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Application of porous-media theory to the investigation of water ADC changes in rabbit Achilles tendon caused by tensile loading

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Abstract

The water apparent diffusion coefficient (ADC) in rabbit Achilles tendon is anisotropic, diffusion-time dependent, and changes as a function of tensile load. Water ADC changes of tendon under mechanical load are thought to be due to the extrusion of water from the more restricted tendon core to a relatively unrestricted bulk phase at the periphery (rim) of the tendon. Tensile loading may influence water ADC values by changing the spatial separation of restricting barriers (e.g., increasing the tendon fibril packing density). To explore this issue, we have applied porous-media theory to the investigation of water ADC changes in rabbit Achilles tendon under two different mechanical loading conditions (a baseline condition with a minimal tensile stress and a second in which the tensile stress was approximately 1 MPa). Diffusion sensitivity was applied in directions parallel and perpendicular to the long axis of the tendon. The short diffusion-time behavior of the resulting time-dependent ADC curves was used to indirectly infer information regarding the average surface area to volume ratio of the space available for molecular diffusion. From these values, we estimated a 40% reduction in volume available for diffusion in the perpendicular direction after tensile loading, but only a 10% reduction in the parallel direction. These differences are consistent with the known geometry of the tendon microstructure and suggest an increase in fibril packing density upon loading. The long diffusion-time behavior of the time-dependent ADC curves was used to indirectly infer the tortuosity of the diffusion pathways through the interstitial space. The tortuosity in the direction perpendicular to the tendon long axis was approximately 2.5 times greater than that in the parallel direction. Stimulated-echo measurement of the ADC values at longer diffusion times resulted in T_1 spin editing of water with shorter T_1 values (and correspondingly lower ADC values). The resulting increase in water ADC with increasing diffusion time was attributed to multiple components arising from the (overlapping) distribution of T_1 values in the core and rim regions of the tendon.

Keywords: Tendon; Tensile loading; Collagen fibril packing; Water ADC; NMR

1. Introduction

Porous-media theory of single-compartment systems has been successfully applied to biological systems [1,2]. The goal of these applications is to non-invasively measure parameters that characterize the connectivity and characteristic structural lengths of the sample. When the system is perturbed, the change in these parameters can yield useful information about how the structural arrangement of the sample changes under the perturbation. Soft tissue, such as tendon, is an ideal candidate for such studies as it is relatively acellular (thus avoiding problems of interpreting results from multi-compartment systems) and the collagen fibers are highly ordered in many cases.

The hydration level of soft tissue is known to be an important contributor to its mechanical behavior [3–5]. There is, however, little direct evidence on the behavior of water in tensile-loaded soft tissues. Previous work has shown that dehydration causes stiffening of soft tissues [6,7] and increases the elastic component of a tissue's

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viscoelastic response [8]. Tissues loaded in tension stiffen with loading and the water content of canine flexor tendons has been shown to decrease after the application of a small (100 g) tensile load and when the load is cycled [8]. Most theoretical modeling of soft tissue in tension [9,10] predicts the development of positive internal pressure that results in water being extruded. This pressure would be the result of the fibrils compressing the extracellular matrix. In this work, we propose to use the determination of the average surface-to-volume ratio of the tendon structures that provide restrictions to the movement of free water and the tortuosity as parameters with which to study the effects of tensile loading on tendon. Specifically, we are looking for evidence that tensile loading decreases the available space for the movement of water in tendon.

The water apparent diffusion coefficient (ADC) in rabbit Achilles tendon is anisotropic, with a larger value in the direction parallel to the long axis of the tendon relative to the perpendicular direction [11]. Furthermore, the water ADC is diffusion-time (t_{dif}) dependent in both of these diffusion directions. The water ADC values change as a function of tensile loading and our initial results suggest that this may, in part, be due to the extrusion of water from the more restricted tendon core to a relatively unrestricted bulk phase at the periphery of the tendon. In addition to water extrusion, however, tensile loading may influence water ADC values by changing the spatial separation of restricting barriers (e.g., changes in tendon fibril packing density). To explore this issue, we have applied porous-media theory to the investigation of water ADC changes in rabbit Achilles tendon caused by variations in mechanical load.

