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RESEARCH ARTICLE

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Rapid volumetric gagCEST imaging of knee articular cartilage at 3 T: evaluation of improved dynamic range and an osteoarthritic population

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Chemical exchange saturation transfer of glycosaminoglycans, gagCEST, is a quantitative MR technique that has potential for assessing cartilage proteoglycan content at field strengths of 7 T and higher. However, its utility at 3 T remains unclear. The objective of this work was to implement a rapid volumetric gagCEST sequence with higher gagCEST asymmetry at 3 T to evaluate its sensitivity to osteoarthritic changes in knee articular cartilage and in comparison with T_2 and $T_{1\rho}$ measures. We hypothesize that gagCEST asymmetry at 3 T decreases with increasing severity of osteoarthritis (OA). Forty-two human volunteers, including 10 healthy subjects and 32 subjects with medial OA, were included in the study. Knee Injury and Osteoarthritis Outcome Scores (KOOS) were assessed for all subjects, and Kellgren-Lawrence grading was performed for OA volunteers. Healthy subjects were scanned consecutively at 3 T to assess the repeatability of the volumetric gagCEST sequence at 3 T. For healthy and OA subjects, gagCEST asymmetry and T_2 and $T_{1\rho}$ relaxation times were calculated for the femoral articular cartilage to assess sensitivity to OA severity. Volumetric gagCEST imaging had higher gagCEST asymmetry than single-slice acquisitions ($p = 0.015$). The average scan-rescan coefficient of variation was 6.8%. There were no significant differences in average gagCEST asymmetry between younger and older healthy controls ($p = 0.655$) or between healthy controls and OA subjects ($p = 0.310$). T_2 and $T_{1\rho}$ relaxation times were elevated in OA subjects ($p < 0.001$ for both) compared with healthy controls and both were moderately correlated with total KOOS scores (rho = –0.181 and rho = –0.332 respectively). The gagCEST technique developed here, with volumetric scan times under 10 min and high gagCEST asymmetry at 3 T, did not vary significantly between healthy subjects and those with mild-moderate OA. This further supports a limited utility for gagCEST imaging at 3 T for assessment of early changes in cartilage composition in OA.

KEYWORDS
cartilage, CEST, gagCEST, human study, knee, osteoarthritis

Abbreviations: B_{1rms}, root mean squared B_1; GAG, glycosaminoglycan; gagCEST, chemical exchange saturation transfer of GAGs; KL, Kellgren-Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome Score; OA, osteoarthritis; qDESS, quantitative double echo in steady state; SPGR, RF-spoiled, gradient echo; $T_{1\rho}$, T_1 in the rotating frame; WASSR, Water Saturation Shift Referencing.

1 | INTRODUCTION

Osteoarthritis (OA) is a progressive and debilitating disease resulting in the degradation of multiple joint tissues. Prior to measurable loss of articular cartilage volume, the earliest changes associated with OA are often characterized by enzymatic activity that alters the quality and composition of cartilage extracellular matrix components including proteoglycans and collagen.[1] Proteoglycan degradation is of particular interest as it is believed to precede the breakdown of collagen microstructure and it continues to be depleted in proportion to the severity of OA.[1,2] Nondestructive in vivo detection of changes in the content and distribution of proteoglycans prior to macroscopic volume loss or structural damage would allow for improved understanding of early cartilage changes associated with the disease.

Advanced MRI techniques provide a strategy to visualize and probe the changing biochemical composition of cartilage, which may enable early detection and monitoring of OA.[3–5] A variety of MRI strategies exist to measure proteoglycan composition within articular cartilage.[6] Negatively charged glycosaminoglycan (GAG) side chains attached to proteoglycans are targeted using techniques such as delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), T_1 in the rotating frame ($T_{1\rho}$), sodium imaging, and chemical exchange saturation transfer of GAGs (gagCEST). GagCEST is distinguished by its theoretically high selectivity to GAG content without the use of exogenous contrast agents or specialized coils or hardware. This technique uses the chemical exchange of saturated exchangeable protons on the hydroxyl groups of GAG molecules and bulk water protons to provide specific contrast and evaluation of cartilage GAG content in vivo.[7]

Prior work on gagCEST imaging at high field strengths suggests that it has potential as a biomarker for cartilage composition. Volumetric gagCEST imaging studies at 7.0 T exhibited highly reproducible results[8–11] that correlate well with other common metrics of assessing proteoglycan composition in healthy and damaged human cartilage, such as sodium and $T_{1\rho}$ imaging.[11,12] Additionally, results at 7 T suggest that gagCEST measures differ between spatial regions in cartilage[8,10,13] and between regions of differing cartilage quality[12]; however, there is limited evidence of the utility of gagCEST imaging at lower field strengths such as 3 T. At 3 T, faster proton exchange and direct saturation effects resulting from reduced separation between the saturation frequency of hydroxyl protons and water protons (Δ1.0 ppm or Δ127.74 Hz) can diminish signal strength and sensitivity to GAG.[14] Additionally, B_0 field variations (~0.6 ppm) within and around the knee affect the magnitude of the gagCEST effects at the saturation frequency.[7,14] When field variations are accounted for, negligible gagCEST measures were observed at 3 T. Later work showed a similarly low average gagCEST asymmetry in the offset range of 0.5-2 ppm of 1.6 ± 0.5% in healthy cartilage as well as a paradoxical increase in gagCEST asymmetry in damaged cartilage.[15] Moreover, many of these gagCEST sequences utilize long imaging times that often limit acquisition to a single slice at 3 T.[14,15] Single-slice gagCEST imaging may not fully characterize variations in articular cartilage composition, obscuring findings such as focal lesions.

The goal of this work was to re-evaluate the potential of gagCEST imaging in knee articular cartilage at 3 T using a volumetric gagCEST sequence with increased gagCEST asymmetry at 3 T and scan times less than 10 min. In particular, we hypothesize that an improved 3D gagCEST method that increases the cartilage gagCEST asymmetry values at 3 T will allow us to measure a decrease in gagCEST between OA patients and healthy controls. We build upon prior work to advance volumetric gagCEST imaging at 3 T,[8,11] altering B_1 parameters to maximize gagCEST asymmetry at 3 T and implementing a long shot T_R to maximize T_1 recovery along with an accelerated readout for volumetric imaging in reasonable scan times. We assess the repeatability of the volumetric gagCEST sequence by evaluating volumetric gagCEST maps of the femoral cartilage in healthy volunteers. Finally, we evaluate femoral cartilage gagCEST asymmetry and T_2 and $T_{1\rho}$ relaxation times of subjects with established OA to those of healthy controls to examine the potential of gagCEST for studying OA at 3 T.

2 | MATERIALS AND METHODS

2.1 | In vivo knee imaging

This study was conducted under an approved university institutional review board protocol. Informed consent was obtained from each volunteer after explaining the study protocol. All subjects were examined in the supine position and the knee was stabilized in a neutral position.

