

RESEARCH ARTICLE

Quantative MRI predicts tendon mechanical behavior, collagen composition, and organization

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Abstract

Quantitative magnetic resonance imaging (qMRI) measures have provided insights into the composition, quality, and structure-function of musculoskeletal tissues. Low signal-to-noise ratio has limited application to tendon. Advances in scanning sequences and sample positioning have improved signal from tendon allowing for evaluation of structure and function. The purpose of this study was to elucidate relationships between tendon qMRI metrics (T1, T2, T1p and diffusion tensor imaging [DTI] metrics) with tendon tissue mechanics, collagen concentration and organization. Sixteen human Achilles tendon specimens were collected, imaged with qMRI, and subjected to mechanical testing with quantitative polarized light imaging. T2 values were related to tendon mechanics [peak stress ($r_{sp} = 0.51$, $p = 0.044$), equilibrium stress ($r_{sp} = 0.54$, $p = 0.033$), percent relaxation ($r_{sp} = -0.55$, $p = 0.027$), hysteresis ($r_{sp} = -0.64$, $p = 0.007$), linear modulus ($r_{sp} = 0.67$, $p = 0.009$)]. T1p had a statistically significant relationship with percent relaxation ($r = 0.50$, $p = 0.048$). Collagen content was significantly related to DTI measures (range of $r = 0.56$ – 0.62). T2 values from a single slice of the midportion of human Achilles tendons were strongest predictors of tendon tensile mechanical metrics. DTI diffusivity indices (mean diffusivity, axial diffusivity, radial diffusivity) were strongly correlated with collagen content. These findings build on a growing body of literature supporting the feasibility of qMRI to characterize tendon tissue and noninvasively measure tendon structure and function. Statement of Clinical Significance: Quantitative MRI can be applied to characterize tendon tissue and is a noninvasive measure that relates to tendon composition and mechanical behavior.

KEYWORDS

Achilles, diagnostic imaging, diffusion tensor imaging, mechanical properties, musculoskeletal imaging

1 | INTRODUCTION

Tendon injuries—including painful degeneration (tendinopathy or tendinosis) and rupture—are common, resulting in reduced occupational and sport performance, and are notoriously slow-healing.^{1–4} Recovery from tendon injury has historically been assessed primarily through outcomes such as self-reported patient satisfaction and clinical re-rupture rates; however, there has been a shift toward incorporating measures capturing multidimensional outcomes (e.g., calf functional performance, tendon structural characteristics, etc.) in individuals with tendon injury.^{5,6} Altered tendon structure has been identified as a characteristic that helps define a subgroup of individuals with tendinopathy.⁷ Tendon characteristics—including parameters like length, cross-sectional area, and mechanical properties—have been assessed using ultrasound and magnetic resonance imaging (MRI) modalities. For example, recent studies have reported promising findings relating to estimation of tendon mechanical properties using ultrasound shearwave elastography.^{8,9} Similar to ultrasound, MRI is noninvasive and does not expose the patient to radiation, supporting its potential use in the assessment of musculoskeletal disease. Additionally, unlike ultrasound imaging, MRI provides more direct insights into tissue composition. Quantitative (quantitative magnetic resonance imaging [qMRI]) measures have provided insights into tissue composition, quality, and function in other musculoskeletal tissues positioning it as a useful early indicator of tissue injury, which would be a meaningful advancement to characterizing tendon tissue.^{10–12} However, limitations in signal-to-noise ratio, e.g., very short echo time (time of echo [TE]), have made applying quantitative measures of tissue quality (e.g., T1p, T2, etc.) a challenge in tendon tissue. Consequently, advancements in qMRI in other musculoskeletal tissues—like cartilage—have been slow to translate to tendon.

As the ability to reliably assess tendon tissue with qMRI has improved, attention has shifted towards improving our ability to understand the physiological interpretation of qMRI metrics. qMRI metrics (T2, T1p, T2*) have been associated with tissue biochemistry in a variety of musculoskeletal tissues.¹³ T2 mapping is the most commonly used qMRI metric in the musculoskeletal literature.¹³ In cartilage, T2 mapping has been linked to early detection of changes in water content and collagen concentration.¹³ T2 has also been used in muscle as a means of assessing water content related to edema and inflammation as well as an indicator of fatty degeneration.¹³ The spin-lattice relaxation time in the rotating frame, T1p, has also been identified as a biomarker for proteoglycan changes in articular cartilage with early osteoarthritis.¹³ There have been limited studies applying qMRI to tendon in contexts in which tissue composition and organization was also measured.^{14,15} Data assisting in physiological interpretation of qMRI metrics in the assessment of tendon tissue would be beneficial in understanding the clinical implications of these measures.