The diffusion-time dependence of the water ADC in biological systems has been reviewed in detail [2] and can provide important structural information about tissue in normal and pathological states. Tissues composed of restricting structures separated by interstitial space can be modeled as a porous medium. Using porous-media theory, structural information about the medium can be obtained from the short diffusion-time behavior of the water ADC, which is proportional to the ratio of the surface area to the pore volume (S/V) [12,13]. In biological systems, where the motion of some fraction of water molecules is always restricted, the ADC(t_{dif}) will be reduced from the bulk unrestricted self-diffusion coefficient, D_0. Mitra et al. [12,13] have shown that in the short t_{dif} regime, ADC(t_{dif}), normalized by D_0, is given by:

$$\frac{\mathrm{ADC}(t_{dif})}{D_0} = 1 - \frac{4}{9\sqrt{\pi}}\frac{S}{V}\sqrt{D_0 t_{dif}}, \quad (1)$$

where (S/V) is the average surface-area-to-volume ratio of the space available for molecular diffusion. The general form of Eq. (1) is a linear equation in $t_{dif}^{1/2}$ with intercept $= D_0$ and slope $\propto (S/V)$; therefore, the initial slope from a plot of ADC(t_{dif}) vs. $t_{dif}^{1/2}$ reflects (S/V) of the system.

At long diffusion times, when all diffusing molecules have sampled an equivalent portion of the confining geometry, the water ADC measurements reflect the "effective" properties of the medium. In this regime, the water ADC (D_{eff}) is related to the tortuosity (T) by:

$$D_{eff} = D_0/T, \quad (2)$$

where D_0 is the bulk unrestricted diffusion coefficient of the interstitial fluid [14]. The tortuosity is a function of the porosity (i.e., the volume fraction of the interstitial space) and the long-range connectivity of the interstitial space.

Our previous investigation involved a preliminary characterization of the time-dependent diffusion properties of tendon water under load [11]. However, the time-dependent diffusion data in those studies were not optimal for also evaluating tendon structural properties using porous-media theory. For example, the estimation of (S/V) requires a sufficient number of short t_{dif} values to accurately calculate the initial slope of a plot of ADC vs. t_{dif}. Furthermore, the long-diffusion-time limit (i.e., the "effective medium" regime) was not achieved in our earlier studies, and thus D_{eff} (and in turn tortuosity from Eq. (2)) could not be estimated. The current investigation will examine both the short- and long-diffusion-time regimes of the water ADC in rabbit Achilles tendon. The goal of this study is to establish if structural parameters derived from porous-media theory provide insights into the changes in tendon fibril packing density in response to tensile loading.

2. Methodology

2.1. Experimental set-up

The preparation of tendons and the method for applying tensile load to them has been described in detail in [11]. Briefly, Achilles tendons were harvested from young (~3 kg) New Zealand white rabbits of either sex that had been sacrificed for other, unrelated research. In order to prevent dehydration, the tendons were excised in a glove-box chamber maintained at 100% humidity. Upon harvesting, samples were placed in a vessel containing perfluoroalkylether (Krytox grade GPL-102 DuPont, Deepwater, NJ) in order to maintain their hydration levels. The use of Krytox insured that there was no proton signal from the bathing solution contributing to the tendon water ADC measurements.

Tendons were mounted in an apparatus (Fig. 1) that oriented them in the magnet bore such that their long axis was parallel to the static B_0 field. The apparatus consisted of a platform that rested in the bore of a gradient insert, and an extension piece (not shown in

