Multi-parametric MRI characterization of inflammation in murine skeletal muscle

Nathan D Bryant[1], Ke Li[1], Mark Does[2], Daniel Gochberg[1], Thomas Yankeelov[1], Jane Park[3,4], and Bruce Damon[1,4]

[1]*Radiology and Radiological Sciences, Vanderbilt University Institute of Imaging Science, Vanderbilt University, Nashville, TN, United States,* [2]*Biomedical Engineering, Vanderbilt University Institute of Imaging Science, Vanderbilt University, Nashville, TN,* [3]*Molecular Physiology and Biophysics, Vanderbilt University Institute of Imaging Science, Vanderbilt University, Nashville, TN, United States,* [4]*Co-Senior Author*

Introduction: The pathophysiology and structural aberrations associated with chronic muscle diseases, such as idiopathic inflammatory myopathies (IIM) and muscular dystrophies (MD), are complex and include inflammation, the loss of membrane integrity, adipose infiltration and fibrosis in the affected muscle tissue (1,2). Advances in MRI have produced an array of noninvasive measures, including T_2, diffusion, magnetization transfer (MT), and dynamic contrast enhancement (DCE) that are sensitive to these biophysical changes. While it is widely accepted that these are valuable parameters in both research and clinical applications, a direct correlation between the MR-based observations and clinical presentation of IIM or MD is not always present (3). The goal of the current study is to elucidate the biophysical basis for these MR-based observations in a simplified animal model of muscle tissue edema. We used a multi-parametric MR approach to investigate inflammation as an isolated pathological feature in the quadriceps muscles of mice 6 to 8 hours after an injection of λ-carageenan.

Methods: Four healthy C57BL/6J mice received a subcutaneous injection of 1% λ-carageenan (w/v in saline; 0.1 ml) to elicit edema in the anterior compartment of the left thigh (4). MRI data were acquired 6 to 8 hours post λ -carageenan injection, at 4.7T on a Varian Direct Drive MR imager/spectrometer using a 38 mm birdcage coil, with the parameters listed in Table 1. All data analyses were performed in MatLab. High resolution, anatomical images (axial) were used to select regions of interest (ROIs) in the vastus lateralis (VL) muscles of the injected limb ("Edema") and were compared to similar regions in the contralateral limb ("Control"). The effect of inflammation on T_2, indices of diffusion [apparent diffusion coefficient (ADC) and fractional anisotropy (FA)], calculated parameters from quantitative magnetization transfer (qMT), and DCE-MR data were assessed. Diffusion tensor imaging (DTI) data were used to calculate ADC and FA parametric maps. Frequency-selective fat saturation was applied to reduce the signal contribution from fat tissue in the diffusion weighted images. T_2 measurements were made by collecting multi-echo, single slice (MESS) images. Signal intensity data from ROIs of edema and control muscle were fitted to mono- and bi-exponential T_2 relaxation curves, as well as multi-component analysis using a non-negative least squares (NNLS) algorithm. Quantitative magnetization transfer (qMT) data were collected using a selective inversion recovery (SIR) imaging sequence using the methods of Li *et al.* 2010 (5). These data were used to calculate parameter maps of R_{1f} (R_1 of free water pool), P_f/P_m (pool size ratios), and k_{mf} (fast exchange rate). Finally, for DCE-MR data collection, 120µl of Magnevist® (0.1 mmol/kg Gd-DTPA) was auto-injected into each mouse through a surgically placed jugular vein catheter, at an infusion rate of 2.4ml/min. (6), as gradient echo images were continuously acquired for 21 minutes. A washout slope was calculated during the 5 min. following the peak in the signal time course, and was interpreted as an estimate of the interstitial volume's capacity to retain the contrast agent.

Table 1. MRI data acquisition parameters. (See text for abbreviations.)