In addition to its potential for estimating tissue composition, qMRI—in particular, diffusion tensor imaging (DTI)—may also be useful to assess tissue organization.¹³ DTI is a noninvasive, magnetic resonance-based technique that characterizes tissue microstructure.¹⁶ The fibrous organization of a tissue restricts the movement of water in

three-dimensions. DTI measures the magnitude and direction (defined by eigenvalues and associated eigenvectors) of water diffusion within the tissue and uses diffusivity and direction of flow to reconstruct the tissue in fibrous tracts.^{16,17} DTI has been applied to muscle,^{18–20} and has recently been developed to enable imaging in tendon.^{21,22} One of the challenges with the clinical application of DTI is that it is unclear how to interpret the results of DTI output metrics. Because tendon collagen organization is such a critical component of tendon mechanical function^{23–26} and DTI may be able to be a surrogate measure for tissue organization, DTI has the potential of being an important addition to the noninvasive assessment of tendon.

The purpose of this study was to elucidate the relationships between qMRI metrics with tendon tissue composition, organization, and mechanics. We hypothesized that tendons with higher T1 and lower T2 mapping as well as greater diffusivity and lower fractional anisotropy on DTI would have more optimal mechanical behavior (i.e., higher linear modulus, lower hysteresis) as well as greater collagen concentration and organization.

2 | METHODS

2.1 | Tissue samples

This is a cross-sectional study of ex vivo human Achilles tendon specimens (Level of Evidence: III). Achilles tendon specimens were collected from lower limbs following amputation ($n = 13$) and from fresh, nonembalmed cadavers ($n = 3$). To be included, living participants needed to be 18 years of age or older and undergoing below knee amputation. Exclusion criteria were diagnosed peripheral artery disease, diagnosis of certain infectious diseases (i.e., human immunodeficiency virus, Hepatitis C, methicillin-resistant *staphylococcus aureus*, and vancomycin-resistant enterococci), or inability to give informed consent for living participants (e.g., due to preoperative medications altering arousal, trauma, etc.). Participants were ambulatory before amputation, however, did use a variety of immobilization (walking boots, etc.) and assistive devices (crutches, canes, wheelchair for long distance ambulation) before amputation. Tendon tissue was harvested, wrapped in saline-soaked gauze, and stored at -80°C . Tendon from cadavers was obtained within 24 h postmortem, harvested, and frozen once received by the study team. This study was performed with the approval from the Washington University School of Medicine in St. Louis Institutional Review Board before performing the study. All tissue was stored in saline-soaked gauze at -80°C and underwent one freeze-thaw cycle before imaging.

2.2 | qMRI

In preparation for imaging, specimens were positioned in a conical tube filled with a 2% agarose mold. Phosphate buffered saline (PBS) was poured around the tendon specimens to ensure adequate hydration during the scan. Prior studies have found that immersion of

tendon in PBS during scanning²⁷ or repeated freeze-thaw cycles²⁸ did not change qMRI values. The conical tubes containing the tendon specimens were positioned in a holder at 55° from the primary axis of the magnetic field with a flexible coil.^{29,30} A T1 weighted image of the entire specimen was taken for anatomy (Figure 1), followed by scans for T1/T2/T1p mapping, and DTI.

MR scans were acquired in a whole-body 3T Siemens Prisma scanner (Siemens Medical Solutions). A 24-channel head coil was the receiver and the body coil was the transmitter. T1, T2, and T1p maps on a pixel-by-pixel basis were obtained at a single, transverse slice³¹ in the middle of the tendon specimen. For T1 weighted measurements, an inversion-recovery (IR) prepared turbo-spin-echo (IR-TSE) sequence was applied with varied time of inversion (TI). The T2 weighted images were acquired using a TSE sequence with a variety of TEs. For T1p imaging, a T1p-prepared gradient-echo sequence was used with three different times of spin locking.³² In addition, DTI was obtained by using an echo planar based DTI sequence. The details of pulse sequence parameters are shown in Table 1.

All T1, T2, and T1p maps were created using a custom-written MATLAB-based analysis program (MathWorks). For T1 maps, a short TR fitting algorithm was used to fit signal intensity of each pixel versus different TI.³³ For T2 and T1p maps, they were computed on a pixel-by-pixel basis using a monoexponential decay equation: as

$M = M_0 \cdot \exp(-TE \text{ or TSL/T2 or T1p})$, where M_0 and M indicate the equilibrium magnetization and T1p-prepared magnetization with the TE or TSL, respectively. Region of interest selection was done manually by a single reviewer.