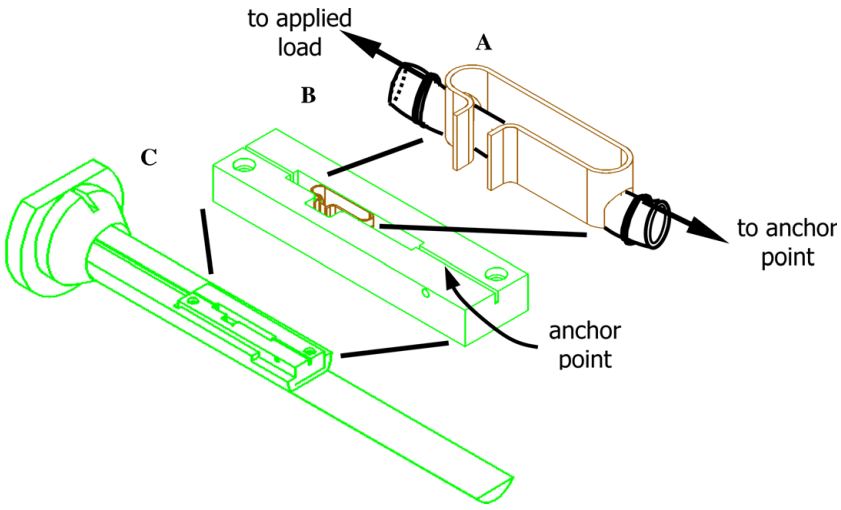

Fig. 1. Mechanical layout of tendon-holding apparatus. The tendon rests in the trough of a Krytox oil-filled well (B) with suture material tied about each end. The suture material passes through slots at each end of the well, which were packed with a Krytox grease to prevent leakage of the bathing oil. The RF coil (with a cutout at each end) fits over the tendon sample (A) and is inductively coupled to a pick-up coil place on the underside of the well (not shown in diagram). The well is secured on a slide (C) that rests in the bore of a ±280 G/cm gradient insert.

Fig. 1) that reached outside the magnet bore to support a pulley system that was used to load the sample. The platform contained a Krytox-filled well that accommodated the RF coil and tendon. Lengths of size #1 braided-silk suture, tied to each end of the tendon, were used to secure the sample in the well and to apply the tensile load to the sample (refer to Fig. 1). The suture lines ran through slots located at each end of the well. These slots were packed with Krytox grade 240-AC grease to prevent leakage from the bathing well over the course of the experiments.

An RF coil was fabricated from copper sheeting and formed into an oblong-shaped, single-turn solenoid with a cutout at each end to allow it to be overlaid on the tendon sample in the well. This coil was inductively coupled to a second coil placed on the underside of the well (not shown in Fig. 1) that connected directly to the transmitter and receiver electronics of the NMR system.

2.2. NMR experimental parameters

NMR experiments were performed with a GE CSI-II 2.0T/45 cm imaging spectrometer (GE NMR Instruments, Fremont, CA) operating at 85.56 MHz for ^{1}H and equipped with a ±280 G/cm gradient insert. Spectroscopic measurements of tissue water ADC were made at 12 diffusion times (over the range of 5.5–400 ms) to characterize the ADC as a function of t_{dif}. At each t_{dif} point, data were acquired with diffusion sensitization parallel and perpendicular to the long axis of the tendon. A combination of diffusion-weighted spin echo (SE) (for t_{dif} values from 5.5–16.5 ms) and stimulated echo (STE) (for t_{dif} values from 21 to 400 ms) pulse sequences were used in the experiment with common parameters of: TR/TE = 1500.0/25.0 ms, SW = ±2000 Hz, 1024 complex digitization points, NEX = 2 (for DW-SE) or 8 (for DW-STE), $\delta = 6.0$ ms, and seven diffusion-gradient strengths (half-sine-shaped gradient pulses) ranging from $g = 2$–14 G/cm, in 2 G/cm increments. Datasets were transferred to an off-line workstation for processing using routines written with IDL (Research Systems, Boulder, CO). Calculation of individual ADC values at each t_{dif} point was made by fitting the natural logarithm of the signal intensity vs. b value $\{[(2/\pi)\gamma g\delta]^2 t_{dif}$, where $t_{dif} = [\Delta - (\delta/4)]$ for half-sine-shaped diffusion-gradient pulses$\}$. Only the initial linear portion of each diffusion-attenuation curve was fitted (i.e., a mono-exponential fit).

2.3. Application of static tensile loads

Static tensile loads were applied to the tendons by hanging a mass from the suture line attached to the sample. Experiments were performed with the samples under two states of tensile loading: first, a baseline condition with a nominal (40 g) mass applied to maintain tautness in the sample (referred to as the 'unloaded' condition); this was followed by a test condition with a 500 g mass applied (referred to as the 'loaded' condition). A load of this magnitude has been shown [11] to result in tensile stresses on the order of 1 MPa in rabbit Achilles tendons, which lies in the range of typical physiological stresses. After transition from the unloaded to the loaded condition, a period of 10 min was allowed for samples to attain a mechanical steady-state before initiation of data acquisition. Experiments were performed once on a given sample.

