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Sensitivity of ACL Volume and T_2* Relaxation Time to Magnetic Resonance Imaging Scan Conditions

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Abstract

Anterior cruciate ligament (ACL) volume and T_2* relaxation times from magnetic resonance (MR) images have been previously shown to predict the structural properties of healing ligaments. We investigated whether MR imaging scan resolution and condition (in vivo, in situ, or ex vivo) affected ACL volume and T_2* relaxation times in intact ligaments. ACLs of 14 pigs were imaged using a 3T scanner and a six-channel flexcoil using at least two of four possible scan conditions: (1) in vivo moderate resolution (n=14); (2) in vivo high resolution (n=7); (3) in situ high resolution acquired within 60 minutes of euthanasia (n=6); and (4) ex vivo high resolution following hind limb disarticulation and one freeze-thaw cycle (n=7). T_2* relaxation times were mapped to the ACL voxels. The total ACL volume was then divided into four sub-volumes ($Vol_{1\text{-}4}$) based on predetermined increasing ranges of T_2* times. ACL T_2* statistics (first quartile, median, and standard deviation (SD)) were computed. Scan resolution had no effect on the total ACL volume, but Vol_1 and first quartile T_2* times decreased with high resolution and in situ/ex vivo scan conditions. The most dramatic differences in T_2* summary statistics were between in vivo moderate and ex vivo high resolution scan conditions that included a freeze-thaw cycle: ACL T_2* SD increased by over 50% in 9 animals, and more than 90% in 4 animals. Our results indicated that T_2*-based prediction models to quantify in vivo structural properties of healing ligaments should be based on high resolution in vivo MR scan conditions.

Conflict of Interest Statement

None of the authors have received financial support from affiliations that may be perceived as having biased the presentation of the data.

Keywords

ACL; MRI; T_2* relaxometry

Introduction

Magnetic resonance (MR) imaging is a valuable tool to monitor soft tissue remodeling non-invasively. T_2* is a measure of MR signal relaxation that is related to the degree of free water bound by collagen (Helms, et al., 2008), with highly organized collagen structures yielding shorter T_2* relaxation times (Williams, et al., 2012). T_2* is particularly well suited for imaging ligament and tendon healing in vivo because collagen re-organization is of interest (Biercevicz, et al., 2015; Weiler, et al., 2001). We have previously demonstrated that a larger volume of the anterior cruciate ligament (ACL) containing short MR T_2* relaxation times predicts the ligament structural properties in a minipig model of bridge-enhanced ACL repair (Biercevicz, et al., 2014). In developing this MR technique, high resolution 512×512 matrix scans were collected in situ to determine the ACL T_2* relaxation times. ACL voxels were then binned into four sub-volumes based on increasing ranges of T_2* relaxation times established a priori to delineate portions of the ligament containing organized versus disorganized collagen. However, it remains unknown to what extent using a more clinically relevant moderate resolution scan, such as a 384×384 matrix, might influence the distribution of T_2* relaxation times in the minipig model, and whether ex vivo scan conditions following a freeze-thaw cycle – if used to store limbs until convenient for pilot testing and/or sequence development (Chang, et al., 2014; Du, et al., 2012; Juras, et al., 2013) – might further influence T_2* relaxation times.

Whereas the binning of ACL voxels based on T_2* relaxation times is an approach that is independent of the range of values within the dataset, the first quartile is a statistical measure that indicates the threshold of the lowest 25% of T_2* relaxation times. Interpreted alongside the median, the first quartile also provides an indication of the spread of values in the dataset. In other words, the binned sub-volume describes how much of the ACL is composed of highly organized collagen (which can account for more than 25% of the total ligament volume), and the T_2* first quartile describes tissue quality and variation within the ACL and is dependent on the values contained within the dataset. Both volume and quality are central elements of our models to predict healing ACL structural properties (Biercevicz, et al., 2013; Biercevicz, et al., 2015; Biercevicz, et al., 2014). In order to extend the in situ-based prediction models to in vivo conditions, we sought to determine whether intact ACL total volume and T_2* relaxation times were sensitive to MR imaging scan conditions by: 1) determining the sensitivity of ACL volume and T_2* relaxation time to MR scan resolution, and 2) exploring whether ACL volume and T_2* relaxation times were different when measured in vivo, in situ or ex vivo after a freeze-thaw cycle in the minipig model.

