Regional differences in anterior cruciate ligament imaging biomarkers: T2 and T2* values

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Summary

Introduction: **Imaging biomarkers such as T2 and T2* relaxation times have been used in a variety of tissues as surrogates of structural components. Before using such techniques to assess ACL tissue, healthy values must be established. The purpose of this study was to characterize T2 and T2* ACL relaxation times in clinically relevant sub-regions in healthy ACLs.**
Methods: **Healthy males (n=20) and females (n=20) were assessed via 3T magnetic resonance imaging. T2 weighted structural imaging was performed to obtain ACL volume. T2 and T2* relaxation imaging were then performed. The ACL was sub-divided into proximal, middle and distal thirds where regional T2 and T2* relaxation times were calculated. A 1 between (sex) and 3 within (sub-region) RMANOVA compared T2 and T2* relaxation times.**
Results: **For the T2 relaxation analysis, the middle sub-region was greater than the proximal sub-region, and males had greater distal sub-region relaxation times than females. T2* relaxation times were significant greater in the distal than proximal and middle sub-regions in males and females.**
Conclusion: **This is the first report of *in vivo* T2 and T2* ACL relaxation times in healthy individuals. Relaxation times were generally less in proximal portions of the ligament suggesting a non-uniform ligamentous structure.**
Level of evidence: IV.

KEY WORDS: knee, MRI, structural composition.

Introduction

Tissues with a high concentration of collagen, such as tendons and ligaments, may be ruptured through acute singular or chronic repetitive loading[1]. Recent attention on the anterior cruciate ligament (ACL) has begun to explain why repetitive non-contact loadings may result in sudden ACL rupture[2]. Given the potential for fatigue failure of the ACL[2], there is a need to develop screening tools to identify characteristics of weaker ligaments which may help to identify those at greatest risk of this type of injury.
The ability to non-invasively assess ligamentous structural properties would benefit both clinical care as well as research environments[3]. Identifying degeneration of pertinent tissues would aid in recognizing deterioration of the ACL which may potentially include erosion of collagen structure and biochemical composition. Quantitative MRI relaxation mapping has allowed for detection of early biochemical changes in cartilage well in advance of the gross morphologic changes[4-6]. Such imaging techniques have been used to evaluate structural characteristics of the Achilles[7] and patellar[8] tendons. Finally, using such quantitative MRI methods has also been suggested in the assessment of chronic ligamentous conditions[9]. Thus, there is the potential for such imaging processes to help determine ligament quality *in vivo*.
T2 and T2* relaxation mapping are quantitative sequences readily available on clinical MRI platforms. T2 relaxation is referred as the transverse relaxation rate[10], with shorter T2 relaxation times in cartilage reflecting denser collagen, more organized collagen ultrastructure, and less free water content[11]. T2* relaxation has been utilized in the study of animal ACL grafts[12,13] and negatively associated with yield load of healing ACL grafts[12]. T2 and T2* relaxation times of the intact posterior cruciate ligament (PCL) have

been reported as baseline values for comparison of acute and chronic PCL injuries[9]. Interestingly, the previous PCL study[9] reported regional differences in T2 and T2* relaxation times. While these findings collectively indicate that T2 and T2* relaxation times may be useful in assessing intrinsic ligamentous properties, T2 and T2* relaxation times have yet to be established for the *in vivo* ACL.

The purpose of this study was to characterize T2 and T2* relaxation times of the *in vivo* ACL in a healthy, active population in clinically relevant regions of interest. With reported ACL collagen density differences between females and males[14], sex was included as a variable. Given previous reports of regional differences in the PCL[9], it was hypothesized that the relaxation times of individual ACL sub-regions would differ and that there would be no difference in relaxation times of individual sub-regions between sexes.