3. Results

Averages of the calculated ADCs from the spectroscopic diffusion experiments ($N = 8$) are plotted in Fig. 2 as a function of the square root of the diffusion time. The plots represent the average ADC values (±SEM) in the parallel and perpendicular directions for tendons in both unloaded and loaded conditions. Each plot exhibits similar $t_{\mathrm{dif}}^{1/2}$ dependence: the initial ADC values decrease linearly to a minimum and then increase and subsequently level off at longer diffusion times. Consistent with our previous findings [11], the ADC is anisotropic with respect to the tendon long axis and tensile loading resulted in an overall increase in the water ADC, irrespective of diffusion direction.

4. Discussion

Information about changes in tendon structure can be extracted from the curves of Fig. 2 by considering the ADC behavior at short diffusion times and its relation to (S/V). Calculation of (S/V) using Eq. (1), however, requires that D_0 be known. D_0 may be estimated by noting that in the limit of $t_{\mathrm{dif}} \to 0$, $\mathrm{ADC}(t_{\mathrm{dif}}) \to D_0$. Consequently, extrapolation of the linear fit of the initial time points of $\mathrm{ADC}(t_{\mathrm{dif}})$ vs. $t_{\mathrm{dif}}^{1/2}$ back to the zero-time intercept would yield D_0. This limit should be generally true as long as the ADC is measured at sufficiently short values of t_{dif}. However, the ability to achieve short t_{dif} values is dependent upon the achievable minimum rise-time and the maximum amplitude of the gradient hardware. The hardware specifications needed to accurately estimate D_0 also depend upon the structure sizes of the sample. Because of these hardware limitations and the size of structures comprising tendon, sufficiently short t_{dif} values were not attained such that the $\mathrm{ADC}_{\perp}$ and $\mathrm{ADC}_{\parallel}$ values converge to a common D_0 value.

Because of our inability to determine D_0, we instead used the slope from the linear fit of the initial time points to indirectly infer (S/V) for each curve. According to Eq. (1), the slope of the $\mathrm{ADC}(t_{\mathrm{dif}})$ vs. $t_{\mathrm{dif}}^{1/2}$ curve is composed of constants and (S/V); therefore, any change in the slope must result from a change in (S/V). Linear regression was performed on the three initial time points of individual tendon $\mathrm{ADC}(t_{\mathrm{dif}})$ vs. $t_{\mathrm{dif}}^{1/2}$ curves to derive slope values that are proportional to (S/V). A weighted-least-squares method was used to take into account differences in the variance associated with each point in the fit [16]. To evaluate the tensile-load-induced effects on structural organization of tendon, a ratio of slopes derived from the unloaded and loaded experimental conditions was calculated for each diffusion direction. We know of no reason why the component representing the surface area (S) of the space available for diffusion in these ratios would vary with tensile loading. If this is the case then the unloaded:loaded (S/V) ratio could serve as an estimate for the change in volume available for