Methods

Animals

Intact ACLs of 14 skeletally mature (16.1 ± 1.1 month old) Yucatan minipigs were imaged using a 3T scanner and a six-channel flexcoil (Prisma; Siemens, Erlangen, Germany). All animal procedures were approved by the Institutional Animal Care and Use Committee. Animals were sedated using telozol with xylazine, and then intubated and maintained under general isofluorane anesthesia during scanning.

MR scan conditions

ACLs were scanned using at least two different MR scan conditions (Table 1). The four conditions were: (1) in vivo "moderate resolution" matrix (n=14); (2) in vivo "high resolution" matrix (n=7); (3) in situ high resolution matrix acquired within 60 minutes of euthanasia (n=6); and (4) ex vivo high resolution matrix following hind limb disarticulation and one freeze-thaw cycle (n=7). All scans were 3D gradient multi-echo sequences, and shared the following parameters: FOV=160×160mm; ST/gap=0.8mm/0mm; TR=29ms; FA=12°. The acquisition matrix of the moderate resolution scan was 384×384 (voxel size of 0.42×0.42×0.8mm) with 6 echoes at TE=2.48, 6.86, 11.24, 15.62, 20.00 and 24.38ms. The acquisition matrix of the high resolution scan was 512×512 (voxel size of 0.31×0.31×0.8mm) and 4 echoes at TE=2.8, 7.88, 12.96 and 18.04ms.

T_2* calculation

ACLs were segmented manually (Mimics v16, Belgium) from the T_2* images by a single segmenter. T_2* relaxation times were then calculated by fitting an monoexponential decay function (Haacke, et al., 1999) to either the 4- or 6-echo sequence on a voxel-wise basis (Figure 1). Residual R^2 values of the 4- and 6-echo T_2* functions were used to quantify goodness of fit between the 4- and 6-echo sequences. All calculations were performed using custom software within Matlab (v2015b, Natick, MA).

ACL volume

The ACL total volume was determined and it was then binned into four ranges of increasing T_2* relaxation times as previously reported (Biercevicz, et al., 2014): Vol_1=0–12.5ms; Vol_2=12.6–25ms; Vol_3=25.1–37.5ms; Vol_4=37.6ms–50ms. In addition to ACL total volume, analyses focused on Vol_1 because this sub-volume contains voxels with the shortest T_2* relaxation times that are representative of more organized collagen and have stronger predictive power in estimating ACL structural properties.

Statistical analyses

ACL T_2* summary statistics (first quartile, median and standard deviation (SD)) were computed. Repeated measures ANOVA were used to test for significant differences in ACL total volume and Vol_1, where these measures were collected for three scan conditions (n=6; Subjects #9–14, Table 1). Paired t-tests were used to evaluate differences between: (1) residual R^2 values of T_2* equation fits of the 6-echo and 4-echo in vivo scan protocols (n=7; Subjects #8-14, Table 1); (2) T_2* summary statistics of high resolution in vivo and in situ

protocols (n=6; Subjects #9-14, Table 1); and (3) T_2* summary statistics of in vivo moderate resolution and ex vivo high resolution protocols (n=7; Subjects #1-7, Table 1). Paired t-tests were adjusted for multiple comparisons ($p<0.017$) using the Šídák-Bonferroni method.

Results

ACL volume and scan condition

Scan resolution had no effect on the ACL total volume ($p=0.3$, Figure 2.A). The mean coefficient of variation between the ACL total volume across in vivo and in situ scan protocols was 1%. However, Vol_1 decreased with increased scan resolution and in situ protocols ($p=0.005$, Figure 2.B).

T_2* equation fit

Mean residual R^2 values (±SD) of the 4-echo and 6-echo T_2* equation fits were 0.91±0.02ms and 0.94±0.01ms, respectively. In vivo high resolution 4-echo scan R^2 values were significantly less than the moderate resolution 6-echo residual R^2 values by 0.03ms (–0.05, –0.02ms; 95% confidence interval (CI)).

