Effectively Measuring Exercise-related Variations in T1ρ and T2 Relaxation Times of Healthy Articular Cartilage

ABSTRACT

Background: Determining the compositional response of articular cartilage to dynamic joint loading using magnetic resonance imaging may be a more sensitive assessment of cartilage status than conventional static imaging. However, distinguishing the effects of joint loading versus inherent measurement variability remains difficult as the repeatability of these quantitative methods is often not assessed or reported.

Purpose: To assess exercise-induced changes in femoral, tibial and patellar articular cartilage composition and compare these against measurement repeatability.

Study Type: Prospective observational study.

Population: Phantom and 19 healthy participants.

Field Strength/Sequence: 3T; 3D fat-saturated spoiled gradient recalled-echo; $T_{1\rho}$- and T_2-prepared pseudo-steady-state 3D fast spin echo.

Assessment: The intra-sessional repeatability of $T_{1\rho}$ and T_2 relaxation mapping, with and without knee repositioning between two successive measurements, was determined in 10 knees. $T_{1\rho}$ and T_2 relaxation mapping of nine knees was performed

before and at multiple time points after a 5-minute repeated, joint-loading stepping activity. Three-dimensional surface models were created from patellar, femoral and tibial articular cartilage.

Statistical Tests: Repeatability was assessed using root-mean-squared-CV (RMS-CV). Using Bland-Altman analysis, thresholds defined as the smallest detectable difference (SDD) were determined from the repeatability data with knee repositioning.

Results: Without knee repositioning, both surface-averaged $T_{1\rho}$ and T_2 were very repeatable on all cartilage surfaces with RMS-CV<1.1%. Repositioning of the knee had the greatest effect on $T_{1\rho}$ of patellar cartilage with the surface-averaged RMS-CV=4.8%. While $T_{1\rho}$ showed the greatest response to exercise at the patellofemoral cartilage region, the largest changes in T_2 were determined in the lateral femorotibial region. Following thresholding, significant (> SDD) average exercise-induced in $T_{1\rho}$ and T_2 of femoral (-8.0% and -5.3%), lateral tibial (-6.9% and -5.9%), medial tibial (+5.8% and +2.9%) and patellar (-7.9% and +2.8%) cartilage were observed.

Data Conclusion: Joint loading with a stepping activity resulted in $T_{1\rho}$ and T_2 changes above background measurement error.

Key Words: Articular Cartilage; MRI; Quantitative Imaging; Repeatability; Exercise; Relaxation Time

INTRODUCTION

Over the last two decades in vivo magnetic resonance imaging (MRI) has increasingly been used to determine the mechanical properties of knee articular cartilage. Previous studies have shown that cartilage loading activities affect the morphology and biochemical composition of articular cartilage and have provided important information on the behaviour of cartilage when exposed to different compressive loads(1–3). $T_{1\rho}$ and T_2 relaxation time mapping techniques allow the assessment of cartilage compositional alterations in response to joint loading as they have been demonstrated to be sensitive to variations in the water and macromolecular content of cartilage(4–6). Normalised changes in $T_{1\rho}$ and T_2 relaxation times of cartilage following different exercise regimes have been shown to be in the order of -2.6% to -14.3% and +3.7% to -12.5%, respectively(2, 3, 7–9). Since the measured changes resulting from joint loading can be small, determining the intra-sessional repeatability of these quantitative measures is essential for reliable assessment of joint loading-related effects on cartilage structure and composition.

A systematic review showed that studies assessing the repeatability of these quantitative relaxation techniques without any joint loading activity have reported root-mean-squared coefficient of variation (RMS-CV) for large regional analysis of $T_{1\rho}$ values in the range of 2.3% – 6.3% and of T_2 values in the range of 2.3% – 6.5%(10). When sub-regional or laminar cartilage analysis was performed, test-retest CVs for $T_{1\rho}$ were up to 19% and for T_2 as high as 22%(10). Intra-sessional repeatability assesses the repeatability of measurements of i) consecutive scans without repositioning and ii) consecutive scans with repositioning of the subject(11). Evaluating the repeatability of consecutive scans without repositioning is important when measuring $T_{1\rho}$ and T_2 at multiple time-points after joint-loading for determining

longitudinal cartilage recovery as previous studies have reported(1, 12, 13).

Healthy cartilage is maintained with regular deformation and compression of the cartilage structure and its extracellular matrix (ECM) through physiological loading, such as experienced during exercise(14, 15). However, both overuse and disuse can have degenerative effects on the cartilage and are important risk factors in the development of osteoarthritis (OA)(15–17). When exposing the cartilage repeatedly to excessive loads, such as may occur during high-impact sports or, to minimal or no load following injury, the cartilage structure and micro-structure begin to break down(15, 18). Morphological changes in articular cartilage volume, thickness and joint space narrowing are not necessarily present in the early stages of OA and may change very slowly during disease progression. Therefore, measuring differences in cartilage deformational responses during or after loading may represent a more sensitive biomarker for detecting the early onset of OA(19, 20).

The aim of this study was to measure the intra-sessional repeatability of both $T_{1\rho}$ and T_2 of knee articular cartilage and to determine if these quantitative relaxation measurement techniques are sensitive to permit effective measurement of short-term cartilage compositional responses after a joint loading activity.

MATERIALS AND METHODS

All imaging was performed on a 3 T MRI system (MR750, GE Healthcare, Waukesha, WI, USA) using an 8-channel transmit/receive knee coil (Invivo, Gainesville, FL, USA). Participant imaging had local ethical approval, and written informed consent was provided by each participant.

Study Procedures

Phantom Repeatability

To assess the test-retest repeatability of the quantitative $T_{1\rho}$ and T_2 relaxation time measurements for a range of relaxation times, two consecutive $T_{1\rho}$ and T_2 relaxation mapping datasets were obtained from a phantom. The phantom consisted of five vials having different $T_{1\rho}$ and T_2 relaxations. Two vials had $T_{1\rho}$ and T_2 relaxation times similar to cartilage (~40 - 50 ms) at 3 T while the relaxation times of the remaining three vials were greater(21, 22). To additionally assess the inter-sessional variability (scanning the same phantom on different days), two further $T_{1\rho}$ and T_2 relaxation mapping datasets were acquired two days later. On each they, the same knee coil and setup was used with the phantom centred in the coil.

Group 1: In Vivo Repeatability Study

To assess the intra-sessional repeatability of $T_{1\rho}$- and T_2-relaxation mapping of cartilage, the right knee of ten healthy participants (five men, five women, mean age 28.9 ± 5.5 years) with no current knee pain symptoms, nor known history of joint disorder was imaged. Imaged knees were unloaded for 15 minutes prior to the imaging session to minimise short-term loading effects on the joint.

The MR session consisted of a sagittal 3D fat-saturated spoiled gradient recalled-echo (3D-FS SPGR) sequence, and sagittal $T_{1\rho}$- and T_2-mapping sequences. For details on pulse sequence parameters used, see section ‘Sequence Parameters’ below. Following repositioning of the participant and imaged knee, two consecutive acquisitions of $T_{1\rho}$- and T_2-mapping were performed using the same pulse sequences as before repositioning (Figure 1A). During knee repositioning, the participants removed their knee from the coil and sat up on the side of the MR table. The coil was

repositioned, followed by participant positioning. The time required for repositioning and the continuation of the imaging protocol was approximately five minutes.

Group 2: Exercise and Recovery Study

A second group were used to assess the magnitude of effect that mild exercise has on $T_{1\rho}$- and T_2-relaxation mapping of cartilage. The right knee of nine healthy participants (five men, four women, mean age 31.6 ± 6.0 years) with no current knee pain symptoms, nor known history of joint disorder was imaged. Imaged knees were unloaded for 15 minutes prior to the imaging session to minimise short-term loading effects on the joint.

The study design consisted of a 3D- FS SPGR sequence, followed by $T_{1\rho}$- and T_2-relaxation imaging before exercise, and at four time-points after exercise to assess cartilage compositional recovery. The standardised exercise protocol involved five minutes of stepping onto a step-stool (height ≈ 24cm) with one leg and stepping down onto the other side of the step-stool with the leg to be imaged (Figure 1B). This resulted in approximately 20 stepping cycles per minute in which the knee joint was repeatedly loaded.

The first post-exercise $T_{1\rho}$- and T_2-mapping sequences were acquired approximately at five and ten minutes after patient positioning, respectively. The post-exercise imaging protocol took approximately 45 minutes.

Sequence Parameters

3D-FS SPGR

The sagittal 3D-FS SPGR sequence parameters were: acquisition time = 6:52 min; field-of-view=150x128x136mm^3, matrix size=512x380x136 zero-fill interpolated to

512x512x136, reconstructed voxel size=0.29x0.29x1 mm^3, TR = 25.8 ms, TE = 6.8 ms, flip angle = 25°, coil acceleration factor (ASSET) = 2, number of excitations (NEX) = 0.7, bandwidth = ±11.9 kHz, with chemical shift selective fat-suppression.

