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Original Research

Effects of Achilles Tendon Immersion in Saline and Perfluorochemicals on T2 and T2*

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Purpose: To determine if immersion of Achilles tendon segments into various solutions improved qualitative delineation of tendon and affected quantitative MR values of T2 and T2*.

Materials and Methods: Six Achilles tendons were dissected, sectioned (proximal, midportion, and distal tensile pieces) and imaged at 3T both at baseline in air and after immersion into saline, Fomblin, and perfluorooctyl bromide (PFOB), respectively, for 24 h. Blinded readers qualitatively assessed the delineation of tendon boundaries and quantitatively Carr-Purcell-Meiboom-Gill (CPMG) T2 and ultrashort echo time (UTE) T2* was calculated. Comparison between images obtained in air and in solution was made.

Results: On qualitative evaluation, all images obtained in air had larger air-tissue susceptibility effects. Mean T2 values of saline, Fomblin, and PFOB groups were 16.1 ± 3.7 , 16.6 ± 2.9 , and 18.8 ± 2.6 ms at baseline in air, and 14.8 ± 4.6 , 15.9 ± 3.0 , and 17.7 ± 3.0 ms after immersion in the fluid, respectively. Mean T2* values of saline, Fomblin, and PFOB groups were 2.0 ± 0.8 , 1.6 ± 0.5 , and 1.5 ± 0.5 ms at baseline in air, and 2.1 ± 0.5 , 1.6 ± 0.5 , and 1.4 ± 0.5 ms after immersion in the fluid, respectively. There was no significant effect of immersion or fluid type on measured T2 or T2* ($P > 0.1$).

Conclusion: These results validate the continued use of these solutions to prevent tendon specimen dehydration and to minimize susceptibility effects.

Key Words: T2; T2*; Achilles tendon; PFOB; fomblin
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MAGNETIC RESONANCE (MR) imaging allows for the noninvasive study of living organisms and nondestructive characterization of tissue. MR imaging of small ex vivo tissue samples often requires long imaging times to achieve high quality images that are comparable to histological methods. Importantly, for accurate correlation of results obtained from ex vivo tissues to in vivo scenarios, tissue preparation and handling should not alter the quantitative measure of interest.

Imaging of small ex vivo samples can be problematic due to susceptibility differences at air-tissue interfaces which create undesirable effects, including difficulty in structure segmentation. Additionally, prolonged exposure of tissue to air can cause dehydration and alteration of tissue biomechanical (1) and MR properties. Various solutions have been used in the literature to circumvent these effects, including perfluorochemicals such as Fomblin (Ausimont, Thorofare, NJ) and perfluorooctyl bromide (PFOB), which demonstrate very low toxicity, a paucity of ¹H nuclei, and improved susceptibility matching with tissue at interfaces (2,3). While abundantly used, the effects of these solutions on MR properties are not well known and there are conflicting results in the literature (4,5), as well as several prior studies showing that formalin changes quantitative MR properties of musculoskeletal tissues (6,7).

We sought to investigate the qualitative and quantitative effects that immersion of tendon into saline, Fomblin, and PFOB would have on two commonly used MR sequences to study the Achilles tendon, T2 and T2*.

MATERIALS AND METHODS

Sample Preparation

Fresh human ankles from six donors (2 males, 4 females; age range = 43–82 years, 67.3 ± 14.1 years, mean \pm standard deviation) were obtained from tissue banks, as approved by our institutional review board. The specimens consisted of the distal portion of the leg, the ankle, and the foot including intact soft tissues. The specimens were immediately deep-frozen at -80°C (Forma Bio-Freezer, Forma Scientific). After a single freeze-thaw cycle, the specimens were allowed

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Table 1
Imaging Parameters for CPMG T2 and UTE T2* Sequences

