EXAMINATION OF DISEASE PROGRESSION IN UPPER AND LOWER LIMB MUSCLES IN BOYS WITH DMD USING FUNCTIONAL CLINICAL ENDPOINTS AND MAGNETIC RESONANCE IMAGING

By

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To my parents
ACKNOWLEDGMENTS

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<tr>
<td>AF</td>
<td>Anterior forearm</td>
</tr>
<tr>
<td>BB</td>
<td>Biceps brachii</td>
</tr>
<tr>
<td>BFLH</td>
<td>Biceps femoris long head</td>
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<tr>
<td>BMD</td>
<td>Becker muscular dystrophy</td>
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<tr>
<td>BRA</td>
<td>Brachialis</td>
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<tr>
<td>DMD</td>
<td>Duchenne muscular dystrophy</td>
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<tr>
<td>DEL</td>
<td>Deltoid</td>
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<td>FID</td>
<td>Free induction decay</td>
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<tr>
<td>GRA</td>
<td>Gracilis</td>
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<tr>
<td>INFRA</td>
<td>Infraspinatus</td>
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<tr>
<td>JHFT</td>
<td>Jebsen Hand Function test</td>
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<tr>
<td>MD</td>
<td>Muscular dystrophy</td>
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<tr>
<td>MFM</td>
<td>Motor function measure</td>
</tr>
<tr>
<td>MG</td>
<td>Medial gastrocnemius</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>PER</td>
<td>Peroneals</td>
</tr>
<tr>
<td>PF</td>
<td>Posterior forearm</td>
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<tr>
<td>PUL</td>
<td>Performance of upper limb</td>
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<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SUB</td>
<td>Subscapularis</td>
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<tr>
<td>SOL</td>
<td>Soleus</td>
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<tr>
<td>STS</td>
<td>Supine to stand</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>-------------</td>
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</tr>
<tr>
<td>$T_1$</td>
<td>Longitudinal relaxation time</td>
</tr>
<tr>
<td>$T_2$</td>
<td>Transverse relaxation time</td>
</tr>
<tr>
<td>TA</td>
<td>Tibialis anterior</td>
</tr>
<tr>
<td>TB</td>
<td>Triceps brachii</td>
</tr>
<tr>
<td>TP</td>
<td>Tibialis posterior</td>
</tr>
<tr>
<td>TFTs</td>
<td>Timed function tests</td>
</tr>
<tr>
<td>VL</td>
<td>Vastus lateralis</td>
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<tr>
<td>6 MWT</td>
<td>Six minute walk test</td>
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EXAMINATION OF DISEASE PROGRESSION IN UPPER AND LOWER LIMB MUSCLES IN BOYS WITH DMD USING FUNCTIONAL CLINICAL ENDPOINTS AND MAGNETIC RESONANCE IMAGING

By
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Chair: Krista Vandenborne
Major: Rehabilitation Science

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder, usually affecting males. It is characterized by progressive weakness of muscles and loss of ambulation around 10-12 years of age, without any steroid treatment. Once boys lose their ambulation, they become heavily dependent on their upper limbs for their activities of daily living; however, the decline in upper limb function is not very well studied. There is no cure for this disease yet and boys with DMD usually die in their second or third decade of life; however, there are many ongoing clinical trials hoping to find a cure for this disease. Unfortunately, several clinical trials have failed. Potential contributing reasons for such failure have been attributed to a lack of both comprehensive natural history data and sensitive outcome measures. Thus, there is a dire need for more natural history studies that describe the disease progression in both upper and lower limbs across a range of ages. Moreover, there is a need for sensitive outcome measures that are not dependent on the motivation of the individuals with DMD and are more robust, objective, and quantitative. DMD patients typically spend most of their life in a wheelchair. Clinical outcome measures that can evaluate disease progression in
this non-ambulatory population would be invaluable in clinical trials. This would enable the non-ambulatory DMD population to participate in clinical trials. Therefore, this doctoral dissertation focuses on three studies where natural history of disease progression was evaluated in both upper and lower limbs in both ambulatory and non-ambulatory individuals with DMD, using functional outcome measures and magnetic resonance imaging.
Muscular dystrophies (MDs) are heterogeneous, genetic muscle disorders\textsuperscript{1-5}. Genetic mapping techniques have helped identify more than 50 diseases, caused by specific gene mutations, which constitute MDs\textsuperscript{1, 6, 7}. Muscle histology has identified changes in fiber size, the presence of necrotic areas in muscle and subsequently, fatty infiltration in MDs\textsuperscript{8-10}. MDs are classified according to their phenotypic features, inheritance pattern, onset age, and the rate of disease progression\textsuperscript{6, 9}. They present clinically with progressive muscle degeneration and weakness, which affects different muscles in different variations\textsuperscript{1, 11-14}. DMD is the most common form of muscular dystrophy among children\textsuperscript{9, 15}.

