnormalities of cartilage, bone, and menisci.\(^{42,43}\) In the current study, both AdT1\(\rho\) and AdT2\(\rho\) were moderately correlated with the PG content and biomechanical properties of the cartilage. AdT1\(\rho\) had the strongest correlation with dynamic modulus. AdT1\(\rho\) could also discriminate 8-week post-ACLT groups from control and contralateral group specimens. Previous multiparametric studies also reported sensitivity of the MR relaxation parameters to degeneration and enzymatic degradation.\(^{9,33,44}\)

There are limitations of this study that need to be kept in mind when drawing conclusions from our results. The thin cartilage in the rabbit knee limited the resolution along the cartilage depth to approximately 10 imaging pixels in the femoral condyles and 15 pixels in the tibias. Another limitation is using specimens after one freeze-thaw cycle, which affects the mechanical properties of articular cartilage.\(^{45}\) This is, however, standard practice and often necessary due to sample logistics. Moreover, this study lacks a true control group with normal cartilage structure. The reference methods (indentation and PLM) used in this study may not be suitable to provide detailed information and distinguish early degeneration in cartilage. Other methods targeting microstructure, such as electron microscopy,\(^{46}\) inflammation,\(^{47}\) and nanoindentation\(^{48}\) are needed to accurately characterize early OA in the tissue. Using a conservative statistical correction approach for multiple comparisons, Dunn-Bonferroni, resulted in the loss of many otherwise statistically significant differences. However, this drawback could be improved by using a larger sample size than we had available here. The age difference between the two control groups might also affect the findings. MRI in this study was performed on a 9.4 T scanner, which is a significantly higher field than that generally used in clinical scanners, thus demanding validation of the measured techniques in vivo at clinical field strengths.
In conclusion, multiple quantitative MRI parameters have been investigated in a posttraumatic ACLT rabbit model. $T_1$, $T_2$, CWT$_{1p}$, and AdT$_{1p}$ relaxation parameters were able to detect compositional changes in cartilage as early as 2 weeks following ACLT. Progressive OA changes in the medial femoral compartment, as detected by most of the studied MRI parameters, could also be quantified at 8 weeks after ACLT. The observed differences between ACL transected and the corresponding contralateral and control group joints, supported by quantitative histology, were mostly significant for the superficial half of the cartilage specimens. Regional differences in MRI parameters at different time intervals suggest that a multiparametric approach may provide a more comprehensive assessment of cartilage tissue status at different degrees of degeneration.

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AUTHOR CONTRIBUTIONS
All authors participated in discussing the results, revised the manuscript critically for important intellectual content, and approved the final version. MTN, MJN, MAF, RKK, SS, and WH developed the initial multiparameter MRI concept and MAF, WH, SS, and RKK designed animal experiments. WH, SS, RKK, MJN, and MTN obtained the funding support. MJN designed the MRI protocol and supervised the MRI experiments. AWK carried out the MRI measurements, analyzed the data, and drafted the manuscript. MAF and SO performed sample collection. SO performed the experiments and analyses of histology and biomechanical measurements. VC, MJN, MTN, MAF, RKK, and SS supervised the analysis of the data and made substantial contributions with interpretation of results and editing of the manuscript. AWK, VC, MJN, and MTN take responsibility for the integrity of the study. All the authors accept the final version of the manuscript and agree with the content.

CONFLICT OF INTEREST
The authors declare that there are no conflict of interest.

DATA AVAILABILITY STATEMENT
The data of the current study is available from the corresponding author on reasonable request.