2.2 | CEST imaging

GagCEST images were acquired with a 3D magnetization prepared spoiled gradient-echo sequence (Figure 1A, Tables 1 and 2). CEST magnetization preparation utilized a 400 ms saturation pulse train (four 99 ms Hanning-windowed pulses with a 1 ms delay—99% duty cycle) and a root mean squared B_1 (B_{1rms}) of 0.9 μT. The saturation amplitude and durations that would maximize gagCEST asymmetry at 3 T were empirically determined by scanning healthy volunteers at varying saturation amplitudes and varying saturation amplitudes. We observed that gagCEST asymmetry was highest at a B_{1rms} of 0.9 and a duration of 400 ms. We then used gagCEST asymmetry values at varying B_1 saturation amplitudes and a duration of 400 ms to determine B_1 inhomogeneity correction factors for gagCEST asymmetry as previously described[16]

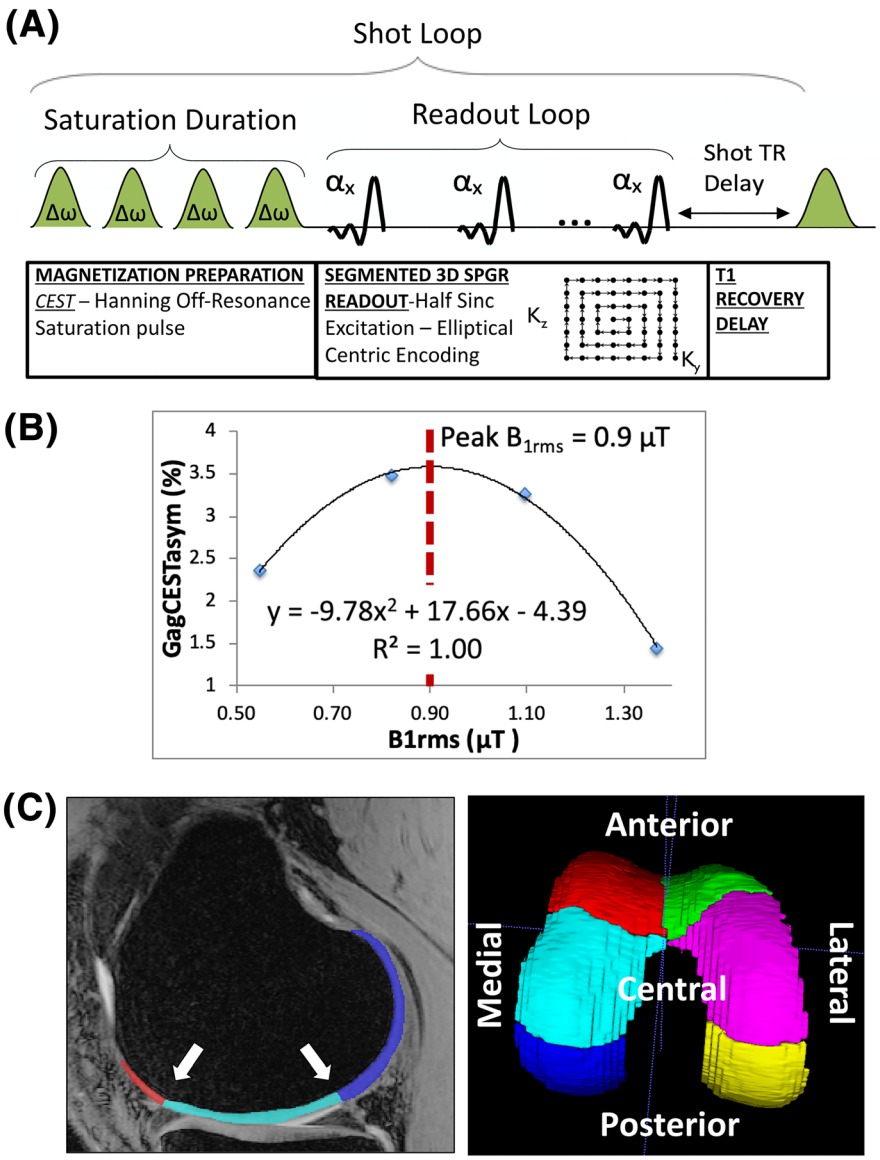

FIGURE 1 A, Pulse sequence diagram for the gagCEST 3D magnetization prepared spoiled gradient-echo sequence. Magnetization preparation used a 400 ms saturation pulse train of four Hanning-windowed pulses with a 1 ms delay. A shot T_R of ~5 T_1 was applied between successive CEST magnetization preparations to allow for T_1 recovery. 3 T gagCEST asymmetry in cartilage was maximized with a saturation B_{1rms} of 0.9 μT and saturation duration of 400 ms. B, With a saturation duration of 400 ms, the dependence of gagCEST asymmetry was evaluated to determine B_1 inhomogeneity correction factors. C, GagCEST asymmetry was evaluated across the entire femoral cartilage as well as anterior, central, and posterior sub-regions in both the medial and lateral compartments of the femur. Sub-regions were segmented based on the outer edges of the meniscus [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 1B). CEST images were acquired with varying saturation offsets from +0.43 to +1.53 ppm and from −0.43 to −1.53 ppm (55-195 Hz) in respective alternating ±0.16 ppm increments. A shot T_R of 5.5 s (~5 T_1) was applied between successive CEST magnetization preparations to allow for T_1 recovery.

To enable correction of field inhomogeneities, both B_1 and B_0 field maps were acquired using the double-angle and Water Saturation Shift Referencing (WASSR) methods, respectively, to use in post-processing for removal of field-inhomogeneity-induced artifacts.[16,17] Double-angle magnetization preparation was applied using a 30° and 60° rectangular pulse followed by a strong crusher gradient to remove transverse magnetization. WASSR magnetization preparation images used a 200 ms saturation pulse train (two 99 ms Hanning-windowed pulses with a 1 ms delay—99% duty cycle) with a B_{1rms} of 0.3 μT from −0.6 to 0.6 ppm with a step size of 0.1 ppm. Finally, an M_0 pulse was acquired with no magnetization preparation to normalize gagCEST asymmetries. WASSR/B_0 and M_0 magnetization preparations utilized shot T_R values of 6.3 and 5.5 s, respectively.

Readout of longitudinally stored magnetization from CEST saturation utilized a segmented, 3D, RF-spoiled, gradient echo (SPGR) sequence with elliptical centric phase encoding. RF excitation used a half-sinc excitation to accelerate readout T_R and T_E after the magnetization preparation. Further, the readout utilized twofold parallel imaging with Autocalibrating Reconstruction for Cartesian Imaging (ARC) in the phase-encode direction. All magnetization preparations used the same readout with parameters listed in Table 1 (3 T) and Table 2 (7 T). For comparison of our 3D volumetric approach to prior methods, identical magnetization preparations with a single-slice 2D centric-encoded SPGR readout was used as previously described,[14] with the parameters listed in Table 1 and Table 2. No fat saturation was used during magnetization preparation or readout. The same 3D readout was utilized for S_0 (no magnetization preparation) and gagCEST, WASSR, and B_1 double-angle magnetization prepared scans.

