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Characterization and mapping of dipolar interactions within macromolecules in tissues using a combination of DQF, MT, and UTE MRI

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Abstract

In the present work it is shown that by combining the double quantum filtered, magnetization transfer (DQF-MT) and the ultra-short TE (UTE) MRI methods it is possible to obtain contrast between tissue compartments based on the following characteristics: (a) the residual dipolar coupling interaction within the biomacromolecules, which depends on their structure, (b) residual dipolar interactions within the water molecules, and (c) the magnetization exchange rate between biomacromolecules and water. The technique is demonstrated in rat tail specimen where the collagenous tissue, such as tendons and the annulus pulposus of the disc are highlighted in these images, and their macromolecular properties along with those of bones and muscles can be characterized. DQF-MT UTE MRI also holds promise because collagenous tissues that typically are invisible in conventional MRI experiments produce significant signal intensities using this approach.

Keywords

DQF; MT; UTE; dipolar interaction; rat tail; tendons; annulus pulposus; intervertebral disc; short T2

Introduction

All commonly used clinical MRI methods employ water magnetization to obtain anatomical and functional information based on diffusion coefficients and relaxation times. However these methods provide only limited information about the properties of biomacromolecules (MM), such as collagen, that are common in extracellular matrix (ECM) and connective tissues like cartilage, ligaments and tendons. In order to obtain a better insight into the properties of the MM content of the tissue, it is desirable to have methods that probe the MM magnetization. Attempts to achieve this goal employed the use of magnetization transfer contrast (MTC). In this method the MM magnetization is fully saturated and the decline in the water signal is monitored. Results depend on the longitudinal relaxation (T_1) of water and its rate of exchange with the MM, and thus give only limited information about the identity or nature of the MM. It was argued, however, that by considering the level of B_1

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and the frequency offset from the water, MTC MR may yield a distribution of T_2 values of the MM (1).

Another method to select the subpopulation of MM-associated protons is with double quantum filtering (DQF) MRI (2-6). This approach enables one to select the protons to be excited on the basis of the strength of their dipolar interactions and thus is more sensitive to the properties of the MM and enabling one to obtain contrast on the basis of these properties. Since water molecules are far more mobile than MM their intramolecular dipolar interactions are largely averaged out. This can be used to suppress the water signal and retain only that of the protons of the MM whose motion is highly restricted, and whose dipolar interactions are not entirely averaged out. Indeed spectroscopic studies of MM such as collagen yielded a spectral width of tens of kHz. Such a large spectral width cannot be detected in MRI even with methods using a very short echo time (TE). In previous studies of neuronal systems this difficulty was solved by using the DQF-MT where the MM transverse magnetization was converted to the longitudinal axis and subsequently, by proton exchange (3), to water, whose magnetization could be used for imaging (6). Though the method provides detailed information about MM in neuronal systems, its application to connective tissues was still restricted by the short water transverse relaxation time (T_2) limiting the use of popular imaging methods such as gradient and spin-echo MRI, and requiring large slice thicknesses and low voxel resolution. These limitations have made the study of MM content and composition difficult. In recent years methods with ultra-short TE (UTE) enabled imaging of tissues with short T_2 like tendons.(7-10) Thus, in the present study, DQF weighted NMR is combined with UTE MRI to obtain images with contrast that distinguishes among tissue compartments on the basis of differences in their MM content.