Sequence	TR [ms]	TE [ms]	FOV [cm]	Matrix	NEX	Imaging Time
T2	2,000	10,20,30,40,50,60,70,80	5	320x256	1	10 min
UTE T2*	100	0.01,0.1,0.2,0.4,0.6,0.8,2,4,10,15,20,30	5	256x256	2	13 min

to thaw for 36 h at room temperature and the entire Achilles tendon from each specimen was then dissected free from surrounding tissues. Each Achilles tendon was precisely sectioned into three segments of the same length (average segment 1.5 cm), corresponding to the proximal, mid, and distal tensile portions. Tendons were placed into a 30-cc plastic syringe and immediately imaged at baseline in air. Thereafter, each proximal tensile piece of tendon was immersed into saline, each mid tensile piece of tendon was immersed into Fomblin, and each distal tensile piece of tendon was immersed into PFOB for 24 hours before MR imaging was performed again at essentially the same slice position. Previous studies have shown that there is regional variability within the Achilles tendon (8) and for this reason, tendon pieces of similar anatomic location were imaged pre and post immersion into a particular medium rather than with a random selection. All specimens were imaged at room temperature.

MR Imaging Acquisition

MR imaging was performed on a clinical 3T MR scanner (Signa HDx, GE Healthcare Technologies, Milwaukee, WI). The system had gradients capable of a slew rate of 150 T/m/s and amplitude of 40 mT/m on each axis. Hardware modifications included the addition of a custom Transmit-Receive (T/R) switch to the receiver preamplifiers for rapid switching at the end of a radiofrequency (RF) excitation pulse to allow for ultrashort echo-time (UTE) imaging with a nominal TE of 8 μ s. Additionally, a home-made 1-inch-diameter birdcage T/R coil was used for signal excitation and reception.

The quantitative imaging protocol was shown in Table 1. This protocol was tailored for measurement of relaxation times rather than structural detail. In samples where auto-prescan failed due to small sample size, manual-prescan was performed, resulting in slight differences in gain which did not alter relaxation parameter measurement. T2 and T2* was measured for each sample under each experimental condition (air, saline, PFOB, or Fomblin). Carr-Purcell-Meiboom-Gill (CPMG) acquisitions were acquired for T2 quantification, where spin echo (SE) signal from eight echoes (TE = 10, 20, 30, 40, 50, 60, 70, and 80 ms, TR = 2000 ms) was subject to a single exponential signal decay model to calculate T2. 2D UTE acquisitions were used for T2* quantification, where UTE signal acquired after a series of TEs (TE = 0.01, 0.1, 0.2, 0.4, 0.6, 0.8, 2, 4, 10, 15, 20, and 30 ms, TR = 100 ms) was subject to a single exponential signal decay model to calculate T2*. For each of the

sequences used in this study, a field of view (FOV) of 5 cm and a slice thickness of 3 mm were prescribed.

Image Analysis

Images before and after immersion into each solution were viewed side by side on a diagnostic picture archiving and communication system (PACS) workstation (AGFA Impax; AGFA Technical Imaging Systems, Ridgefield Park, NJ) and the set of images with the most sharp/distinct tendon interface was determined qualitatively and in consensus by blinded readers (E.Y.C. and J.D., with 2 and 9 years of experience with quantitative musculoskeletal imaging).

T2 and T2* values were obtained using Levenberg-Marquardt fitting algorithm developed in-house. The analysis algorithm was written in MATLAB (The Mathworks Inc., Natick, MA) and was executed offline on axial images obtained with the protocols described above. The mean intensity within each of the ROIs was used for subsequent curve fitting. Five different ROIs were fitted to determine the average T2 and T2* relaxation times for the Achilles tendon using a single component curve fitting model. T2 was derived through exponential fitting of the equation: $S(TE) \propto \exp(-TE/T2) + \text{constant}$. T2* was derived through exponential fitting of the equation: $S(TE) \propto \exp(-TE/T2^*) + \text{constant}$.

