## 2152

## Collagen composition and content-dependent contrast in porcine annulus fibrosus using double-quantum filtering combined with magnetization-transfer and UTE MRI

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**Introduction**: Recently, it was demonstrated that by combining double quantum filtering (DQF) and magnetization transfer (MT) and Ultra-Short TE (UTE) MRI (DQF-MT/UTE) (1) it is possible to obtain image contrast that distinguishes between tendons and annulus fibrosus in rat tail intervertebral disc (IVD) on the basis of differences in their collagen content. However, due to limited spatial resolution it was not clear whether the source of this contrast was the difference in the amount of collagen or in the type of collagen that constitute these tissues. Here we address this problem by using DQF-MT/UTE μMRI in the larger porcine annulus fibrosus.

**Materials and Methods**: We concatenate DQF-MT pulse sequence and UTE MRI sequence:  $90^{\circ}-\tau/2-90^{\circ}-t_{DQ}-90^{\circ}-\tau/2-90^{\circ}-t_{LM}$ -UTE, where  $\tau/2$  is the creation/reconversion time, and  $t_{DQ}$  and  $t_{LM}$  are the DQ coherence and longitudinal magnetization evolution time intervals, respectively. This approach enables one to select the population of protons to be excited on the basis of the strength of their dipolar interactions,  $\omega_D$ , making the sequence sensitive to the properties of the macromolecules and enabling one to obtain contrast from collagen-associated protons.  $\mu$ MRI was performed on a 14T Bruker BioSpin scanner with an Avance III console and a micro2.5 imaging probe. Fresh porcine annulus fibrosus was obtained after sacrifice, soaked in saline, cut into two sections, and placed in a tube with the inner section (located close to the nucleus pulposus) stacked under the outer section.

**Results:** Figs. 1 show sagittal slices of porcine annulus fibrosus. In the upper row of Fig. 1 the three images to the right demonstrate the dependence of the DQF-MT/UTE MRIs of porcine annulus fibrosus on  $\tau/2$ , while the exchange time  $t_{LM}$  was kept constant (200ms). The image on the left side of the upper row is a non-weighted UTE. This latter image reflects the water proton density and hardly shows any contrast, since the  $T_2$  (20-30ms) for this tissue is much longer than the TE (200µs). On the other hand the DQF-MT/UTE MRIs show a decline in signal intensity from the outer layers towards the inner ones. As can be seen from the figure the decay of the DQF-MT/UTE images as a function of  $\tau/2$  occurs on time scale of tens of µs and thus reflects directly macromolecular magnetization decay such as that of collagen.

**Discussion**: The observed contrast in the DQF-MT/UTE MRIs can arise from two sources: (a) higher amount of collagen in outer layers of the annulus fibrosus than in inner layers; and (b) differences in collagen type between the outer and inner layers (2). The small variations in contrast in the DQF-MT/UTE MRIs with  $\tau/2$  suggest that the dynamics of the rigid part of the collagen is not the source of the observed contrast. The plot shown in Fig. 1 demonstrates this fact. Within the two tissue sections we labeled four ROIs (A-D in the UTE image), plotted their average intensities as a function of  $\tau/2$ , and fitted them to a Gaussian decay given by:  $Aexp(-2(\tau/2t_{deacv})^2)+B$ . The resulting decay times,  $t_{deacv}$ , were on a times

scale of tens of  $\mu$ s, typical of macromolecules, but were very similar for all ROIs. However, the ratio of the average intensity in regions A and D is about four. This ratio cannot be explained solely by the collagen content (2) in these two ROIs. Thus, we also examined the possibility that the MT efficiency is different in the various ROIs. Spectroscopic studies using DQF-MT NMR pulse sequence (the UTE module in the DQF-MT/UTE method is replaced by a single 90° pulse) (1) have shown that the MT efficiency in region A to be twice that in region D. The differences in the MT efficiencies are interpreted by the decline of the ratio of collagen type I to type II on going from the outer into the inner layers of the annulus fibrosus.

**Conclusions:** Taken together, the variation in collagen content and MT efficiency can explain the observed intensity variation in porcine annulus fibrosus images.





Fig. 1. Images of annulus fibrosus obtained by DQF-MT/UTE (1). TE=0.2ms, slice=1mm, FOV=1.5cm, Matrix size=128X128

References:

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- 2. Eyre DR. et al. Biochem J 1976;157:267-270.