**Duchenne Muscular Dystrophy**

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder, caused by out-of-frame mutations in the dystrophin gene, leading to an absence of the dystrophin protein\textsuperscript{16-19}. It has an incidence of 1 in 3500-5000 live male births\textsuperscript{13, 16, 20-23}. It mostly affects males, who are diagnosed between the ages of 3 - 5 years when their motor milestones are delayed compared to those of their peers\textsuperscript{9, 13, 24}. Females are non-symptomatic carriers most of the time, but in 5% - 22% of the cases they exhibit clinical symptoms ranging from mild muscle weakness to severe cardiac involvement\textsuperscript{25-29}. DMD is diagnosed by increased serum creatine kinase (CK) levels, muscle biopsy, genetic testing, and observed clinical features\textsuperscript{2, 13, 30, 31}.

**Inheritance of DMD**

DMD is inherited in X-linked recessive fashion. Since females have two X chromosomes, in the event there is a mutation in one of them, they still have the second
X chromosome to produce a normal dystrophin gene. Therefore, female carriers are usually unaffected, barring rare cases of abnormal X chromosome inactivation. Males, on the other hand, have only one X chromosome. Thus, mutation in their X chromosome would always result in DMD. Therefore, two-thirds of the DMD cases are the result of an X-linked recessive pattern and one-third of cases are due to spontaneous mutations.

Pathogenesis of DMD

The dystrophin gene, the largest gene known, is present on the Xp21 chromosome. It is approximately 2.2-2.3 megabases in size. Various mutations can occur on this gene, such as deletions (60% - 65%), duplications (5% - 10%), and point mutations (remaining cases). The “reading-frame hypothesis” states that in DMD, there are out-of-frame mutations that cause early stoppage of protein translation and leads to an absence of dystrophin. This characteristic of DMD differentiates it from Becker Muscular Dystrophy (BMD), where in-frame mutations occur and form abnormal but partially functional dystrophin protein. The dystrophin protein is normally present in the inner surface of sarcolemma. The main functions of dystrophin are stabilizing muscle cell membranes and linking internal cytoskeleton to extracellular matrix. Dystrophin acts like a shock absorber during contraction of the muscle. In DMD, due to the absence of dystrophin, there is an abnormal permeability and fragility of cell membranes, which increases the chance of muscle damage at any given stress level and is particularly pronounced in high force muscle contractions.
The various mechanisms involved in muscle damage are mechanical damage, increase in intracellular calcium level, impairment of signaling molecules, and increase in immune cells. They are described as below:

Mechanical damage: Dystrophin helps in the transmission of force between intracellular cytoskeleton and extracellular matrix. A lack of dystrophin causes increased stress on cell membrane, even with normal muscle contractions\textsuperscript{6, 19, 38, 41, 42}. It also leads to an increase in the permeability of the muscle membrane\textsuperscript{41, 42}.

Increase in intracellular calcium level: Damage of a muscle membrane causes an increase in intracellular calcium concentration\textsuperscript{6, 19, 20, 38, 41-43}. This leads to proteases activation, especially of calpains, which further causes necrosis of fibers\textsuperscript{6, 19, 38, 42}.

