

DMD_D.2.2.004

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Evaluation of Muscles by Magnetic Resonance Imaging (MRI)

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1. OBJECTIVE

The study of muscle diseases has evolved from a classical time in which a diagnosis was based on clinical and pathologic features, to a modern period when muscle biopsies were further characterized through histo- and cytochemical techniques, to the current era of molecular diagnosis. With the advent of sophisticated molecular techniques, DMD can be diagnosed non-invasively without the need for muscle biopsy. As a result, baseline and follow-up pathologic data are not typically available to assess disease progression or response to therapy. Other surrogate biomarkers must be utilized to ensure that results of treatment trials are interpreted appropriately.

2. SCOPE AND APPLICABILITY

Most DMD natural history studies have included measurements of muscle strength, joint contractures, and timed function tests. Results from these tests are used to track disease progression and offer insight on clinical milestones, such as the loss of ambulation and the need for ventilatory support. Magnetic resonance imaging (MRI) has been used increasingly to provide meaningful data on the natural history and response to therapy of a number of diseases, including DMD.¹⁻³ Studies have also been done in GRMD.⁴⁻¹¹

3. CAUTIONS

Dogs must be anesthetized. A magnetic resonance imaging unit is required. See METHODS and EVALUATION AND INTERPRETATION OF RESULTS (below) for guidance on interpretation.

4. MATERIALS

Separate personnel are utilized for anesthetic management, the imaging procedure, and image analysis. The imaging procedure was first done with a Siemens 3 Tesla (T) Allegra Head-only System and subsequently with a 3 T Siemens MAGNETOM Trio with Tim Whole Body System, both available through the University of North Carolina-Chapel Hill (UNC-CH) Biomedical Research Imaging Center. Dogs are now imaged with a 3T Siemens Verio MRI unit at Texas A&M. Our animal imaging protocol (**Table 1**) was originally based on one used previously in DMD patients at UNC-CH (Fan J, Howard J, and Lin W, unpublished observations) and has subsequently been modified (see 5.3 and reference 8 for details). This protocol provides excellent anatomic resolution, thus allowing region-of-interest measurements of MRI parameters. Image analysis has been done through the UNC Neuro Image Research and Analysis Laboratories (NIRAL) using the software program ITK-SNAP (<http://www.itksnap.org>)¹² and Insight Segmentation and Registration Toolkit (ITK, <http://www.itk.org>), which provides semi-automatic segmentation of medical images by using image interpolation methods, manual delineation, and image navigation. We have focused on the proximal pelvic limb.

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Table 1. Pulse Sequences Used in GRMD MRI									
	TR (ms)	TE (ms)	FOV (mm)	Thickness (mm)	#Slices	Matrix	Orientation	Time	Resolution (mm)
T2w	3,000	406-409	256	1	160	256x256	transverse	16'38"	1X1X1
T2fs	3,000	406-409	256	1	160	256x256	transverse	16'38"	1X1X1
T2 Value #1	3,040	7-70; Δ7	256	2	200	256x256	transverse	7'10"	1X1X2
T2 Value #2	3,170	20-100; Δ20	230	7	300	256x256	transverse	7'28"	1X1X7
PD	200	2.43, 8.49, 12.13, 15.77	256	5	16	256x256	transverse	9'18"	1x1x5

TR = Repetition time; TE = Echo time; FOV = Field of View; T_{2w} = T2-weighted; T_{2fs} = T2-weighted fat-suppressed; PD = Point Dixon.

5. METHODS

5.1. Anesthetic protocol

20-30 minutes prior to anesthesia induction:

- Pre-anesthetic agents:
 - Atropine sulfate (0.04 mg/kg, IM)
 - Acepromazine maleate (0.02 mg/kg, IM) for dogs weighing greater than 5 kg
 - Butorphanol tartrate (0.4 mg/kg, IM)

Anesthetic induction:

- Anesthetic agents:
 - Propofol (up to 3 mg/kg, IV – slowly!) for dogs weighing greater than 5 kg
 - Isoflurane or sevoflurane (to effect, inhaled) (avoid masking down)

Anesthetic monitoring:

During anesthesia, ECG, heart and respiratory rate, blood pressure, end tidal (Et)CO₂, and saturation of hemoglobin by peripheral oxygen (SpO₂) are monitored continuously with a pulse oximeter (Cardell Multiparameter Monitor 9405, Minrad International, Inc, Orchard Park, NY). These values, as well as capillary refill time and anesthetic setting, are recorded every 15 minutes.

Anesthetic recovery:

Monitor affected and carrier dogs closely during anesthetic recovery until fully awake and in sternal recumbency.

- Naloxone (up to 0.4 mg/kg, SQ) for rapid recovery; given in ½ dose increments (1st dose given while the dog is still intubated and breathing O₂; 2nd dose, if necessary, after extubated and/or if respiration drops below 7 breaths per minute).