T1ρ Mapping

$T_{1\rho}$ maps were obtained with a sagittal $T_{1\rho}$-prepared pseudo-steady-state 3D fast spin echo (PSS 3D-FSE) sequence using a rotary-echo spin-lock preparation to minimise B_1 non-uniformity effects(23, 24). Images were acquired using the following parameters: acquisition time = 5:23 min; matrix = 320x256 zero-fill interpolated to 512x512; FOV = 160x144 mm^2; reconstructed voxel size = 0.31x0.31x3 mm^3; flip angle = 90°; TR = 1580 ms; spin lock time (TSL) = 1, 10, 20, 35 ms; 72 slices per TSL; echo train length = 45; NEX = 0.5; and bandwidth = ±62.5 kHz. The $T_{1\rho}$ maps were created using a log-linearised least-squares algorithm to fit a mono-exponential decay function to the signal intensities

$$M(TSL) = M_0 \cdot e^{-TSL/T_{1\rho}} \quad (1)$$

Where *M(TSL)* is the signal intensity of the $T_{1\rho}$-weighted image at a specific *TSL* and M_0 is the initial magnetisation / signal intensity. $T_{1\rho}$ relaxation times > 130ms in $T_{1\rho}$ maps were excluded from analysis to avoid partial volume effects with synovial fluid(25, 26).

T_2 Mapping

T_2 maps were obtained with a sagittal T_2-prepared PSS 3D-FSE sequence using a composite 90_x - 180_y - 90_x pulse train for T_2-preparation(23, 27). Images were acquired

using the following parameters: acquisition time = 5:25 min; matrix = 320x256 interpolated to 512x512; FOV = 160x144 mm^2; reconstructed voxel size = 0.31x0.31x3 mm^3; flip angle = 90°; TR = 1580 ms; TEs = 6.5, 13.4, 27.0, 40.7 ms; 72 slices per TE; echo train length = 45; NEX = 0.5; and bandwidth = ±62.5 kHz. The T_2 maps were created using a log-linearised least-squares algorithm to fit a mono-exponential decay function to the signal intensities

$$M(TE) = M_0 \cdot e^{-TE/T_2} \quad (2)$$

Where *M(TE)* is the signal intensity of the T_2-weighted image at a specific *TE* and M_0 is the initial magnetisation / signal intensity. As with $T_{1\rho}$, T_2 relaxation times > 100ms in T_2 maps were excluded from analysis to avoid partial volume effects with synovial fluid(25, 26).

Imaging Analysis

Phantom Repeatability

Mean relaxation times from all five vials of the phantoms were determined using rectangular regions-of-interest (ROIs) placed on two central sequential slices of the sagittal $T_{1\rho}$ and T_2 maps.

In Vivo Surface Analysis

All $T_{1\rho}$- and T_2-weighted images were rigidly registered to the high-resolution 3D-FS SPGR images using the Elastix toolbox(28) before calculating the respective quantitative maps.

Surface-based analysis (3D Cartilage Surface Mapping, 3D-CaSM) of femoral, tibial and patellar cartilage was performed using the freely available Stradwin software version 5.4a (University of Cambridge Department of Engineering, Cambridge, UK, now freely available as 'StradView' at http://mi.eng.cam.ac.uk/Main/StradView/)(29). After creating sparse manual cross-sections (on every 2^{nd} – 4^{th} sagittal slice) of the patella, tibia, and femur including their surrounding cartilage on the 3D-FS SPGR datasets, a triangulated surface mesh object of each segmented bone-cartilage structure was automatically generated using shape-based interpolation and the regularised marching tetrahedra method(30). Following cartilage thickness calculation and the generation of inner and outer cartilage surfaces, these surfaces were used to analyse the registered quantitative $T_{1\rho}$ and T_2 maps. At each vertex, the $T_{1\rho}$ and T_2 values along a perpendicular line between inner and outer surface (surface normal) were sampled and averaged.

Canonical (average) femoral, tibial and patellar meshes were created from all participants to be able to compare the $T_{1\rho}$ and T_2 value distributions between participants. Canonical surfaces were calculated from all participants involved in the exercise and recovery imaging. All quantitative surface data from both the repeatability and exercise-recovery cohorts were mapped onto the canonical surface following surface registration. Canonical surface generation and the subsequent registration and mapping of the individual surfaces was performed using the freely available wxRegSurf software version 18 (University of Cambridge Department of Engineering, Cambridge, UK, freely available at http://mi.eng.cam.ac.uk/~ahg/wxRegSurf/). The full 3D-CaSM analysis pipeline is illustrated in Figure 2.

Statistical Analysis

Phantom Repeatability

Coefficients of variation (CVs) were calculated from the two successive repeatability scans on each day ($CV_{Phant,Day1}$, $CV_{Phant,Day2}$) for all five vials using

$$CV = \frac{\sigma}{\mu} \quad (3)$$

With σ being the within-vial standard deviation and μ the within-vial mean of measurements. The intra-phantom variability was evaluated by calculating the CV from the mean and standard deviation of the relaxation values obtained from both days ($CV_{Phant,All}$).

Group 1: In Vivo Repeatability Study

The intra-sessional repeatability of $T_{1\rho}$ and T_2 acquisitions was assessed by calculating root-mean-square average coefficients of variation (RMS-CV) from the surface-averaged $T_{1\rho}$ and T_2 measurements of all participants for femoral, medial tibial, lateral tibial and patellar cartilage surfaces. The RMS-CV between repeatability measurements 1 (before repositioning) and 2 (first measurement following repositioning) were calculated ($RMS\text{-}CV_{S1\text{-}S2}$) to evaluate the effects of knee repositioning on repeatability. The RMS-CV between measurements 2 and 3 (with no repositioning between both measurements) were determined to assess repeatability without knee repositioning ($RMS\text{-}CV_{S2\text{-}S3}$).

The smallest detectable difference (SDD)(31) was calculated as the repeatability coefficient from the ±95% confidence intervals from a Bland-Altman analysis(32) of all surface vertices of the repeatability data for all four cartilage surfaces and for both $T_{1\rho}$ and T_2.

Group 2: Exercise and Recovery Study

To determine the effects of the dynamic joint-loading stepper activity on mean MR relaxation times of entire cartilage surfaces, linear mixed-effects models with timepoint as a fixed effect and participant as a random effect for each surface/parameter combination were created. For all statistical analysis, a level of significance of 0.05 was used.

The upper ($+1.96 \cdot \sigma$) and lower ($-1.96 \cdot \sigma$) limits of agreement as determined from the ±95% confidence intervals of the Bland-Altman plots of the repeatability data were used to establish thresholds.

Exercise-induced changes in vertex-wise $T_{1\rho}$ and T_2 relaxation times greater than the SDD signify variations which have a 95% probability of representing a true change rather than a variation due to measurement error(33). Thresholds were determined for all four cartilage surfaces of interest. The determined thresholds were applied to the canonical surface data to only present cartilage regions undergoing a statistically significant exercise-induced compositional change at each surface vertex.

Vertex-wise percentage changes in $T_{1\rho}$ ($\%T_{1\rho}$ change) and T_2 ($\%T_2$ change) following exercise were calculated as the normalised change in cartilage relaxation time measurements

$$\%T_{relax} = 100 \cdot \frac{T_{relax,post} - T_{relax,pre}}{T_{relax,pre}} \quad (4)$$

Where $T_{relax,post}$ is the relaxation time measurement at a post exercise timepoint and $T_{relax,pre}$ is the relaxation time measurement prior to exercise.

The variability of $T_{1\rho}$ and T_2 relaxation values during cartilage compositional recovery following exposure to the mild stepping exercise was assessed only in the cartilage regions determined as regions experiencing significant exercise responses.

RESULTS

Phantom Imaging

The phantom test-retest repeatability on both days ($CV_{Phant,Day1}$, $CV_{Phant,Day2}$) was ≤2.29% for $T_{1\rho}$ and ≤0.74% for T_2 relaxation time measurements for all five vials. The CVs for the two phantoms having relaxation times comparable to cartilage were ≤0.64% for $T_{1\rho}$ and ≤0.21% for T_2. The inter-sessional repeatability ($CV_{Phant,All}$) calculated from all phantom repeatability scans over both days was ≤2.94% and ≤1.43% for $T_{1\rho}$ and T_2 relaxation time measurements, respectively. The measured relaxation times and determined CVs are listed in Supplementary Table 1.