Impairment of signaling molecules: Dystrophin supports signaling molecule neuronal nitric oxide synthase (nNOS), through its dystrophin-glycoprotein complex (DGC)\textsuperscript{6, 42}. nNOS forms nitric oxide (NO), whose primary function is to maintain vascular tone by reacting with free radicals\textsuperscript{42}. Absence of dystrophin causes downregulation of nNOS and its displacement into cytoplasm from the plasma membrane\textsuperscript{19, 38, 42}. This leads to reduced production of NO and causes muscle damage associated with free radicals\textsuperscript{20, 42, 44}. It also leads to vasoconstriction and ultimately ischemia of the muscle\textsuperscript{19, 38, 44}.

Increase in immune cells: Increased inflammation is also considered to play a role in the pathogenesis of DMD. There are increased levels of CD4+ and CD8+ T cells found in the dystrophic muscles, causing more muscle damage and fibrosis\textsuperscript{20, 30, 38, 45}.

Boys with DMD are born with normal muscle function\textsuperscript{30}. As they age, there is decrease in functional mass and a degeneration of the muscle fibers, followed by
fibrofatty tissue replacement. There is appearance of fibers with central nuclei, the marker of regeneration. By the time a boy reaches his teenage years, reduced muscle fibers are present and fibrofatty tissue is highly prevalent\textsuperscript{6, 30}.

**Clinical Features of DMD**

DMD patients exhibit progressive muscle weakness, from the proximal to distal direction\textsuperscript{12, 16, 46-49}. Boys with DMD exhibit waddling gait, clumsiness, flat feet, and an inability to walk until 18 months of age\textsuperscript{27, 30}. When they start walking around 18 – 24 months of age, boys have a wide-based gait, difficulty running, and increased lordosis in the spine due to weakness in the hip extensors\textsuperscript{2, 13, 30, 48}. Moreover, they struggle with getting up from the floor, jumping, walking on their toes, and they fall frequently\textsuperscript{48}. They also often exhibit head lag when they sit up from a lying position due to weakness of neck flexor muscles\textsuperscript{13, 48}. When these boys stand up from a supine position, they tend to place their hands on their knees, exhibiting what is referred to as the “Gowers’ sign”\textsuperscript{2, 13, 30}. They also exhibit enlargement of calf muscles referred to as pseudo hypertrophy, when fatty tissue replaces degenerated muscle\textsuperscript{32}.

DMD boys have increased difficulty walking and lose the ability to both rise from the floor and climb stairs\textsuperscript{27}. Boys typically lose the ability to walk by the age of 10 – 12 years\textsuperscript{16, 20} without steroid treatment. However, they can still sustain their posture\textsuperscript{27}. Scoliosis is usually seen, which affects their respiration and worsens as a boy becomes increasingly non-ambulatory\textsuperscript{27, 48, 49}. Upper limb functions decline at a later stage which leads to difficulty in performing activities of daily living such as eating, bathing, brushing teeth, etc. At this point they also find it difficult to sustain their posture\textsuperscript{27}.

Other clinical features, such as joint contractures, are usually first seen in the ankles and then in the hips, knees, elbows, and wrists. Long bone fractures due to falls
and osteoporosis are other orthopedic complications present in boys with DMD\textsuperscript{48, 49}. Impaired cognition, attention deficit hyperactive disorder, autism spectrum disorder, and obsessive compulsive disorder have also been reported in some cases\textsuperscript{13, 50}. As the disease progresses, boys become increasingly dependent on others for assistance with their activities of daily living\textsuperscript{51}. Dilated cardiomyopathy is usually seen after the age of 10 years and is evident in all patients of 18 years or older\textsuperscript{48, 52}. As DMD boys age, respiratory and cardiac problems continue to worsen, ultimately leading to their death\textsuperscript{16, 20, 27, 30, 48}.