Materials and methods

Study population and design

Healthy, recreationally active participants (20 males and 20 females) were recruited from local universities to participate in this study (Tab. I). Inclusion criteria were: 1) current engagement in sport activities at least 2 hours per week; and 2) no lower extremity injury in the last 6 months. Participants were excluded if they had: 1) previous history of injury to the capsule; ligament, or menisci of either knee; 2) any vestibular or balance disorder; and 3) any metal or implanted medical device in the body. Informed consent was obtained from all individual participants included in the study. Each participant attended a MRI testing session consisting of T1 and T2 weighted magnetic resonance imaging (MRI) along with T2 and T2* relaxation mapping imaging on the left knee. Participants were instructed to avoid high intensity activities 24 hours prior to testing. The Marx activity rating scale[15] quantified participant activity level. Participants self-rated running, cutting, decelerating, and pivoting activities each as 0 (less than once per month), 1 (once per month), 2 (once per week), 3 (2-3 times per week) or 4 (4 or more times per week), resulting in a score from 0 to 16. All procedures of the study met ethical standards[16].

Image acquisition

MRI data were acquired using a 3T Siemens Tim Trio scanner (Erlangen, Germany) and a 15 channel knee coil (Siemens Erlangen, Germany). T2-weighted, multiplanar MRI scans [repetition time (TR)=1300 ms; excitation time (TE)=39 ms; Flip angle (FA)=160°; FOV=150x150 mm; voxel size=0.5×0.5×0.5 mm] were used for ACL morphometric measures. The T2 relaxation imaging was performed using spin echo data sets with the following previously reported parameters: repetition time (TR)=3040 ms; excitation time (TE) at 13.8, 27.6, 41.4, 55.2, and 69 ms; flip angle (FA)=180°; voxel size, FOV=160 x160 mm; voxel size=0.4×0.4×3.0 mm[17]. T2* relaxation was performed using gradient echo data sets with the following previously reported parameters: repetition time (TR)=1000 ms; excitation time (TE) at 8.26, 10.28, 12.3, 14.32, 16.34, 18.36, 20.38, 22.4, 24.42, 26.44, 28.46 and 30.48 ms; flip angle (FA)=90°; FOV=280 x280 mm; voxel size=0.5×0.5×3.0 mm[3,18].

Image analyses

All morphometric segmentations of data were performed by a single investigator. The entire ACL region of interest (ROI) was calculated per the methods of Chaudhari et al.[19] using ITK-SNAP software[20] (http://www.itksnap.org/pmwiki/pmwiki.php). First, the ACL contouring of each sagittal slice was done manually using a digitizing tablet (Wacom DTK1300; Wacom Co, Kazo, Japan). All slices that were contoured were spatially summed to create an ACL ROI that was calculated using the software (Fig. 1). For the establishment of intra-tester reliability and precision of the ACL volume ROI in 10 pilot participants was measured twice at least a week apart [$ICC_{3,1}$ (SEM)=0.97 (36.1) mm^3].

Next 3 ACL sub-regions ROIs were ascertained. The sagittal slice which showed the clearest image of Blumensaat's line was selected. From this image a line was drawn from the most proximal pixel included the segmented ACL ROI to the most distal pixel included in the ACL ROI. Following previous work investigating T2 and T2* values of the posterior cruciate ligament[9], the ligament was segmented and was then divided in proximal, middle, and distal thirds (Fig. 2).

Using customized Matlab scripts (Mathworks Inc, USA), the voxel-wise T2 and T2* relaxation maps

Table I. Participants' Descriptive Statistics (Mean ± Standard Deviation).

	Males (N=20)	Females (N=20)
Age (yrs)	23.3 ± 2.9*	21.3 ± 2.3
Height (cm)	180.4 ± 6.7*	166.9 ± 7.7
Weight (kg)	84.0 ± 10.9*	61.9 ± 7.2
Marx Activity-Rating Score	9.2 ± 4.1	10.7 ± 3.9

*significantly greater than males.