TABLE 1 Volumetric 3D and 2D single-slice gagCEST scan parameters at 3 T

		3 T—volumetric 3D CEST				3 T—single-slice 2D CEST			
		M_0	CEST	WASSR	B_1	M_0	CEST	WASSR	B_1
Magnetization preparation	Saturation amplitude (B_{1rms}) (μT)	No magnetization preparation	0.9	0.3	30 and 60° rectangular pulses (separate) followed by a strong crusher gradient to remove transverse magnetization	No magnetization preparation	0.9	0.3	30 and 60° rectangular pulses (separate) followed by a strong crusher gradient to remove transverse magnetization
	Saturation duration (ms)		400	200			400	2000	
	Duty cycle (%)		99	99			99	99	
	Frequency offsets (ppm)		±0.43 to ±1.53	0 to ±0.6			±0.43 to ±1.53	0 to ±0.6	
	Frequency step size (ppm)		±0.13	±0.1			±0.13	±0.1	
	Shot T_R (s)	5.5	5	6.3	6.3	6			
Readout	Readout	3D segmented elliptical centric SPGR				2D centric encoded SPGR			
	Field of view (cm)	14.0				14.0			
	Matrix	160 × 160				160 × 160			
	T_R (ms)/T_E (ms)	3.2/1.4				4.6/2.1			
	Flip angle (°)	5				10			
	Slices	28				1			
	Slice thickness (mm)	3				3			
	Bandwidth (Hz/pixel)	781.25				781.25			
	Fat saturation	None				None			
	Mag. preps/full 3D *k*-space readout	3	3	2	2	1			
	Acceleration	2× (in plane)				None			
	Scan time (min:s)	0:22	4:49	3:17	0:35	0:06	1:42	1:30	0:18
	Total scan time (min:s)	9:03				3:36			

2.3 | Additional quantitative imaging techniques

To compare our gagCEST technique with other metrics sensitive to cartilage composition at 3 T, volunteers were also scanned with a quantitative double echo in steady state (qDESS) acquisition[18] and a $T_{1\rho}$-prepared magnetization-prepared pseudo-steady-state 3D fast spin-echo sequence (CubeQuant-$T_{1\rho}$),[19] to generate quantitative T_2 and $T_{1\rho}$ relaxation time maps, respectively, of the femoral cartilage. The qDESS images were acquired using T_R = 26 ms, T_E = 7.6 ms for the first echo and 43 ms for the second echo, resolution = 0.2734 mm × 0.2734 mm × 1.5 mm, and scan time of 5 min, 32 s. CubeQuant-$T_{1\rho}$ images were acquired with an alternating phase spin-lock preparation (500 Hz), an echo train length of 45, partial *k*-space acquisition using 0.5 averages, resolution of 0.3125 mm × 0.3125 mm × 1.5 mm, T_R = 1301 ms, and four time spin-lock

TABLE 2 Volumetric 3D and 2D single-slice gagCEST scan parameters at 7 T

		7 T—volumetric 3D CEST				7 T—single-slice 2D CEST			
		M_0	CEST	WASSR	B_1	M_0	CEST	WASSR	B_1
Magnetization preparation	Saturation amplitude (B_{1rms}) (μT)	No magnetization preparation	1.8	0.3	30 and 60° rectangular pulses (separate) followed by a strong crusher gradient to remove transverse magnetization	No magnetization preparation	1.8	0.3	30 and 60° rectangular pulses (separate) followed by a strong crusher gradient to remove transverse magnetization
	Saturation duration (ms)		500	200			500	200	
	Duty cycle (%)		99	99			99	99	
	Frequency offsets (ppm)		±0.6 to ±1.4	0 to ±0.5			±0.6 to ±1.4	0 to ±0.5	
	Frequency step size (ppm)		±0.13	±0.08			±0.13	±0.08	
	Shot T_R (s)	7				8	8	8	10
Readout	Readout	3D segmented elliptical centric SPGR				2D centric encoded SPGR			
	Field of view (cm)	14.0				14.0			
	Matrix	160 × 160				160 × 160			
	T_R (ms)/T_E (ms)	3.8/1.4				4.9/2.1			
	Flip angle (°)	5				10			
	Slices	28				1			
	Slice thickness (mm)	3				3			
	Bandwidth (Hz/pixel)	781.25				781.25			
	Fat saturation	None				None			
	Mag. preps/full 3D *k*-space readout	3	3	3	3	1			
	Acceleration	2× (in plane)				None			
	Scan time (min:s)	0:26	5:10	5:10	0:46	0:16	1:52	1:52	0:20
	Total scan time (min:s)	11:32				4:20			

durations of 1, 10, 30, and 60 ms for a total scan time of 5 min, 13 s.[20] Readout of longitudinally stored $T_{1\rho}$ magnetization was done with a 3D variable flip-angle fast spin-echo readout.

2.4 | Quantitative image analysis

GagCEST analysis was performed using custom MATLAB scripts (MathWorks, Natick, MA, R 2017b). CEST data was corrected for B_0 and B_1 field inhomogeneities as described previously.[16,17] B_0 maps were used to generate corrected CEST images (±1.0 ppm) using the WASSR method. Voxels where B_0 inhomogeneities were greater than ±0.55 ppm were outside of the range that could be corrected for and were excluded from

the analysis. Similarly, B_1 maps were created from two images obtained using preparation square pulses with flip angles of 30° and 60°. A B_1 calibration curve for cartilage tissue was developed from in vivo human tibiofemoral cartilage CEST data at varying saturation amplitudes (Figure 1B) and used in conjunction with B_1 maps to correct for B_1 inhomogeneities.[16] CEST asymmetry due to GAG was calculated from the normalized B_0-corrected signal intensity at ±1.0 ppm, the chemical shift of GAG hydroxyl protons, using

$$\text{gagCEST}_{\text{asymmetry}} = \frac{S_{-1.0\text{ ppm}} - S_{+1.0\text{ ppm}}}{S_0} \times 100\% \quad (1)$$

where S_0 is the reference dataset acquired without saturation and $S_{\pm 1.0\text{ ppm}}$ is the B_0-corrected signal intensity of images acquired with saturation offsets of ±1.0 ppm.

T_2 relaxation times were calculated for articular cartilage using extended phase graph modeling of the relationship between the two DESS echoes as previously described.[18] $T_{1\rho}$ relaxation times were calculated from CubeQuant-$T_{1\rho}$ images using a nonlinear monoexponential fit on a pixelwise basis without inclusion of the additive constant.

For quantitative mapping of knee femoral cartilage, cartilage was manually segmented using the first echo of the DESS image (ITK-SNAP, Version 3.6.0)[21] for analysis. The center of mass of the total segmented cartilage volume was used to automatically divide the mask in half and identify medial and lateral portions of the femoral cartilage. The outer edges of the menisci were used to further divide each portion into anterior, central, and posterior regions for a total of six sub-regions over the cartilage surface (Figure 1C).

The mask was rigidly registered and resampled to the gagCEST image resolution, manually adjusted as necessary, and applied to calculate gagCEST asymmetry maps. For analysis of DESS images, the mask was applied directly to select T_2 relaxation times of the femoral cartilage. For the CubeQuant-$T_{1\rho}$ scans, all volumes acquired at different spin lock times were first registered to the volume acquired at spin lock time = 1 ms to correct for potential subject motion between the different scans. These images were subsequently registered to DESS images using Elastix (Version 4.8)[22] focusing on the masked femoral cartilage, implementing affine transformations and resampling using B spline interpolations with a factor of three.

For visualization, quantitative gagCEST asymmetry, T_2, and $T_{1\rho}$ maps were projected into the 2D plane using methods described previously.[23] Briefly, a cylinder was fit through the femoral cartilage and angular projections from the center were used to create bins with 1° increments. Voxel data from the cartilage within each bin was averaged and the 2D projection map of the averaged voxel data was created by plotting angular bin versus slice number.