Theoretical background and pulse sequences

The dipolar interaction between nuclei is a function of their distance ($1/r^3$) and the orientation of the internuclear vector with respect to the external magnetic field. In well-ordered systems this interaction will lead to a well-resolved spectrum. In systems where the orientations of the internuclear vectors are partially ordered or randomly distributed the spectrum lineshape will vary between a Pake doublet and a Gaussian, respectively. Moreover, dynamic processes such as reorientation and chemical exchange cause reduction of the observed dipolar interaction, whose extent depends on the rate and the anisotropy of the motion. For MM, such as collagen in connective tissues, for which the motions are slow or anisotropic and where the chemical exchange has no effect, dipolar interactions between protons are not reduced significantly (having order parameters in the range 0.3-1.0) and their size is in the range of tens of kHz. Such a large interaction manifests itself with spin dynamics on a time scale of a few tens of μ s. On the other hand for bound water molecules the intramolecular dipolar interactions are significantly reduced as a result of their fast isotropic or anisotropic reorientational motion, their exchange with the bulk water and the exchange among binding sites with different average orientations. Furthermore, proton exchange among water molecules causes an additional reduction of the proton-proton intramolecular dipolar interaction (3). The latter process is affected by temperature (3), pH (Eliav, Seo and Navon unpublished results) and buffer concentration (11). It has been shown that ^1H water NMR spectra in ordered systems such as collagen fibers and tendons where proton exchange among water molecules was suppressed either by cooling or by reducing the amount of free water, result in a splitting due to residual dipolar interactions (3,12,13). For water typical residual dipolar interactions are less than 1kHz and thus the spin dynamics occurs on a time scale of many hundreds of μ s. When the rate of the proton exchange among the water molecules is larger than the residual dipolar interaction one obtains coalescence of splitting and the line width is given by $1/T_2 = \omega_D^2/\kappa$ where ω_D is the dipolar interaction and κ is the proton exchange rate (3).

Dipolar interaction leads to the formation of high rank tensors such as $I_{x1}I_{z2}$ (1 and 2 indicate the interacting protons) that can be selected by DQF (14). It was further shown that intensity of this observed signal may depend on the dipolar interaction, ω_D , making it possible to distinguish among compartments by these interactions (3,6).

However, the population of immobile or restricted MM associated protons that can be selected and excited using the DQF would not be sufficient in themselves to produce a strong MR signal suitable for MRI acquisition due to their very large ω_D that causes a very fast decay of the MM transverse magnetization. Thus, another step then is necessary to amplify the signal due to the MM associated protons. This is done using MT approach. Therefore, the pulse sequence used to study the effects of the residual dipolar interaction in tissues, to gain information about the water intramolecular dipolar interaction and MM content, and to obtain a new and measurable contrast with MRI is the DQF-MT MRI sequence shown in Fig. 1. In Fig. 1 gradient echo (GE) is used as an imaging block. However, in the following we also show results where 2D ultra short TE (2D-UTE) is used for image formation. For spectroscopic studies the imaging module was replaced by a single 90° pulse followed by signal acquisition.

In the DQF-MT pulse sequence single-rank single quantum coherences, $T_{1,\pm 1}$ (given by $\mp I_{\pm}/2$) are obtained after the application of the first 90° pulse and evolve during the creation time, $\tau/2$, to become second rank tensors, $T_{2,\pm 1}$ (which consist of product operators such as $I_{\pm}^1 I_{\pm}^2$). These are filtered through double quantum coherences, $T_{2,\pm 2}$ ($I_{\pm}^1 I_{\pm}^2/2$) by suitable phase cycling (2-6,14). The filtered tensors revert to $T_{2,\pm 1}$ by the application of the third pulse, and then evolve during the second $\tau/2$ (reconversion) period into $T_{1,\pm 1}$. These tensors are further transformed to $T_{1,0}$ by the 4th 90° pulse and by the last 90° pulse into the observable tensor $T_{1,-1}$ to yield an in-phase spectrum (i.e., similar to a spectrum obtained in a single-pulse free induction decay (FID) experiment) with an intensity proportional to $M_{eq} \sin^2(\omega_D \tau/2)$. For creation time ($\tau/2$) values that fulfill the condition $\omega_D \tau/2 \ll 1$, the amplitude of the observed signal is proportional to $M_{eq} \omega_D^2$ (M_{eq} – is the magnetization at equilibrium, reference 3). This dependence on the interaction and number of protons makes it possible to distinguish between spectra originating from different compartments which, as discussed above, may be distinguished either by the water's intramolecular residual dipolar interaction within the compartment or by the dipolar interaction of its MM. Similarly in MRI, on the basis of the same differences among the compartments, this pulse sequence yields a new type of contrast.