Effect of in vivo scan resolution

Median ACL T_2* relaxation times were not different between in vivo moderate and high resolution scans (Table 2 & Figure 3.A). First quartile ACL T_2* relaxation times were lower for in vivo high resolution scans (Table 2 & Figure 3.B).

Effect of in vivo versus in situ/ex vivo scan protocols

Compared to in vivo high resolution scans, in situ high resolution scan median and 1st quartile ACL T_2* relaxation times were decreased but T_2* SD was not different (Table 2, Column 2). However, significant differences were observed between in vivo moderate resolution scans and ex vivo high resolution scans after a freeze-thaw cycle (red line pairs in Figure 4 B–C.). ACL T_2* SD increased by over 50% in 9 animals, and more than 90% in 4 animals (Table 2, Figure 4.B).

Discussion

The purpose of this study was to determine whether the ACL total volume and T_2* relaxation times were sensitive to MR scan resolution and in vivo, in situ, and ex vivo scan conditions. Our results suggest that scan resolution and scan condition influence ACL T_2* relaxation times, which in turn could affect the predictive models for ACL structural properties (Biercevicz, et al., 2014). Based on T_2* relaxation time standard deviations and first quartile values, the magnitude of differences between scan conditions investigated increased in the following order:

In vivo high resolution scans versus in situ high resolution scans
<
In vivo moderate resolution versus in vivo high resolution scans
<
In vivo moderate resolution scans versus in situ high resolution scans
<
In vivo moderate resolution scans versus ex vivo high resolution scans after a freeze- thaw cycle

Whereas differences in ACL total volume depended largely on user segmentation reproducibility, Vol_1 was dependent on both segmentation and T_2* relaxation times. The decrease in Vol_1 suggests a redistribution of ACL sub-volumes that occurred with different MR scan conditions. A post-hoc investigation of T_2* inter-quartile ranges revealed that volume and scan condition (in vivo versus in situ, and moderate resolution versus high resolution) affected the distribution of T_2* relaxation times across the four bins. Compared to the in vivo moderate resolution condition, the in vivo high resolution scan increased the interquartile range of T_2* times by a mean of 2.3ms. This finding suggests that the higher resolution scan condition resulted in a greater spread of T_2* relaxation times, particularly at higher T_2* values that correspond to $Vol_{3\text{-}4}$ sub-volumes. As an example, Figure 5 shows the difference in binned ACL sub-volumes for the same subject circled in Figure 2 and illustrates the shift in the number of voxels assigned to Vol_3 and Vol_4. Although the magnitude of the shift in voxels assigned to these sub-volumes is small in healthy ACLs, the difference in distribution may be more consequential in repaired ACLs where the number of voxels assigned to these sub-volumes would, theoretically, be greater during the early healing phases when collagen fibers are initially less organized (Frank, et al., 1999; Proffen, et al., 2013).

Greater partial volume effects that average high and low T_2* relaxation times from adjacent tissue regions may explain the smaller inter-quartile range and higher T_2* first quartile values associated with the moderate resolution scans shown in Figure 3.B, and the Vol_1 sub-volumes in Figure 2. Thus moderate scan resolutions may mask localized reductions in T_2* relaxation times and truncate the dynamic range of ACL T_2* values that are detectable using higher resolution scans, both of which may be important for monitoring ligament remodeling in vivo (Biercevicz, et al., 2014; Weiler, et al., 2001). Despite these limitations, the differences in binned voxel sub-volumes as a percentage of total ligament volume between moderate and high resolution scans shown in Figure 5 were ≤5% across the four sub-volumes in healthy ACL, which suggest that 384×384 is an acceptable level of resolution, but is not ideal.