2.5 | Single-slice and volumetric comparisons

Three healthy volunteers were scanned at both 3 T and 7 T to compare gagCEST asymmetry maps from single-slice and volumetric 3D sequences. Images were acquired using a 3 T whole-body system (GE Healthcare, Waukesha, WI) with a flexible 16-channel receive coil (NeoCoil, Pewaukee, WI) and a 7 T whole-body system (GE Healthcare) using a 28-channel receive-transmit knee coil (Quality Electrodynamics (QED), Mayfield, OH). A similar slice from the middle of the medial and lateral femoral condyles was selected from the volumetric sequence and the single-slice image for comparison. The average gagCEST asymmetry of the entire cartilage surface and of each of the six cartilage sub-regions was determined.

2.6 | Repeatability analyses

To assess the repeatability of the volumetric gagCEST asymmetry calculations in femoral cartilage at 3 T, three healthy volunteers (demographic information in Table 3) were scanned three times in succession. Before each scan and between scans, subjects were instructed to be weight bearing for 5 min to simulate conditions prior to standard scanning. The average gagCEST asymmetry map for the entire femoral cartilage and for each of the cartilage sub-regions was calculated for each of the three scans. A coefficient of variation (standard deviation/mean) was computed to compare the between-scan variability in average gagCEST asymmetry for each subject. The average coefficient of variation across the three volunteers is reported.

2.7 | OA cohort and healthy control study

A cohort of 42 volunteers was used to assess the feasibility of a 3D gagCEST sequence at 3 T to study osteoarthritic changes in knee femoral cartilage. All subjects had a BMI less than 35 and no history of meniscectomy or anterior cruciate ligament injury or repair. Ten healthy volunteers with no diagnosis of OA and total Knee Injury and Osteoarthritis Outcome Scores (KOOS) greater than 90 were compared with 32 individuals

TABLE 3 Scan volunteer demographic information

Study	Cohort	Number of subjects	Sex	Age (years)	BMI (kg/m^2)	KOOS
Volumetric and single slice	Healthy	3	F = 0	22.5 ± 2.1	24.9 ± 0.8	100 ± 0
Repeatability	Healthy	3	F = 3	26.3 ± 4.5	24.4 ± 3.7	98.4 ± 1.9
Osteoarthritic and healthy Volunteers	Younger healthy	5	F = 3	24.8 ± 4.0	24.6 ± 2.6	99.0 ± 1.6
	Advanced healthy	5	F = 3	57.2 ± 10.8	22.9 ± 2.5	96.2 ± 4.9
	KL 1	10	F = 7	63.9 ± 6.6	25.8 ± 4.4	62.6 ± 10.9
	KL 2	12	F = 9	61.3 ± 8.5	27.5 ± 2.9	63.8 ± 12.1
	KL 3	10	F = 5	61.6 ± 10.3	25.7 ± 4.1	69.1 ± 7.3

with medial knee OA (Table 3). Healthy individuals were subdivided into "young" (24.8 ± 4.0 years, n = 5) and "advanced" (57.2 ± 10.8 years) cohorts to examine the effect of age on gagCEST values. Disease severity in the OA population was assessed using radiographs and subjects were classified into three cohorts (Table 3) based on Kellgren-Lawrence (KL) grade: KL 1, KL 2, and KL 3. KL grading was performed by a musculoskeletal radiologist with more than 20 years of experience (GEG). Volunteers were scanned using the volumetric 3D gagCEST sequence described above as well as with DESS and CubeQuant-$T_{1\rho}$. The mean and standard deviation gagCEST asymmetry (%) and T_2 and $T_{1\rho}$ relaxation times (ms) were calculated for the entire femoral cartilage surface and for each of the six sub-regions of the femoral cartilage.

2.8 | Statistical analysis

The average gagCEST asymmetry values over the entire cartilage surface and in cartilage sub-regions for single-slice and multislice methods were calculated to compare between the methods at 3 T as well as at 7 T using a Wilcoxon sign-rank test (α = 0.05). Comparisons between cartilage sub-regions in healthy and OA subjects, categorized by KL grade, were made using a general linear model with a Bonferroni post hoc correction for multiple comparisons (α = 0.05). A Spearman's rank correlation coefficient (rho) was used to examine correlation between gagCEST asymmetry and T_2 and $T_{1\rho}$ relaxation times and rates $R_2 = 1/T_2$ and $R_{1\rho} = 1/T_{1\rho}$, as well as between gagCEST asymmetry, T_2, and $T_{1\rho}$ parameters and both the total KOOS score and KOOS pain sub-score for the subjects (α = 0.05). Statistical analysis was conducted using Minitab software (Minitab, State College, PA, Version18.1).

3 | RESULTS

3.1 | Single-slice and volumetric comparison

At 7 T, the average gagCEST asymmetry for all cartilage of a single slice within the volumetric acquisition (5.22 ± 2.49%) was significantly higher than that for a similar slice acquired using a single-slice acquisition (3.41 ± 1.60%, $p < 0.001$). This was also true at 3 T, where the average gagCEST asymmetry was 3.65 ± 2.21% for the volumetric acquisition and 2.39 ± 1.63% for the single-slice acquisition ($p < 0.001$). GagCEST asymmetry was significantly different between the 2D and 3D methods in the central region of the cartilage at 3 T (p = 0.0313), and in both the central and posterior regions of the cartilage at 7 T (p = 0.0313 for both). Visually, the asymmetry maps appear smoother using the volumetric acquisition compared with a single-slice map (Figure 2). Regions of high gagCEST asymmetry on volumetric 7 T images likewise appear on volumetric 3 T images.

3.2 | Repeatability at 3 T

The gagCEST asymmetry maps for the femoral cartilage for a representative subject are shown for each of three repeated volumetric scans at 3 T (Figure 3). The average gagCEST asymmetry scan-rescan coefficient of variation was 6.8% for measures over the entire cartilage surface and 8.8% for sub-region measurements.

3.3 | Osteoarthritic cohort and healthy control study

Forty-two volunteers were included in a sample cohort study to assess the utility of volumetric gagCEST mapping for observing osteoarthritic changes in articular cartilage at 3 T. Subject demographic information is provided in Table 3.