Once only the longitudinal magnetization of the protons with ω_D typical of the largest dipolar interactions in MM is selected at $t_{LM}=0$ dipolar interaction will induce spin diffusion between these protons and others that were not selected (4). This process reaches a pseudo equilibrium state, typically within 1-2ms, where the observed spectrum resembles that of the MM obtained after a single 90° pulse for samples where the water signal doesn't interfere (e.g., samples where water was replaced by D_2O). Since $\tau/2$ was selected to be compatible with MM dipolar interaction but too short to enable excitation of the water (whose ω_D is much smaller than that of the MM) at the stage of pseudo equilibrium a very small water signal is observed. For t_{LM} periods longer than 1-2ms MT between the MM and the water takes place. In the following discussion we re-define the time of pseudo equilibrium as $t_{LM}=0$. For the case where at pseudo equilibrium only the MM magnetization is retained the following conditions apply $m_{zw}(t_{LM}=0)=0$ and $m_{zp}(t_{LM}=0) \neq 0$. In the following discussion m_{zw} and m_{zp} are the longitudinal magnetizations of the water and the MM, respectively that were obtained after the DQ filter (immediately after pseudo-equilibrium was established). Thus, it is the only the transfer of the magnetization, $m_{zp}(t_{LM}=0)$, to the water during the t_{LM} period can be observed in the experiment shown in Fig. 1. In previous studies of tendons (4,6) it was shown that the transfer mechanism between the MM and water is dominated by

proton exchange between them. And since the two most visible compartments in the images to be described below constitute of collagen it seems to us that it can be assumed that proton exchange is the most important transfer process in the rat tail. From the mathematical prospective while carrying out phase cycling the effect of the phases of the r.f pulses that preceded the time interval t_{LM} is to multiply $m_{zp}(t_{LM}=0)$ by the phase $e^{i\varphi_R}$ (where $\varphi_R = \pm 2(\varphi_1 + \varphi_2) \pm 2(\varphi_3 + \varphi_4)$) (see Eq. 2a) and by $e^{-i\varphi_R}$ during acquisition (see Eq. 2b). For a single site the MM magnetization, m_{-p} ($m_{-p} = m_x - im_y$), obtained after the last pulse is given by Eq. 2a:

$$m_{-p} = \frac{\sqrt{2}}{2} \left\{ m_{zp}(t_{LM}=0) e^{\frac{-t_{LM}}{T_1}} e^{i\varphi_R} + m_{eq}^p \left(1 - e^{\frac{-t_{LM}}{T_1}} \right) \right\} \quad [2a]$$

$$m_{-p} = \frac{\sqrt{2}}{2} \left\{ m_{zp}(t_{LM}=0) e^{\frac{-t_{LM}}{T_1}} + e^{-i\varphi_R} + m_{eq}^p \left(1 - e^{\frac{-t_{LM}}{T_1}} \right) \right\} \quad [2b]$$

$$m_{-p} = \frac{\sqrt{2}}{2} m_{zp}(t_{LM}=0) e^{\frac{-t_{LM}}{T_1}} \quad [2c]$$

In this expression there are two contributions to m_{-p} : one originating from the magnetization immediately after the fourth 90° pulse that is multiplied by the phase $e^{i\varphi_R}$ and another one from the magnetization formed during the interval t_{LM} by longitudinal relaxation, which is not multiplied by that phase. During acquisition, following the last pulse, both contributions to the signal are multiplied by the phase $e^{-i\varphi_R}$ (Eq. 2b). As a result of the phase cycling on φ_R the second term in Eq 2b, which depends on the longitudinal relaxation and does not depend on the transverse magnetization spin dynamics within the MM, is eliminated (Eq. 2c).

For exchange between two sites it was shown in previous study (4) that under the initial conditions mentioned above ($m_{zw}(t_{LM}=0)=0$, $m_{zp}(t_{LM}=0)=0$) and the fast exchange limit ($k \gg 1/T_1$), where k is the sum of the MT rates from the MM to the water and that of the reverse reaction, the detected magnetizations of the water and the MM, m_{-w} and m_{-p} respectively, are given in Eq. 3:

$$\begin{aligned} m_{-p} &= \frac{\sqrt{2}}{2} m_{zp}(t_{LM}=0) \left(p_p e^{\frac{-t_{LM}}{T_1}} + p_w e^{-kt_{LM}} \right) \\ m_{-w} &= \frac{\sqrt{2}}{2} m_{zp}(t_{LM}=0) p_w \left(e^{\frac{-t_{LM}}{T_1}} - e^{-kt_{LM}} \right) \\ \frac{1}{T_1} &= p_p R_{1p} + p_w R_{1w} \end{aligned} \quad [3]$$