Statistical Analysis

Statistical analyses were performed with Excel (version 2011, Microsoft Corporation, Redmond, WA) and R software, version 2.10.1 (2009) (R Foundation for Statistical Computing, Vienna, Austria). First, data was summarized for each solution and each imaging parameter. To determine effects of immersion (baseline versus postimmersion) and the type of immersion fluid (saline, Fomblin, PFOB) on T2 and T2*, two-way repeated measures ANOVA was used. Next, linear regression between the baseline and the postimmersion T2 and T2* values was performed to determine intraclass correlation, and to compare correlation coefficients and the slopes for each fluid type (9). To determine the agreement between the baseline and postimmersion T2 and T2* values, Bland-Altman analysis was performed (10). Bias and limits of agreement were determined. For all statistical analyses, *P*-values less than 0.05 were considered significant.

RESULTS

On qualitative evaluation, all images obtained in solution had a more distinct tendon interface compared

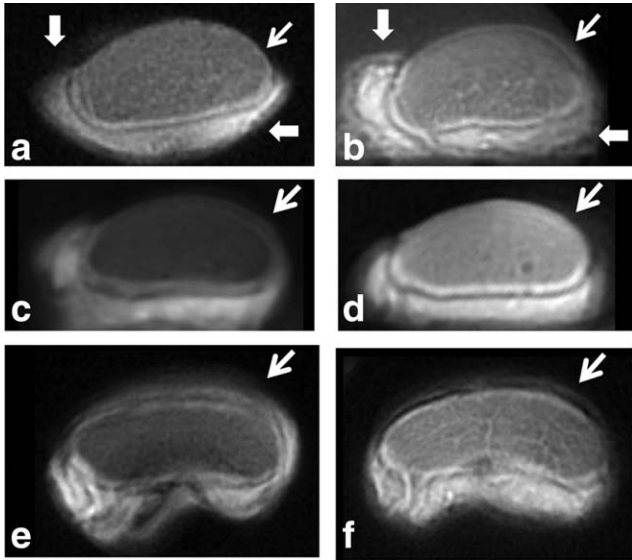


Figure 1. Representative UTE-T2* images (TE 8 μ s) obtained in air (a,c,e) and immersed into saline (b), Fomblin (d), and PFOB (f). In each case, the posterior margin of the Achilles tendon is better delineated on imaging after immersion into solution compared with imaging in air (thin arrows). After immersion into saline (B), fat appears macerated and enlarged (block arrows) compared with imaging in air (A), although quantitative values of tendon remained the same.

with that obtained in air (Fig. 1). Also of note, fat that was attached to the dissected tendon demonstrated a noticeable change after soaking in saline for 24 hours. Specifically, immersion of fat into saline created a macerated and expanded appearance (Fig. 1b), which was less apparent for samples soaked in Fomblin or PFOB. The tendon did not qualitatively appear affected, however.

Quantitatively, neither immersion nor type of immersion fluid had significant effect on the measured T2 or T2* values (Table 2; Fig. 2).

Linear regression of SE T2 values (Fig. 3a) suggested moderately strong intraclass correlation ($r = 0.66$), and when analyzed separately for each fluid type, there was no significant difference between correlation coefficients (each $p = 0.15$ or greater) or slopes (all $p = 1.0$). Linear regression of UTE T2* values (Fig. 3c) suggested a strong intraclass correlation ($r = 0.85$) and no significant difference between correlation coefficients (all $P = 1.0$) or slopes (all $P = 1.0$) of different fluid types.

Bland-Altman analysis for SE T2 values (Fig. 3b) suggested a small positive bias of 1.0 ms, and limit of

confidence ($1.96 \times$ standard deviation) of 5.5 ms. In comparison, Bland-Altman plot of UTE T2* values (Fig. 3d) suggested negligible bias of -0.02 ms and limit of confidence of 0.64 ms. These results suggested that there is very little bias in T2 or T2* values due to immersion of the samples in various fluids tested in this study.

