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***In vivo* assessment of time dependent changes of T2* in medial meniscus under loading at 3T: A preliminary study**

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Abstract

Due to the internal structure of the knee joint, the ability to characterize and quantify the dynamic response of the meniscal tissue directly *in vivo* is highly problematic. The main purpose of this study was to investigate the behaviour of the meniscus under loading conditions. Four healthy young females were included. To obtain T2* values in the meniscus, the vTE sequence was used with 10 echoes ranging from 0.8 to 10.1 ms. Submillisecond first echo time is a great advantage of vTE sequence allowing for precise mapping of relatively short T2*. The two-parametric least squares fitting procedure was used to calculate T2* pixel-wise. A custom-made diamagnetic apparatus was developed to simulate stress conditions on the lower limb in a conventional MR scanner. vTE T2* was performed in five consecutive scans, 6:10 min apart. Three different compartments of the medial and lateral meniscus were segmented. The differences at the different time-points were calculated. A constant increase of T2* times after compression was statistically significant in the anterior horn of the medial meniscus. T2* mapping with variable echo time sequence might be a satisfactorily sensitive technique to detect the changes of meniscus physiology under loading conditions *in vivo*.

Keywords

Meniscus; Loading; MRI; vTE; T2* changes

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Conflict of interests

We declare that we have no financial or personal relationships with other people or organizations that could inappropriately influence (bias) our work.

Introduction

In the knee joint, the meniscus is considered to be a secondary knee stabilizer. It is an important component of the structure of the knee, especially in the long-term preservation of correct function of the knee joint. Its importance was also confirmed by the high correlation between the degree of meniscal degeneration and degree of articular cartilage degeneration (Sun et al., 2010). The meniscus is also one of the most frequently injured parts of the knee joint (Ford et al., 2005), therefore it is important to know more about the behaviour. Due to the internal structure of the knee joint, the ability to view changes in the macromolecular composition directly, in its normal position, *in vivo*, is highly problematic. Non-invasive imaging of the knee meniscus without the use of contrast agents is more difficult compared to articular cartilage. MRI is an imaging method that enables the demonstration of pathological changes in the human body. Despite the lower signal intensity of the knee meniscus, MRI is considered the best non-invasive imaging method (Braun and Gold, 2012).

As stated by Rauscher et al. (2008) in their study, T2 relaxation times reflect more closely the changes in the meniscus compared to T1rho relaxation time, whereas, in the articular cartilage (AC), the opposite is true. T2 values as an indirect biomarker of meniscal structure provide information about the interaction of water molecules and the structure of the ECM, especially the interactions based on the content, orientation, and anisotropy of collagen (Bae et al., 2010; Fragonas et al., 1998; Liess et al., 2002; Mosher et al., 2005; Rauscher et al., 2008). The recently introduced, three-dimensional, spoiled gradient echo (SPGR) sequence with a variable echo time scheme (3D vTE Cartesian SPGR – also referred to as vTE) minimizes the TE. T2* relaxation times in the healthy meniscus of a middle age person range from 7 to 8 ms (Juras et al., 2013; Williams et al., 2012) and T2 times are approximately 11 ms (Rauscher et al., 2008; Stehling et al., 2011; Zarins et al., 2010). Higher values have been reported for the medial meniscus (Chiang et al., 2013; Subburaj et al., 2012; Tsai et al., 2009; Zarins et al., 2010). Further, T2* times are lower in the white zone and they increase in proximity to the red zone (Chiang et al., 2013; Tsai et al., 2009). Aging and gender (both noted to have a significant effect on proteoglycan synthesis in human menisci) may also be factors that could affect the diagnostic value of quantitative meniscal T2* measurements (McAlinden et al., 2001; Tsai et al., 2009). Several studies have shown the potential of dynamic loading response of the cartilage to determine its functional properties either in healthy volunteers (Nishii et al., 2008) or patients with osteoarthritis (Souza et al., 2010) and cartilage transplantations (Juras et al., 2009). Non-invasive nature of biochemical MRI provides a tool for describing the functional processes and understands the mechanisms of various pathologies. This knowledge can be extended to other connective tissues, such as meniscus; however, the different function and structure of meniscus might result in different response to the loading and the pathologies might manifest differently. There are only few studies analyzing the response of meniscus to the loading. Calixto et al. (2015) have studied the response of the meniscus in the osteoarthritic knee, the results demonstrated different response of degenerated meniscus compared to healthy one in regard to T2 values. We believe it would be interesting to the community to know what is the response of the meniscus to the loading on a longer time scale. To the best of our knowledge,

the behaviour of the meniscus under loading at multiple time-points has not yet been investigated.