With $m_{zp}(t_{LM}=0) = m_{eq}^p \sin^2(\omega_D^p \tau / 2) e^{-\tau/T_2}$, m_{eq}^p , ω_D^p and T_2 are the MM equilibrium magnetization, intramolecular dipolar interaction and transverse relaxation respectively, $R_{1p} = 1/T_{1p}$, $R_{1w} = 1/T_{1w}$ with T_{1p} , T_{1w} being the longitudinal relaxation times of the MM and water, respectively. The fractions of the protons residing on the MM and in water are given by p_p and p_w , respectively. From Eq. [3] it is clear that during MT the water signal is given by a difference of two exponentials, one that characterizes the build up due to an exchange process and another that describes the decay due to longitudinal relaxation (with rate given by $1/T_1$). Experimentally this was confirmed in previous studies for the MM and the water signal (4, 6) and in the current study for the MM (see Figs. 3, 4) where it is shown that for long t_{LM} the signal is decaying off. More generally longitudinal relaxation will be observed

as a decay process if prior to its monitoring phase cycling that select passage through the transverse plan is implemented.

Along with the changes in the water signal the MM magnetization is decaying bi-exponentially. It is worth noting that for $t_{LM}/T_1 \ll 1$ Eq. [3] shows that the sum of the water and MM magnetizations equal $m_{zp}(t_{LM}=0)$. However, for widely used conditions in studies of tissues: $t_{LM} \gg 1/k$, $t_{LM} \ll T_1$ and $p_w \approx 1$, the water signal is proportional to $m_{zp}(t_{LM}=0)$. This result is summarized in Eq. [4]:

$$m_{zw}(t_{LM} \text{ at max. intensity}) = m_{zp}(t_{LM}=0) \quad [4]$$

It is worth noting that under the conditions that makes Eq. 4 valid the MT between the MM and the water has reached chemical equilibrium but the effect of longitudinal relaxation is negligible. For the case of selective excitation of the water, expressions similar to those in Eq. [3] are obtained by interchanging the subscripts p and w .

From the above discussion it follows that the DQF-MT sequence enables the experimenter by judicious choice of τ and t_{LM} to obtain MRI contrast that reflects differences among various compartments that are on the basis of: (a) the amount of the MM protons (b) MM dipolar interactions and (c) exchange rates between the MM and the water. In the following sections we demonstrate for several systems the effect of the tissue properties such as order, MM content and exchange rate on the images obtained with the pulse sequences DQF and DQF-MT (Fig. 1).

Experimental

Spectroscopic NMR and MRI experiments were conducted on 14.7T Bruker micro-imager. For imaging studies the pulse sequence shown in Fig. 1 was used either with GE as shown in the figure or with commercially available 2D ultra-short TE pulse sequence (Bruker's program 2DUTE running within Bruker's operating system Paravision version 5.1) (7-10). For spectroscopic purposes the MRI module was replaced by a single 90° pulse. The hard 90° pulses shown in Fig. 1 were $22\mu s$. The ranges of values examined for τ and t_{LM} were $5\text{-}400\mu s$ and $1\text{-}600ms$ respectively. The UTE method was optimized by comparison with gradient echo (GE) images of a porcine spinal cord that has a T_2 relaxation time longer than the TE used in the GE method (3.1 ms). The combined DQF and UTE experiments were performed on the part of the rat tail that is the closest to the animal's body. It was skinned, immersed in perfluoropolyether (Fomblin LC/8, Solvay Solexis, Italy) and then placed in 10mm Shigemitsu tube (Shigemitsu, Japan) to reduce susceptibility artifacts. The samples were aligned with their cylindrical symmetry axis parallel to the magnetic field.

Results and Discussion

NMR Spectroscopy

In Fig. 2 spectra of rat tail obtained by using DQF-MT are shown as a function of the creation/reconversion periods, $\tau/2$ at very short ($10\mu s$) t_{LM} . Two types of spectra are evident. For $\tau = 70\mu s$ a very broad component with a line width larger than 10 kHz is observed (Fig. 2a), while for longer time scales a single peak of ~ 900 Hz line width emerges, (Fig. 2b) reaching a maximum intensity for $\tau/2$ in the range of $300\text{-}400\mu s$. The width of this peak is maintained over a large range of creation/reconversion time ($25\text{-}600\mu s$) and thus can be attributed to a single species.