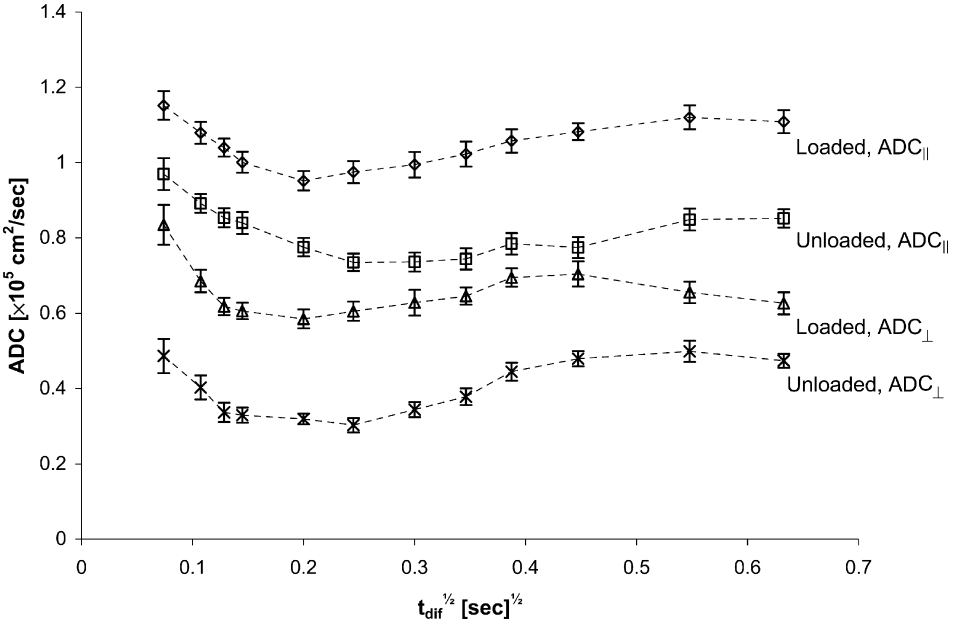

Fig. 2. Plots of the NMR-measured apparent diffusion coefficient (ADC) of tendon water as a function of the square root of the diffusion time ($N = 8$, mean ± SEM). Diffusion sensitization was applied parallel and perpendicular to the tendon long axis for each of the two tensile load conditions: nominal 40 g mass to maintain tautness (unloaded) and 500 g mass (loaded) (- - - lines connecting the data points are only to guide the eye). The ADC increase observed at the long t_{dif} points is interpreted as a T_1 spin-editing effect from the use of the STE pulse sequence (which was used to acquire the last 9 t_{dif} points). The signal from the population of water molecules with relatively short T_1 values (and correspondingly shorter ADC's) is reduced as the TM period (during which T_1 relaxation occurs) in the STE pulse sequence is increased to lengthen the diffusion time.

diffusion between the unloaded (V^{U}) and loaded (V^{L}) states:

$$\frac{(S/V)^{U}}{(S/V)^{L}} = \frac{V^{L}}{V^{U}}. \quad (3)$$

The average ratios and associated 95% confidence intervals for both diffusion directions are reported in Table 1. The relatively wide confidence intervals noted in Table 1 reflect the large variance in ADC values between tendon samples. We hypothesize that the primary reason for this variability is due to differences in initial tissue hydration.

It should be emphasized that in generating these results, the diffusion-attenuation curves were fitted with a single exponential rather than a multi-exponential function. This procedure ignores the multi-component nature of the tendon tissue and the fact that the diffusion-attenuation data at each t_{dif} point was acquired with varying degrees of diffusion weighting (i.e., the range of b values differed at each t_{dif} point). Since the total tendon water signal has multiple components (at least 2–3), each with associated M_o, ADC(t_{dif}), T_1, and T_2 values [15], fitting the data with a multi-exponential model was not considered appropriate, particularly given the relatively small number of b values that were used. Consequently, only the initial linear portion of the diffusion-attenuation curve was fitted using a mono-exponential model in order to treat the data in the most consistent manner. This approach also reduced errors in the calculation of ADC values at longer diffusion times (which employed a larger range of b values), where the diffusion-attenuation curve is more likely to be non-mono-exponential.

Using a simple calculation, the effect of extruded water on the measured ADC resulted in a roughly constant offset over the range of t_{dif} values used to estimate the slope used to determine (S/V). In this calculation, extruded water and tendon water were assumed to be non-exchanging. Furthermore, extruded water was assumed to have the diffusion coefficient of free water at room temperature and comprise 20% of the total tendon water. The tendon-water ADC value used in the calculation was the unloaded value measured at each diffusion time. Performing a mono-exponential fit to the sum of the modeled tendon and extruded water signals resulted in an offset in ADC value that varied by less than 5% over the relevant range of t_{dif}. Because of this, the measured slope is expected to reflect a consistent contribution from tendon water. Consequently, differences in slope upon loading should still be indicative of changes in the tendon structure.

Table 1
Averages of the unloaded:loaded (S/V) ratios (±95% confidence interval) obtained from individual tendon samples ($N = 8$) are summarized for both diffusion directions.