Therefore, the goal of this study was to investigate the alterations in T2* in the human knee meniscus *in vivo* under continuous loading.

Materials and methods

Subjects

Four healthy volunteers were included in this study (four females, 25–30 years of age). The participants did not suffer from degenerative damage of the cartilage and growth plates were closed.

The inclusion criteria were: BMI less than 25 kg/cm²; no chronic disease; no long-term medication or nutritional replacement; and no feeling of instability of the knee joint. The participants must have been asymptomatic for more than 30 days before measurement, the ICRS (International Cartilage Repair Society) score to evaluate both knee joints had to be more than 90, and there had to have been no signs of damage or meniscus tear in physiotherapy evaluations. Contraindications to the MR examination had to be excluded before the measurements in the MR scanner. After morphological measurements on MR, the resulting findings were further excluded: meniscus tearing, liquid inside the knee, ligamentous abnormalities, and evidence of the absence of the meniscus or the discoid meniscus. Morphological evaluation was performed by the radiologist (S.T.) with 20 years of experience. All volunteers provided written, informed consent before inclusion into the study.

Simulation of the loading technique

A diamagnetic apparatus was designed in the Laboratory of Biomechanics of Extreme Load (labBEZ) at the Charles University in Prague. This simple yet robust equipment can simulate stress conditions on the lower limb in a conventional MR scanner. It consists of a board with four holes. Through these holes pass four bars with air rubber bands attached to the ends. These rubber bands are attached to a harness. Loading conditions are simulated by tightening each rubber band to a pre-determined extension value. Prior to load induction using this apparatus, the resultant force vectors and centre of pressure in the chosen segment of the foot after long-term static load must be quantified.

The dynamometric insoles, PEDAR[®]-X, were used to quantify the force vectors on the foot (Fig. 1). These insoles can record a pressure distribution map for the interaction between foot segments and the pad (Gerych et al., 2013).

During MR imaging, the volunteers were measured in the supine position, and the examined knee of the non-dominant leg was fully extended. The foot of the examined leg was secured in a neutral rotational position by fixation on the foot-rest of the sliding foot-plate. Foot plate was prepared from resultant calculation force vector and its centre of pressure. After the foot was properly placed on the plate, rubber bands were tightened by pre-determined extension value to simulate loading conditions in the standing position. In the knee coil, the measured

leg was firmly secured by rigid foam and sand bags. These were placed around the knee in the coil to prevent knee movements.

MR image acquisition

All measurements were performed on a 3 T MR whole-body scanner (Magnetom Trio; Siemens, Erlangen, Germany) using a Tx/Rx eight-channel knee coil (*In vivo* Corp, Gainesville, FL, USA).

First, a morphological T2 true fast imaging with steady-state free precession (TRUFI) sequences were used in the supine position without loading (Table 1). vTE sequences were used to measure T2*. Baseline (no load) and four consecutive scans, performed 6:10 min apart, were obtained (Table 1).

MR image analysis

Images from the vTE sequence (Fig. 2) were analyzed using a custom-written script in IDL 6.3 (Interactive Data Language, Research Systems, Inc., Boulder, CO, USA).

A mono-exponential fitting procedure was employed on all MR data sets on a pixel-by pixel basis. A two-parametric function was used to fit the signal intensity:

$$S(TE) = S_0 * \exp\left(-\frac{TE}{T2^*}\right)$$

where S_0 represents the signal intensity at a TE of ≈ 0 ms, $T2^*$ corresponds to the actual $T2^*$ (mono-exponentially calculated $T2^*$).

Three different compartments of the lateral meniscus (LM) and medial meniscus (MM) were segmented (lateral anterior (LM ant), lateral body (LM body), lateral posterior (LM post) and medial anterior (MM ant), medial body (MM body), medial posterior (MM post)). Regions of interest were defined by an orthopaedic surgeon with 10 years of experience. ImageJ was used to segment the meniscus on the images from the 9th echo time (Fig. 3). ImageJ is an open source image processing program designed for scientific multidimensional images. This program is highly extensible, with thousands of plugins and scripts for performing a wide variety of tasks, and a large user community.