Dataset	Sequence	TR/TE (ms)	Matrix	Slices	Additional Parameters
Anatomical	FSEMS	2000/12	256 x 256	7	NEX: 4, Slice thickness: 2mm, Freq. Selective Fat Sat.
DTI	FSEMSDW	1200/25	128 x 96	7	10 DWI directions, b-values: 12 and 500 s/mm^2, Freq. Selective Fat Sat., ETL: 2
T2	MESS	2000/9-688	128 x 128	1	Echoes:1-32 (ESP:9ms), Echoes:3-40 (ESP:50ms)
qMT	SIR	6000/10	64 x 64	1	See (Li *et al.* 2010[5])
DCE	GEMS	10/3.1	128 x 128	1	250 images, NEX: 4, Interval: 5.1s, (Loveless *et al.* 2011[6])

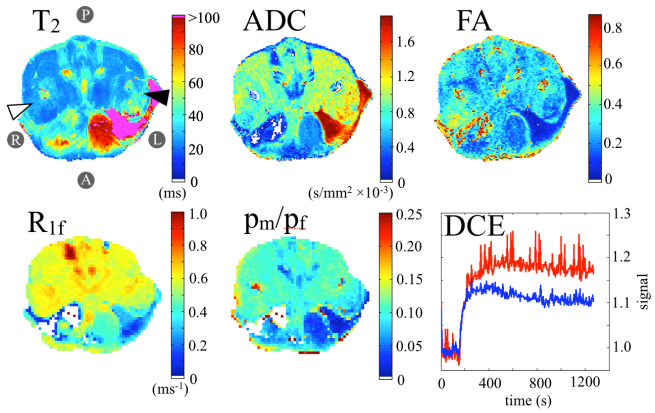

Figure 1. Representative parameter maps for T_2, ADC, FA, R_{1f}, and p_m/p_f in mouse quadriceps muscles. The right limb served as a healthy control (open arrowhead), while the left limb has edema (closed arrowhead). The lower right panel shows the time course of normalized DCE signal intensity for control (blue) and edematous (red) VL muscle.

Results and Discussion: The most consistent indicator of edema was an increase in T_2 (Monoexponential T_2: Figure 1 and Table 2). Bi-exponential fits of the data reveal a second, long T_2 component in the edematous muscle [Control: 24.6±0.46 ms (99%), vs. Edema: 25.4±1.12 ms (88%) + 119.7±17.9 (12%)]. This suggests that the global increase in T_2 is largely due to changes in the extracellular compartment. The hyper-intense region seen on the outside of the left limb is due to fluid accumulation (subcutaneous) after the λ-carageenan injection. Compared to the control limb, a significant increase in ADC was seen in the edematous muscle and the changes were variable depending on the intensity of the inflammation response in each individual mouse. There was also a decrease in p_m/p_f ratio as the free water pool increased in the edematous tissue. We also observed a decreasing trend in R_{1f} in the inflamed muscle, but it was not significant. k_{mf} displayed a trend to move with changes in R_{1f}, but this parameter was particularly sensitive to noise and changes were small and not significant (data not shown). Finally, we observed a longer retention of contrast agent (a less negative washout slope) in the DCE data; this also suggests an increased interstitial volume in the edematous muscle (Figure 1, Table 2). Taken together, these data provide a much more detailed account of the effects of edema on skeletal muscle than any one parameter alone.

Table 2. A multi-parametric comparison of healthy control skeletal muscle and edematous muscle in the Vastus Lateralis of the quadriceps. (‡ p<0.01, * p<0.05)

	T_2 (ms) Mono-exponential	ADC ($\times 10^{-3}$ s/mm^2)	FA	p_m/p_f	R_{1f} (ms^{-1})	Washout$_{Slope}$ ($\times 10^{-5}$)
Control	25.3 ± 1.0	1.18 ± 0.09	0.21 ±0.04	0.12 ± 0.01	0.54 ± 0.08	-7.75 ± 1.54
Edema	37.9‡ ± 4.6	1.35* ± 0.08	0.20 ±0.03	0.06* ± 0.01	0.41 ± 0.07	-3.64* ± 0.85

Conclusion: These studies provide a basis for understanding how inflammation, in isolation, influences the quantitative MRI parameters that are commonly used or proposed to be used to characterize muscle disease so that additional studies, using more complex models of muscle disease, will be able to be fully and properly interpreted.

References [1]Wing *et al.* 2002 AJR 179(4): 989-997, [2]Tidball *et al.* 2005. Curr Opin Rheumatol 17(6):707-713, [3]Park *et al.* 2001 Curr Opin Rheumatol 3(4):334-345, [4]Fan *et al.* 2008 NMR Biomed. 21(6):566-573, [5]Li *et al.* MRM 2010. 64(2):491-500, [6]Loveless *et al.* 2011 MRM (In Press).