Group 1: In Vivo Repeatability Study

The intra-sessional repeatability RMS-CV for in vivo relaxation time measurements averaged over the entire femoral, medial tibial, lateral tibial and patellar cartilage surfaces are listed in Table 1. The determined mean ± standard deviation (SD) of $T_{1\rho}$ relaxation times of repeatability scan 1 from all participants in group 1 for femoral,

lateral tibial, medial tibial and patellar cartilage surfaces were 50.1 ± 2.6 ms, 44.0 ± 3.3 ms, 44.0 ± 4.0 ms and 51.2 ± 3.5 ms. Mean ± SD of T_2 relaxation times for femoral, lateral tibial, medial tibial and patellar cartilage surfaces were 37.2 ± 1.6 ms, 32.0 ± 1.5 ms, 32.0 ± 2.3 ms and 35.5 ± 2.9 ms.

Knee repositioning showed the greatest effect on the mean surfaced-averaged $T_{1\rho}$ relaxation time values of the patellar cartilage (51.2 ms → 54.8 ms, RMS-CV_{S1-S2} = 4.8%) and the mean surfaced-averaged T_2 relaxation times of the lateral tibial cartilage (32.0 ms → 32.9 ms, RMS-CV_{S1-S2} = 2.0%).

The Bland-Altman plots for vertex-wise $T_{1\rho}$ and T_2 repeatability measurements with knee repositioning of all four cartilage surfaces under investigation are shown in Figure 3A and 3B, respectively.

The determined SDD and 95% limits of agreement from the Bland-Altman plots of all four cartilage surfaces and both compositional MRI methods are listed in Table 2.

Group 2: Exercise and Recovery Study

The $T_{1\rho}$ and T_2 relaxation times averaged over whole femoral, lateral tibial, medial tibial and patellar cartilage surfaces are illustrated in Figure 4. The determined mean baseline $T_{1\rho}$ relaxation times from the exercise-recovery cohort for femoral, lateral tibial, medial tibial and patellar cartilage surfaces were 50.9 ± 3.6 ms, 44.3 ± 4.5 ms, 44.9 ± 3.7 ms and 51.2 ± 8.9 ms. Mean baseline T_2 relaxation times for femoral, lateral tibial, medial tibial and patellar cartilage surfaces were 38.0 ± 2.0 ms, 34.4 ± 2.3 ms, 32.9 ± 3.0 ms and 34.6 ± 4.2 ms. There was a statistically significant group-averaged change of T_2 of the lateral tibia over time (b [95% CI] = -0.43 [-0.83, -0.04], $p < 0.05$). No other surface/parameter combination demonstrated a statistically significant change over time at the group level. There was significant variation in change over

time between participants for medial tibial $T_{1\rho}$ (SD [95% CI] = 1.04 [0.62,1.75], $p < 0.05$). The results of the linear mixed-effects models for each region are provided in Supplementary Table 2.

Figures 5 and 7 highlight the cartilage regions experiencing statistically significant changes in $T_{1\rho}$ and T_2 relaxation times following the mild stepping exercise, respectively. Correspondingly, Figures 6 and 8 illustrate the alteration ('recovery') in participant-averaged femoral $T_{1\rho}$ and T_2 percentage ($\%T_{1\rho}$ and $\%T_2$) changes determined from the four post-exercise measurements (scans 2 – 5) and the one pre-exercise baseline measurement (scan 1). Plots illustrating the variations in average lateral tibial, medial tibial and patellar $\%T_{1\rho}$ and $\%T_2$ changes are shown in Supplementary Figure 1 – 3, respectively.

Table 3 shows the total number of vertices of each canonical cartilage surface and the percentage of cartilage surface area covered in regions experiencing changes (increases and decreases) in $T_{1\rho}$ ($T_{1\rho}$-%SC) and T_2 (T_2-%SC) relaxation time measurements above the determined measurement errors.

Average $\%T_{1\rho}$ change of -7.9 ± 5.5 % and $\%T_2$ change of +2.8 ± 8.6 % were determined from all canonical patellar cartilage areas experiencing a significant change in relaxation times immediately following exercise. For the canonical femoral cartilage surface, average $\%T_{1\rho}$ and $\%T_2$ changes of -8.0 ± 4.9 % and -5.3 ± 2.3 % were observed in response to exercise, respectively. Average $\%T_{1\rho}$ and $\%T_2$ changes determined from all canonical lateral tibial cartilage regions displaying significant responses to exercise were -6.9 ± 3.2 % and -5.9 ± 2.8 %, respectively. Average medial tibial cartilage $\%T_{1\rho}$ change of +5.8 ± 5.2 % and $\%T_2$ change of +2.8 ± 9.5 % were determined.

The highest negative normalised change of -25.5 % was observed in the patellar cartilage $T_{1\rho}$ followed by -17.3 % in femoral cartilage $T_{1\rho}$ and -15.0 % in lateral tibial cartilage T_2. The largest positive normalised change of +28.4 % was displayed in the patellar cartilage T_2 followed by +15.7 % in medial tibial cartilage T_2 and +12.1 % in medial tibial cartilage $T_{1\rho}$.

When looking at cartilage compositional recovery following exercise and comparing the surface $\%T_{1\rho}$ and $\%T_2$ changes calculated from first post exercise measurements with the $\%T_{1\rho}$ and $\%T_2$ changes determined from last post exercise measurements, patella cartilage $\%T_{1\rho}$ change recovered by 15% while the T_2 'recovered' by 171%. The overall femoral cartilage $\%T_{1\rho}$ change dropped by 13% and the $\%T_2$ change increased by 2% compared to the initial, first post exercise percentage change. While the lateral tibial cartilage $\%T_{1\rho}$ change decreased by 15% of its initial value, the medial tibial $\%T_{1\rho}$ change increased by 1%. The overall $\%T_2$ change of both lateral and medial tibial cartilage increased by 12% and 50% compared to their initial values, respectively.

DISCUSSION

This work determined the effects of a mild dynamic stepping exercise on the MR relaxation times of cartilage surfaces related to variation in biochemical composition.

The intra-sessional repeatability coefficients-of-variation for $T_{1\rho}$ and T_2 in this study were lower than or comparable to those determined in previous studies(10). When looking at the surface-averaged $T_{1\rho}$ and T_2 repeatability measurements without knee repositioning, both $T_{1\rho}$ and T_2 were very repeatable on all surfaces. Repositioning of the knee had the greatest effect on the $T_{1\rho}$ relaxation time measurements of patellar cartilage. During repositioning the knee joint experienced bending which could lead to

larger changes in cartilage composition at the patellofemoral cartilage contact areas though friction than at the tibiofemoral areas. Averaging of relaxation times over large surfaces could mask these effects on the femoral cartilage surface due to its greater size in comparison to the smaller patellar surface. However, knee repositioning did not show a similarly strong effect on the patellar T_2 relaxation time measurements. This could be a consequence from the time delay (≈10 minutes) required for patient positioning, localisation and $T_{1\rho}$ data acquisition before the T_2 acquisition started and therefore allowing compositional recovery during this time period.

In this study, 3D surface analysis was performed to help gain a better insight into how different cartilage regions respond to and recover from exercise. When averaging the $T_{1\rho}$ and T_2 measurements over the entire femoral, lateral tibial, medial tibial and patellar cartilage surfaces, no statistically significant exercise-related changes were determined when comparing the pre-exercise scan with the first post-exercise scan. As a previous study has also reported, determining mean relaxation time changes from individual slices or across large regions-of-interest may mask significant focal changes(34). When the individual vertex-wise relaxation times measurements in this study were re-gridded onto a canonical surface, significant exercise-related focal changes in $T_{1\rho}$ and T_2 were observed. Although individual participants showed different cartilage compositional response to the exercise performed, cartilage regions experiencing compositional responses consistent across all participants became evident. By thresholding the exercise-related changes in MR relaxation time measurements with the predetermined threshold limits from the repeatability measurements, cartilage regions undergoing significant responses to the mild dynamic joint-loading activity were highlighted.

Since greater overall normalised changes were seen with $T_{1\rho}$ than with T_2 relaxation time measurements, $T_{1\rho}$ may be a more sensitive biomarker for detecting compositional cartilage responses to joint-loading activities. The $\%T_{1\rho}$ changes of patellar (-7.9%), femoral (-8.0%) and lateral tibial (-6.9%) cartilage and the $\%T_2$ changes of femoral (-5.3%) and lateral tibial (-5.9%) cartilage observed in this study are comparable with those seen in previous studies. Mosher et al showed a $\%T_2$ change of approximately -2.5% to -3.2% in femoral and -1.3% to -3.6% in lateral tibial cartilage following a 30-minute running activity(35). Similarly, Subburaj et al demonstrated a $\%T_{1\rho}$ change of -4.1% to -14.3% and a $\%T_2$ change of -3.0% to -9.3% in femoral, tibial and patellar cartilage following running for 30 minutes(2). The joint movements during the stepping activity performed in this study are comparable to the movements during the stair activity carried out in the study by Chen et al(3). Similarly, the 5-minute stepping activity performed in this study showed a greater effect on patellofemoral cartilage $T_{1\rho}$ relaxation times than on those of femorotibial cartilage, especially in the region of patellofemoral cartilage contact.