Statistical analysis

In all $T2^*$ maps, means and standard deviations (SD) were calculated, independently for each meniscal region, and separately for each time-point. A hierarchical linear model (HLM) was used in order to consider multiple measures per patient. To determine the main effects of loading on the changes in MR relaxation times (Comp0 = no compression [baseline], comp1 [+6:10 min], comp2 [+12:20 min], comp3 [+18:30], comp4 [+24:40]), mean scores over five time-points were analyzed. P -value < 0.05 was considered to indicate significant results. For calculation, SPSS (IBM, Armonk, NY, USA) version 21.0 was used.

Results

An example of a resulting T2* map for each time-point is shown in Fig. 3.

Mean values of T2* relaxation times with standard deviations for each measurement are shown in Table 2. The evaluation of the T2* changes in each part of the meniscus after each time of loading is shown in the third column for each compression in percent.

When we compared T2* changes between the no-compression state (Comp0) and after 24:40 min of loading (Comp4), we could evaluate the effect of loading on the meniscus after a longer time.

Using HML, the statistical significance was found in time $\times$ compartment; specifically in medial meniscus anterior horn. The results for each area are shown in Table 3.

The pattern of a gradient T2* increase was observed only in the medial meniscus anterior horn with significance level $p < 0.027$ (Fig. 4).

T2* relaxation times after loading increased in MM ant, LM post and LM body, but changes were not statistically significant. In other regions, T2* is stable or slightly decreasing, but this trend was not statistically significant. Generally speaking, increased loading time did not reveal the same effect on the different areas of the meniscus.

Discussion

The purpose of this study was to characterize and quantify the dynamic response of the deep layer of the meniscal tissue to axial loading using T2*, acquired by vTE sequences capable of very low TE (0.8 ms). The main objective was to test our hypothesis about the possibility for the non-invasive detection of changes in the deeper layers of tissue through T2* relaxation times. There are previous studies that have shown that T2* can be used to evaluate differences in the meniscus in different disease states (Juras et al., 2013). According to this research, we focused to detect changes of T2* based on loading time.

The results from this study support the following conclusions. First, the results from T2* relaxation times support the presumption that there is the tendency toward elevation of the T2* times after loading applied to healthy meniscal tissue in MM ant, LM post and LM body. The T2* difference was statistically significant in the anterior horn of the MM, where the T2* time increased after the first three time-points (6 min, 12 min, 18 min) ($p < 0.027$). Increased T2* times between each measurement ranged from 3.3% to 7.4%. Increase in the LM was not statistically significant. The small changes in T2* may be due to changes in the water distribution in various parts of meniscus under load, *i.e.*, water moves from more loaded (or more deformed) to adjacent less loaded (less deformed or even undeformed) parts of meniscus. This could explain the small increase of T2* in LMbody and MMant and the decrease in LMpost and MMbody.

Stehling et al. (2011) studied T2 values in the menisci after a marathon. All nine marathon runners showed a significant increase in T2 values after competition in all meniscus compartments ($p < 0.0001$), which may indicate changes in the biochemical composition of

meniscal tissue. Subburaj et al. (2012) studied meniscal behaviour after 30 min of running. Runners were also measured without any load. In their study the T2 times were increased in all regions except the posterior horn of the medial meniscus, but the changes were not significant.

In both studies, T2 in these runners increases probably because of the meniscus swelling (increase of water content) or/and temporary loosening of the collagen matrix. Authors did not solve the question about meniscal behaviour under compression. They would probably see a decrease of T2, what is not consistent with the results of our study.

The discrepancy between previously published results and the results of this study might arise from the fact that T2* decay in the meniscus is bi-exponential. If there was a significant contribution to short component alteration during the loading phase, it was not possible to detect it with our mono-exponential approximation. Unfortunately, the echo times number and range needed for a reliable bi-component T2* fitting is impossible in *in vivo* loading conditions because the total scan time would become intolerable for the subjects.