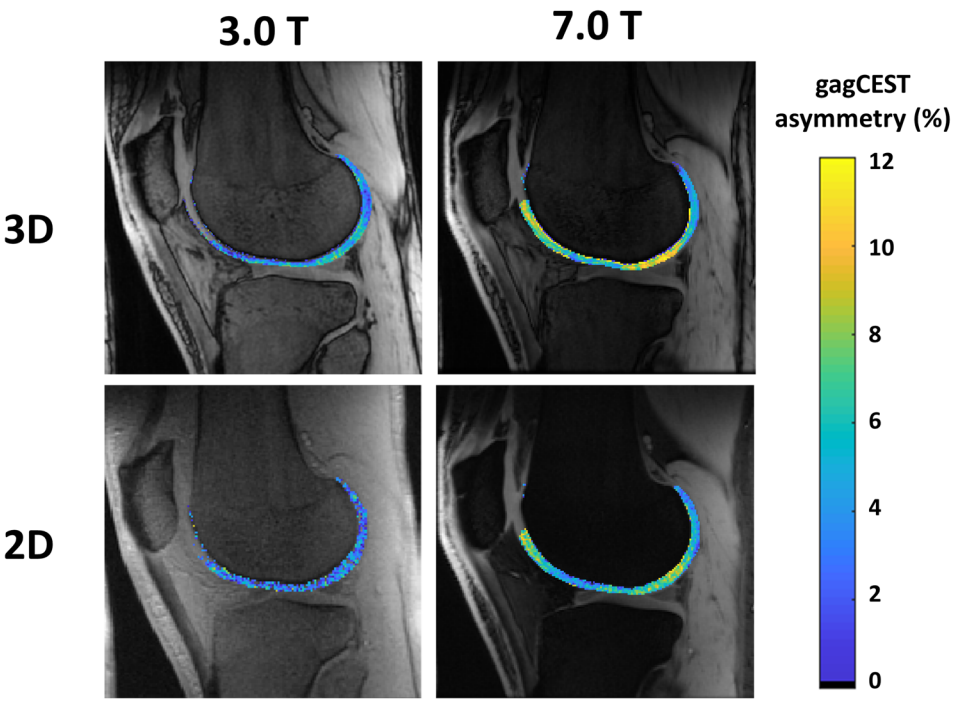

FIGURE 2 Representative gagCEST asymmetry maps from healthy volunteers scanned at both 3 T and 7 T with a 3D multislice and a 2D single-slice protocol. Average gagCEST asymmetry values were calculated across the entire cartilage surface and within anterior, central, and posterior regions for a single slice in the lateral and medial (not shown) compartments. GagCEST asymmetry maps are overlaid on the first echo of the qDESS scan (fat saturation from a spatial spectral excitation pulse) [Colour figure can be viewed at wileyonlinelibrary.com]

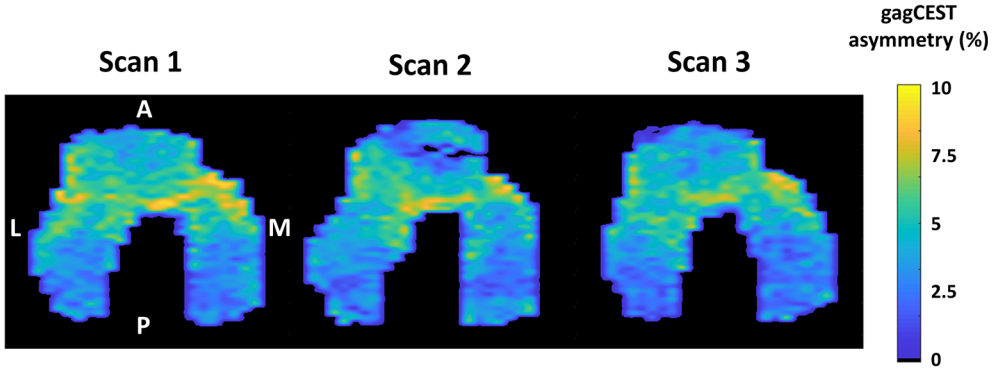

FIGURE 3 Representative 2D projections of the gagCEST asymmetry maps for the femoral cartilage of a healthy volunteer scanned three times at 3 T in sequence. Volunteers stood and were repositioned between scans. The average scan-rescan coefficient of variation was 6.8% across the entire cartilage surface and 8.8% across all six sub-regions [Colour figure can be viewed at wileyonlinelibrary.com]

Cartilage gagCEST asymmetry over the entire cartilage surface did not differ significantly between the younger and advanced healthy cohorts (p = 0.655). The gagCEST asymmetry values of all healthy volunteers showed no significant differences compared with those of individuals with medial compartment OA (Figure 4), both when considered as an average across the whole femoral surface (p = 0.310) and when anterior, central, and posterior regions of the medial and lateral halves of the femur were considered individually (Figure S1).

Additionally, there was no correlation between gagCEST asymmetry and the total KOOS score (rho = 0.051, p = 0.424) or the KOOS pain score (rho = 0.090, p = 0.152). There was no correlation between the gagCEST asymmetry and T_2 (rho = –0.063, p = 0.693) across the entire cartilage surface, or between gagCEST asymmetry and $T_{1\rho}$ relaxation times (rho = –0.150, p = 0.342) (Figure S2).

Differences between healthy and osteoarthritic subjects were observed in both the T_2 and $T_{1\rho}$ relaxation times (Figure 5). Between the younger and advanced healthy cohorts, no significant differences were observed in T_2 relaxation times (p = 0.986) or $T_{1\rho}$ relaxation times (p = 0.670). T_2 relaxation times averaged over the entire cartilage surface were significantly elevated in KL groups 1-3 compared with the healthy volunteers (p < 0.001). The elevation in T_2 relaxation times in osteoarthritic subjects compared with healthy controls was significant in the medial and lateral anterior sub-regions (p = 0.047 for both) and the medial posterior sub-region (p = 0.014) of the cartilage surface (Figure S3). There was a significant correlation between T_2 and the total KOOS score (rho = –0.1810, p = 0.004) but not the KOOS pain score (rho = –0.112, p = 0.075). There was no significant correlation between R_2 and the total KOOS score (rho = 0.222, p = 0.158) or the KOOS pain score (rho = 0.128, p = 0.420). Average $T_{1\rho}$ relaxation times were significantly elevated in KL groups 1 and 3 compared with the healthy controls (p < 0.001). Significant differences in $T_{1\rho}$ relaxation times were present only in the posterior sub-regions of the medial (p = 0.030) and lateral (p = 0.022) portions of the cartilage surface, where KL 3 subjects had higher $T_{1\rho}$ relaxation times than young healthy subjects (Figure S4). There was a significant relationship

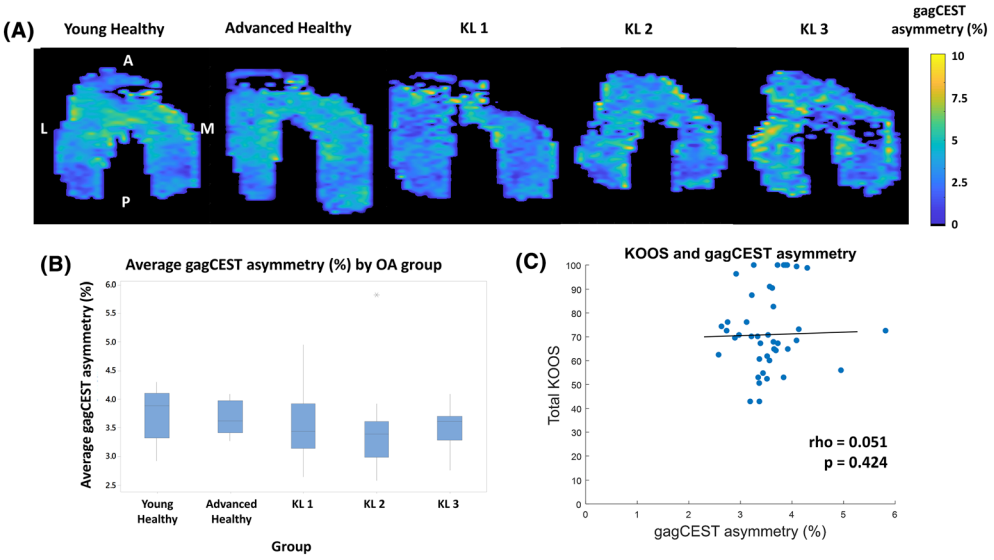

FIGURE 4 A, Representative gagCEST asymmetry maps of femoral cartilage of young healthy, advanced healthy, and osteoarthritic subjects (subdivided into groups KL 1-3). B, There was no significant difference in the average gagCEST asymmetry between young and advanced healthy cohorts ($p = 0.655$) or between healthy and osteoarthritic cohorts ($p = 0.310$) (outlier marked with asterisk). C, There were no significant correlations between gagCEST asymmetry values and total KOOS scores (Spearman's rho = 0.051, $p = 0.424$) [Colour figure can be viewed at wileyonlinelibrary.com]

between $T_{1\rho}$ and total KOOS (rho = −0.332, $p < 0.001$) and with the KOOS pain score (rho = −0.249, $p < 0.001$), as well as between $R_{1\rho}$ and total KOOS (rho = 0.567, $p < 0.001$) and KOOS pain score (rho = 0.436, $p = 0.004$).