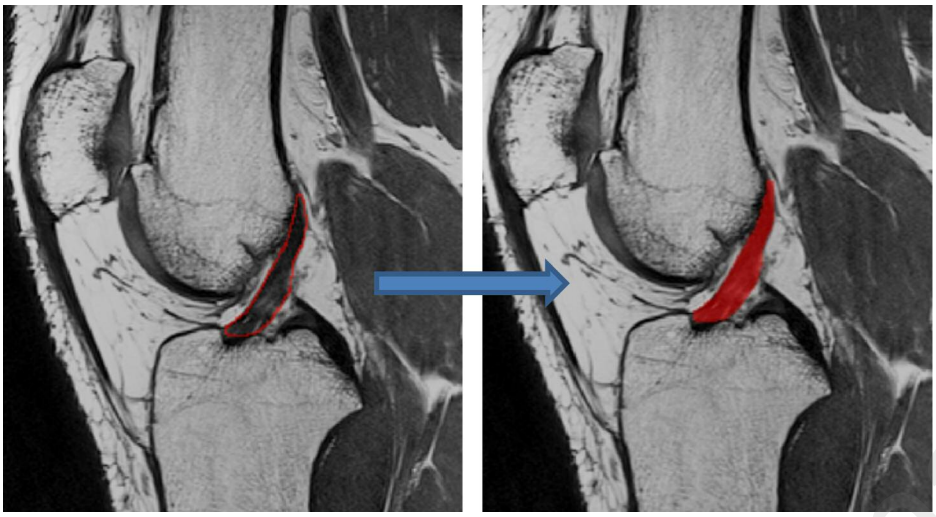

Figure 1. Manual ACL segmentation and resultant area (shaded) on sagittal image.

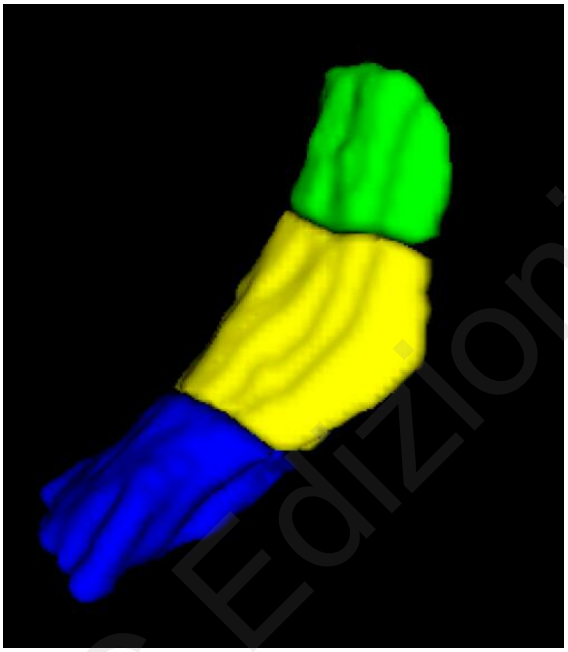

Figure 2. Proximal (green), middle (yellow), and distal (blue) one-third ACL sub-regions.

were calculated using the signal intensity (SI) relationship from all five echo times of the T2 or all twelve echo times of theT2* relaxation imaging sequence. Equation: $SI(TE)=S_o \exp(-TE/T2orT2^*)$, where SI(TE) are the voxel-specific SIs for the various echo times (TE) and where S_o is the signal intensity at the initial TE[3,17,18]. To isolate ligament specific T2 or T2* values, the calculated T2 or T2* relaxation map was registered to the structural imaging sequence using Slicer 3D Software (https://www.slicer.org/) and the mean T2 (Fig. 3a) or T2* relaxation value of the voxels (Fig. 3b) included in the 3D ACL volume, proximal sub-region, middle sub-region, and distal sub-region described above were included for analyses.

Statistical analysis

Independent sample t-tests assessed differences in demographics between sexes. To address the hypothesis of no difference in relaxation times across the three calculate sub-regions or sex, a 1 between (sex) and 3 within (sub-region) RMANOVA was performed for T2 and T2* relaxation times. Post-hoc Bonferroni pair-wise comparisons were performed. The alpha level for all analyses was set priori at equal or less than .05. All calculations were performed using the SPSS statistical software (version 21.0; IBM Corp, Armonk, NY).