DISCUSSION

In this pilot study, we have found that MR imaging of ex vivo tendon immersed into saline, Fomblin, and PFOB, improves boundary delineation and does not significantly affect T2 and T2* measurements compared with imaging in air. These findings are important because they validate the continued use of these solutions to study ex vivo tendon by decreasing tissue dehydration, decreasing susceptibility differences and improving segmentation.

Although there are numerous studies that have used various solutions to circumvent these adverse effects, there has been a paucity of studies confirming that solution immersion does not alter quantitative values. Specifically, saline, Fomblin, and PFOB have been used in the study of a variety of tissues in the body (2,3,11–16), but to our knowledge only two studies have evaluated the effect of perfluorochemicals on quantitative MR measures (4,5), both of which have used tissues after formalin fixation. Formalin modifies collagen residues and forms new cross-links, thereby altering both biomechanical and quantitative MR properties of musculoskeletal tissues. For instance, formalin has been shown to greatly stiffen the matrix of tendon (17) and reduce T2 values of cartilage (6,7) and tendon (18). For this reason we do not use formalin fixation in our quantitative studies of musculoskeletal tissues.

Our study has several limitations. First, the sample size is small, limiting the generalizability of these results. However, the mean T2* values seen in our sample were similar to those previously reported (19,20), suggesting that our sample was representative. Second, our study was performed with human tendons as a precursor to future work we will conduct with this tissue. It is likely that our results are not generalizable to other tissues with different porosity and absorption characteristics, including fatty tissues as shown by the qualitative differences noted after immersion into saline. Third, although all efforts were made to perform imaging at the same

Table 2
T2 and T2* Results Before and After Immersion Into Solution

	Mean Value in Air (Baseline)[ms]	Standard Deviation [ms]	Mean Value Post-Immersion [ms]	Standard Deviation [ms]
Saline T2	16.09	3.73	14.77	4.62
Saline UTE-T2*	1.97	0.82	2.08	0.54
Fomblin T2	16.58	2.91	15.89	2.89
Fomblin UTE-T2*	1.57	0.53	1.59	0.53
PFOB T2	18.77	2.58	17.70	3.03
PFOB UTE-T2*	1.46	0.51	1.40	0.49

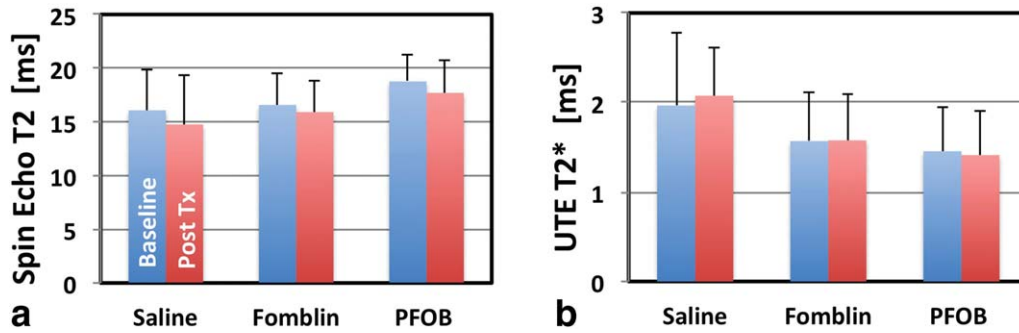


Figure 2. Bar graphs demonstrating mean and standard deviation values of spin echo T2 values (a) at baseline in air, and postimmersion in saline, Fomblin, and PFOB. UTE T2* values (b) at baseline in air, and postimmersion in saline, Fomblin, and PFOB. There was no significant difference between values between pre- and postimmersion for any group.

slice position in air and after sectioning and placement into solution, minor differences in registration and minor tissue deformation due to handling are possibilities. Fourth, as previously mentioned, mono-exponential analyses were used in this study although tendon demonstrates multi-exponential decay. However, the best fit curve for our data was mono-exponential. When we used a bi-component fitting model (21), the longer component was consistently less than 5% for all samples. This is likely due to uncorrected susceptibility causing rapid decay at longer echo times with the clinically compatible UTE technique. In contrast, some authors that have shown the multi-exponential decay behavior of tendon have used CPMG sequences on an NMR spectrometer, which is much less sensitive to susceptibility due to the refocusing pulses (22,23). Juras et al used a bi-exponential fitting model on Achilles tendon with a clinically compatible technique, but the in vivo samples in their study are not directly comparable to ours (8). Additional studies to explain these variations are necessary. Finally, con-

sensus interpretation was used in this study and intra- and interobserver reliability could not be assessed.