	Average $(S/V)^{U}/(S/V)^{L}$ ratio ± 95% CI
$ADC_{\perp}$	0.6 ± 0.3
$ADC_{\parallel}$	0.9 ± 0.4

Initial slopes obtained from the linear fit of the initial t_{dif} points of the ADC(t_{dif}) curves were used to estimate (S/V) for each experimental condition. Given the assumption that S does not change upon load application, the calculated average unloaded:loaded (S/V) ratios reflect the change in volume available for diffusion following application of the tensile load. The average ratio for the perpendicular diffusion direction experiences a larger decrease from unity relative to the parallel direction. This observation is consistent with a structural model of tendon in which the volume available for diffusion is reduced in the perpendicular direction after tensile loading.

The average, calculated volume ratios in Table 1 are both less than one, which implies a decrease in the local volume following tensile loading (in this calculation, unity would indicate no change in volume). We note that the decrease in average ratio for the perpendicular direction is greater than that of the parallel direction (a 40% decrease relative to a 10% decrease, respectively). A difference in the load-induced ratio change between parallel and perpendicular diffusion directions is not unexpected given the tendon geometry. In the direction parallel to the tendon long axis, collagen fibrils are largely oriented parallel to one another and offer fewer barriers to diffusion than in the perpendicular direction. Tensile loading may straighten the crimped collagen fibrils that exist in tendon under rest [17], but the length scale of the crimp is much larger than that of the RMS displacement of the diffusing water molecules. Therefore, the space available for diffusion in the parallel direction is expected to be relatively unchanged after loading.

From electron microscopy images, collagen-fibril diameters in rabbit Achilles tendon were previously shown to have an average value on the order of ~0.15 μm [11]. The ADC values in the plots of Fig. 2 can be used to calculate the 1D mean molecular displacement for a given t_{dif}. For example, with ADC values at the third t_{dif} point ($t_{dif} = 16.5$ ms), the Einstein relation predicts a 1D mean-squared displacement of $(2t_{dif}ADC)^{1/2}$, which is 5.9 μm for the loaded $ADC_{\parallel}$ case and 3.3 μm for the unloaded $ADC_{\perp}$ case. These displacement values are an order of magnitude larger than the mean collagen fibril diameter, which implies that diffusing water molecules have traversed distances far greater than the fibril diameter. Therefore, the ADC measurement averages over the fibril perpendicular length scale. Because of this, the slope changes observed here imply structural changes on length scales greater than that of the fibril diameter. This finding is of interest because it demonstrates how the measurement of spectroscopic water diffusion provides a tissue-wide, aggregate estimate of fibril packing changes associated with tensile loading.

Estimating T using Eq. (2) was hampered by several issues. First, as was the case for determining (S/V), the

calculation of T requires an estimate for D_0. As discussed above for (S/V), gradient hardware limitations and sample structure size preclude reliable estimates of D_0. Second, upon tensile loading, the extruded bulk water component also results in an overall ADC increase in the curves in both diffusion directions. This contributes to a change in D_{eff} (and hence T) that obscures the changes in D_{eff} that arise from tensile loading alone. Third, the initial minimum in the ADC(t_{dif}) vs. $t_{\text{dif}}^{1/2}$ curves is followed by a subsequent increase in ADC at longer diffusion times. This trend may be due to a change in the relative spin populations contributing to the NMR signal with increasing $t_{\text{dif}}^{1/2}$ (as discussed in more detail below).

In other biological [1,2] as well as non-biological systems [14,18], the ADC(t_{dif}) vs. $t_{\text{dif}}^{1/2}$ curve usually declines to a single plateau region in the long-time diffusion limit that reflects the "effective" average properties of the porous medium (and, in turn, T). By contrast, the tendon ADC(t_{dif}) vs. $t_{\text{dif}}^{1/2}$ curves appear to exhibit two different "plateau regions" as a function of diffusion time. The first plateau is represented by a range of minimum ADC(t_{dif}) values in the curves which are statistically similar (~4–6 values for the curves shown in Fig. 2). The onset of the first plateau region for the $\text{ADC}_\perp$ curves occurs at earlier $t_{\text{dif}}^{1/2}$ values relative to the $\text{ADC}_\parallel$ curves. This is because the "effective medium" regime is reached at shorter t_{dif} values due to the greater degree of restriction experienced by water molecules diffusing perpendicular to the long axis of the tendon. Following the first plateau, ADC(t_{dif}) increases with increasing $t_{\text{dif}}^{1/2}$ and then levels off at a second higher plateau at longer diffusion times.