Reproducibility of qMRI has been reported in tendon in prior studies,^{15,34,35} however, reproducibility of this technique was also established within our laboratory group in bovine tendon. Ten deep digital flexor tendons were positioned in the scanner (Prisma 3T) at 55° relative to the primary axis of the magnet, imaged, removed from the scanner, and then repositioned in the scanner and imaged again. Between scan error was 2.5% for T1, 7.0% for T2, and 4.5% for T1p. T1 relaxation time was higher in the repeat scan ($p = 0.02$), but this scan had the lowest percentage of error within the three output parameters, so the significant p value likely does not reflect a clinically meaningful difference between scans. There were no differences between scans for T2 ($p = 0.74$) and T1p ($p = 0.18$) (Figure 2).

2.3 | Tendon mechanics and composition assessment

After scanning, tendons were refrozen and stored for mechanical testing. Tendons were thinned to approximately 1 mm thick in the

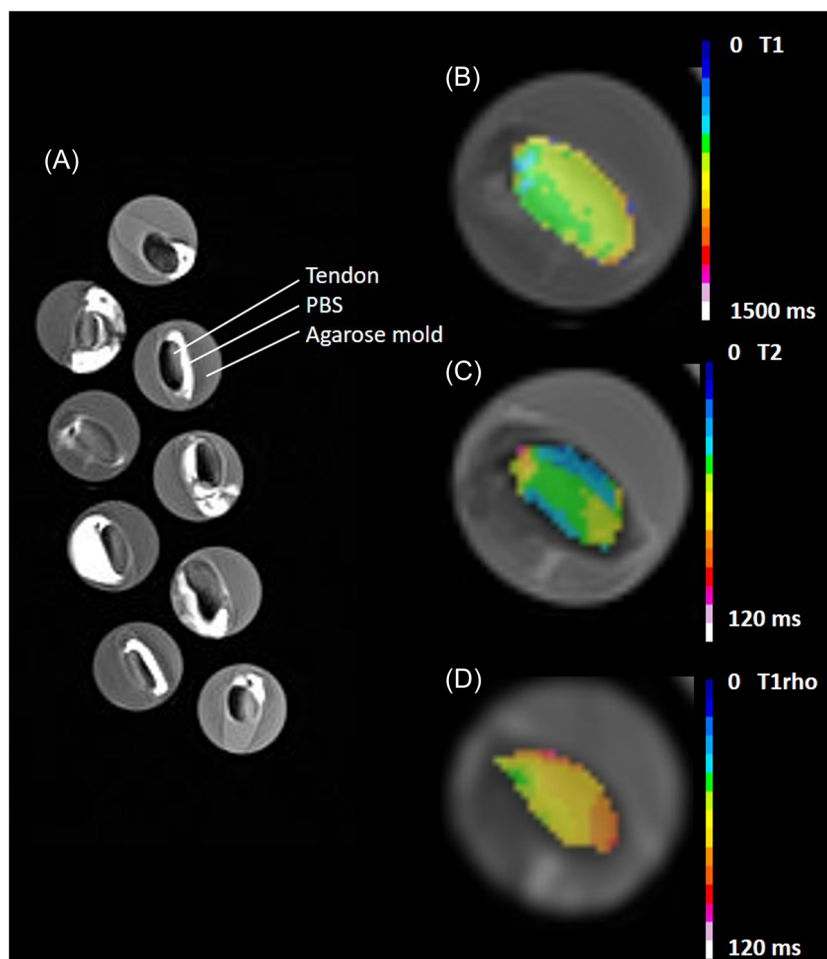
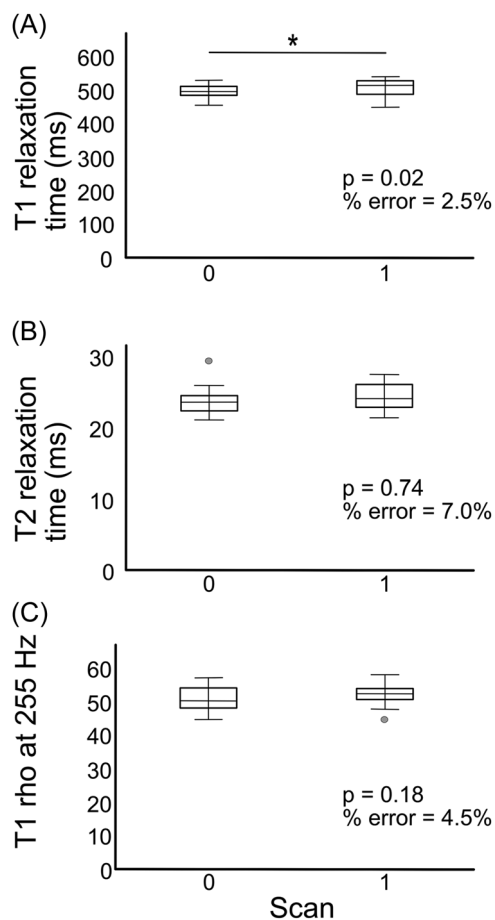


FIGURE 1 Representative image of tendons in agarose mold on transverse, T1 weighted image (A). Representative T1 (B), T2 (C), and T1p (D) maps.