To investigate the extent that T_2* relaxation times were sensitive to the more clinically applicable scan conditions versus the conditions that we have used previously in our research (Biercevicz, et al., 2015; Biercevicz, et al., 2014), T_2* relaxation times derived from in vivo 384×384 matrix resolution were compared to those derived from in situ 512×512 matrix resolution scan conditions. Scanning ACLs at high resolution in situ within 60 minutes of euthanasia led to decreased and more variable T_2* relaxation times compared to in vivo moderate resolution scans. Whereas whole-ligament median T_2* relaxation times

were not different (Figure 3.A), first quartile values decreased (Figure 3.B) and T_2* standard deviation increased significantly (black line pairs in Figure 4.B). We believe that these results reflect rapid localized changes in T_2* characteristics post-mortem, and may be the result of decreased but variable tissue temperature (Petren-Mallmin, et al., 1993). It is important to point out that even though these particular changes in T_2* statistics were statistically significant, the magnitude of the differences between in vivo and in situ high resolutions scans were at, or below, the temporal resolution of our scanner (2.8 ms). Therefore, these differences may not be relevant.

The combination of increasing scan resolution coupled with greater variation in tissue temperature with ex vivo scan conditions after a freeze-thaw cycle resulted in significant, and likely clinically relevant (up to 90%), increases in T_2* standard deviation and decreases in first quartile T_2* values, but not median, T_2* relaxation times. These results suggest that the absolute T_2* relaxation times acquired ex vivo are likely lower and more variable than values obtained in vivo at a moderate scan resolution. Therefore model equations for predicting ligament structural properties using ex vivo scans obtained after a freeze-thaw cycle may not reflect in vivo conditions accurately given that the prediction models are based on the distribution of ACL T_2* relaxation times.

The primary limitation of this study was that not all MR conditions were applied to the same subjects, which would have enabled analyses that investigated the interaction effects between scan resolution and in vivo, in situ, and ex vivo conditions. Nevertheless, repeated measures of ACL volume and paired analyses of T_2* summary statistics demonstrated significant differences in these outcome measures as a result of different scan resolutions and conditions. Although great care was taken to ensure that the stifle and flexcoil positions were standardized inside the scanner bore for all scans, these parameters were not identical. Despite this limitation, the approach we chose parallels clinical protocols for this model. The consistency in ACL total volume between in vivo and in situ scans that were acquired after subjects were removed and then repositioned in the scanner 60 minutes after euthanasia (without limb disarticulation) supports our view that positioning had little effect on our final outcome measures. Finally, the number of echoes used in the exponential decay model in determining T_2* relaxation times was different between moderate and high resolution scans. For higher resolution scans, the number of echoes that could be collected was reduced to accommodate the inherent limitations of our scanner. The difference in mean residual R^2 values between the 4- and 6-echo exponential decay function fit was two orders of magnitude smaller than the resolution of our system (2.8ms), confirming that any differences in T_2* relaxation times due to the number of echoes used are negligible.

In summary, the MR scan resolutions tested had little effect on ACL total volume but truncated the dynamic range of T_2* values, which may mask localized reductions in T_2* relaxation times, which are important for monitoring ligament remodeling in vivo. Scanning ex vivo at a high resolution following one freeze-thaw cycle amplified the differences in T_2* relaxation times associated with scan resolution alone, and resulted in more variable T_2* times within the ACL. Therefore, prediction models to quantify in vivo ACL structural properties longitudinally should be based on high resolution in vivo MR scan conditions.

Acknowledgments

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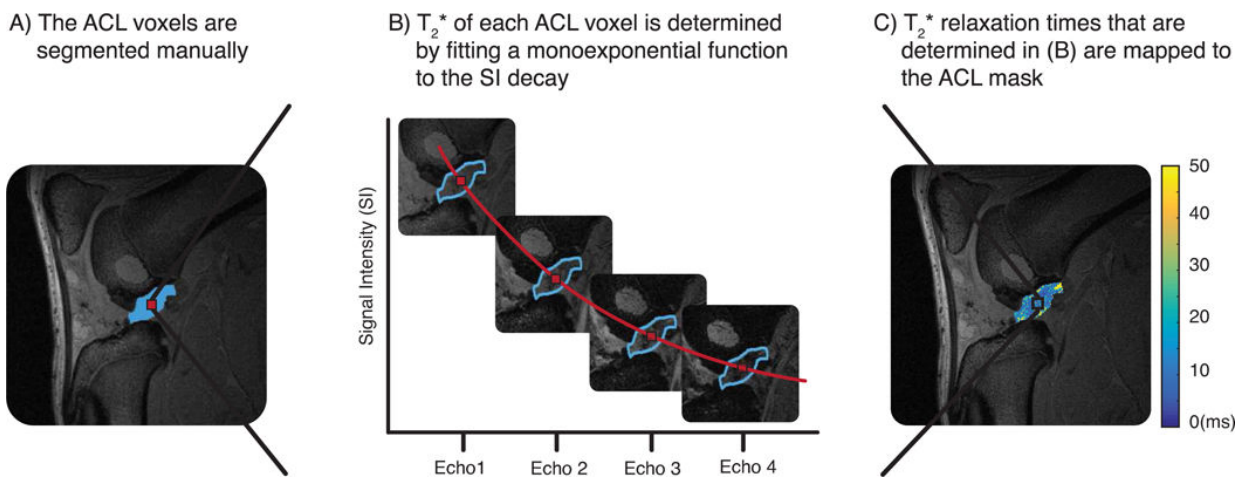