In our study, the feasibility of the meniscus dynamic response has been validated using vTE T2* mapping. Submillisecond first echo time is a great advantage of vTE sequence allowing for precise mapping of relatively short T2* in menisci. This is, in our mind, the advantage over T2 mapping which has technological limits for decreasing the first echo time (Calixto et al., 2015). T2* are useful in acquiring the fast-decaying MR signal from meniscal tissue and assure sufficient resolution within short and clinically adequate scan times. Thanks to the calculation and the display of the T2* maps it is possible to detect the changes in the collagen component of the ECM noninvasive and more precisely to detect the changes in the water content (distribution of interstitial water in the solid matrix) which are not normally visible in conventional MRI images (Nishii et al., 2008; Welsch et al., 2008). The use of T2* provides the opportunity for faster imaging times and potentially will provide greater spatial resolution with 3-D techniques.

We found a relatively high standard deviation in T2* values. It is caused mainly due to low SNR in menisci and so the low precision of signal intensities used for calculation of T2*. This is due to the inhomogeneity of meniscal tissue, the differences in the individual regions, organization collagen fibers, proteoglycan content, and vascularization. A higher standard deviation (around 25–35%) in healthy meniscus has also been shown in many previously published works (Chiang et al., 2013; Juras et al., 2013; Nishii et al., 2008; Rauscher et al., 2008; Williams et al., 2012).

The limitations of our study have to be acknowledged. The number of subjects was relatively low; only seven healthy subjects could be examined and only four of them were consistent to complete the follow-up MRI examination. As a result, our study was not able to investigate the influence of loading on meniscal structure in general. Although the number of subjects was low, statistical significance was achieved, as the results were consistent between the volunteers, *i.e.* all four subjects demonstrated similar T2* increase patterns at each time-point.

In our study, we analyzed the entire meniscus without considering zonal variations. This may have been a limitation, and future studies will be required to analyze T2* values in the anatomic sub-regions of the menisci.

Conclusion

In this study, the dynamic response of the meniscus to loading was validated using vTE T2* mapping. The sub-millisecond first echo of this technique is a great advantage of the vTE sequence, which enables precise mapping of the relatively short T2* in the menisci. This study showed that vTE T2* is capable of detecting the biomechanical process in the anterior horn of medial meniscus during loading, as well as describing the trend in different times of loading.

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Fig. 1.

Quantification of plantar pressure using the dynamometric insoles PEDAR®-X (Novel GmbH, Munich, Germany) in cooperation with the workplace Sports Research Institute of Czech Armed Forces (CASRI) in Prague (<http://casri.cz/web/>).

Insoles are able to measure plantar pressure at a pre-set frequency 100 Hz. From measured values in the standing position during 60 s, the resultant force vector and its coordinates on the feet were calculated. The mean value of the force vector in the standing position was used to simulate loading on the non-dominant leg in the supine position.

Fig. 2.

Representative sagittal 3.0 T source image (TR/TE:18 ms/9.114 ms) obtained from the medial femoral/tibial compartment. a) The resulting image from the 18th slice after 12:20 min of loading (Comp2) shown at an echo time TE = 9.114 ms (9th echo from 10 created); b) The resulting T2* map for the 18th slice after 12:20 min of loading (Comp2) obtained by mono-exponential fitting. Gray shades in each pixel correspond to the relaxation time T2*.

Fig. 3. Medial meniscus anterior and posterior horn. An example of mono-exponential T2* maps. Evaluation of similar sections without loading (A = Comp0) and with loading (B = Comp1; C = Comp2; D = Comp3; E = Comp4). On each ROI, the border of the meniscus and AC is omitted due to the partial volume effect (caused by insufficient resolution), and thus, the deep part of the meniscus could be studied. Note that all maps are overlaid on the morphologic image and pseudo-grayscale-coded with the corresponding bar in the lower right corner on each image.

Fig. 4.

The different regions of the meniscus without compression (Comp0, $t = 0$ min) and under compression (Comp1, $t_1 = 6:10$ min; Comp2, $t_2 = 12:20$ min; Comp3, $t_3 = 18:30$ min; and Comp4, $t_4 = 24:40$ min). The Y – axis represents T2* in milliseconds. a – statistical significance ($p < 0.05$).

Table 1

Details of the sequences used in the measurements.