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REFERENCES
1. Nelson F, Billinghurst RC, Pidoux I, et al. Early post-traumatic osteoarthritis-like changes in human articular cartilage following rupture of the anterior cruciate ligament. *Osteoarthritis Cartilage*. 2006;14:114-119.
2. Eckstein F, Wirth W, Lohmander L, Hudelmaier M, Frobell R. Five-Year followup of knee joint cartilage thickness changes after acute rupture of the anterior cruciate ligament. *Arthritis Rheumatol*. 2015;67:152-161.
3. Li G, Moses JM, Pappannagari R, Pathare NP, Defrate LE, Gill TJ. Anterior cruciate ligament deficiency alters the in vivo motion of the tibiofemoral cartilage contact points in both the anteroposterior and mediolateral directions. *J Bone Joint Surg Am*. 2006;88:1826-1834.
4. Chaudhari AM, Briant PL, Bevill SL, Koo S, Andriacchi TP. Knee kinematics, cartilage morphology, and osteoarthritis after ACL injury. *Med Sci Sports Exerc*. 2008;40:215-222.
5. Mäkelä JTA, Rezaeian ZS, Mikkinen S, et al. Site-dependent changes in structure and function of canine articular cartilage 4 weeks after anterior cruciate ligament transection. *Osteoarthritis Cartilage*. 2014;22:869-878.
6. Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, Rodan GA, Duong LT. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. *Bone*. 2006;38:234-243.
7. Yoshioka M, Coutts RD, Amiel D, Hacker SA. Characterization of a model of osteoarthritis in the rabbit knee. *Osteoarthritis Cartilage*. 1996;4:87-98.
8. Lozano J, Saadat E, Li X, Majumdar S, Ma CB. Magnetic resonance $T_1p$ imaging of osteoarthritis: a rabbit ACL transection model. *Magn Reson Imaging*. 2009;27:611-616.
9. Rautiainen J, Nissi MJ, Liimatainen T, Herzog W, Korhonen RK, Nieminen MT. Adiabatic rotating frame relaxation of MRI reveals early cartilage degeneration in a rabbit model of anterior cruciate ligament transection. *Osteoarthritis Cartilage*. 2014;22:1444-1452.
10. Abramson SB, Attur M. Developments in the scientific understanding of osteoarthritis. *Arthritis Res Ther*. 2009;11:227.
11. Guermazi A, Alizai H, Crema MD, Trattnig S, Regatte RR, Roemer FW. Radiographic assessment of cartilage degeneration in osteoarthritis. *Osteoarthritis Cartilage*. 2015;23:1639-1653.
12. Thüring J, Linka K, Itskov M, et al. Multiparametric MRI and computational modelling in the assessment of human articular cartilage properties: a comprehensive approach. *BioMed Res Int*. 2018;2018. 9460456-12.
13. Berberat JE, Nissi MJ, Jurvelin JS, Nieminen MT. Assessment of interstitial water content of articular cartilage with $T_1$ relaxation. *Magn Reson Imaging*. 2009;27:727-732.
14. Nissi MJ, Rieppo J, Töyräs J, et al. T2 relaxation reveals spatial collagen architecture in articular cartilage: a comparative quantitative MRI and polarized light microscopic study. *Magn Reson Med*. 2001;46:487-493.
16. Akella SV, Reddy Regatte R, Gougoutas AJ, et al. Proteoglycan-induced changes in T1p-relaxation of articular cartilage at 4T. Magn Reson Med. 2001;46:419-423.

17. Sepponen RE, Pohjonen JA, Sipponen JT, Tanttu JI. A method for T1 rho imaging. J Comput Assist Tomogr. 1985;9:1007-1011.

18. Garwood M, Delabarre I. The return of the frequency sweep: designing adiabatic pulses for contemporary NMR. J Magn Reson. 2001;153:155-177.

19. Limatainen T, Sorce DJ, O’connell R, Garwood M, Michaeli S. MRI contrast from relaxation along a fictitious field (RAFF). Magn Reson Med. 2010;64:983-994.

20. Troyer H. The effect of short-term immobilization on the rabbit knee joint cartilage. A histochemical-study. Clin Orthop Relat Res. 1975;107:249-257.

21. Gröhn OHJ, Mäkelä HI, Lukkarinen JA, et al. On-and-off-resonance T1p MRI in acute cerebral ischemia of the rat. Magn Reson Med. 2003;49:172-176.

22. Li X, Kuo D, Theologis A, et al. Cartilage in anterior cruciate ligament-degraded tissue. Magn Reson Imaging. 2012:20:407-422.

23. Ojanen SP, Finnilä MAJ, Mäkelä J, et al. Anterior cruciate ligament transection of rabbits alters composition, structure and biomechanics of articular cartilage and chondrocyte deformation 2 weeks post-surgery in a site-specific manner. J Biomech. 2019:98:109450.

24. DeMay BS, Bai X, Howard L, et al. Septin filaments exhibit a dynamic, paired organization that is conserved from yeast to mammals. J Cell Biol. 2011;193:1065-1081.

25. Rieppo J, Halikainen J, Juvelin JS, Kiviranta I, Helminen HJ, Hyttinen MM. Practical considerations in the use of polarized light microscopy in the analysis of the collagen network in articular cartilage. Microsc Res Tech. 2008;71:279-287.

26. Laverty S, Girard C, Williams J, Hunziker EB, Prötzker K. The OARSI histopathology initiative-recommendations for histological assessments of osteoarthritis in the rabbit. Osteoarthritis Cartilage. 2010;18:553-565.