We not only observed regions experiencing significant decreases but also significant increases in relaxation time measurements immediately following exercise, especially in medial tibial $T_{1\rho}$ and T_2, and patellar T_2. Farrokhi et al also demonstrated a slightly increased $\%T_2$ relaxation time change of 0.3% of healthy patellar cartilage following 50 deep knee bends(7). Gatti et al showed an increased medial femoral $\%T_2$ change after participants bicycled for approximately 45 minutes(9). Areas of increased normalised change could result from water redistribution rather than expulsion, increasing the water content and decreasing collagen and proteoglycan concentrations in these regions.

Various compositional 'recovery' time-courses were determined for the four different cartilage surfaces. While patellar cartilage volume has been shown to recover in an almost linear fashion following 100 knee bends, we did not observe this linear recovery pattern in patellar cartilage composition(1). Overall, we only observed a drop in compositional normalised change in four instances (%$T_{1\rho}$ change of patellar, femoral and lateral tibial cartilage; %T_2 change of patellar cartilage) while in the other four instances (%$T_{1\rho}$ change of medial tibial cartilage; %T_2 change of femoral, medial and lateral tibial cartilage) an increase in normalised change was observed during the recovery period (post-exercise scan 2 → scan 5). Cartilage morphology (thickness, volume), independent of cartilage health state, has been shown to recover almost fully in about 45-90 minutes following 30(36) and 100 knee bends(1) and a 30 minute(13) and 20 km run(12). Based on our results, the focal compositional changes appear to require more time to return to baseline. More cartilage surfaces experienced some degree of compositional recovery in $T_{1\rho}$ compared to T_2, suggesting that the proteoglycan concentration is recovering faster due to water uptake than the changes in the collagen network after cessation of dynamic joint-loading.

The stepping exercise performed in this study is mild and of short duration. This exercise type was chosen as it is thought to be feasible and extendable for use in patients with early stage knee joint disease and minimal accompanying pain. Knowledge of the effects that deformational loads have on cartilage structure and biochemical composition are important when evaluating clinical imaging studies aiming at determining differences in healthy and diseased cartilage. Differences in cartilage compositional MR relaxation time measurements between healthy and osteoarthritic cartilage have been shown to be in the range of 2 - 13% for $T_{1\rho}$ and 1 - 12% for T_2 for large regional analysis(21, 25, 37). As the disease-induced

compositional changes in cartilage reflected in $T_{1\rho}$ and T_2 measurements can be of the same order, and appear in similar cartilage regions, as exercise-induced changes, it is important to mitigate these effects when conducting clinical OA trials. A 3D surface analysis provides the possibility of spatially localising the deformational and compositional effects of joint loading on articular cartilage and could also assist in determining the regions most prone to exhibit cartilage degeneration(29).

Limitations

As the number of participants in the repeatability and exercise-recovery groups was limited, a larger sample size would increase the precision of the study results. A major limitation to in vivo studies assessing cartilage response to different joint-loading activities is that the compositional behaviour of cartilage cannot be determined immediately after cessation of the exercise but only some short time after as time is required to position the participant back in the MRI system and for acquiring the data. Additionally, the $T_{1\rho}$ and T_2 relaxation time mapping data were not acquired simultaneously but sequentially. Although both sequences are fast spin-echo based sequences, the T_2 mapping was always performed about six minutes after $T_{1\rho}$ during which time further compositional recovery could take place preventing an exact comparison between $T_{1\rho}$ and T_2 results. A sequence capable of simultaneous $T_{1\rho}$ and T_2 acquisition, such as the sequence proposed by Li et al(38), could help address this issue.

CONCLUSION

We have shown that exercise-related changes in cartilage $T_{1\rho}$ and T_2 relaxation times exceed measurement error and can reliably be determined when using the described 3D-CaSM analysis approach. Based on the results presented here, we hypothesise that mapping of cartilage $T_{1\rho}$ and T_2 relaxation times are measuring

dissimilar compositional features as similar cartilage regions showed different $T_{1\rho}$ and T_2 responses to exercise. However, while complete morphological recovery has previously been shown, the question of when, whether and how the different cartilage regions recover completely from compositional variations following joint loading activities persists.

REFERENCES

1. Eckstein F, Tieschky M, Faber S, Englmeier KH, Reiser M: Functional analysis of articular cartilage deformation, recovery, and fluid flow following dynamic exercise in vivo. *Anat Embryol (Berl)* 1999; 200:419–424.

2. Subburaj K, Kumar D, Souza RB, et al.: The acute effect of running on knee articular cartilage and meniscus magnetic resonance relaxation times in young healthy adults. *Am J Sports Med* 2012; 40:2134–2141.

3. Chen M, Qiu L, Shen S, et al.: The influences of walking, running and stair activity on knee articular cartilage: Quantitative MRI using T1 rho and T2 mapping. *PLoS One* 2017; 12:1–15.

4. Akella SVS, Regatte RR, Gougoutas AJ, et al.: Proteoglycan-Induced Changes in T1ρ-Relaxation of Articular Cartilage at 4T. *Magn Reson Med* 2001; 46:419–423.

5. Nissi MJ, Töyräs J, Laasanen MS, et al.: Proteoglycan and collagen sensitive MRI evaluation of normal and degenerated articular cartilage. *J Orthop Res* 2004; 22:557–564.

6. Regatte RR, Akella SVS, Lonner JH, Kneeland JB, Reddy R: T1rho mapping in human osteoarthritis (OA) cartilage: Comparison of T1rho with T2. *J Magn Reson Imaging* 2006; 23:547–553.

7. Farrokhi S, Colletti PM, Powers CM: Differences in patellar cartilage thickness, transverse relaxation time, and deformational behavior: A comparison of young women with and without patellofemoral pain. *Am J Sports Med* 2011; 39:384–391.

8. Cha JG, Lee JC, Kim HJ, et al.: Comparison of MRI T2 relaxation changes of knee articular cartilage before and after running between young and old amateur athletes. *Korean J Radiol* 2012; 13:594–601.

9. Gatti AA, Noseworthy MD, Stratford PW, et al.: Acute changes in knee cartilage transverse relaxation time after running and bicycling. *J Biomech* 2017; 53:171–177.

10. MacKay JW, Low SBL, Smith TO, Toms AP, McCaskie AW, Gilbert FJ: Systematic review and meta-analysis of the reliability and discriminative validity of cartilage compositional MRI in knee osteoarthritis. *Osteoarthr Cartil* 2018; 26:1140–1152.

11. Jordan CD, Monu UD, McWalter EJ, et al.: Variability of CubeQuant T1rho, Quantitative DESS T2, and Cones Sodium MRI in Knee Cartilage. *Osteoarthr Cartil* 2014; 22:1559–1567.

12. Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB: Recovery of the menisci and articular cartilage of runners after cessation of exercise: Additional aspects of in vivo investigation based on 3-dimensional magnetic resonance imaging. *Am J Sports Med* 2008; 36:966–970.

13. Van Ginckel A, Verdonk P, Victor J, Witvrouw E: Cartilage status in relation to return to sports after anterior cruciate ligament reconstruction. *Am J Sports Med* 2013; 41:550–559.

14. Bertrand J, Held A: Role of Proteoglycans in Osteoarthritis. In *Cartil Vol 2 Pathophysiol*. Edited by Grässel S, Aszódi A. Springer International Publishing; 2017.

15. Sun HB: Mechanical loading, cartilage degradation, and arthritis. *Ann N Y Acad Sci* 2010; 1211:37–50.

16. Simon LS: Osteoarthritis: A review. *Clin Cornerstone* 1999; 2:26–37.

17. Buckwalter JA, Martin JA: Osteoarthritis. *Adv Drug Deliv Rev* 2006; 58:150–167.

18. Ismail HM, Vincent TL: Cartilage Injury and Osteoarthritis. In *Cartil Vol 2 Pathophysiol*. Edited by Grässel S, Aszódi A. Springer International Publishing; 2017.

19. Burstein D, Bashir A, Gray ML: MRI techniques in early stages of cartilage disease. *Invest Radiol* 2000; 35:622–38.

20. Oei EHG, van Tiel J, Robinson WH, Gold GE: Quantitative Radiologic Imaging Techniques for Articular Cartilage Composition: Toward Early Diagnosis and Development of Disease-Modifying Therapeutics for Osteoarthritis. *Arthritis Care Res (Hoboken)* 2014; 66:1129–1141.