Figure 1.

T_2* relaxation time was calculated for each voxel contained in the ACL. (A) The ACL is segmented (solid blue shade in (A) and blue trace in (B)) to create a 3D volume. For each ACL voxel (shown as a red square in (A) and (B), and a clear square in (C) (not to scale for illustration purposes)), a monoexponential function (Haacke, et al., 1999) is fitted to the MRI signal intensity decay associated with each echo. (C) T_2* relaxation time for each ACL voxel is then mapped back to the 3D segmentation volume.

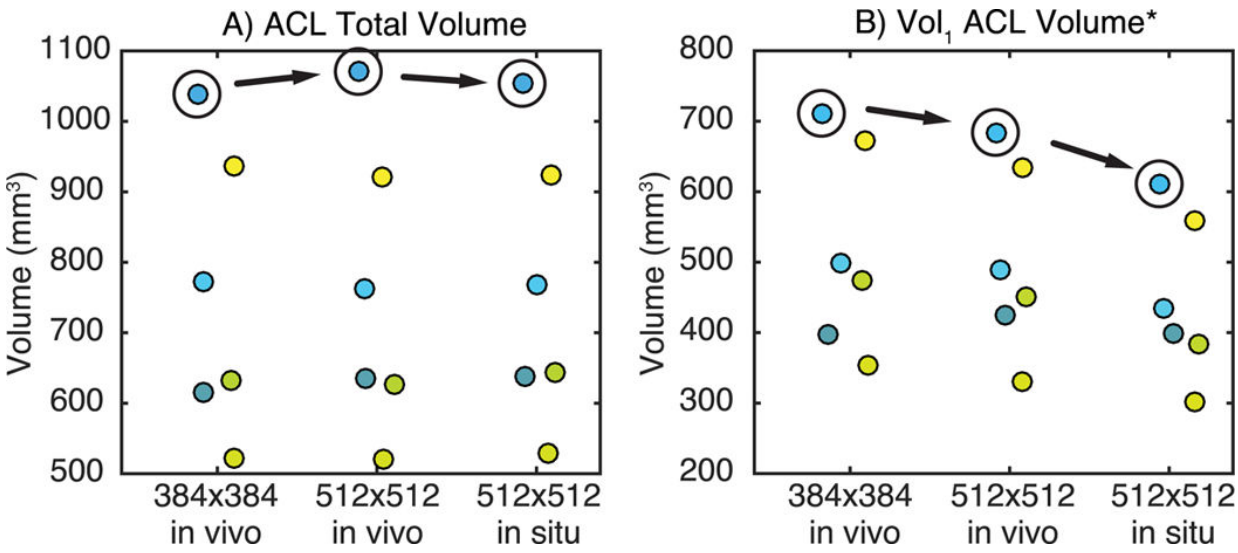

Figure 2.

ACL total volume (A) and Vol_1 (B). Markers are color-coded by animal to illustrate the within-subject consistency in segmented ACL volumes across scan conditions. Subject-specific color is the same for (A) and (B). As an example, ACL Volume and Vol_1 values are circled for one subject, in blue. Vol_1 was significantly different across scan conditions, denoted by "*" (repeated measures ANOVA, n=6).