By setting $\tau/2$ in the range of $5\text{-}80\mu s$ (typical for MM transverse magnetization, 4,6) and examining the dependence on t_{LM} one obtains the spectra shown in Fig. 3. Two major

physical processes are noted in Fig. 3 (See also ref 4). On a time scale shorter than 0.8ms fast spin diffusion within the MM causes the spectrum to converge to an unchanging shape for $t_{LM} > 0.8\text{ms}$ but its amplitude decays off with a concomitant increase of a narrow peak which is attributed to water. This assignment is based on previous spectroscopic studies on tendon where a similar peak of $\sim 900\text{Hz}$ width was attributed to the water since it disappeared upon replacing the H_2O in the sample with D_2O (4). This peak reaches its maximum intensity at $t_{LM} = 100\text{ms}$. The intensity of the MM decayed by a factor of about 4 for $t_{LM} = 12\text{ms}$ and by at least 8 for $t_{LM} = 100\text{ms}$. Since no narrow peak is observed at short t_{LM} it is clear that the water peak seen at $t_{LM} = 100\text{ms}$ originates solely from magnetization transfer from the MM. It is evident from Fig. 3b that during the exchange process two peaks of different line widths both smaller than 1kHz are formed. One of the peaks has a width of 900Hz while the other one has a line width of 70Hz ($T_2 \sim 4.5\text{ms}$). The 900Hz line resembles the water signal in tendon ($T_2 \sim 350\mu\text{s}$). An estimate of 1-1.5s for T_1 of the above two component were obtained from the decay of the signals shown in Figs. 3b and 4b. The narrower of the two peaks may be identified with the water signal in the annulus fibrosus since its T_2 was reported to be 19ms (15) which is compatible with the width reported here taking into account possible inhomogeneity and susceptibility effects. Contribution to this peak from the nucleus pulposus is expected to be very small since it contains small amounts of collagen while proteoglycans represent the major fraction of the nucleus' MM content. These proteoglycans do not contribute to the DQF-MT since their high mobility reduces their residual dipolar interaction. This assignment is further supported by the imaging results to be described in the next section.

MRI

In Fig. 4 images of an axial slice through a disc of the most superior portion of the rat tail, are obtained by gradient echo (GE), UTE and their weighting by DQF-MT. In the GE images with TE of 2.86ms the tendons are absent. Only the annulus fibrosus and the nucleus pulposus are visible as well as the thin layers of the para-, epi- and peri-tendineum (16) surrounding the tendons. The UTE method makes the tendons appear with positive contrast reflecting the fact that the weighting by T_2 is less important than in GE. Since the duration of the gradients used for the imaging are 2ms (both for GE and UTE) too long to image macromolecules such as collagen which dephase on a time scale shorter than $80\mu\text{s}$ the contrast obtained by UTE is mostly due to the proton density of the water molecules. However, the short TE diminishes the contrast between the annulus fibrosus and nucleus pulposus as well as between the tendons and their surrounding tissue i.e. the SNR of the tendons, annulus fibrosus and muscles are similar (Fig. 4). This shortcoming was addressed by combining UTE with DQF-MT (bottom of Fig. 4) with the creation/reconversion times set to 300ns . In this image only the tendons are visible, thus confirming that the peak of 900Hz width (Fig. 1) is due to residual dipolar interactions of bound water in tendons. It is worth noting that para-tendineum (16) that is in close proximity to the tendons, is absent from the DQF-MT weighted UTE MRI although present in the UTE images. As explained in theoretical background section combining DQF-MT and UTE yields images with contrast that reflects differences in MM characteristics. For rat-tail it is expected to distinguish between various types of collagen that constitute the tendons and the annulus fibrosus, e.g. Type I and Type II collagens. In Fig. 5 we show UTE MRIs weighted by a DQF-MT sequence. As can be seen from Fig. 5 only two parts of the rat tail are clearly visible: the tendons and the annulus, while there is some contribution from muscles in the images obtained for $\tau/2 = 10\mu\text{s}$. For this value of $\tau/2$ and t_{LM} in the range of $50\text{--}600\text{ms}$ the ratio of the SNR of tendon and annulus fibrosus is 1.6 i.e. very different from the equal value observed in the UTE images. Furthermore, since spectroscopic measurements show that for $\tau/2 = 10\mu\text{s}$ the contribution of intramolecular dipolar interaction of the water is negligible and the contrast in the UTE images very small we attribute the contrast between the tendon and