Results

On average, males were significantly older (1.9 yrs), taller (13.5 cm), and had greater mass (22.1 kg) than females. There was no significant difference in Marx Activity scores (Tab. I). Summary statistics (mean, SD, min-max, and 95%CI) of all variables are presented in Table II. For the T2 relaxation analysis there was a significant interaction of sub-region and sex (P=0.053) that demonstrated that males had greater distal sub-region T2 relaxation times than females (Fig. 4). Also, there was a main effect for sub-region (P=0.037) that demonstrated greater middle sub-region T2 relaxation times than the proximal sub-region. There was no main effect for sex (P=0.722). For the T2* relaxation analysis there was a significant a main effect for sub-region (P<.001) that demonstrated that the distal sub-region T2* relaxation times were significant greater than the proximal and middle sub-regions (Fig. 5). There was no significant interaction of sub-region and sex (P=0.103) or main effect for sex (P=0.219).

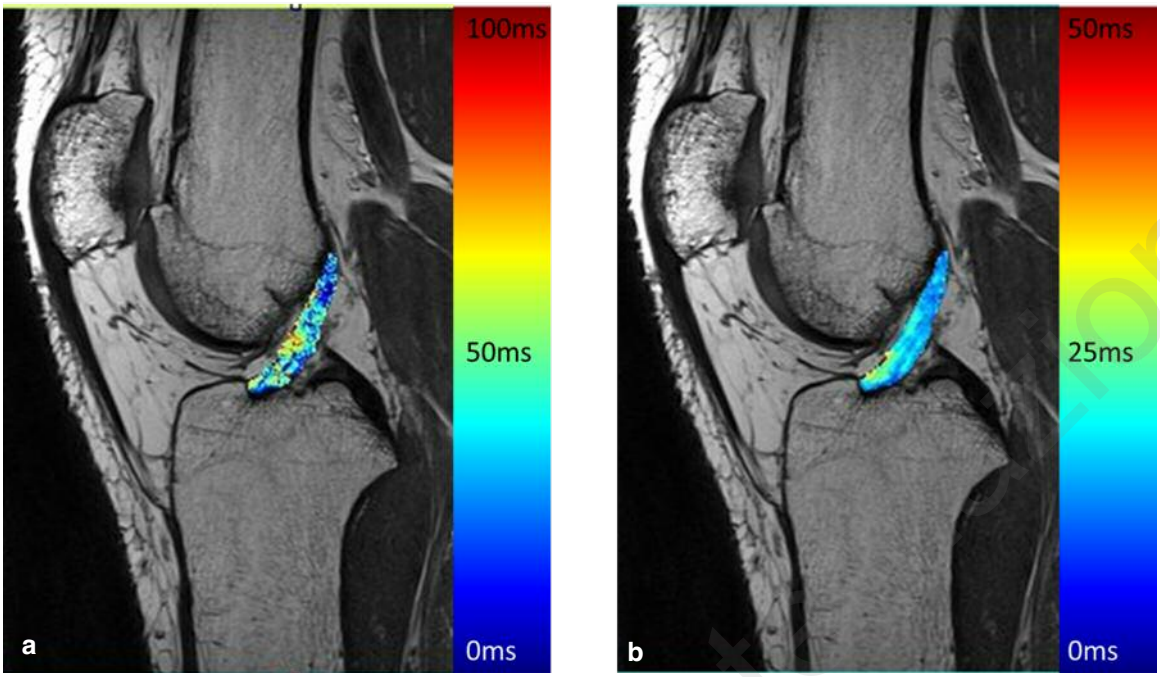

Figure 3. (a) T2 relaxation map of ACL; (b) T2* relaxation map of ACL.

Table II. T2 and T2* ACL Relaxation Times (ms) by Region and Sex.