TABLE 1 Imaging parameters for tendon ex vivo imaging

T1, T2, T1p, DTI	T1 maps	T2 maps	T1p map	DTI
Sequence	2D/IR-TSE	2D/TSE	2D/T1p-prep GE	Resolve DTI
TR (ms)	3000	3000	3000	3000
TE (ms)	8.5	10	1.33	36
TI, TE, or TSL (ms)	TI: 50, 100, 250, 400, 550, 700, 850, 1000, 1500, 2000	TE: 10, 29, 30, 40, 50, 60, 70, 80, 90, 100	TSL: 30, 44, 54	N/A
DTI directions/b values	N/A	N/A	N/A	12/0, 400 mm ²
Echo train length/segment	7	10	61 (segment)	60
Number of averages	1	1	5	1
Matrix (frequency)	256	256	256	120
Matrix (phase)	128	128	122	60
Field-of-view (FOV) (mm × mm)	200 × 100	200 × 100	200 × 100	200 × 100
Slice thickness (mm)	8	8	8	8
Pixel Bandwidth (Hz)	590	225	910	1040
Pixel size (mm × mm)	0.78 × 0.78	0.78 × 0.78	0.78 × 0.82	1.67 × 1.67

Abbreviation: DTI, diffusion tensor imaging.

**FIGURE 2** Between scan repeatability of T1 (A), T2 (B), and T1p (C) measures in ex vivo bovine deep digital flexor tendon

frontal plane using a freezing-stage sliding microtome. Cross sectional area of thinned samples was measured using a noncontact laser scanning device (LJ-V7080; Keyence).³⁵ Samples were gripped with sandpaper and clamped using a custom fixture, submerged in PBS, and subjected to uniaxial tensile testing with a mechanical tensile test system (574LE2; TestResources) with strain beads attached to the tendon surface for optical strain tracking. Tensile testing included: application of a 0.1 MPa preload, 10 cycles of preconditioning to 6% strain, stress-relaxation at 6% strain for 10 min, a 1-min rest period, and a ramp to failure at a rate of 1% strain per second. Mechanical testing variables included in data analysis were hysteresis (from last cycle of preconditioning); peak stress, equilibrium stress, and percent relaxation (from stress relaxation testing); and linear modulus (calculated using bilinear curve fitting of the ramp-to-failure test).

Quantitative polarized light imaging was performed during mechanical testing (Figure 3).^{23,36,37} This technique leverages the natural birefringence of tendon to measure collagen alignment and organization.^{36,37} Circularly polarized light is shone through the tendon during mechanical testing and is captured by a division-of-focal-plane polarization-sensitive digital camera.³⁶ Degree of linear polarization (DoLP) ranges from 0 to 1, with larger values indicating greater alignment. The average value over the region of interest (AVG DoLP) used in analysis. The region of interest was defined as the area within the strain-tracking beads. Angle of polarization (AoP) reflects the average angle the collagen is positioned within a given pixel. Because the collagenous structure of tendon is primarily aligned with the axis of tension, the standard deviation of the angles (STD AoP) for each pixel within the region of interest was used in analysis as an

indicator of consistency of alignment over the region of interest. The maximum AVG DoLP and minimum STD AoP were used for analysis, representing the stage of the mechanical test protocol when collagen was most aligned.

Following tensile testing, the portion of tendon used during mechanical testing was digested. Collagen content was then quantified using a hydroxyproline assay and normalized to wet weight.³⁸

2.4 | Statistical analysis

Data were inspected for parametric assumptions using the Shapiro–Wilk test and visual inspection of Q-Q plots. Relationships between qMRI variables and tendon mechanical/structural variables were tested using Pearson correlation. The variables T2 map and STD AoP did not meet parametric assumptions, so Spearman's rho is reported for the strength of relationship between variables in these cases. Strength of relationships between variables were interpreted^{39,40} based on correlation coefficient as weak (r : 0.1 to <0.3), moderate (r : 0.3 to <0.5), and strong ($r \geq 0.5$).⁴¹

Effect size estimates for relationships between qMRI parameters and tendon composition/organization were not available to inform a robust a priori power analysis. A priori power analysis (G*Power^{39,40}) estimated that 11–26 specimens would be required to detect relationships with large ($p = 0.5$) to very large ($p = 0.7$) effect size with 80% statistical power. Therefore, given the challenges associated with obtaining these specimens, we planned to collect as many specimens that were available within a reasonable timeframe to allow specimens to be tested and analyzed at the same time, with a minimum of 11 specimens.

3 | RESULTS

Tissue specimens were collected from donors (10 male, 6 female) a mean (SD) of 54.7 (8.2) years of age. Causes for amputation included Charcot deformity ($n = 5$), osteomyelitis/wound infection/other infection ($n = 4$), and trauma/complications following orthopaedic surgery ($n = 4$). Descriptive statistics for qMRI metrics are displayed in Table 2.