4 | DISCUSSION

This work implemented rapid and volumetric gagCEST mapping at 3 T and examined the sensitivity of this methodology to cartilage changes associated with OA. The methodology was shown to be reproducible and provided higher gagCEST asymmetry values compared with prior methods at 3 T. However, results did not show a significant difference in gagCEST asymmetry values between OA patients and healthy controls.

The presented 3D volumetric gagCEST sequence was developed to optimize gagCEST asymmetry in cartilage at 3 T while allowing for whole-joint gagCEST knee imaging in clinically reasonable scan times of less than 10 min. There are several differences to this 3D method compared with previous approaches.[7–9,11–13] First, a 3D approach maximizes signal for whole-joint coverage compared with prior 2D methods. Another key difference is the delay times between sequential CEST magnetization preparations. Many other approaches have crushed all magnetization and used a short T_1 recovery time (around 1 s) before playing a CEST saturation pulse and reading out a much shorter segment of *k*-space.[9,13] While this approach allows for high-frequency *k*-space to be acquired closer to the magnetization preparation, it does so at the expense of signal. By waiting only 1 T_1, this approach sacrifices about 40% of the starting magnetization. In addition to the decreased SNR, this results in a smaller pool of unsaturated bulk water protons available to exchange with CEST saturated hydroxyl protons from GAG, which results in a decrease in the CEST effect, as saturated protons build up in the bulk water pool. While less of a concern at 7 T, at 3 T this is amplified by the further large decrease in bulk water magnetization due to the high direct water saturation, resulting in not only low SNR images but minimal bulk water magnetization for CEST exchange and therefore low CEST asymmetry. Waiting for full recovery allows for maximum exchange of CEST saturated protons with unsaturated bulk water protons and thus maximizes the CEST effect.

The approach described in this work uses a long shot T_R, roughly five times the T_1 of cartilage, to allow for over 99% of magnetization to recover between subsequent magnetization preparations. While this requires long readout train lengths to maintain clinically reasonable scan times, an elliptic centric readout, coupled with short readout T_R and parallel imaging, allows most of the low-frequency data to have a strong CEST weighting. While outer *k*-space data may have less CEST weighting, which may induce some blurring in gagCEST maps, this high-frequency data is expected to have limited effect on gagCEST asymmetry measurements. This is similar to DCE keyhole techniques, which acquire the center of *k*-space dynamically and utilize static or lower-temporal resolution information for the outer parts of *k*-space, which have a limited effect on SNR. Further, we also incorporated half-sinc excitation to minimize T_E. The shorter excitation pulse, in addition to improving cartilage signal, also reduces the readout T_R. In combination with parallel imaging, this allows for more of the low-frequency, center *k*-space data to be collected shortly following the magnetization preparation with the 3D approach. Finally, the saturation amplitude (B_1) and duration were chosen to further maximize cartilage gagCEST asymmetry at 3 T with this approach.

This 3D volumetric gagCEST method showed elevated gagCEST asymmetry values compared with 2D single-slice acquisitions as well as with previously reported results in healthy volunteers at 3 T.[13,14] Volumetric gagCEST asymmetry values at 7T were similar to some previously reported values.[8,10,14] In addition to increased gagCEST asymmetry values, 3D gagCEST maps demonstrated a smoother, less noisy appearance