	Males			Females		
	Mean±SD	min-max	95% CI	Mean±SD	min-max	95% CI
T2 - proximal	53.4±12.7	28.9-73.8	47.4-59.3	54.9±12.9	27.7-78.2	48.8-60.9
T2 - middle	57.7±7.5	43.0-67.5	54.2-61.2	58.8±7.9	45.8-76.9	55.1-62.4
T2 - distal	59.0±8.7	42.7-70.7	54.9-63.1	53.6±10.5	34.8-69.9	48.7-58.5
T2* - proximal	18.5±4.0	13.9-30.6	16.6-20.4	16.7±2.3	10.8-20.7	15.6-17.8
T2* - middle	18.8±2.6	15.9-25.1	17.6-20.0	17.5±1.8	12.9-20.1	16.6-18.3
T2* - distal	19.7±3.0	11.8-25.0	18.3-21.1	20.1±3.6	12.3-25.9	18.4-21.8

Discussion

This paper provides baseline ACL quantitative MRI relaxation mapping values in healthy females and males similar in age and activity level to populations at increased risk of ACL injury. This study specifically describes sex-specific baseline T2 and T2* values, which have been hypothesized to be related to ligamentous strength and structure[17,21], to serve as comparison values for *in vivo* studies of ACL injury risk. The following discussion will address our primary findings of sex and regional differences in T2 and T2* relaxation values of the intact ACL and discuss potential clinical implications of these novel data.

For T2 relaxation, the interaction of region and sex demonstrated that males had greater T2 relaxation times than females in the distal region. When males and females were pooled the proximal T2 relaxation times were less that the mid region. Taken together this may be interpreted that the collagen structure and orientation is not uniform throughout the ACL and this non-uniformity may be sex-specific. Similarly, T2 and T2* *in vivo* assessment of the human PCL revealed increase relaxation times in the distal sub-region[9]. Such regional variation of T2 relaxation times have also been reported in tendon tissue[22]. These regional differences may be due to increased complexity or non-uniformity of tissue that could be related to degenerative changes[9]. However, we are unable to locate any work that has directly assessed the relationship of degenerative ligamentous tissue and T2 relaxation times.