Our pilot study demonstrates the need for additional confirmatory studies to better understand the differences between ex vivo and in vivo measurements. Multiple studies using quantitative MRI on tendon have found differences between cadaveric samples and live patients (8,19,24), with proposed reasons including the number of freeze-thaw cycles, tension of samples, and influences of various temperatures. Ongoing work is being performed in our group to study many of these variables.

In conclusion, in this pilot study, we have not found significant differences in T2 and T2* values on Achilles tendon at baseline and postimmersion into saline, Fomblin, and PFOB. Additionally due to the improved tendon interface visualization in solution, these findings validate the continued use of these solutions to prevent specimen dehydration and to minimize susceptibility effects.

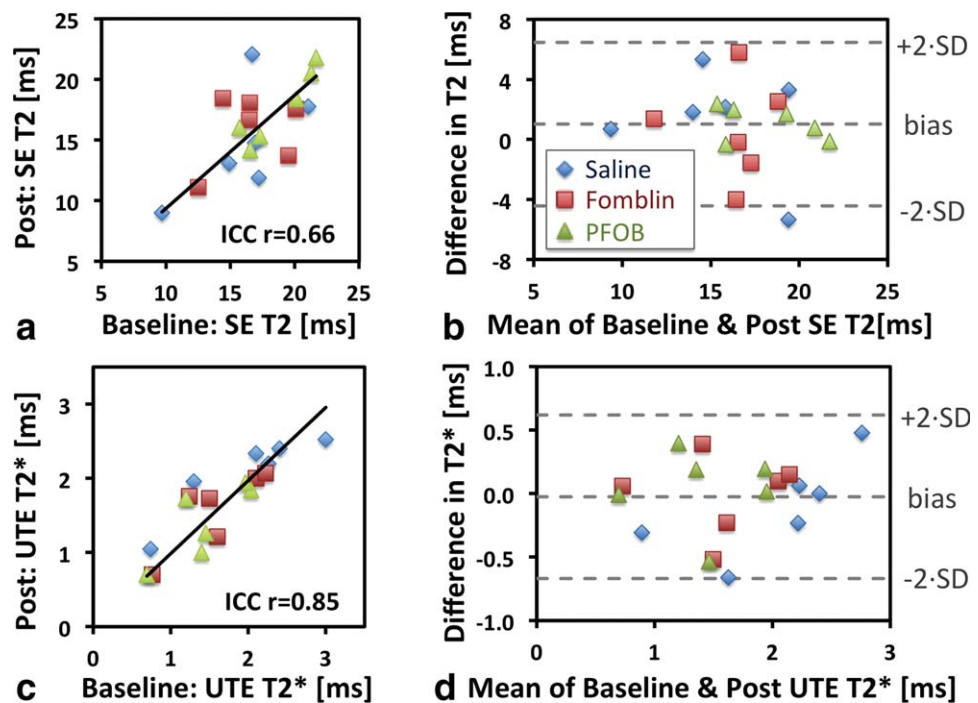


Figure 3. Linear regression between baseline and postimmersion values obtained using spin echo T2 (a) and UTE T2*(c) sequences showed moderately strong ($r = 0.66$) and strong ($r = 0.85$) intraclass correlation, respectively. Bland-Altman analysis for spin echo T2 (b) and UTE T2* (d) sequences suggest very little bias (1.0 and -0.02 ms, respectively) due to immersion of samples in fluids.

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