21. Li X, Ma CB, Link TM, et al.: In vivo T1rho and T2 Mapping of Articular Cartilage in Osteoarthritis of the Knee Using 3 Tesla MRI. *Osteoarthr Cart* 2007; 15:789–797.

22. Jordan CD, Saranathan M, Bangerter NK, Hargreaves BA, Gold GE: Musculoskeletal MRI at 3.0 T and 7.0 T: A comparison of relaxation times and image contrast. *Eur J Radiol* 2013; 82:734–739.

23. Chen W, Takahashi A, Han E: 3D Quantitative Imaging of T1rho and T2 (Abstract). *Proc Annu Meet ISMRM* 2011; 19:231.

24. Charagundla SR, Borthakur A, Leigh JS, Reddy R: Artifacts in T1ρ-weighted imaging: Correction with a self-compensating spin-locking pulse. *J Magn Reson* 2003; 162:113–121.

25. Souza RB, Kumar D, Calixto N, et al.: Response of knee cartilage T1rho and T2 relaxation times to in vivo mechanical loading in individuals with and without knee osteoarthritis. *Osteoarthr Cartil* 2014; 22:1367–1376.

26. Van Rossom S, Smith CR, Zevenbergen L, et al.: Knee cartilage thickness, T1ρ and T2 relaxation time are related to articular cartilage loading in healthy adults. *PLoS*

One 2017; 12:1–16.

27. Levitt MH, Freeman R: Compensation for pulse imperfections in NMR spin-echo experiments. *J Magn Reson* 1981; 43:65–80.

28. Klein S, Staring M, Murphy K, Viergever MA, Pluim JPW: elastix: A Toolbox for Intensity-Based Medical Image Registration. *IEEE Trans Med Imaging* 2010; 29:196–205.

29. MacKay JW, Kaggie JD, Treece GM, et al.: Three-Dimensional Surface-Based Analysis of Cartilage MRI Data in Knee Osteoarthritis: Validation and Initial Clinical Application. *J Magn Reson Imaging* 2020:1–13.

30. Treece GM, Prager RW, Gee AH: Regularised marching tetrahedra: improved iso-surface extraction. *Comuters Graph* 1999; 23:583–598.

31. Lassere M, Boers M, Van Der Heijde D, et al.: Smallest detectable difference in radiological progression. *J Rheumatol* 1999; 26:731–739.

32. Altman DG, Bland JM: Measurement in Medicine : The Analysis of Method Comparison Studies. *Stat* 1983; 32:307–317.

33. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1:307–310.

34. Waterton JC, Solloway S, Foster JE, et al.: Diurnal variation in the femoral articular cartilage of the knee in young adult humans. *Magn Reson Med* 2000; 43:126–132.

35. Mosher TJ, Liu Y, Torok CM: Functional cartilage MRI T2 mapping: evaluating the effect of age and training on knee cartilage response to running. *Osteoarthr Cartil* 2010; 18:358–364.

36. Ginckel A Van, Witvrouw E: Acute Cartilage Loading Responses After an In Vivo Squatting Exercise in People With Doubtful to Mild Knee Osteoarthritis: A Case-Control Study. *Phys Ther* 2013; 93:1049–1060.

37. Wang L, Chang G, Xu J, et al.: T1rho MRI of menisci and cartilage in patients with osteoarthritis at 3T. *Eur J Radiol* 2012; 81:2329–2336.

38. Li X, Wyatt C, Rivoire J, et al.: Simultaneous Acquisition of T1ρ and T2 Quantification in Knee Cartilage : Repeatability and Diurnal Variation. *J Magn Reson Imaging* 2014; 39:1287–1293.

Tables

Table 1 - Root-mean-squared coefficients of variation (RMS-CV) for in vivo $T_{1\rho}$ and T_2 repeatability measurements. For RMS-CV calculation, the vertex-wise $T_{1\rho}$ and T_2 measurements were averaged over whole femoral, lateral tibial, medial tibial and patellar cartilage surfaces. Between repeatability scans 1 and 2, the knee was repositioned (RMS-CV $_{S1\text{-}S2}$). Repeatability scans 2 and 3 were obtained successively and without knee repositioning (RMS-CV$_{S2\text{-}S3}$).

Cartilage	$T_{1\rho}$		T_2	
Surface	RMS-CV$_{S1\text{-}S2}$ [%]	RMS-CV$_{S2\text{-}S3}$ [%]	RMS-CV$_{S1\text{-}S2}$ [%]	RMS-CV$_{S2\text{-}S3}$ [%]
Femoral	0.15	0.24	0.99	0.10
Lateral Tibial	0.26	0.03	2.03	0.30
Medial Tibial	0.41	0.90	1.37	1.09
Patellar	4.81	0.05	1.39	0.22

Table 2 - Determined smallest detectable differences (SDD) and ±95% limits of agreement from Bland-Altman analysis for both $T_{1\rho}$ and T_2 and for all cartilage surfaces.

Cartilage Surface	$T_{1\rho}$		T_2	
	SDD [ms]	+/- 95% limits of agreement [ms]	SDD [ms]	+/- 95% limits of agreement [ms]
Femoral	3.4	+3.6 / -3.2	1.9	+2.5 / -1.4
Lateral Tibial	2.6	+2.4 / -2.9	1.5	+2.4 / -0.6
Medial Tibial	2.2	+2.4 / -2.0	2.5	+3.2 / -1.8
Patellar	4.8	+8.7 / -0.8	1.6	+2.3 / -0.8

Table 3 – The total number of canonical surface vertices from all four cartilage surfaces and the percentage of surface covered by cartilage regions experiencing changes in $T_{1\rho}$ ($T_{1\rho}$-%SC) and T_2 (T_2-%SC) above the measurement error in response to exercise.

Cartilage Surface	Total Number of Surface Vertices	$T_{1\rho}$ - %SC	T_2 - %SC
Femoral	3694	8.1	23.0
Lateral Tibial	916	11.4	76.7
Medial Tibial	999	44.0	3.0
Patellar	1093	39.5	36.2

Figure Legends

Figure 1 - Summary of MR sessions performed. **A:** In vivo assessment of intra-sessional repeatability of cartilage $T_{1\rho}$ and T_2 mapping. After having the participant sit and keep the imaged knee in an unloaded state for approximately 15 minutes prior to imaging, initial $T_{1\rho}$ and T_2 relaxation mapping was acquired. Following knee repositioning, two successive $T_{1\rho}$ and T_2 relaxation mapping measurements were acquired. **B:** In vivo assessment of the change in cartilage composition following mild exercise. The imaged knee (green) was kept in an unloaded state for approximately 15 minutes before acquiring the initial $T_{1\rho}$ and T_2 relaxation measurements. Following mild exercise, four repeats of $T_{1\rho}$ and T_2 relaxation mapping measurements were acquired to evaluate cartilage compositional change and recovery following exercise.

Figure 2 - Summary of 3D-CaSM analysis pipeline illustrated for femoral cartilage surface. The 3D-FS SPGR datasets (**A**) were used to creating sparse manual contouring (on every 2^{nd} – 4^{th} sagittal slice) of the patella, tibia, and femur including their surrounding cartilage (**B**). Following the generation of unique triangulated surface mesh objects of each cartilage surface (**C**) and for each participant, canonical cartilage surfaces were calculated (**D**). All the quantitative surface data ($T_{1\rho}$ and T_2) from both the repeatability and exercise-recovery groups were mapped onto the canonical surface following surface registration (**E**).

Figure 3 – **A**: Bland-Altman plots showing the difference in $T_{1\rho}$ measurements with knee repositioning between repeatability acquisition 1 and 2 (blue circles) against their mean values. **B**: Bland-Altman plots showing the difference in T_2 measurements with knee repositioning between repeatability acquisition 1 and 2 against their mean values. The dotted lines represent the 95 % limits of agreement; the solid line is the overall mean difference from all difference measurements.

Figure 4 - $T_{1\rho}$ (top) and T_2 measurements (bottom) averaged over whole femoral, medial tibial, lateral tibial and patellar cartilage surfaces for all exercise recovery scans. Each colour represents an individual participant with the black curve representing the mean average trend (loess) of all participants with shaded 95% confidence intervals. Between the baseline scan (timepoint 0) and the first post-exercise scan (timepoint 1), the participant performed a stepping activity dynamically loading the imaged knee for 5 minutes. The first post-exercise $T_{1\rho}$- and T_2-mapping sequences were acquired approximately five and ten minutes after patient positioning, respectively. The last post-exercise $T_{1\rho}$- and T_2-mapping sequences (timepoint 4) were acquired approximately 35 and 40 minutes after patient positioning, respectively. The acquisition of the post-exercise imaging protocol took approximately 45 minutes.