27. Ranstam J. Repeated measurements, bilateral observations and pseudoreplicates, why does it matter? Osteoarthritis Cartilage. 2012;20:473-475.

28. Liu Z, Hu X, Man Z, Zhang J, Jiang Y, Ao Y. A novel rabbit model of early osteoarthritis exhibits gradual cartilage degeneration after medial collateral ligament transaction outside the joint capsule. Sci Rep. 2016;6:34423.

29. Madry H, Luyten FP, Facchini A. Biological aspects of early osteoarthritis. Knee Surg Sports Traumatol Arthrosc. 2012;20:407-422.

30. Kiviranta I, Juvelin J, Tammi M, Säämänen A, Helminen HJ. Weight bearing controls glycosaminoglycan concentration and articular cartilage thickness in the knee joints of young beagle dogs. Arthritis Rheumatol. 1987;30:801-809.

31. Ojanen SP, Finnilä MAJ, Reunamo AE, et al. Site-specific glycosaminoglycan content is better maintained in the pericellular matrix than the extracellular matrix in early post-traumatic osteoarthritis. PLoS One. 2018;13:e0196203.

32. Gushue DL, Houck J, Lerner AL. Rabbit knee joint biomechanics: motion analysis and modeling of forces during hopping. J Orthop Res. 2005;23:735-742.

33. Nissi MJ, Saio EN, Tiitu V, et al. Multi-parametric MRI characterization of enzymatically degraded articular cartilage. J Orthop Res. 2016;34:1111-1120.

34. Findlay DM, Kuliwaba JS. Bone–cartilage crosstalk: a conversation for understanding osteoarthritis. Bone Res. 2016;4:16028.

35. Williams AA, Titchenal MR, Do BH, Guha A, Chu CR. MRI UTE-T2* shows high incidence of cartilage subsurface matrix changes 2 years after ACL reconstruction. J Orthop Res. 2019;37:370-377.

36. Bashir A, Gray ML, Boutin RD, Burdstein D. Glycosaminoglycan in articular cartilage: in vivo assessment with delayed gDTPA(2-)–enhanced MR imaging. Radiology. 1997;205:551-558.

37. Bashir A, Gray M, Hartke J, Burdstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med. 1999;41:857-865.

38. Xia Y. Relaxation anisotropy in cartilage by NMR microscopy (μMRI) at 14-μm resolution. Magn Reson Med. 1998;39:941-949.

39. Akella SV, Regatte RR, Wheaton AJ, Borthakur A, Reddy R. Reduction of residual dipolar interaction in cartilage by spin-lock technique. Magn Reson Med. 2004;52:1103-1109.

40. Regatte RR, Akella SV, Lonner J, Reddy R. T1p relaxation mapping in human osteoarthritis (OA) cartilage: comparison of T1p with T2. J Magn Reson Imaging. 2006;23:547-553.

41. Su F, Hilton JF, Nardo L, et al. Cartilage morphology and T1p and T2 quantification in ACL-reconstructed knees: a 2-year follow-up. Osteoarthritis Cartilage. 2013;21:1058-1067.

42. Casula V, Nissi MJ, Podlipská J, et al. Elevated adiabatic T1p and T2p in articular cartilage are associated with cartilage and bone lesions in early osteoarthritis: a preliminary study. J Magn Reson Imaging. 2017;46:678-689.

43. Kajabi AW, Casula V, Nissi MJ, et al. Assessment of meniscus with adiabatic T1p and T2p relaxation time in asymptomatic subjects and patients with mild osteoarthritis: a feasibility study. Osteoarthritis Cartilage. 2018;26:580-587.

44. Hämínen N, Rautiainen J, Rieppo L, Saarakkala S, Nissi MJ. Orientation anisotropy of quantitative MRI relaxation parameters in ordered tissue. Sci Rep. 2017;7:9606.

45. Kennedy EA, Tordonado DS, Duma SM. Effects of freezing on the mechanical properties of articular cartilage. Biomed Sci Instrum. 2007;43:342-347.

46. Changoo A, Nelea M, Méthot S, et al. Structural characteristics of the collagen network in human normal, degraded and repair articular cartilages observed in polarized light and scanning electron microscopies. Osteoarthritis Cartilage. 2011;19:1458-1468.

47. Hedbom E, Häuselmann H. Molecular aspects of pathogenesis in osteoarthritis: the role of inflammation. Cell Mol Life Sci. 2002;59:45-53.

48. Ebenstein DM, Kuo A, Rodrigo JJ, Reddi AH, Ries M, Pruitt L. A no-noindention technique for functional evaluation of cartilage repair tissue. J Mater Sci. 2004;19:273-281.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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