3.1 | Relationships between qMRI metrics and tendon mechanical behavior and structure

T2 values were significantly correlated to tendon mechanics, including hysteresis ($r_{sp} = -0.64$, $p = 0.007$), linear modulus ($r_{sp} = 0.67$, $p = 0.009$), peak stress ($r_{sp} = 0.51$, $p = 0.044$), equilibrium stress ($r_{sp} = 0.54$, $p = 0.033$), and percent relaxation ($r_{sp} = -0.55$, $p = 0.027$) (Figure 4). T1p had a statistically significant relationship with percent relaxation ($r = 0.502$, $p = 0.048$). There were no other statistically significant relationships observed between T1 map and T1p and tensile mechanics, nor were there any statistically significant

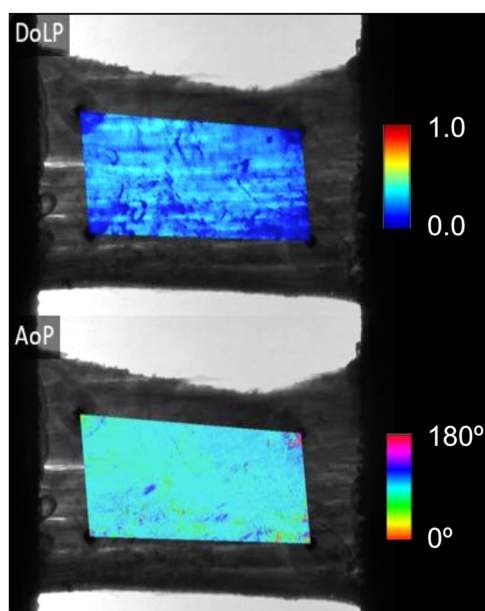
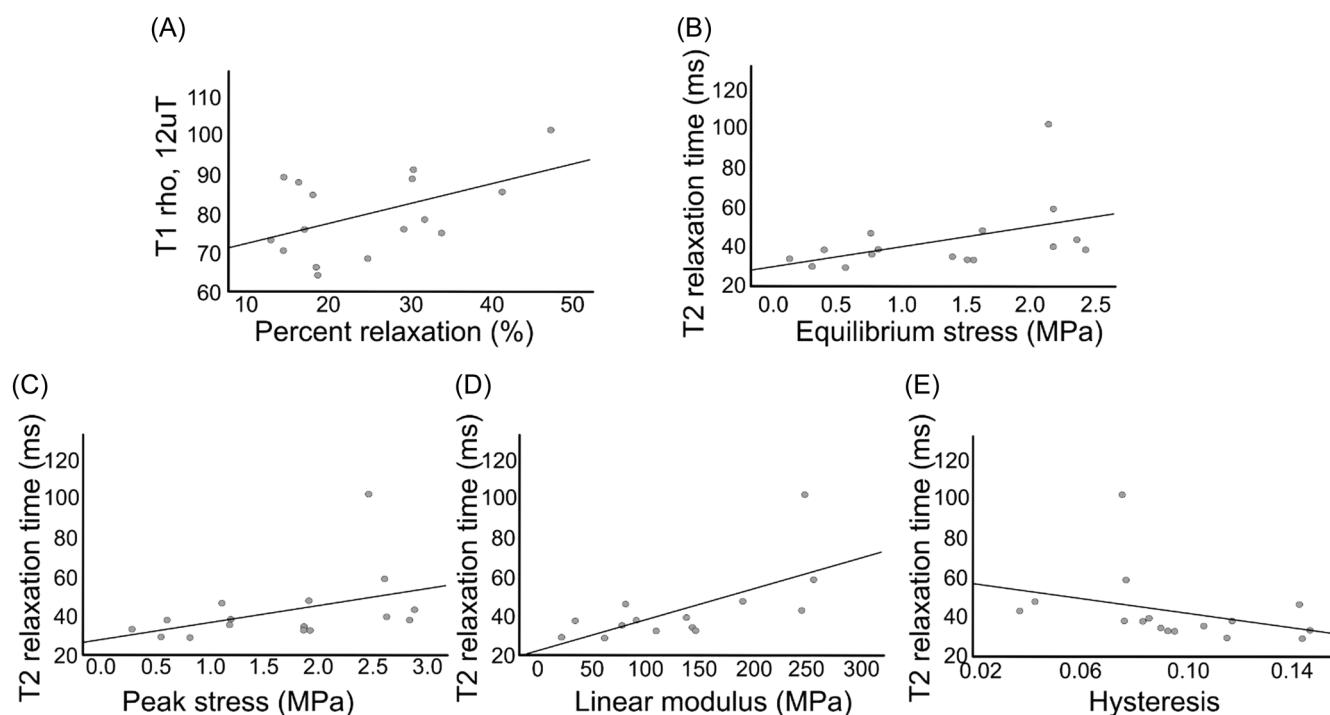


FIGURE 3 Representative tendon on quantitative polarized light imaging (QPLI) showing degree of linear polarization (DoLP, top) and angle of polarization (AoP, bottom).