Figure 5 - Participant-averaged $T_{1\rho}$ difference maps from (**A**) patellar, (**B**) femoral, (**C**) lateral and medial tibial cartilage surfaces. The difference maps were calculated by subtracting the average pre-exercise measurement from all four post-exercise recovery measurements (left to right: 1. Post – Pre; 2. Post – Pre; 3. Post – Pre; 4. Post – Pre). Cartilage regions experiencing decreases in $T_{1\rho}$ are specified in red, and regions with an increase in $T_{1\rho}$ compared to the pre-exercise measurement are specified in blue. Only regions experiencing

changes larger than the determined thresholds from the repeatability scans are colour-coded. Other areas have been thresholded to zero.

Figure 6 - Plot showing the normalised change in participant-average femoral $T_{1\rho}$ ($\%T_{1\rho}$ change) determined from the four post-exercise measurements (scans 2 – 5) and the one pre-exercise baseline measurement (scan 1). $\%T_{1\rho}$ change at each vertex was calculated as 100*(post-pre/pre) and then averaged. The black solid line represents the collective $\%T_{1\rho}$ change from all areas experiencing a significant change (increase and decrease) between a post-exercise timepoint and pre-exercise measurement. Below the plot is a table containing $\%T_{1\rho}$ change mean ± standard deviation (range) [%] from all vertex-wise calculated normalised changes in the areas experiencing significant variations.

Figure 7 – Participant-averaged T_2 difference maps from (**A**) patellar, (**B**) femoral, (**C**) lateral and medial tibial cartilage surfaces. The difference maps were calculated by subtracting the average pre-exercise measurement from all four post-exercise recovery measurements (left to right: 1. Post – Pre; 2. Post – Pre; 3. Post – Pre; 4. Post – Pre). Cartilage regions experiencing decreases in T_2 are specified in red, and regions with an increase in T_2 compared to the pre-exercise measurement are specified in blue. Only regions experiencing changes larger than the determined thresholds from the repeatability scans are colour-coded. Other areas have been thresholded to zero.

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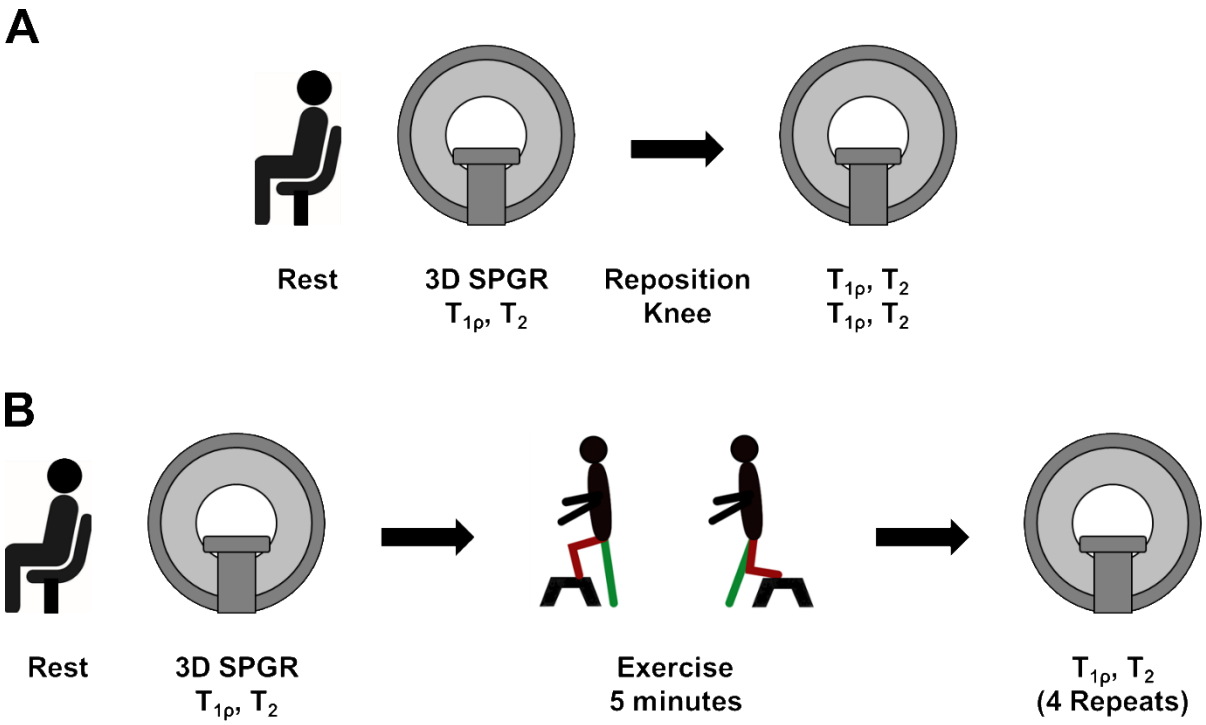
Figures



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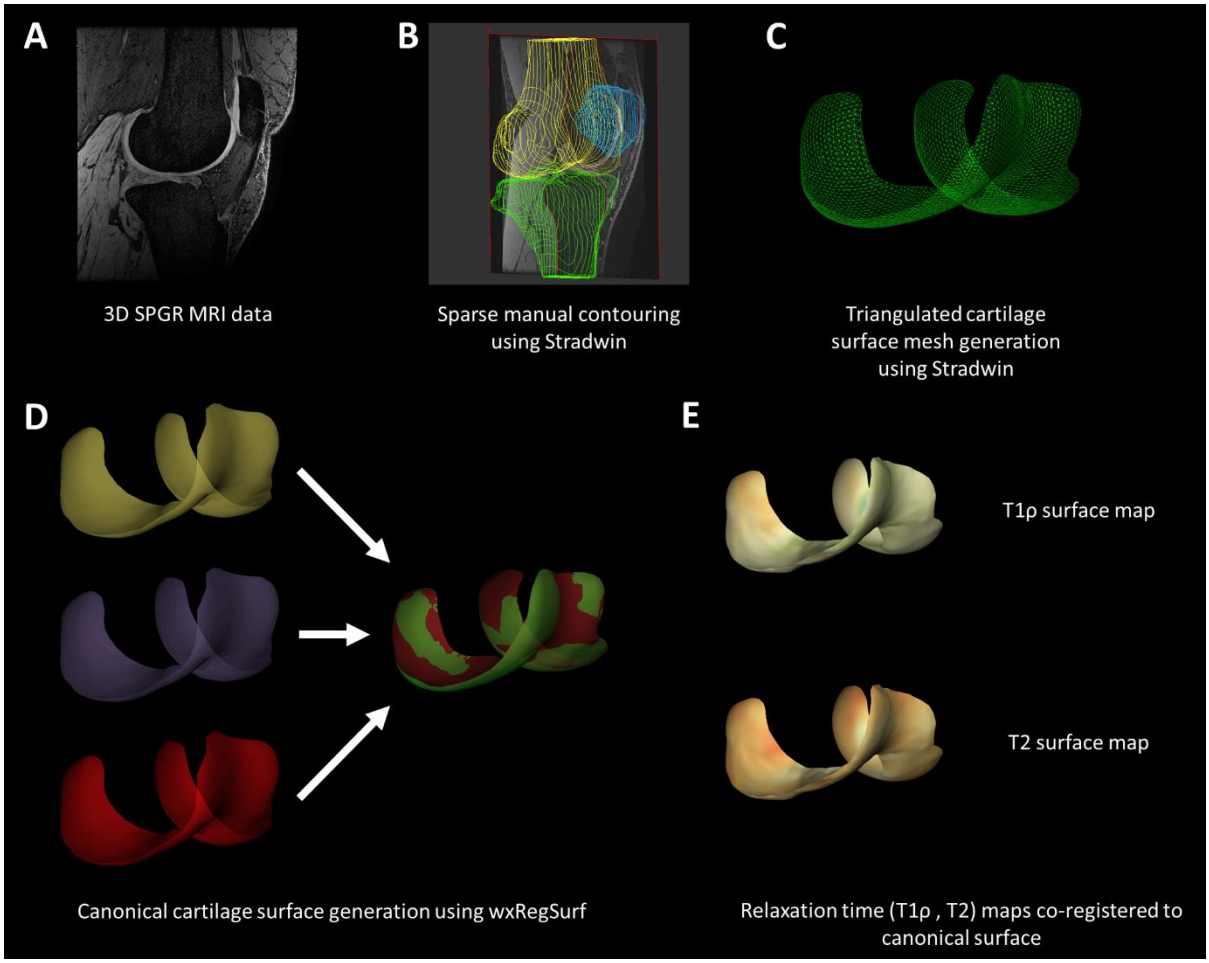