**Standard of Care for DMD**

DMD is currently incurable but many experimental therapeutic strategies are currently in clinical trials\textsuperscript{20, 41}. The current management of DMD focuses on the multi-disciplinary approach for treating symptoms and improving the quality of life and function\textsuperscript{49, 53-55}.

**Musculoskeletal management.** Contractures are commonly seen in these patients, so appropriate physical therapy is essential to maintain range of motion. In physical therapy, stretching, proper positioning of the joints, and range of motion exercises are recommended in order to prevent contractures. Orthoses can be used either to prevent or minimize contractures. Spinal corset can be used to reduce scoliosis. In a few cases, surgery can be considered as an option to correct contractures or scoliosis\textsuperscript{6, 49, 54}. There is not enough evidence to suggest the type of exercise that may be beneficial in this population\textsuperscript{54, 56}. Swimming is recommended for aerobic conditioning of the muscles\textsuperscript{49, 54}. Since fractures are commonly seen in these patients, splints or casts can be used for fracture healing\textsuperscript{54}. Vitamin D and calcium supplements can be considered for maintaining bone health\textsuperscript{48, 49}. 19
**Respiratory management.** Airway clearance techniques, including manual and mechanical assisted cough, can be used to prevent respiratory complications. Respiratory infections can be treated with antibiotics and chest physiotherapy\textsuperscript{48, 49, 57, 58}. Non-invasive ventilation is suggested when patients start experiencing hypoventilation\textsuperscript{48, 49, 54, 57, 59}.

**Cardiac management.** Angiotensin-converting-enzyme (ACE) inhibitors are considered to be the first line drugs in cardiac management\textsuperscript{12, 20, 48, 49, 54, 58}. Beta blockers can be used with ACE inhibitors for even more beneficial results\textsuperscript{12, 58}.

**Glucocorticoids therapy.** Glucocorticoids have been shown to delay the loss of ambulation in DMD patients by 1-2 years\textsuperscript{60-62}. They also reduce the chance of scoliosis\textsuperscript{54}. Currently, prednisone with a daily dose of 0.75 mg/kg or deflazacort with a daily dose of 0.9 mg/kg are the recommended drugs\textsuperscript{4, 27, 48, 49, 55, 63}. Weight gain, behavioral problems, vertebral and long bone fractures, stunted growth, puberty delay, hypertension, and cataracts are some of the common side effects seen in glucocorticoid treated boys\textsuperscript{6, 20, 24, 39, 49, 64}.

**Experimental Therapeutic Strategies**

There are many experimental therapeutic strategies in preclinical and clinical trials that look promising for slowing down disease progression in DMD in the future. Below is a review of some of the major strategies.

**Stem cell therapy.** Muscle stem cells (myoblasts) have a capacity for differentiation into muscle cells. Promising results were seen when myoblasts were transplanted into immunosuppressed mdx mice, a mouse model for DMD\textsuperscript{6, 11, 19, 48}. Unfortunately, these results could not be replicated in boys with DMD because of their
immune responses\textsuperscript{6, 19}. Another limitation of myoblasts is that they do not travel long distances in muscles and die soon after injection\textsuperscript{24}.

**Gene replacement therapy.** The large size of the dystrophin gene and the relatively limited packaging capacity of viral vectors necessitate the creation of micro and mini-dystrophin genes for delivery\textsuperscript{19, 20, 65, 66, 67}. Recombinant adeno associated viral (rAAV) vectors are used to carry micro and mini-dystrophins\textsuperscript{11, 19, 48}. This therapy showed successful results in mdx mice, but failed to replicate the results in boys with DMD. The reason behind its failure is the immune responses shown by boys with DMD\textsuperscript{19, 20}. Recently, a study showed that delivery of micro-dystrophin using adeno associated viral vector could reduce inflammation and fibrosis in skeletal muscles of DBA/2J-mdx mice, a severe mouse model for DMD\textsuperscript{68}; though this therapy is not tested clinically yet.