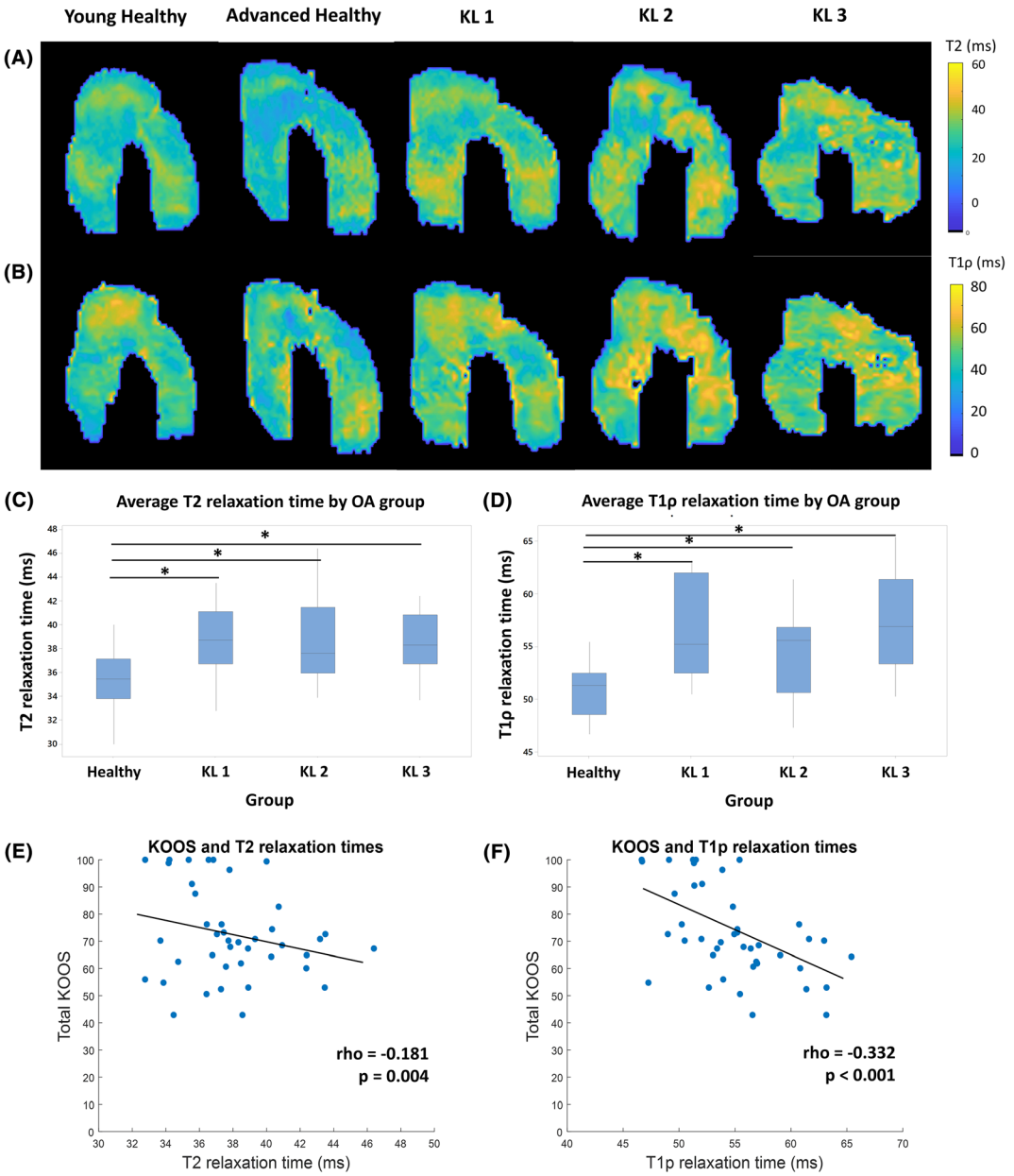

FIGURE 5 A, B, Representative T_2 (A) and $T_{1\rho}$ (B) maps of femoral cartilage of young healthy, advanced healthy, and osteoarthritic subjects (subdivided into groups KL 1-3). Note the different scales. C, D, Average relaxation times in the OA cohort were significantly elevated compared with those in both healthy cohorts (*$p < 0.001$). E, F, There was a significant correlation between T_2 and total KOOS score (Spearman's rho = –0.1810, $p = 0.004$, E) and between $T_{1\rho}$ and total KOOS score (Spearman's rho = –0.332, $p < 0.001$, F) [Colour figure can be viewed at wileyonlinelibrary.com]

compared with those acquired with 2D methods. Further, this method allowed for full knee coverage with 28 slices in less than 10 min, which demonstrates translational potential for incorporation into clinical protocols. This was a considerable improvement over single-slice acquisitions, which only acquire a single slice in roughly a third of the time. Repeated acquisitions of the volumetric sequence at 3 T showed repeatable gagCEST asymmetry maps in healthy volunteers, with an average scan-rescan coefficient of variation of less than 7% across the whole cartilage surface. The average coefficient of variation is 6.4% for T_2 relaxation times from qDESS and is 4.6% for CubeQuant-$T_{1\rho}$ relaxation times.[19]

Though our technique yields high gagCEST asymmetry at 3 T and allows for volumetric whole-knee gagCEST imaging, there remained insufficient sensitivity to differentiate between cartilage in healthy volunteers and in individuals at various stages of OA disease progression. While we did not observe significant differences in gagCEST asymmetry between healthy and osteoarthritic cohorts, others have found significantly higher gagCEST measures at 3 T in populations with chondromalacia and cartilage repair compared with healthy controls.[15] Of note, the authors calculated an average of the gagCEST asymmetry between 0.5 and 2.0 ppm, which may have resulted in a different driver of asymmetry measures

compared with our measure calculated only at 1.0 ppm. It is difficult to discern what might have led to significant differences in gagCEST values, though the increases in gagCEST measures in subjects with cartilage damage and chondromalacia run counter to the expected decrease in GAG content with cartilage degeneration. Proteoglycan content is known to decrease with OA severity. While KL grading does not directly indicate proteoglycan content, KL grading has been associated with changes in the dGEMRIC index, which is related to proteoglycan content.[24] We had therefore hypothesized that gagCEST, as another measure of proteoglycan content, would decrease with increasing KL grade, reflecting the progressive proteoglycan loss in OA. However, there were no significant differences in the average gagCEST asymmetry of healthy and osteoarthritic cohorts. Differences among cohorts were more readily visualized with T_2 and $T_{1\rho}$ mapping, where relaxation times were significantly elevated in the osteoarthritic groups compared with healthy subjects. While it is difficult to compare absolute values across vendors and acquisition methods, the relaxation times fall within the range of prior work and the trends between groups are in agreement with previous studies of OA cartilage changes at 3 T.[25,26] Both T_2 and $T_{1\rho}$ relaxation times also showed significant correlations with non-radiographic KOOS scores.

Despite the higher gagCEST asymmetry values obtained at 3 T using this technique, practical constraints of CEST imaging at 3 T may still limit its ability to visualize differences in cartilage biochemical composition. At 3 T, the reduced chemical shift between GAG hydroxyl protons and bulk water protons increases direct water saturation, which in turn reduces the number of unsaturated protons in the bulk water pool to exchange with saturated GAG hydroxyl protons. This is the primary challenge of gagCEST imaging at 3 T and limits the saturation power that optimizes gagCEST asymmetry. The optimal saturation power that we determined at 3 T (B_{1rms} = 0.9 μT) was roughly half of the optimized saturation power at 7 T (B_{1rms} = 1.8 μT). This likely means that there is a considerable reduction in saturation efficiency at 3 T compared with 7 T. However, at the same time, we also have reduced magnetization in the bulk water pool. Increasing saturation power, while providing an increase in saturation efficiency, would result in a further reduction in bulk water magnetization that outpaces the gain in saturation efficiency in its effect on gagCEST asymmetry, in addition to increasing the noise of CEST magnetization prepared images and the resulting gagCEST asymmetry map. Finally, the reduced chemical shift also means that gagCEST asymmetry measures are more susceptible to B_0 inhomogeneity. While we correct for this inhomogeneity using the WASSR method, errors in the correction as small as a few hertz, which might not have much effect at 7 T (chemical shift = ~300 Hz), may have a substantial effect at 3 T (chemical shift = ~125 Hz) due to the differences in direct water saturation effects at this reduced offset.

It is necessary to point out several other limitations of our study. Single-slice 2D and volumetric gagCEST scans had different readout parameters, including readout acceleration and scan times, which may have led to differences in gagCEST asymmetry as well as the noise in the gagCEST maps. Additionally, the 3D readout did not implement flip angle modulation, which may have led to some blurring in the magnetization prepared scans. There were no regions of full thickness cartilage loss in the subjects included in this study; however, in many scans, voxels in the trochlear groove were excluded from analysis during post-processing steps where field inhomogeneities were outside of the range that could be corrected for based on the data acquired during the scan. This region has been reported previously to have poor reproducibility related to poor CEST spectral fits originating from field inhomogeneities or sometimes patellar movement.[9,13] We did not apply corrections for any bias introduced by the loss of voxels, but could expect this impact to be similar in all subjects since they are related to field inhomogeneities. Cartilage subregion analysis was performed to examine defined regions that each contain smaller field variations. Methods of minimizing field variations within and around articular cartilage will improve the value of volumetric gagCEST imaging of the knee. These challenges may have contributed to the lack of sensitivity in the measured cartilage gagCEST asymmetry values to different stages of OA. In this study, osteoarthritic grading was performed using radiographs, which may not appropriately capture changes in soft tissues such as cartilage. Future studies using biochemical or arthroscopic evaluations of cartilage health may improve the ability to assess the sensitivity of gagCEST to cartilage at different stages of OA. Alternative volumetric gagCEST strategies could also be implemented to improve sensitivity to GAG.[9,10] Our method maximized gagCEST asymmetry and minimized scan time; however, other methods with longer scan times or lower gagCEST values might prove more sensitive to cartilage GAG content and thus provide more clinically meaningful measurements.