The increase in ADC(t_{dif}) at longer diffusion times can be explained by the partial elimination of one population of water molecules due to spin editing based on T_1 value. The T_1 spin-editing effect is peculiar to the DW-STE pulse sequence in which the diffusion-time increase is achieved through a concomitant increase in the TM period between the second and third 90° RF pulses. Since the z-magnetization is inverted during the TM period, it will experience T_1 decay. Consequently, the observed signal will be weighted to the distribution of water molecules having the longest T_1 values. If the T_1 and ADC values are correlated (i.e., the component with the longer T_1 relaxation time also has a higher ADC value because of fewer restrictions to diffusion), then T_1 spin editing would be expected to result in an ADC increase at longer diffusion times.

Preliminary MRI studies have shown that the T_1 value for rabbit Achilles tendon has multiple components arising from the (overlapping) distribution of T_1 values in the core and rim regions [15]. The mean T_1 value of the core is ~20% shorter relative to the rim of the tendon. In the same study, the mean core $\text{ADC}_\perp$ value is approximately half that of the rim value, while the difference in the parallel direction is negligible. The larger difference in $\text{ADC}_\perp$ between the respective core and rim components, coupled with the relatively shorter T_1 values in the core, supports our contention that T_1 spin editing is responsible for the ADC increase at longer diffusion times. It should also be noted that the $\text{ADC}_\perp$ curves exhibit a more significant increase in ADC at longer diffusion times (under both loaded and unloaded conditions) relative to the $\text{ADC}_\parallel$ curves. In this case, the 50% difference in the $\text{ADC}_\perp$ value would be consistent with T_1 spin editing having a relatively larger effect in that direction.

The calculation of T from Eq. (2) requires an estimate for D_0, which could not be determined in these studies. However, as was the case for determining (S/V), an estimate of the relative tortuosity for unloaded tendon can be obtained. The quantity $T_\perp/T_\parallel$ can be estimated from Eq. (2):

$$\frac{D_{\text{eff}}^{\parallel,\text{unl}}}{D_{\text{eff}}^{\perp,\text{unl}}} = \frac{T^\perp}{T^\parallel} \approx \frac{0.75}{0.3} = 2.5, \tag{4}$$

where the D_{eff} values were obtained from the first plateau of the respective unloaded curves. Data from the first plateau was used in order to exclude the influence of the T_1 spin-editing effect on the resulting ratio. This calculation was not performed for the loaded case since there is no clear plateau in D_{eff} before the T_1 spin-editing effect becomes significant. Furthermore, since by definition the tortuosity is sensitive to the value at which the ADC becomes constant, any offset due to the presence of extruded water signal will introduce error into the estimate for T. Because there is no way to determine exactly how much water is extruded in the present experiments, the tortuosity ratio cannot be reliably estimated in the loaded case. The anisotropy in T (as seen in Eq. (4)) is not unexpected given the tendon geometry. In the direction perpendicular to the tendon long axis, the close parallel packing of collagen fibrils impose significantly more barriers to water diffusion relative to the parallel orientation. The more tortuous nature of the perpendicular diffusion pathways accounts for the relatively smaller RMS displacement of water molecules in that direction.

The water ADC in rabbit Achilles tendon is anisotropic, diffusion-time dependent, and changes as a function of tensile load. Although water extrusion is partially responsible for the water ADC changes in response to mechanical loading, increased tendon fibril packing density may also play a role. Porous-media theory provides useful structural parameters for interpreting changes in tendon fibril packing density in response to tensile loading. The space available for molecular diffusion is related to (S/V). Changes in (S/V) reflect changes in the tendon fibril packing density and are consistent with the known geometry of the tendon microstructure. Tortuosity is related to the ease with which water molecules can negotiate the diffusion

pathways through the interstitial space of a porous medium. In this study, the tortuosity ratio reflects the inherent anisotropic nature of rabbit Achilles tendon. Finally, the interpretation of NMR spectroscopic ADC measurements of tendon water is hampered by the multi-component nature of the sample. Preliminary MRI studies have demonstrated overlapping distributions of T_1 and ADC values in the core and rim regions of rabbit Achilles tendon [15]. Further MRI studies should be helpful for interpreting the separate contributions of these components to the response of rabbit Achilles tendon under mechanical load.

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