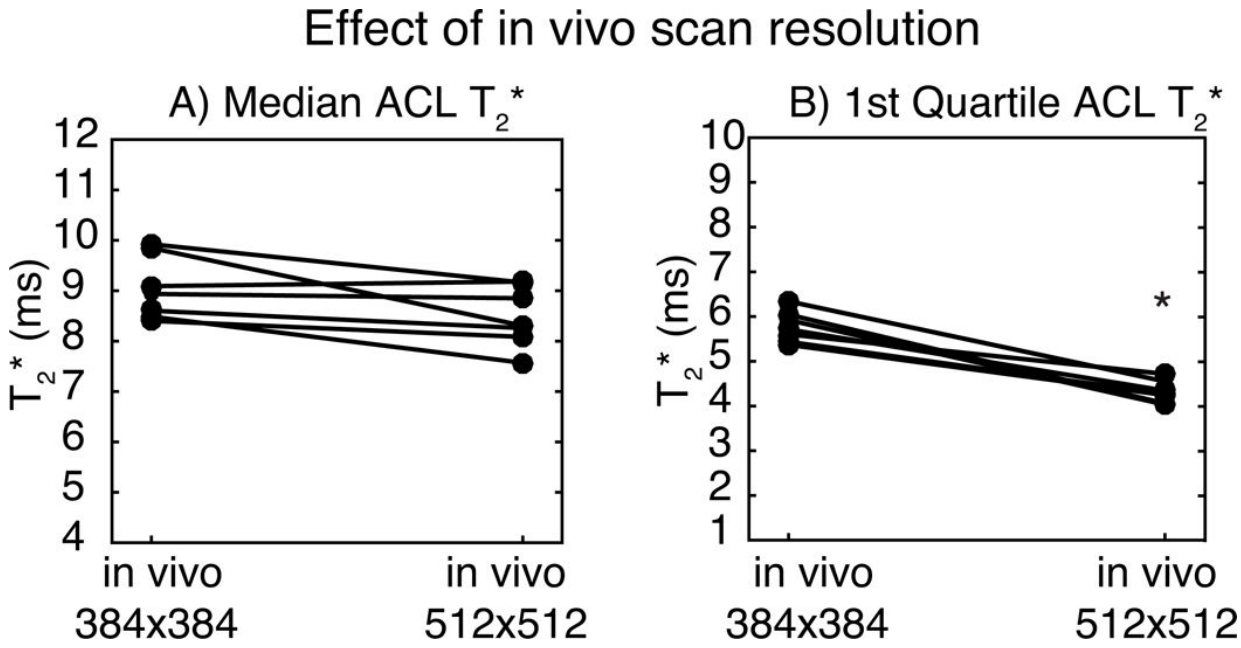

Figure 3.

Paired comparisons of (A) Median ACL T_2* values and (B) 1st quartile values. "*" indicates significant decrease in the quartile of lowest T_2* values (paired t-test, $p \leq 0.05$; n=7).