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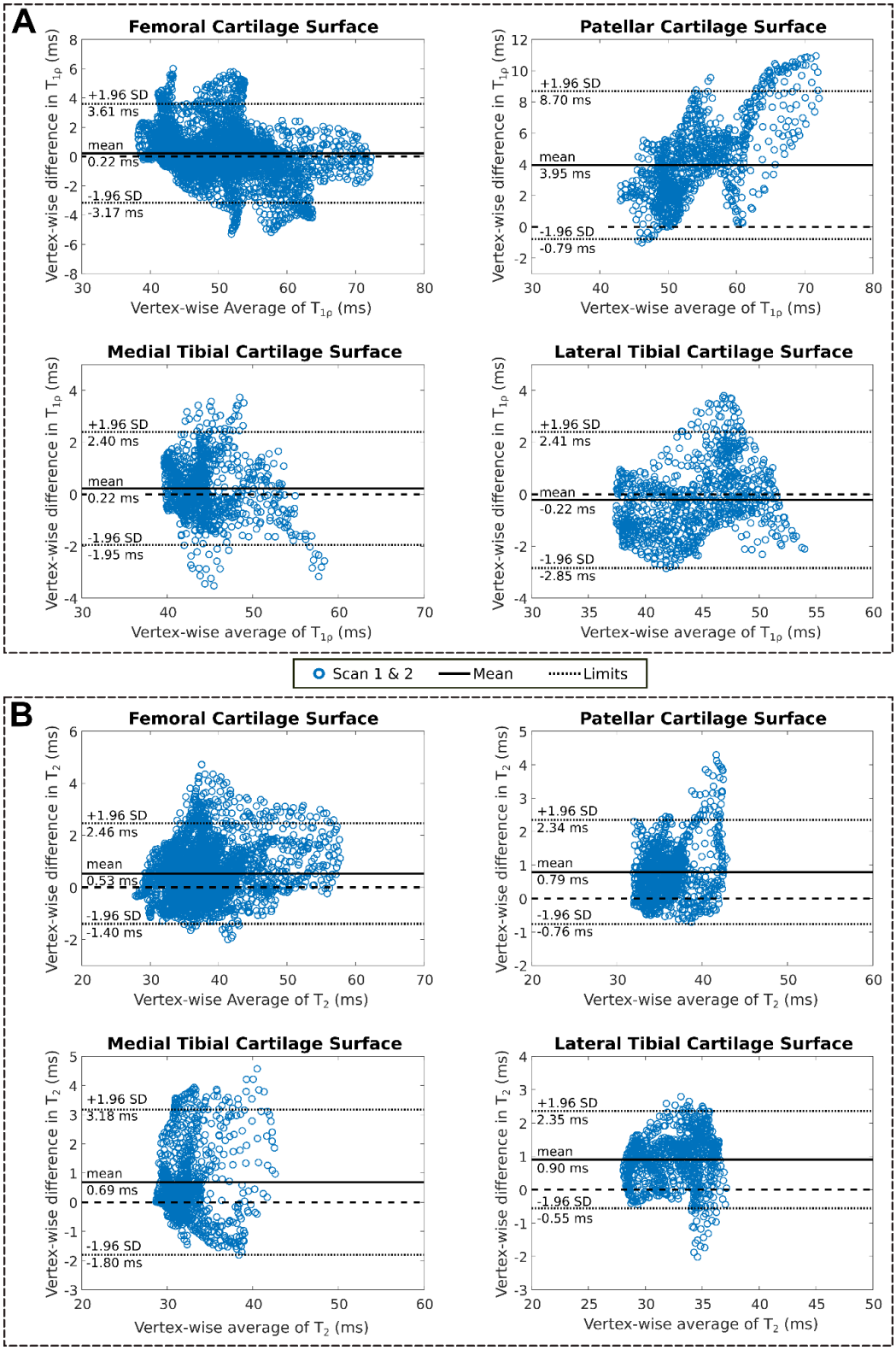

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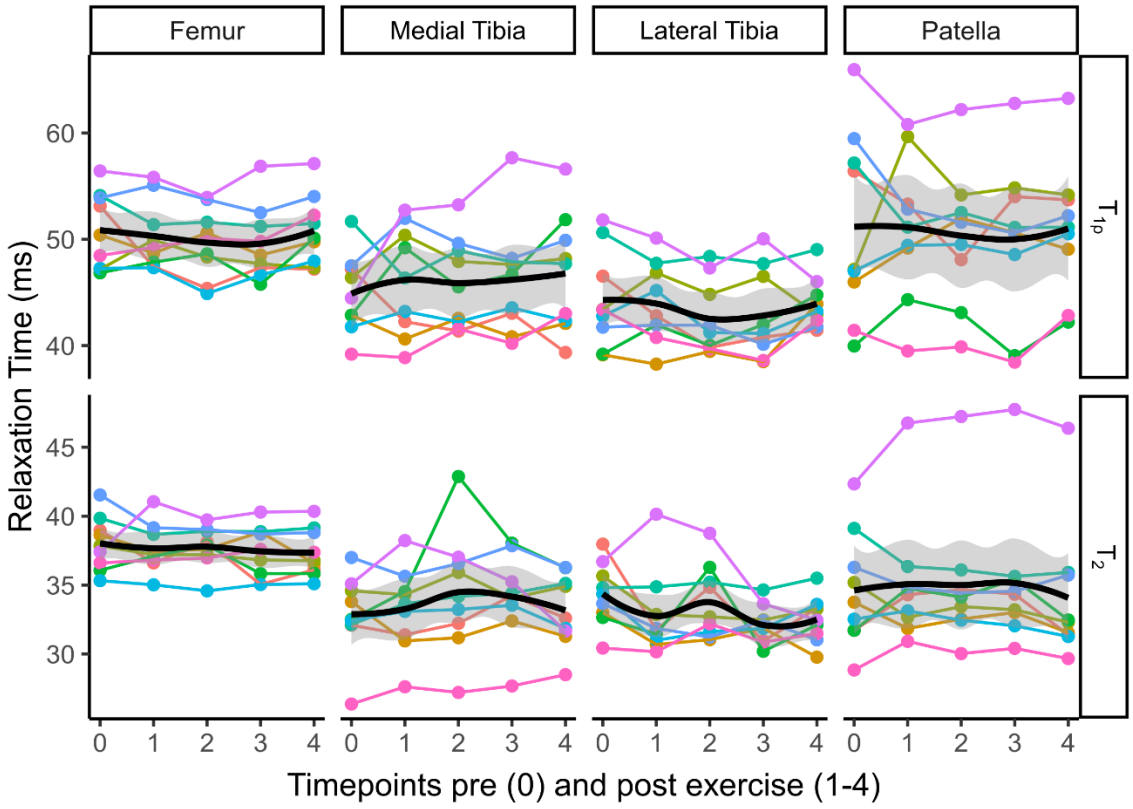

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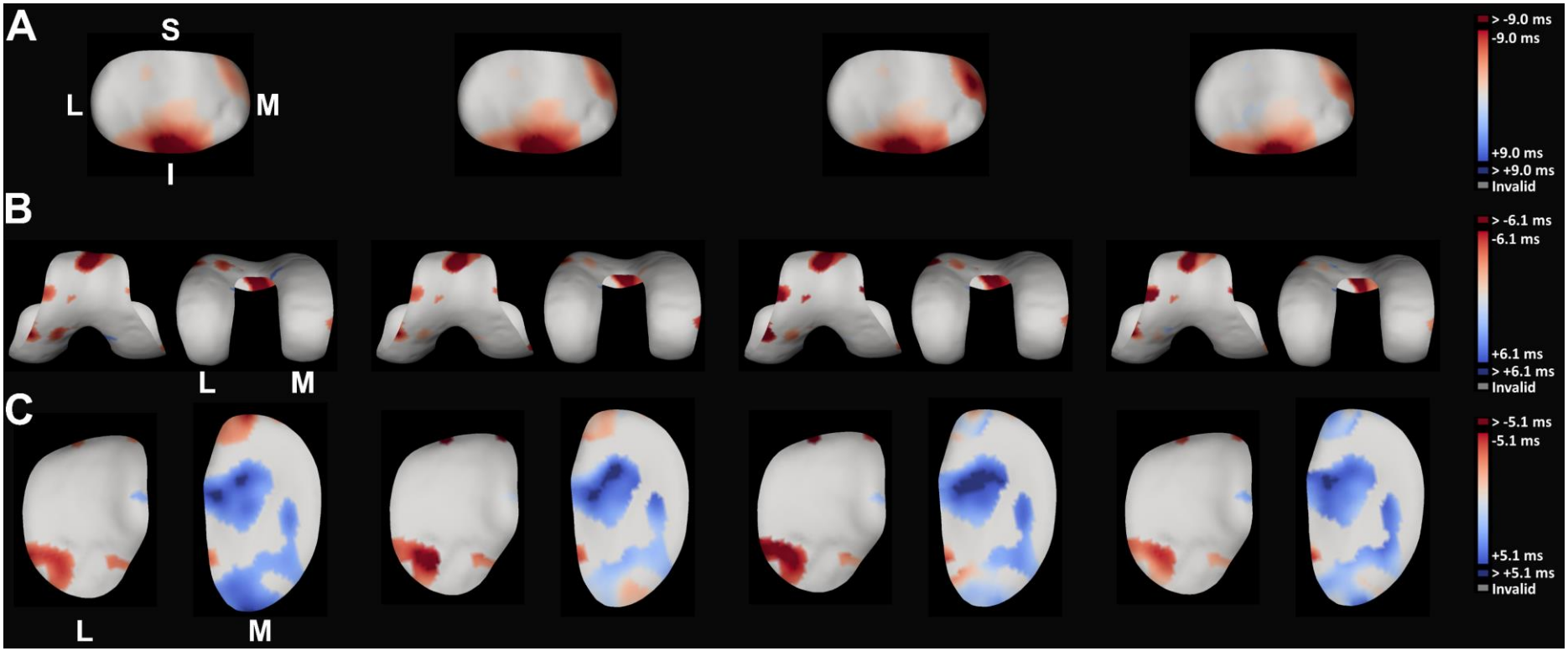