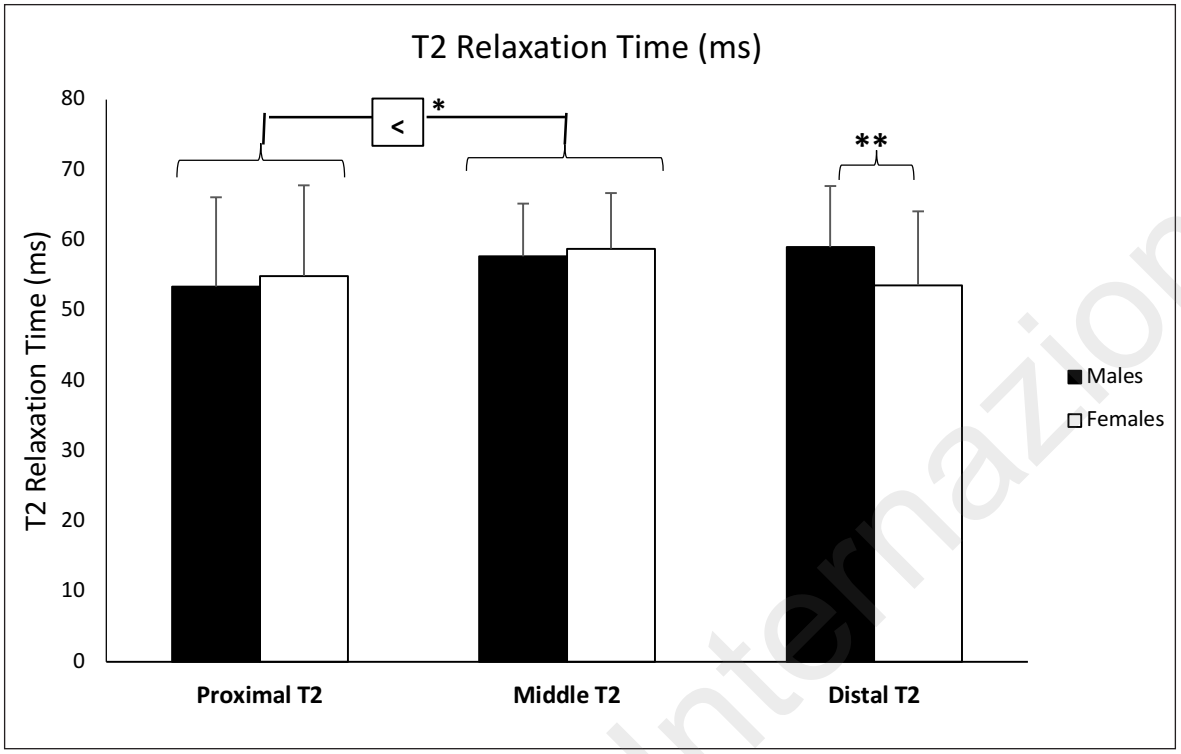

Figure 4. ACL T2 relaxation times by sex and region. **Males had significantly greater distal sub-region T2 relaxation times than females (P=0.053). * Greater middle sub-region T2 relaxation times than the proximal sub-region (P=0.037).

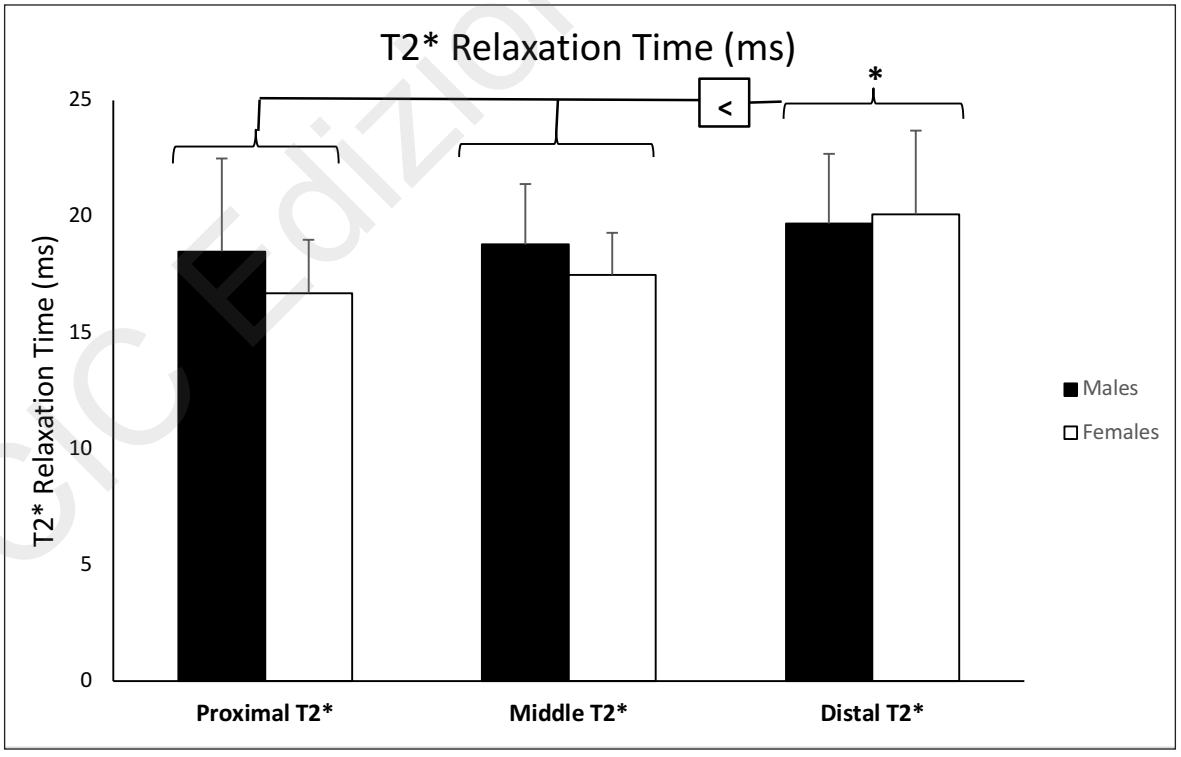

Figure 5. ACL T2* Relaxation Times by sex and region, * Distal significantly larger (P<.001) than proximal and middle sub-regions.