MR Centre of Excellence (Medical University of Vienna) 10th–11th April 2015	TR/TE (ms)	FOV (mm)	Matrix	Voxel size	Pixel bandwidth	Number of slices	Flip angle (°)	Acquisition time (min)
T2 TRUFI 3D_we_cor_p3_iso_384	8.84/3.8	144 × 159	348 × 384	0.4 × 0.4 × 0.4	200	288	28	5:37
Comp0–Comp4 xd_vTE_we_T2star_0.7 × 0.7 × 1.3_10 echoes	18/0.8; 2.128; 3.126; 4.124; 5.122; 6.12; 7.118; 8.116; 9.114; 10.112	131 × 218	184 × 304	0.7 × 0.7 × 1.3	322	64	13	6:10

Note: TRUFI sequences were used for morphologic evaluation. vTE sequences were used to acquire T2* relaxation times while minimizing TE, and thus, enable the evaluation of changes in the meniscus without loading and under loading conditions. Parameters were based on Deligianni et al. (2012).

Table 2

Meniscal T2* measurements at each time–point (Comp0–Comp4) in the medial meniscus (MM) and the lateral meniscus (LM) in the anterior horn (ant), the posterior horn (post), and the body.

Loading	Comp0		Comp1			Comp2			Comp3			Comp4		
Meniscal part/T2* value	mean T2*	SD	mean T2*	SD	Comp1–Comp0 (%)	mean T2*	SD	Comp2–Comp1 (%)	mean T2*	SD	Comp3–Comp2 (%)	mean T2*	SD	Comp4–Comp3 (%)
MM ant	4.69	±0.37	4.84	±0.58	3.3	5.20	±0.53	7.3	5.42	±0.64	4.4	5.23	±0.70	−3.6
MM post	5.63	±0.35	5.71	±0.34	1.3	5.66	±0.39	−0.8	5.75	±0.48	1.6	5.85	±0.52	1.8
MM body	5.92	±0.34	5.13	±0.46	−13.3	5.66	±0.34	10.4	5.39	±0.58	−4.8	5.09	±0.46	−5.5
LM ant	5.78	±0.39	5.86	±0.57	1.2	5.87	±0.50	0.3	5.79	±0.50	−1.4	5.87	±0.57	1.5
LM post	5.33	±0.57	5.84	±0.45	9.5	6.00	±0.60	2.8	5.83	±0.54	−2.8	5.55	±0.51	−4.7
LM body	5.07	±0.50	5.07	±0.36	0.0	5.33	±0.51	5.2	5.27	±0.57	−1.0	5.28	±0.64	0.1

Note: SD – standard deviation for each area. Third column in Comp1, Comp2, Comp3, Comp4 shows percentage changes in T2* relaxation times between each time–point. Comp1–Comp0 shows T2* changes between Comp1 and Comp0 evaluated in percentages. A positive value means an increase of T2 * time, a negative value of its decrease after 6:10 time of loading.

Table 3

The differences of T2* in different time–points during the loading.

Parameter	<i>p</i> -value	INTERACTIONS		<i>p</i> -value
TIME	0.263	MM ant	LM ant	0.000 ^a
TIME × LOCATION	0.000 ^a		LM post	0.545
MM ant	0.027 ^a		LM body	1.000
MM post	0.250		MM post	0.0121
MM body	0.760		MM body	0.058
LM ant	0.317	MM post	LM ant	0.165
LM post	0.589		LM post	1.000
LM body	0.228		LM body	1.000
			MM ant	0.121
			MM body	0.000 ^a
		MM body	LM ant	0.000 ^a
			LM post	0.000 ^a
			LM body	0.121
			MM ant	0.058
			MM post	0.000 ^a
		LM ant	LM post	0.083
			LM body	0.012 ^a
			MM ant	0.000 ^a
			MM post	0.165
			MM body	0.000 ^a
		LM post	LM ant	0.083
			LM body	1.000
			MM ant	0.545
			MM post	1.000
			MM body	0.000 ^a
		LM body	LM ant	0.012 ^a
			LM post	1.000
			MM ant	1.000
			MM post	1.000
			MM body	0.121

TIME – T2* in all parts of meniscus in different time points (Comp0 to Comp4); TIME × LOCATION – T2* separately in parts of meniscus in different time points; MM – medial meniscus; ant – anterior; post – posterior; body – meniscal body. *p*-value < 0.05 was considered to indicate significant results.

^aStatistical significance. INTERACTIONS – relationship between parameters in hierarchical linear model.