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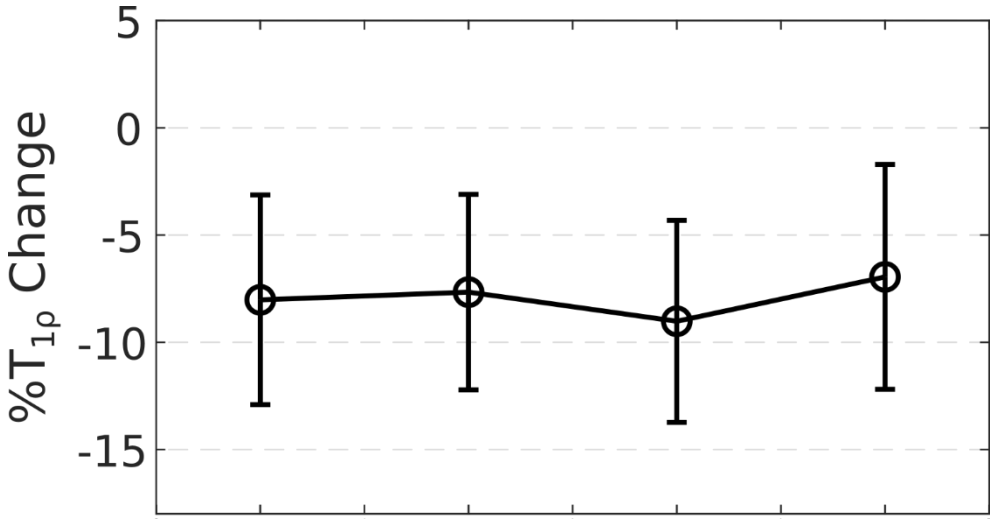

Scan 1 & 2	Scan 1 & 3	Scan 1 & 4	Scan 1 & 5
-8.0 ± 4.9	-7.7 ± 4.6	-9.0 ± 4.7	-7.0 ± 5.2
(-17.3, 7.6)	(-17.4, 6.8)	(-20.6, 5.7)	(-17.0, 8.5)

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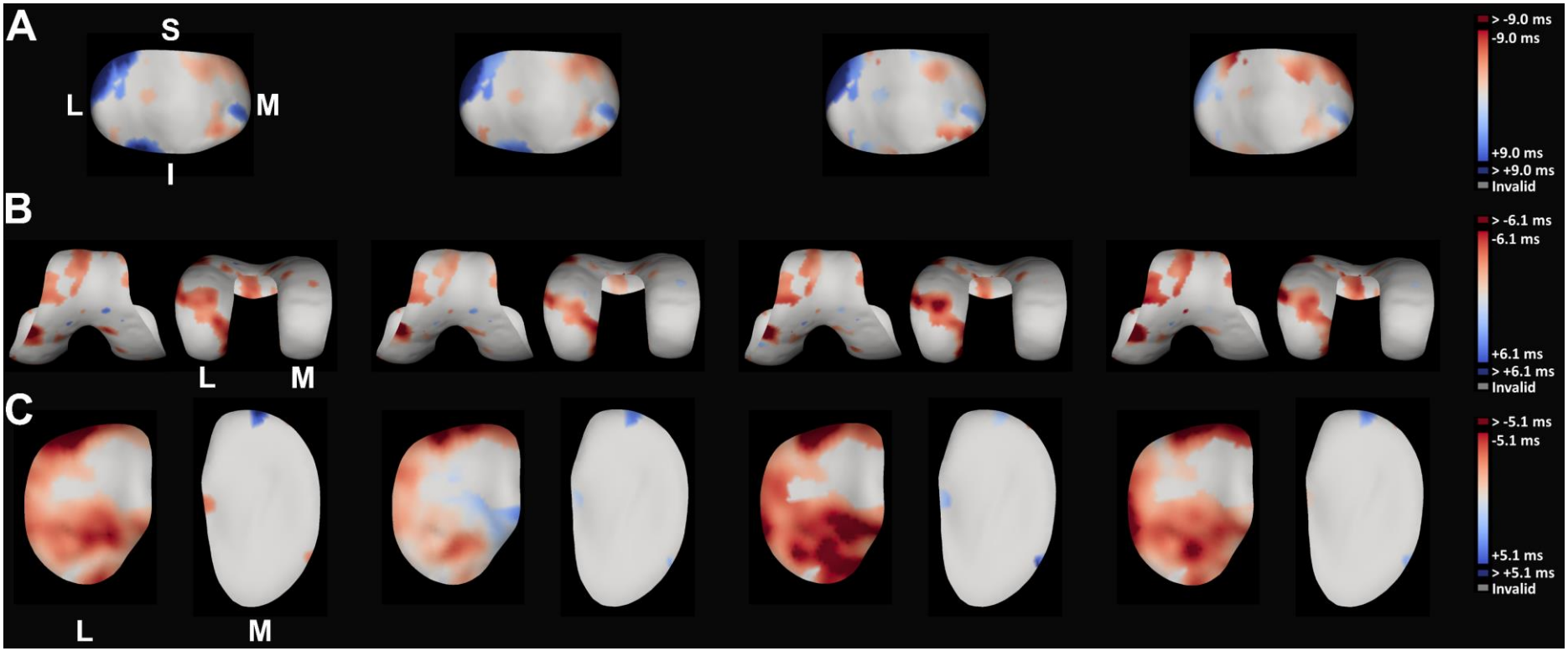

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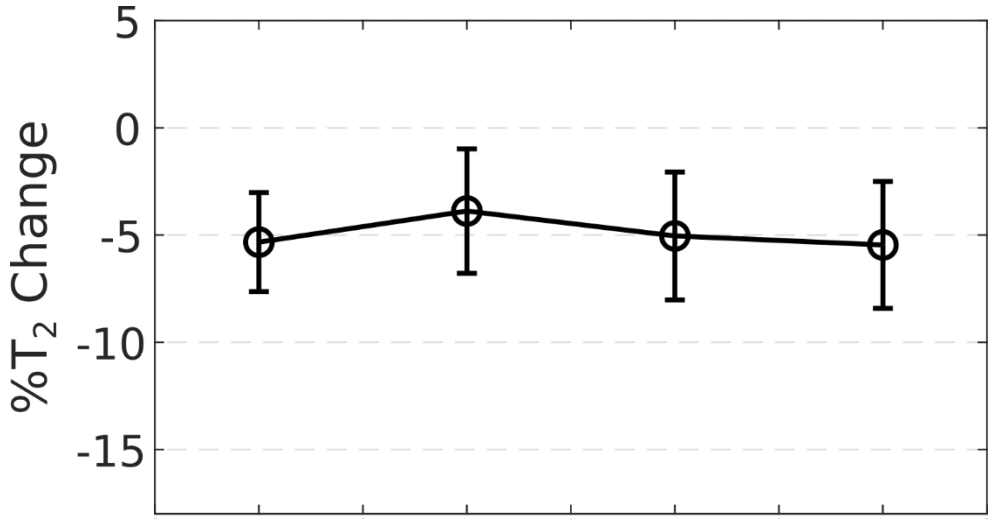

Scan 1 & 2	Scan 1 & 3	Scan 1 & 4	Scan 1 & 5
-5.3 ± 2.3	-3.9 ± 2.9	-5.0 ± 3.0	-5.5 ± 3.0
(-12.9, 7.6)	(-12.6, 5.8)	(-14.8, 5.2)	(-17.6, 4.4)

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