This work explored the utility of gagCEST imaging of osteoarthritic cartilage changes at 3 T with a new volumetric technique. The technique allows for rapid volumetric imaging of the knee with improved gagCEST asymmetry values and good repeatability. However, gagCEST imaging with this methodology was unable to detect differences in cartilage composition between a healthy cohort and a population with mild to moderate OA. This further supports a limited utility for gagCEST imaging at 3 T for assessment of early changes in cartilage composition in OA.

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REFERENCES

1. Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. *Arthritis Res*. 2001;3(2):107-113.
2. Bertrand J, Cromme C, Umlauf D, Frank S, Pap T. Molecular mechanisms of cartilage remodelling in osteoarthritis. *Int J Biochem Cell Biol*. 2010;42(10):1594-1601. https://doi.org/10.1016/j.biocel.2010.06.022
3. Matzat SJ, Kogan F, Fong GW, Gold GE. Imaging strategies for assessing cartilage composition in osteoarthritis. *Curr Rheumatol Rep*. 2014;16(11):462–477. https://doi.org/10.1007/s11926-014-0462-3
4. Li X, Cheng J, Lin K, et al. Quantitative MRI using $T_{1\rho}$ and T_2 in human osteoarthritic cartilage specimens: correlation with biochemical measurements and histology. *Magn Reson Med*. 2011;29(3):324-334.
5. Guermazi A, Alizai H, Crema MD, Trattnig S, Regatte RR, Roemer FW. Compositional MRI techniques for evaluation of cartilage degeneration in osteoarthritis. *Osteoarthr Cartil*. 2015;23(10):1639-1653. https://doi.org/10.1016/j.joca.2015.05.026
6. Link TM, Neumann J, Li X. Prestructural cartilage assessment using MRI. *J Magn Reson Imaging*. 2017;45(4):949-965. https://doi.org/10.1002/jmri.25554
7. Ling W, Regatte RR, Navon G, Jerschow A. Assessment of glycosaminoglycan concentration in vivo by chemical exchange-dependent saturation transfer (gagCEST). *Proc Natl Acad Sci U S A*. 2008;105(7):2266-2270. https://doi.org/10.1073/pnas.0707666105
8. Krishnamoorthy G, Nanga RPR, Bagga P, Hariharan H, Reddy R. High quality three-dimensional gagCEST imaging of in vivo human knee cartilage at 7 Tesla. *Magn Reson Med*. 2017;77(5):1866-1873. https://doi.org/10.1002/mrm.26265
9. Brinkhof S, Nizak R, Khlebnikov V, Prompers JJ, Klomp DWJ, Saris DBF. Detection of early cartilage damage: feasibility and potential of gagCEST imaging at 7T. *Eur Radiol*. 2018;28(7):2874-2881. https://doi.org/10.1007/s00330-017-5277-y
10. Schreiner MM, Zbýň Š, Schmitt B, et al. Reproducibility and regional variations of an improved gagCEST protocol for the in vivo evaluation of knee cartilage at 7 T. *Magn Reson Mater Phys Biol Med*. 2016;29(3):513-521. https://doi.org/10.1007/s10334-016-0544-5
11. Kogan F, Hargreaves BA, Gold GE. Volumetric multislice gagCEST imaging of articular cartilage: optimization and comparison with $T_{1\rho}$. *Magn Reson Med*. 2017;77(3):1134-1141.
12. Schmitt B, Zbyn S, Stelzeneder D, et al. Cartilage quality assessment by using glycosaminoglycan chemical exchange saturation transfer and Na MR imaging at 7 T. *Radiology*. 2011;260(1):257-264. https://doi.org/10.1148/radiol.11101841/-/DC1
13. Schleich C, Bittersohl B, Miese F, et al. Glycosaminoglycan chemical exchange saturation transfer at 3T MRI in asymptomatic knee joints. *Acta Radiol*. 2016;57(5):627-632. https://doi.org/10.1177/0284185115598811
14. Singh A, Haris M, Cai K, et al. Chemical exchange saturation transfer magnetic resonance imaging of human knee cartilage at 3T and 7T. *Magn Reson Med*. 2012;68(2):588-594.
15. Rehnitz C, Kupfer J, Streich NA, et al. Comparison of biochemical cartilage imaging techniques at 3 T MRI. *Osteoarthr Cartil*. 2014;22(10):1732-1742. https://doi.org/10.1016/j.joca.2014.04.020
16. Singh A, Cai K, Haris M, Hariharan H, Reddy R. On B_1 inhomogeneity correction of in vivo human brain glutamate chemical exchange saturation transfer contrast at 7T. *Magn Reson Med*. 2013;69(3):818-824. https://doi.org/10.1038/jid.2014.371
17. Kim M, Gillen J, Landman BA, Zhou J, Peter CM. WAter Saturation Shift Referencing (WASSR) for chemical exchange saturation transfer experiments. *Magn Reson Med*. 2009;61(6):1441-1450. https://doi.org/10.1002/mrm.21873.WAter
18. Sveinsson B, Chaudhari AS, Gold GE, Hargreaves BA. A simple analytic method for estimating T_2 in the knee from DESS. *Magn Reson Imaging*. 2017;4(38):63-70.
19. Jordan CD, McWalter EJ, Monu UD, et al. Variability of CubeQuant $T_{1\rho}$, quantitative DESS T_2, and cones sodium MRI in knee cartilage. *Osteoarthr Cartil*. 2014;22(10):1559-1567. https://doi.org/10.1016/j.joca.2014.06.001
20. Charagundla SR, Borthakur A, Leigh JS, Reddy R. Artifacts in $T_{1\rho}$-weighted imaging: correction with a self-compensating spin-locking pulse. *J Magn Reson*. 2003;162(1):113-121. https://doi.org/10.1016/S1090-7807(02)00197-0
21. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *NeuroImage*. 2006;31(3):1116-1128.
22. Klein S, Staring M, Murphy K, Viergever M, Pluim J. Elastix: a toolbox for intensity-based medical image registration. *IEEE Trans Med Imaging*. 2010;29(1):196-205.
23. Monu UD, Jordan CD, Samuelson BL, Hargreaves BA, Gold GE, McWalter EJ. Cluster analysis of quantitative MRI T_2 and $T_{1\rho}$ relaxation times of cartilage identifies differences between healthy and ACL-injured individuals at 3T. *Osteoarthr Cartil*. 2017;25(4):513-520. https://doi.org/10.1016/j.joca.2016.09.015
24. Williams A, Sharma L, McKenzie CA, Prasad PV, Burstein D. Delayed gadolinium-enhanced magnetic resonance imaging of cartilage in knee osteoarthritis: findings at different radiographic stages of disease and relationship to malalignment. *Arthritis Rheum*. 2005;52(11):3528-3535. https://doi.org/10.1002/art.21388
25. Atkinson HF, Birmingham TB, Moyer RF, et al. MRI T2 and T1ρ relaxation in patients at risk for knee osteoarthritis: a systematic review and meta-analysis. *BMC Musculoskelet Disord*. 2019;20(1):182-199. https://doi.org/10.1186/s12891-019-2547-7
26. Regatte RR, Akella SVS, Lonner JH, Kneeland JB, Reddy R. $T_{1\rho}$ mapping in human osteoarthritis (OA) cartilage: comparison of $T_{1\rho}$ with T_2. *J Magn Reson Imaging*. 2006;23(4):547-553. https://doi.org/10.1002/jmri.20536

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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