**Stop codon read through drugs.** Aminoglycoside antibiotics like gentamicin help in reading through premature stop codons. Gentamicin has its application in nonsense mutations in the dystrophin gene\textsuperscript{11, 19, 24}, but its major disadvantage is that it causes toxicity in DMD patients\textsuperscript{19, 24, 55}. PTC124 (ataluren) is another synthetic drug that can read through premature stop codons, thus allowing full translation and formation of functional protein\textsuperscript{19, 20, 48, 49, 58}. It is a well-tolerated drug and is also used for nonsense mutations\textsuperscript{24, 48}. Unfortunately, in phase 2b of its clinical trial, ataluren failed to show a significant effect in its primary outcome measure – the six minute walk test (6 MWT) which caused the clinical trial to be terminated\textsuperscript{19, 24, 49}. Significant effect was seen in DMD patients who received a low dose of the drug, after subsequent post hoc analysis\textsuperscript{24}. Based on this, ataluren has been conditionally approved in Europe for DMD.
patients and started its phase 3 clinical trial\cite{20,24}. Recently, phase 3 clinical trial results demonstrated that ataluren showed a 15 m improvement in the 6 MWT from baseline to 48 weeks but with no statistical significance\cite{55,69,70}. The FDA had initially refused to review the drug’s application, stating that the drug does not show efficacy\cite{55,71}, however, they have recently agreed to conduct a review.

**Exon skipping drugs.** Exon skipping drugs use anti-sense oligonucleotides to skip exons in pre-mRNA, restoring the reading frame of dystrophin. These drugs form a partially functional dystrophin thus converting DMD to BMD\cite{20,24,48,49}. Successful results have been observed in mdx mice\cite{19}. Drisapersen and eteplirsen are two of the drugs in clinical trials for DMD patients. Both of them skip exon 51 and can be used in 13% of DMD population\cite{19,24}. Drisapersen failed to show significant improvement in the 6 MWT in its phase 3 clinical trial over a 48 week treatment\cite{24,70,72,73}, which has led to the discontinuation of further clinical trials. Eteplirsen showed promising results when tested on 12 patients in phase 2 trial and it is currently completing a phase 3 clinical trial\cite{20}. Recently, the FDA has granted conditional approval to eteplirsen\cite{55,74-76}.

**Utrophin upregulation.** Utrophin is structurally very similar to dystrophin\cite{6,11,19,20,24,70}. It is thought that utrophin can substitute for dystrophin deficiency, when it is upregulated\cite{6,11,30,48}. Utrophin upregulation has shown effective results in mdx mice and the golden retriever muscular dystrophy, a dog model for DMD\cite{19}. Clinical trials for the drug SMT C1100 are ongoing to upregulate utrophin expression in patients with DMD\cite{19,20,24,48}. A phase 1 clinical trial showed that this drug is well tolerated by boys with DMD\cite{55}. The drug is currently in phase 2 of its clinical trial\cite{55,62}. 

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**Phosphodiesterase 5A (PDE5A) inhibitors.** Absence of dystrophin leads to the displacement of neuronal nitric oxide synthase (nNOS) from the dystrophin glycoprotein complex (DGC), resulting in decreased nitric oxide (NO) production. Reduction in NO leads to muscle ischemia. PDE5A inhibitor drugs like sildenafil and tadalafil have a vasodilatory effect that could help reduce muscle ischemia. After showing promising results in mdx mice, a clinical trial was initiated. Unfortunately, sildenafil showed no improvement in cardiomyopathy in a phase 2, double-blind, randomized study of patients in DMD.

**NF-κB inhibitors.** Activation of NF-κB results in the degradation of muscle proteins, which produces pro-inflammatory mediators like cytokines and chemokines. Activated NF-κB is seen in patients with DMD. The drug Edasalonexent (CAT-1004) has been developed to inhibit the NF-κB pathway and is currently in a phase 1/2 clinical trial. This drug was shown to be safe and well-tolerated in patients with DMD in phase 1 of its clinical trial. However, it was recently reported that the drug did not show significant improvement in boys with DMD after 12 weeks of treatment. Its long-term effect is currently being investigated.