annulus to differences in the collagen type or content. This conclusion is consistent with the known difference in the collagen composition of tendons and annulus (17). Tendons mostly consist of collagen type I while the annulus contains both types I and II collagen (18) with the former type being abundant in the outer part of annulus and Type II in the inner part, closer to the nucleus pulposus. Examining the dependence of the images on the creation/reconversion periods, $\tau/2$, we see that the signals from the tendons, annulus and muscles decay off on the time scale of tens of μs . However, the extent of their intensity fall-off resulting from increasing $\tau/2$ from 10 to 40 μs was different, namely by factors of 1.0, 2.0, and 1.5 for tendons, annulus fibrosus, and muscles respectively. Thus, we conclude that on creation/reconversion time scale of tens of μs it is the MM spin dynamics that dominates the MRI signal. This conclusion is further supported by the fast decay of the DQ coherence during the time interval t_{DQ} occurring on a times scale shorter than $t_{DQ}=80\mu\text{s}$. This fast decay can be the sole result of spin systems where two dipolar interactions are involved. For instance dipolar interaction between protons in one CH_2 group and protons in another CH_2 group or in CH or CH_3 groups. It is worth noting that the lack of decay of the tendon's signal may be the result of a combined effect of a decay of the MM magnetization compensated by a signal buildup due to the intramolecular dipolar interaction of the water. This buildup may be more significant for the tendon relative to the other tissues in the rat tail owing to the larger residual interaction of the water in the tendon. From spectroscopic studies the contribution from the water residual intramolecular interaction at $\tau/2=40\mu\text{s}$ can be as large as 40% as was estimated in the following way: Upon changing $\tau/2$ from 10 μs to 40 μs with $t_{LM}=1\mu\text{s}$ the water signal increases by a factor of 3.5 while the MM intensity decreases by a factor of 3. Since the amount of magnetization that can be transferred to the water is proportional to MM magnetization a simple calculation yields that at least 40% of the water signal in tendon acquired with $\tau/2=40\mu\text{s}$ and $100\text{ms}<t_{LM}<300\text{ms}$ should be attributed to a buildup due to water residual intramolecular dipolar interaction. Since the water residual intramolecular interaction in the annulus and in the muscle is small the water DQF-MT signal buildup at $\tau/2=40\mu\text{s}$ due to this interaction can be neglected. Thus the difference in the decay of the signals as a function of $\tau/2$ between these two tissues is solely due to differences in their MM structures.

For short creation times, the dependence on the exchange period, t_{LM} , shows a maximum at 200ms and it is similar for tendons and the annulus. This similarity may suggest that the exchange with water proceeds through similar functional groups present on the collagen backbone, i.e., protons of hydroxyproline or hydroxylysine. Further demonstration of the capability of DQF-MT when combined with UTE to distinguish between tissues on the basis of their MM differences is shown in Fig. 6 where an axial slice through a vertebrae located in the middle between two discs is shown. As in the case of the disc the contrast between the various tissues in the DQF-MT weighted UTE images is very different from the one obtained by UTE. For the UTE experiment SNR's of 6.8, 8.8 and 7.2 were obtained for the tendon, muscle and bone respectively (Fig. 6). The addition of the DQF-MT weighting with $\tau/2=10\mu\text{s}$ eliminates the contribution from the bone marrow, and sets the SNRs (for eight scans) of the tendons, muscles, and vertebrae to 8.7, 4.6 and 2.9, respectively. Using the same arguments as in the discussion of the results obtained with $\tau/2=10\mu\text{s}$ for the disc we may conclude that differences between the various tissues reflect differences in the MM contents and structure. Examining the dependence of the images on the creation/reconversion periods, $\tau/2$, show that tendons, muscles and vertebrae decay on the time scale of tens of μs indicating that the decay is dominated by the MM magnetization. However, the extent of the intensity decay as a result of increasing $\tau/2$ from 10 to 40 μs is different: 1.3, 1.9 and 1.5 for tendons, muscles and vertebrae respectively, reflecting differences in the MM structure.

Conclusions

In the present work it is demonstrated that combining a DQF-MT “filter” with UTE MRI produces image contrast that is based on the residual dipolar interactions either within the water molecules or within the macromolecules. It is further demonstrated that this new type of contrast makes it possible to distinguish between and among various compartments within the rat tail on the basis of differences in their macromolecular properties. The prospect of possibly being able to distinguish between and among different types of collagen may have far-reaching consequences in diagnostic applications, particularly in orthopedics, where there is a dearth of successful MR methods for imaging ECM, intervertebral discs, cartilage, bone, tendons and ligaments.