TABLE 2 Descriptive statistics for quantitative MRI (qMRI) metrics in all specimens ($n = 16$)

qMRI metric	Minimum	Maximum	Mean	Standard deviation
T1 map (ms)	472	671	585	67
T2 map (ms)	29	102	43	17
T1p (ms)	64	101	79	10
Fractional anisotropy	0.23	0.55	0.38	0.08
Mean diffusivity ($\times 10^{-3}$ mm ² /s)	0.52	0.86	0.71	0.10
Axial diffusivity ($\times 10^{-3}$ mm ² /s)	0.71	1.17	0.93	0.12
Radial diffusivity ($\times 10^{-3}$ mm ² /s)	0.43	0.77	0.61	0.09

Abbreviation: qMRI, quantitative magnetic resonance imaging.

**FIGURE 4** Scatterplots showing relationships between qMRI metrics [T1p (A) and T2 (B–E) and tendon tensile mechanics]. Percent relaxation and hysteresis are displayed as decimals and not percentages. qMRI, quantitative magnetic resonance imaging

relationships between T1 map, T2 map, or T1p and tendon compositional or organizational measures (Table 3).

Collagen content was significantly correlated to DTI measures—including mean diffusivity (mean diffusivity [MD]; $r = 0.62$, $p = 0.011$), axial diffusivity (axial diffusivity [AD]; $r = 0.62$, $p = 0.011$), and radial diffusivity (radial diffusivity [RD]; $r = 0.56$, $p = 0.024$) (Figure 5). There were no other statistically significant relationships between DTI metrics (MD, AD, RD, and fractional anisotropy [FA]) and tensile mechanical behavior or composition (Table 3). FA was significantly correlated with STD AoP ($r = 0.53$, $p = 0.035$). There were no other statistically significant relationships between collagen organization on QPLI and qMRI metrics (Table 3).

4 | DISCUSSION

This study comprehensively assesses quantitative MRI measures, including DTI, and identifies their relationships with tendon mechanics and composition. While these relationships were explored ex vivo to allow for the direct measurement of tendon composition and tensile mechanics, elucidating relationships between tissue appearance on noninvasive, quantitative, diagnostic imaging modalities and physical measures is an important step in being able to translate findings from bench to clinic. We found that T2 values from a single slice of the tendon midportion of human Achilles tendons were strongly correlated to all tendon tensile mechanical metrics evaluated

TABLE 3 Correlation table showing relationships between quantitative MRI metrics and tendon tensile mechanics, collagen organization, and composition measures

	T1 map	T2 map ^a	T1p	FA	MD	AD	RD
Peak stress	0.25	0.51 ^b	-0.39	-0.22	0.07	0.08	0.04
Equilibrium stress	0.25	0.54 ^b	-0.38	-0.25	0.10	0.11	0.07
Percent relaxation	-0.22	-0.55 ^c	0.50 ^b	0.27	-0.22	-0.26	-0.15
Hysteresis	-0.47	-0.64 ^c	0.15	0.31	-0.15	-0.07	-0.17
Linear modulus	0.33	0.67 ^c	0.04	-0.21	-0.07	-0.11	-0.07
AVG DoLP, maximum	0.18	0.11	-0.18	-0.34	0.25	0.20	0.25
STD AoP ^c , minimum	-0.43	-0.30	0.43	0.53 ^b	-0.40	-0.36	-0.47
Collagen/weight	0.04	0.28	0.02	-0.43	0.62 ^b	0.62 ^b	0.56 ^b