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5.2. Position the dog in ventral recumbence, with the pelvic limbs extended caudally into the gradient coil (gradient strength 40mT/m) within the MRI gantry (**Figure 1**). Care should be taken to position the limbs symmetrically so as to facilitate side-to-side comparison of the images. Note, in the case of inevitable asymmetry, MRI image software can be used to align the limb in 3-D planes.



Figure 1. Anesthetized dog positioned in sternal recumbence with the pelvic limbs in the MRI coil/gantry.

5.3. T₂-weighted image sequences without (T₂w) and with fat saturation (T₂fs) are acquired using a variable-flip-angle turbo spin echo (TSE) sequence. The time between the excitation pulse and the center of k-space is 400 ms. Importantly, the contrast is not determined only by the TE (400 ms), but also by the flip angle evolution scheme. Although a traditional TSE sequence would have very little signal at 400 ms, the variable flip angle sequence is similar in principle to hyper-echo. The hyper-echo reduces the specific absorption rate (SAR), while the variable flip angle sequence allows long TE times.^{13,14} A multi-spin-echo T₂ (MSE-T₂), using a ten-echo Carr-Purcell-Meiboom-Gill sequence, is acquired to calculate the T₂ value map. Analysis of the images is completed in three modules: muscle segmentation, pre-processing, and biomarker analysis. As a prerequisite, we first segment the major proximal pelvic limb muscles in the MRI images. All proximal pelvic limb muscles are segmented but only five slices at the midfemur are analyzed and averaged.^{8,10}

5.4. Images have been uploaded via a password-protected procedure to the NIRAL. Investigators have remote access to the datasets via encrypted secure shell log-in directly to the NIRAL file server. All datasets, as well as derived data computed as part of the processing procedure, are maintained on RAID 5 disk storage to protect against single disc failures. Data are backed up daily, with bi-weekly offsite storage of the backup tapes.

5.5. Image analysis has been done through the NIRAL using the software program ITK-SNAP (<http://www.itksnap.org>)¹² and Insight Segmentation and Registration Toolkit (ITK, <http://www.itk.org>). We initially manually segmented individual muscles of the proximal pelvic limb and averaged a total of five consecutive images at the level of the mid-femur. However, we have found that a relatively straightforward interpolation method provides data more representative of the entire muscle.⁸

6. EVALUATION AND INTERPRETATION OF RESULTS

Principal MRI changes in DMD include an increase in T₂ and decrease in T₁ relaxation times due to accumulation of fat in affected muscles and an associated increase in whole body fat and decrease in muscle mass. Objective grading systems,^{17,18} and quantitation of parameters such as contractile vs. non-contractile tissue content,³ allow data to be

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compared over the course of the disease. Results have correlated with those of clinical functional tests. One serial study suggested that MRI is more sensitive than functional tests in predicting disease progression.¹⁸ MRI has also been used to monitor DMD disease progression in treatment trials.^{19,20} MRI changes correlate with pathologic lesions.²¹

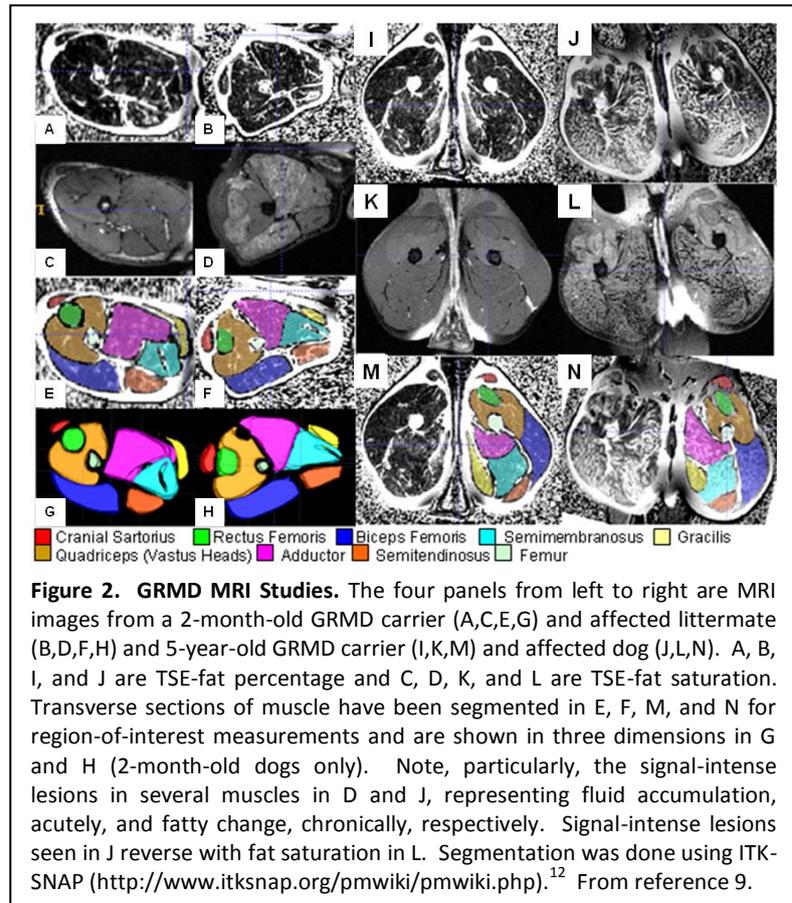
The potential role of MRI as a biomarker in GRMD has been reported in both natural history and preclinical papers.⁴⁻¹¹ In the first study, the thoracic limbs of 2-month-old GRMD dogs scanned at 4 T had an abnormally high T2-weighted/T1-weighted signal ratio, greater T2-weighted image heterogeneity, and more pronounced signal enhancement post-gadolinium contrast.⁴ These same authors extended this work to assess distal muscles of both the pelvic and thoracic limbs at 2, 4, 6, and 9 months.⁷ Standard and fat-saturated T1, T2, and proton density-weighted images, as well as gadolinium enhancement, were assessed. GRMD muscles were variably affected and, when compared with normal, had increased T1 and T2 values and more intense gadolinium enhancement. Consistent with our own findings (below), the differential between normal and GRMD findings did not progress significantly with age and there was minimal increase in fat signal.