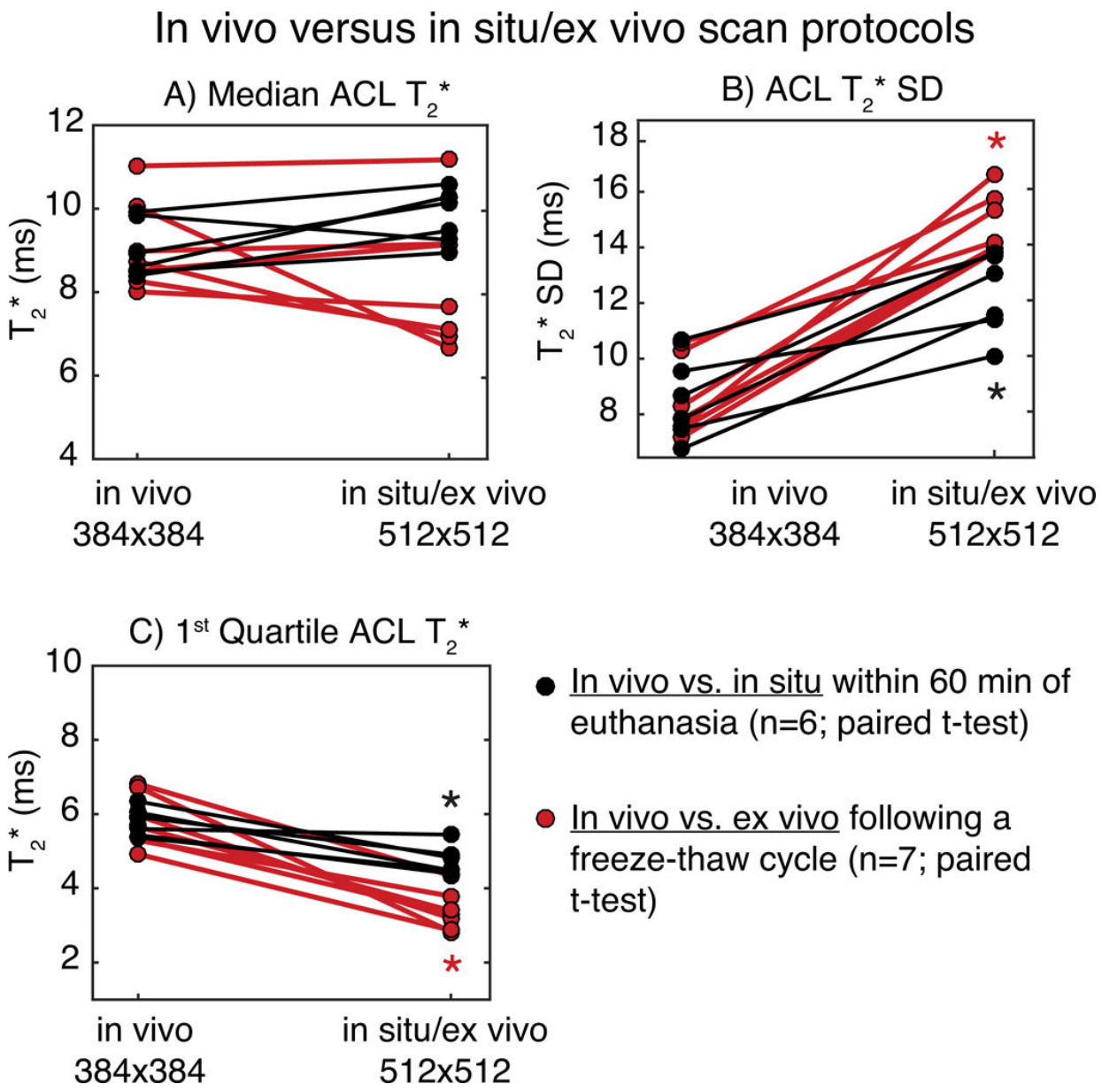

Figure 4.

Paired comparisons of T_2* summary statistics determined from moderate resolution in vivo scans, and high resolution scans collected either in situ (black line pairs) or ex vivo after a single freeze-thaw cycle (red line pairs). "*" indicates T_2* values were significantly different between scan conditions ($p \leq 0.017$).