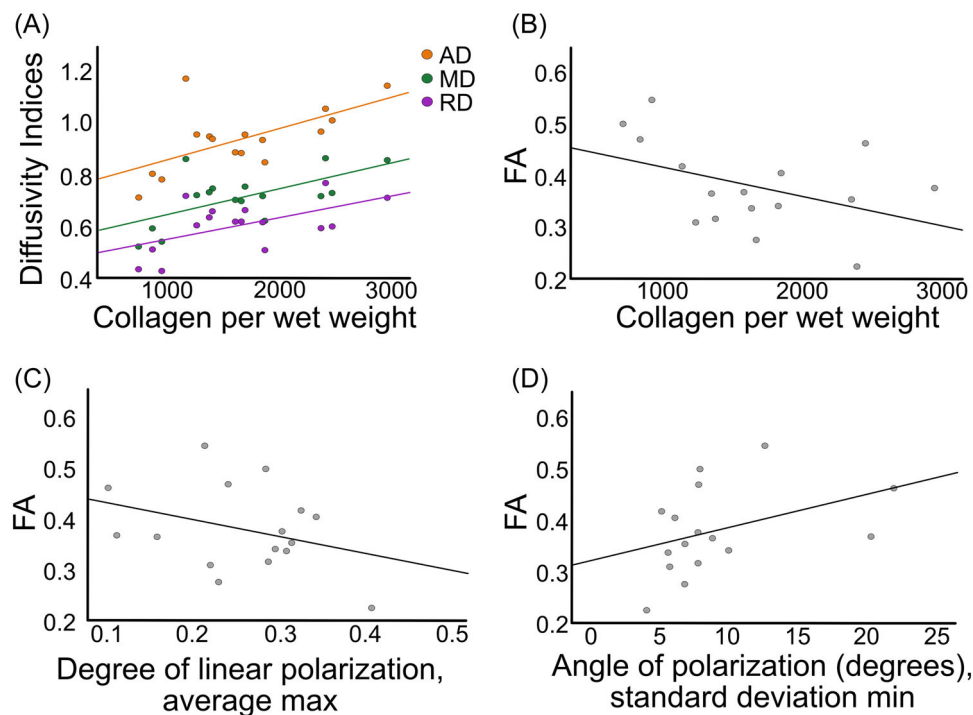
Note: Values reflect Pearson correlation coefficient unless otherwise noted.

Abbreviations: AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; MRI, magnetic resonance imaging; RD, radial diffusivity.

^aIndicates Spearman's rho.

^bIndicates $p < 0.05$.

^cIndicates $p < 0.01$.

**FIGURE 5** Scatterplots showing relationships between diffusivity indices (A) and fractional anisotropy (B) with collagen concentration as well as fractional anisotropy and measures of collagen organization on quantitative polarized light imaging (C, D). Diffusivity indices are in $\times 10^{-3} \text{ mm}^2/\text{s}$, collagen per wet weight is in ng/mg.

in this study. Additionally, DTI diffusivity indices (MD, AD, RD) were all strongly related to collagen content. These findings build on a growing body of literature supporting the feasibility of qMRI to characterize tendon tissue and its potential use as an assessment tool of tendon structure and function.^{14,15,29,34,42}

Experimental data in tendon are becoming available to better understand the physiological implications of qMRI metrics in tendon

tissue. In tendons, T2* has most commonly been used to assess composition changes and is thought to reflect early collagen changes.^{13,17} A study by Bachmann, et al.,¹⁵ tracked changes in T1 and T2* mapping in ex vivo bovine tendon treated with collagenase or induced glycation. This study found that treatment with collagenase increased both T1 and T2* map values compared to control tendons, while induced glycation (via ribosylation) increased T2* but

not T1 values.¹⁵ In vivo studies of human tendon have found higher T2 signal in individuals with rotator cuff tendinopathy^{48,49} that may relate to patient outcomes,⁴⁷ and studies in the Achilles tendon have also reported an increase in T2⁴⁵ and alterations in DTI parameters in individuals with tendon pathology.^{22,33}

T2 is an indicator of fluid and collagen content within musculoskeletal tissues,¹³ including tendon tissue.¹⁴ A study in rabbit tendon found higher T2 values in tendon indicate greater water content and less organized collagen on the Bovin scale.¹⁴ In human tendon tissue, T2 mapping values have been observed to increase in the presence of Achilles and supraspinatus tendinosis^{46,48} and with tendon tear and repair.^{47,49} Further, reduction in T2 value over time after tendon repair has been associated with improvement in patient outcome⁴⁷ and improved tendon composition and organization.¹⁴ Our study found that higher T2 values were associated with larger linear modulus values and reduced hysteresis. This finding was counter to what we had anticipated based on the literature of in vivo tendinopathic patellar tendons,⁴⁵ and may be due to participants not having tendinopathy or tendon injury. Because collagen content was normalized to wet weight, which is predominantly water in tendon, it may also be that relationships were driven primarily by water content influencing the direction of correlations.

Relationships between T2 values and mechanical properties observed in this study were strong, but in a direction opposite to our hypothesis. Because increased T2/T2* signal has been associated with tendon injury,^{14,45} we had hypothesized that higher T2 values would be related to impaired mechanical properties (lower linear modulus and greater hysteresis). It may be that T2 measures are associated with tendon mechanical function, but the direction of these relationships may be different in the context of tendon injury or in the presence of presumed chronically impaired tendon homeostasis (as is the case with diabetes). The donors in this study did not have active tendon injury and were ambulatory at time of surgery, though tendon loads would likely have been altered before surgery (due to offloading recommendations from orthopaedic complications or to promote wound healing). Additionally, 63% (10/16) of the specimens came from diabetic individuals, so tendon glycation and impaired homeostasis may be a consideration. This said, a prior study of induced enzymatic tendon digestion and glycation found both digestion and glycation increased the T2* signal, which was found to correlate with tendon mechanical properties.¹⁵ It is possible that relationships between T2 values and mechanical properties observed in prior studies in injured individuals and in our study of individuals with uninjured but likely disrupted tendon homeostasis, while strong, may not translate to the relationships observed in otherwise healthy individuals.

