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Appendix E1

MRI and ^{1}H-MR Spectroscopy Acquisition

MRI and ^{1}H-MR spectroscopy data were acquired from the shoulder, upper arm, forearm, thighs, and lower legs from three centers using either a 3T Achieva Quasar Dual MRI instrument (Philips, Best, the Netherlands), a 3T Magnetom TIM Trio MRI instrument (Siemens, Erlangen, Germany), or a 3T Magnetom Verio MRI instrument (Siemens, Erlangen, Germany). Radiofrequency coil configurations differed between centers. Upper extremity measures were performed using either a SENSE eight channel flex coil (invivo), a Siemens six channel body Array Flex coil, or an 18 channel Siemens Body Matrix coil were used. Thigh and lower leg data were acquired using a SENSE eight channel flex coil, two-channel surface coil, a body matrix array coil, or transmit-receive extremity coil.

The dominant arm was defined as the arm that the participant (or parent) indicated that they use to write. For shoulder scans, the coil was centered at or slightly superior to the anterior axillary fold and covered the top of the shoulder. For the upper arm, the coil was centered at approximately 1/2 of the distance from the elbow joint (lateral epicondyle) to the shoulder joint (acromion process). For the forearm, the coil was centered over the proximal 1/3 of the forearm muscles. To allow comparisons between upper and lower extremity muscles, MRI T2 data were acquired from lower extremity muscles as described previously for the thigh and lower leg (6–8,14,15,20). The participants typically viewed a movie during the MRI/^{1}H-MR spectroscopy examination.

MRI T2-weighted multislice spin echo (SE) axial images were acquired from each region (4–6 slices, 7 mm slices, 3.5 mm gap; 16 TE's, 20–320 msec evenly spaced; TR = 3 s). Multipolar gradient echo (GE) images were acquired in two sets of 4 different echo times (TR = 430; TE, Philips: initial TE 3.54 and 4.6 ms, delta 4.6 ms; Siemens, initial TE 3.59 and 4.78; delta 4.78 ms) over 13–25 axial slices (4 mm slice thickness, 2 mm gap) with a 20° flip angle. Single voxel ^{1}H-MR spectroscopy data were acquired to measure fat fraction and $^{1}H_2O$ T2 using stimulated-echo acquisition mode (STEAM) from the deltoid and biceps brachii (4 repeated TE's nonlinearly spaced from 11–243 ms; TR = 9 s; NA = 4) (8). Voxel size was optimized for each individual's muscle size (biceps brachii: 2340 ± 1266 mm^3; deltoid 4689 ± 2625 mm^3).

The field of view (FOV) was optimized for each participant, and axial slices were oriented perpendicular to the long bone (humerus, radius, femur, tibia). Generally, the FOV was centered on the maximal cross-sectional area of the extremity region. For the shoulder region, the center of the FOV in the foot-head direction was located halfway between the most distal portion of the deltoid on the upper arm (insertion of the deltoid on the tuberosity of the humerus bone) and the top of the humeral head. For the upper arm region, the FOV was centered between the most distal portion of the deltoid and the epicondyles of the humerus. For the forearm, the FOV was centered on the widest girth of the forearm. The thigh and lower leg FOV were prescribed as described previously (8). The total scanning was performed over two sessions (upper and lower extremity) performed within 24 hours, with each session taking approximately 60 minutes.

After the MRI and ^{1}H-MR spectroscopy examination was complete, each participant performed upper extremity functional evaluation tests of his dominant arm consisting of the Performance of Upper Limb (PUL), version 2.0 (11,16,17), the Brooke Upper Extremity Scale (18), and strength testing using MyoGrip and MyoPinch (Institute of Myology, Ateliers Laumonier, France) (19). The tests were administered by trained evaluators who followed standardized operating procedures and gave instructions to the participants. The PUL test (PUL versus 2.0) consists of 22 items, testing the three different segments of the arm: shoulder (high PUL), upper arm (mid PUL), and forearm (Distal PUL) (11). Total scores ranged from 0–42, with a score of 0 implying no use of the arms/hands, and a score of 42 implying fully functional arms, including the ability to lift one kg above shoulder height. The Brooke Upper Extremity Scale consists of grades 1–6, with grade 1 indicating the ability to fully abduct the arms until the hands touch above the head and grade 6 indicating an inability to raise the hands to the mouth and no functional use of the hands (18). Both MyoGrip and MyoPinch are sensitive tools that have been designed to measure strength in the individuals with DMD (19). Participants were asked to squeeze the tool as hard as possible with their full hand (MyoGrip) or pinch with their thumb (MyoPinch). Both of these strength tests were performed three times with a 30 second break after each trial. If the third trial had the highest force, additional trials were completed to ensure that the participant's maximal effort had been recorded. The highest value of all trials was measured.

Quality Assurance Procedures and Analyses Landmarks

The protocols were standardized between centers by: 1) developing standardized methods at the start of the study with an investigators meetings in which personnel from all acquisition centers attended; 2) developing detailed written instructions describing participant set-up, sequence details, voxel and FOV placement; and 3) MRI and ^{1}H-MR spectroscopy system operators were certified for this study by: attending training sessions (in person or webinar), demonstrating appropriate voxel placement with a powerpoint assignment and successfully scanning a quality assurance phantom (8) and a practice volunteer participant. During the study, data acquired were continually reviewed during biweekly conference calls to ensure acceptable data quality and consistency of acquisitions among centers. Sequences utilized in this study have also been validated across centers using phantoms and traveling participants in which leg/thigh were imaged (8).

During imaging analyses, the landmark chosen for the deltoid was the most distal slice in which the lateral head of triceps brachii was visible, and two slices proximal to that. The landmark for the upper arm muscles (biceps brachii, triceps brachii) was the most distal slice in which the deltoid was visible, and two slices distal to that. The landmark for the forearm analysis was the most distal slice in which the anconeus was visible, and two slices distal to that. For the lower extremity, the center slice was in the region in which the most proximal slice that the flexor digitorum longus was visually present for the lower leg and the biceps femoris short head for the thigh. The muscles of interest were carefully drawn within the borders to avoid any potential contamination of intermuscular fascia. In some scans, blood flow caused phase-encoding artifacts in the T2-weighted spin echo images. These artifacts produced regions of low-confidence T2 values in the calculated T2 maps. Since the phase-encoding direction during acquisition was anterior-posterior, these regions were confined to distinct columns in the T2 maps. These regions were identified by having a trained reader (H.A. [7 years experience with MRI analysis], K.V. [22 years], R.J.W. [10 years], S.C.F. [14 years], W.T. [11 years]) visually

inspect each T2 map and note the left and right boundaries of the column of pixels affected by the blood flow artifact. Pixels within the blood-flow artifact regions that were also included in muscle regions of interest were excluded from the final average T2 calculation.

The manual tracing was double analyzed by analyzers blinded to each other's results, and when the data did not reach quantitative agreement a third analysis was performed. Quantitative agreement was reached when *either* the absolute difference in fat fraction was 2% or less, or the intrareader coefficient of variation was 5% or less. When the data did not reach quantitative agreement a third analysis was performed. The average of the two closest data sets were incorporated. The analyzers were blinded to the group that the participants were in (ie, control/DMD and age group), and to the other analyzer's muscle outlines. MRI T2 and chemical shift-encoded imaging data were segmented on an ongoing basis as part of the readers' daily responsibilities.