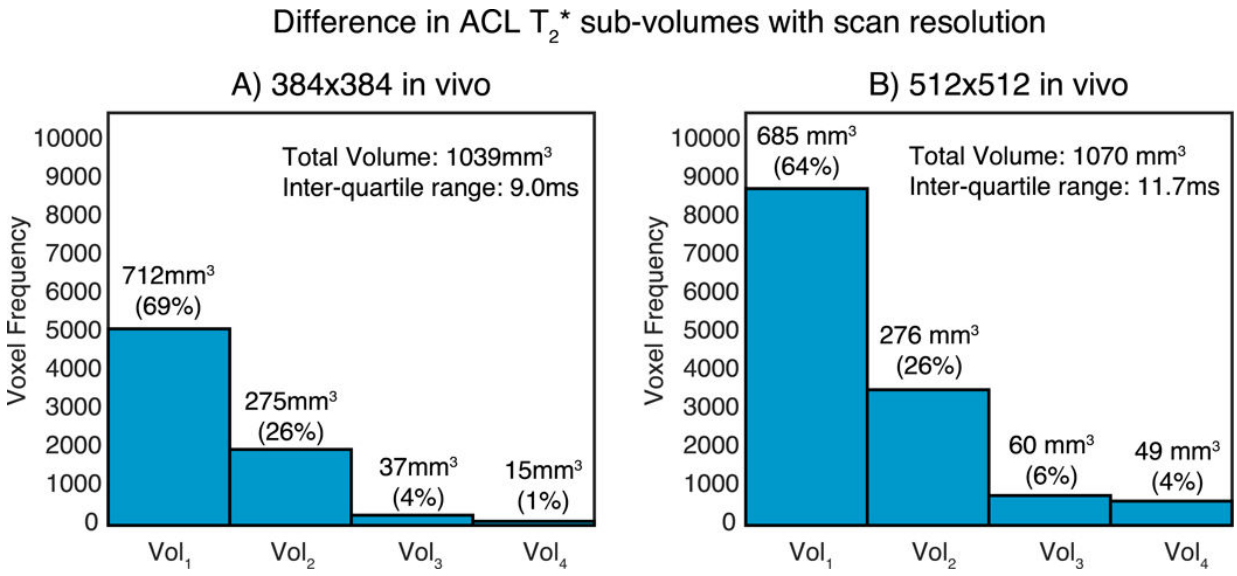

Figure 5.

An example of differences in ACL sub-volumes (and the equivalent percentages of total volume) for the same subject circled in Figure 1. Compared to the moderate resolution 384×384 scan (A), the high resolution scan (B) leads to a larger inter-quartile range, and more voxels assigned to ACL Vol_3 and Vol_4 sub-volumes that contain voxels with higher T_2* values.

Table 1

Distribution of animals across the scanning conditions investigated.

Animal #	*In vivo* 384×384	*In vivo* 512×512	*In situ* 512×512	*Ex vivo* 512×512
1	✓			✓
2	✓			✓
3	✓			✓
4	✓			✓
5	✓			✓
6	✓			✓
7	✓			✓
8	✓	✓		
9	✓	✓	✓	
10	✓	✓	✓	
11	✓	✓	✓	
12	✓	✓	✓	
13	✓	✓	✓	
14	✓	✓	✓	

Table 2

Paired analyses of ACL T_2* summary statistics. T_2* summary statistics from animals #1–7 (n=7) were used to test for differences between in vivo 384×384 scans and ex vivo 512×512 scans (column 4). Summary statistics from animals #8–14 (n=7) were used to test for differences between in vivo scan resolutions (column 1). Summary statistics from animals #9–14 (n=6) were used to test for differences between in vivo and situ protocols (columns 2–3). For paired analyses involving animals #8–14, alpha was adjusted to account for multiple comparisons. Significant differences are in bold.

	Effect of Scan Resolution		**Effect of In Vivo vs In Situ**		**Effect of Combining Change in Resolution and Scan Condition**		**Effect of a Freeze-Thaw Cycle**	
	In vivo 384×384 vs In vivo 512×512		***In vivo 512×512 vs In situ 512×512***		***In vivo 384×384 vs In situ 512×512***		***In vivo 384×384 vs Ex vivo 512×512***	
	Mean Difference (95% CI)	**p-value**	**Mean Difference (95% CI)**	**p-value**	**Mean Difference (95% CI)**	**p-value**	**Mean Difference (95% CI)**	**p-value**
Median T_2* (ms)	–0.6 (–1.1, –0.04)	p=0.04	**1.4 (1.0, 1.8)**	**p=0.0002**	0.8 (0.1, 1.6)	p=0.067	–0.8 (–2.1,0.5)	p=0.17
T_2* Standard Deviation (ms)	**2.4 (1.5, 3.3)**	**p=0.0007**	1.6 (0.03, 3.1)	p=0.05	**3.8 (2.2, 5.3)**	**p=0.0014**	**6.4 (4.8, 7.9)**	**p<0.0001**
First Quartile T_2* (ms)	**–1.5 (–1.9, –1.1)**	**p=0.0002**	**–0.4 (–0.8, –0.1)**	**p=0.014**	**–1.0 (–1.6, –0.5)**	**p=0.004**	**–2.4 (–3.1, –1.7)**	**p=0.0002**