Whereas T2 measures related to tendon mechanics, DTI measures related to collagen content (MD, AD, and RD values) and alignment (FA value). Prior studies have reported values for DTI acquired for in vivo tendons.^{22,33} DTI values observed in the present study were 1–2 standard deviations lower than healthy Achilles tendons reported previously in vivo.²² Higher amounts of diffusivity were associated with greater collagen concentration. This aligns with

data from a study by Wengler, et al.,²² which found tendinopathic tendons (presumed to have lower collagen content when compared to healthy tendons) to have lower AD, MD, and RD than healthy tendons. Higher FA was associated with greater variability in collagen alignment. FA ranges from 0 to 1, with diffusion more aligned with the primary axis of diffusion being closer to 1. Therefore, we had hypothesized that there would be a negative relationship between FA and STD AoP. While the data did not support this hypothesis, it is important to note that QPLI has the resolution to account for collagen alignment at length scales larger than the molecular level (likely the fiber level and above), so it may be that DTI and QPLI are assessing tissue isotropy at different length scales and are not directly analogous. Additional research, perhaps leveraging accessibility of tendons from animal models, may be helpful in further elucidating these relationships to improve our understanding of the physiologic interpretation of DTI measures in tendon tissue.

We observed a relationship between T1p and percent relaxation, but did not observe any other statistically significant relationships between T1 and T1p and tendon mechanics, collagen content, or collagen organization. To date, limited studies have investigated T1 and T1p mapping in tendon tissue.^{42,43,46,52,55,56} T1 values observed in the present study align with those reported in the literature, which range from just under 500 ms to about 800 ms.^{43,46,52,55,56} In one study reporting T1p values in tendon tissue to date, mean values ranged from about 3–5 ms. However, scans were not performed with the Achilles tendon positioned at the magic angle, which may significantly underestimate T1p values (T1p behaves more or less like T2).⁴² Given the limited application of T1 and T1p to tendon tissue, additional studies incorporating T1 and T1p measures would be helpful in better understanding the utility of these metrics in understanding tendon structure and function. In particular, comparing T1p to proteoglycan content may help in explaining the relationship observed with percent relaxation.

There are a few limitations in this study. Due to the challenges associated with acquiring these samples, a relatively moderate number of specimens were obtained which limits our ability to detect more subtle relationships. Additionally, tendons were imaged ex vivo which may alter the observed tendon characteristics due to alterations in hydration or interactions with surrounding tissues compared to in vivo conditions. Again, due to limitations in sample size, we were unable to account for the influence of age or other donor demographics on tendon appearance on MRI or mechanics via study design or statistical analysis. This may be of concern as T2 mapping values have inconsistently been found to associate with age in uninjured tendon.^{44,50} We performed a retrospective analysis to identify whether age or body mass index were significantly correlated with qMRI measures. Only T1 was significantly related to body mass index ($r = 0.671$, $p = 0.012$). No other statistically significant relationships were observed. Finally, to identify if there are concerns regarding differences between tissues obtained from living compared to deceased donors, we performed t-tests comparing the mechanical properties of the tendon specimens obtained from cadavers to those obtained from living donors. There were no significant differences

between groups, and cadaveric tissue fell within 1 standard deviation of the mean of specimens from living donors for all parameters.

Despite these limitations, this study builds on a growing body of literature demonstrating the ability to apply qMRI to tendon tissue and supporting its use as a noninvasive measure that relates to tendon composition and mechanical behavior. qMRI metrics provide some indication of tendon mechanics and content and, to some extent, organization (when incorporating DTI). Importantly, this is the first study to report relationships in human tissue between qMRI and assays directly assessing collagen concentration and tendon tensile behavior.

AUTHOR CONTRIBUTIONS

Research design: Jennifer A. Zellers, Simon Y. Tang, Spencer P. Lake, Michael J. Mueller, Mary K. Hastings, Jie Zheng. **Data acquisition:** Jennifer A. Zellers, Reika McNish, Masoud Edalati, Jeremy D. Eekhoff, Jie Zheng. **Data analysis:** Jennifer A. Zellers. **Data interpretation:** all authors. **Drafting manuscript:** Jennifer A. Zellers, Reika McNish, Jie Zheng. **Critical manuscript revision:** all authors. All authors have read and approved the final submitted manuscript.

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