An additional study of 3-month-old GRMD dogs showed increased signal intensity on T2-weighted images in which the fat signal was suppressed, increased T2 values, and greater enhancement with gadolinium, all consistent with inflammation associated with early necrosis.⁵ T2 signal was decreased in GRMD dogs treated systemically with morpholinos compared to age-matched untreated dogs, supporting a role for MRI as a biomarker in preclinical studies.⁶ Another study showed that increased T2 signal could be used to track sites of AAV-micro-dystrophin construct injection.²²

We have assessed MRI in GRMD dogs both longitudinally and at single time points.⁸⁻¹⁰ Consistent with prior studies, signal-intense lesions, presumably corresponding to fluid accumulation in necrotic lesions, have been seen on fat suppressed, T2-weighted images in younger dogs, while increased fat deposition has been seen at later ages (**Figure 2**). The severity of these changes has varied among muscles. We have focused our studies on the 3-12 month age period, since this time frame has most commonly been used for preclinical studies by our group and others (see reference 9 for discussion). The principal parameters assessed have included muscle volumes, T2 mapping values, and several texture analysis features, including a first-order intensity histogram texture feature (entropy) and two high order run length matrix features (short run emphasis [SRE] and run length non-uniformity [RLN]).¹¹ These texture analysis features are assessed as potential markers of patchy lesions such as necrosis.^{8,10,15,16} Based on the mathematical model, we refer to short run emphasis as the Small Lesion Index (SLI) and non-uniformity as the Heterogeneity Index (HI). Both SLI and HI use the run-length matrix method. Compared to histogram-based biomarkers that use intensity data only, the run-length matrix method also takes into account the spatial distribution and intensity of the voxels. A gray-level 'run' is defined as a set of consecutive voxels of similar intensity level in a given direction within a predefined similarity range. This is run in a 3-dimensional matrix and is intended to detect lumps of hyper-intensity in MRI.

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In a natural history study, the proximal pelvic limbs of ten GRMD and eight normal dogs were scanned at 3, 6, and 9–12 months of age.¹⁰ Several MRI imaging and texture analysis biomarkers were quantified in seven muscles. Almost all MRI biomarkers readily distinguished GRMD from control dogs; however, only selected biomarkers tracked with longitudinal disease progression. The biomarkers that performed best were full-length muscle volume and a texture analysis biomarker, termed heterogeneity index. The biceps femoris, semitendinosus and cranial sartorius muscles showed differential progression in GRMD versus control dogs.



MRI features in GRMD dogs showed dynamic progression that was most pronounced over the 3- to 6-month period. Volumetric biomarkers and water map values correlated with histopathological features of necrosis/regeneration at 6-months.

We have seen increased signal intensity in muscles associated with an apparent innate immune response in dogs treated with AAV-9 and a codon-optimized human mini-dystrophin.²³ Another study in our lab demonstrated changes in texture features subsequent to treatment with a proprietary NF- κ B inhibitor.¹¹

Potential Advantages/Disadvantages of the Methodology

Advantages

MRI is a non-invasive technique that can be used serially to quantitate end-points of both acute (edema) and chronic (fat deposition, volumetric changes) disease.

Disadvantages

Instrumentation and “scanner time” for sequential studies are expensive. There is considerable variation among MRI units and protocols, which could limit comparison of data. Results of MRI have not been correlated well with functional and pathologic studies.

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