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Abbreviations

DQF	double quantum filter
ECM	extracellular matrix
GE	gradient echo
MM	macromolecules
MT	magnetization transfer
MTC	magnetization transfer contrast
TE	time to echo or echo time
UTE	ultra-short TE
FID	free induction decay

Figure 1.

Schematic presentation of the DQF-MT MRI pulse sequence using the gradient-echo imaging modality. The duration of 90 hard pulses was 22 μ s. The phase cycling was eight steps given by: $\phi_1=\phi_2=0^{\circ} 90^{\circ} 180^{\circ} 270^{\circ}$; $\phi_4=\phi_3-90^{\circ}=0^{\circ} 0^{\circ} 0^{\circ} 0^{\circ} 90^{\circ} 90^{\circ} 90^{\circ} 90^{\circ}$; $\phi_5=0^{\circ}$; $\phi_R=0^{\circ} 180^{\circ} 0^{\circ} 180^{\circ} 180^{\circ} 0^{\circ} 180^{\circ} 0^{\circ}$

Figure 2. Dependence of ^1H rat tail spectrum on creation/reconversion times, $\tau/2$, using the pulse sequence shown in Fig. 1 (DQF-MT) where the imaging modality was replaced by a single 90° pulse. The shown spectra are drawn on two spectral ranges (a) the tens of kHz range suitable to display the MM spectra (b) the few kHz range suitable to display the water molecules spectra. $t_{LM}=1.5\mu\text{s}$.

Figure 3. Dependence of ^1H rat-tail spectrum on exchange time t_{LM} using the pulse sequence shown in Fig. 1 (DQF-MT) where the imaging modality was replaced by a single 90° pulse. The shown spectra are drawn on two spectral ranges (a) the tens of kHz range suitable to display the MM spectra (b) the few kHz range suitable to display the water molecules spectra. The creation/reconversion time $\tau/2$ was $10 \mu\text{s}$.

T= tendons
 A= Annulus Fibrosus
 N= Nucleus Pulposus
 M= Muscle

DQF-MT+UTE, $t_{LM}=1\mu s$, $\tau/2=300\mu s$

Figure 4.

Images of axial slice of rat-tail disc obtained using gradient echo, UTE and DQF-MT combined with UTE. Combining DQF-MT with gradient echo gave no image for TE of 2.86ms as the tendons signal has already decayed away. For UTE-related methods the TE was 0.14ms, matrix size is 256×256 , slice thickness 1.86mm. The selective r.f pulse duration was 0.1ms. The dead time after echo slice was $2\mu s$. TR=2 s, number of averages is 8. The creation/reconversion time, $\tau/2$, in the DQF-MT weighted UTE image was $300\mu s$. $t_{LM}=1.5\mu s$.

Figure 5.

Images of axial slice of rat-tail through disc that was obtained using DQF-MT combined with UTE are shown as a function of creation/reconversion time ($\tau/2$) and exchange time (t_{LM}). The matrix size 256×256 . Slice thickness 2.1mm. The selective r.f. pulse duration was 0.1ms. The dead time after echo slice was $2\mu s$. $TR=2$ s, number of averages =8. The SNR of the tendons (t) was calculated as average of the SNRs of all the tendons and similarly the SNR for annulus (a) was calculated by taking into account all the area of this tissue.

T – tendon
M- muscle
BM- bone marrow
V- vertebrae

Figure 6.

Images of axial slice in a middle point between two disc of rat-tail that was obtained either by using UTE (upper image) or by DQF-MT combined with UTE (bottom line). The latter images are shown as a function of creation/reconversion time ($\tau/2$) for exchange time that yields maximum intensity ($t_{LM}=100\text{ms}$). The matrix size 256×256 . Slice thickness 2.1mm. The selective r.f pulse duration was 0.1ms. The dead time after echo slice was $2\mu\text{s}$. $\text{TR}=2\text{ s}$, number of averages =8 The SNR of the tendons (t) was calculated as average of the SNRs of all the tendons and similarly the SNR for annulus (a) was calculated by taking into account all the area of this tissue.