For T2* relaxation both sexes display greater T2* relaxation times in the distal region. The distal region was in large part spatially related (Fig. 2) to the portion of the ACL distal to the notch outlet. As greater T2* values are suggested to be indicative of a less organized extracellular matrix[9], this may suggest that this region may be more affected by loading than the proximal and middle region. The premise that this region may have different intrinsic characteristics is partially supported by findings of the majority of incidences of partial ACL disruption occurred in either the distal or middle ACL sub-regions[23].

While the current study provides baseline T2 and T2* relaxation values of healthy ligamentous tissue, it is unknown as to whether such values are representative of the structural components of healthy ligaments. Structural composition of cadaver ACLs has been associated with failure load[14,24] which indicates that intrinsic ACL properties may play a role in ACL injury. Previous work has reported T2* relaxation times of human cadaver ACL[18]. However, use of such cadaver values for *in vivo* human comparisons are limited due to significant differences in the physiologic field that affect obtained mapping values[18].

Studies validating T2 and T2* relaxation times to tissue properties have focused largely on articular cartilage tissue[25-27]. When comparing the structure of articular cartilage to ligamentous tissue, both tissues have an abundance of water with an extracellular matrix[28-31]. While 65-80% of cartilage is composed of water, type II collagen accounts for 10-20% of wet weight and proteoglycans account for 10-20% of the wet weight[30,31]. Conversely, slightly less of the ligament is composed of water (~67% of wet weight) with type I and type III collagen accounting for ~25% of the wet weight and the other being small amounts of elastic, proteoglycans and glycoproteins[29,32]. While articular cartilage and ligament share structural similarities that warrant initial investigation of T2 and T2* relaxation imaging in ligament, detailed ligamentous validation studies are need to fully validate and understand the utility of such imaging in ligaments.

While T2 and T2* relaxation times are thought to represent somewhat similar intrinsic properties there is still some uncertainty as to this. T2 and T2* relaxation times have been shown to be related in articular cartilage[33-35]. However, an *in vitro* study reported a negative relationship between T2 and T2* relaxation times[36]. Thus there does not appear to be a consensus of the relationship between T2 and T2* relaxation times. Taken with the non-congruent T2 and T2* values of the current investigation, this suggests that T2 and T2* relaxation times may be representing different structural/biochemical properties in tissue.

Comparison of T2 and T2* relaxation times between the cartilage and ligaments help us to understand the utility of assessing T2 and T2* measures in various tissues. In knee cartilage measures, T2 relaxation times have been reported around 58.3 ±14.4 ms *in vivo*[33] and 51.9 ± 9.2 ms *in vitro*[36] while T2* relaxation times were around 22.5 ± 7.7 ms[33] *in vivo* and 20.3 ± 10.3 ms[36] *in vitro*. The present study reported that mean T2 relaxation times ranging from 53.4-59.0 ms and mean T2* relaxation times from 16.7-20.1 ms. While mean values are similar between previous cartilage research and currently reported values of ligamentous tissue, we are still unaware of work that has fully investigated the relationship T2 and T2* measures to ligamentous tissue components. Hence, future investigations of histologic studies and imaging biomarkers in healthy ligaments are needed.

The present study was limited by lack of direct validation with ligamentous histology. It is important to note that the previous T2 and T2* findings are related to the collagen characteristics and water content as studied in articular cartilage[4,5,37-39]. With regard to ACL tissue, existing work is limited to the T2 and T2* relaxation times relationships in cadaver or reconstructed animal ligamentous biomechanics[12,17,18]. Additionally, although activity-rating scores were similar between sexes (Tab. I), participants had a wide range of physical activity levels which may affect ligamentous structural composition[40].

Conclusion

This study provides baseline *in vivo* T2 and T2* relaxation mapping values in relevant sub-regions of the ACL in a younger, active population. Significantly greater T2 and T2* relaxation times in the distal subregion suggests a non-uniformity in ligamentous structure. This study provides a quantitative method to obtain *in vivo* ACL biomarkers using a MRI platform. Current data can serve as reference for future comparisons with pathologic or high-risk individuals. Future histologic studies are needed to determine the extent of which structural components of ligament are sensitive to T2 and T2* relaxation times.

Conflict of interest

No relationships/conditions/circumstances present potential conflict of interest.

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