**Myostatin and insulin growth factor-1.** Myostatin inhibits muscle growth. Myostatin antibodies have been used in reducing muscle pathology in mdx mice but similar results were not found in DMD patients. In a recent phase 2 clinical trial, myostatin inhibitors showed a trend of maintaining performance on the 6 MWT in the treatment group, but no statistical difference could be found between placebo and treated groups. Insulin growth factor-1 (IGF-1) helps in muscle growth. Its use in increasing the size of muscle fiber is currently in clinical trial.
**Histone deacetylase inhibitors.** Histone deacetylase (HDAC) inhibitors help in the regeneration of muscle and reduce fibrosis and fatty infiltration in mdx mice. Givinostat, a HDAC inhibitor, has shown to improve muscle growth in mdx mice. This drug is starting a phase 2 clinical trial\textsuperscript{20, 76}. It is assumed that this drug delays the progression of DMD in patients at an early stage of the disease\textsuperscript{79}.

**CRISPR/Cas9.** CRISPR/Cas9 is used for gene editing, where Cas9 nuclease is used to cleave DNA sequences with the help of a single guide RNA\textsuperscript{80}. It has already been shown that this method can re-establish expression of dystrophin in patient-derived induced pluripotent stem cells (iPSCs) and in murine iPSCs\textsuperscript{81}. It can restore partial dystrophin expression and enhance muscle function in mdx mice\textsuperscript{80, 82-84}. However, its efficacy and safety have not been tested in patients with DMD so far\textsuperscript{81}, though it is projected that this method can treat about 80\% of the patients\textsuperscript{62}.

CRISPR/Cas9 gene editing has several challenges such as unknown efficacy, the specificity of delivery vehicles, and the possibility of off-target effects. Translatability of the majority of in vitro research to in vivo delivery methods in the clinic is another challenge. Immune responses to delivery vehicles and Cas9 peptides may also be a hurdle, as with any gene therapy. These challenges need to be overcome in future\textsuperscript{85}.

**Summary**

Muscular dystrophies are heterogeneous disorders, characterized by progressive muscle weakness. DMD is one of the most common forms of muscular dystrophy and mostly affects boys. DMD is caused by the absence of the dystrophin protein, which leads to abnormal membrane permeability and fragility of the cell membrane. This permeability and fragility make the cell membrane more susceptible to damage. In the early stages, the affected muscles undergo numerous cycles of damage and repair, but
in later stages, there is a progressive replacement by fat and fibrotic tissue. Boys with DMD exhibit progressive muscle weakness in the proximal to distal direction, which leads to gradual functional decline. There is no known cure for DMD, and glucocorticoids are the only drug which has been shown to delay loss of ambulation by 1-2 years. Many drugs in clinical trials have shown success pre-clinically in animals but have shown minimal success in humans. There are significant barriers in the treatment of DMD. It is worth noting that use of a single drug may not be successful in treating DMD and it may be helpful to administer multiple drugs, targeting different pathways, for successful treatment of DMD\textsuperscript{66}. Moreover, most of the clinical trials target ambulatory patients with DMD, thus excluding roughly two-thirds of DMD patients who are no longer able to walk.
DMD is currently incurable, but many potential therapies are in clinical trials\textsuperscript{20, 86}. As described in the previous chapter, these therapies have been successful pre-clinically in animals and are in different phases of clinical trials. All these clinical trials need sensitive and reliable outcome measures that can detect the disease progression and the potential effects of therapeutic drugs. These clinical outcome measures should also have the ability to detect changes even in young DMD patients. Because DMD is a heterogeneous and rare disease, the design of clinical trials is often more difficult\textsuperscript{87}. A lack of sensitive clinical outcome measures makes it even more challenging. This chapter will review the existing outcome measures being used in clinical trials and it will also describe the potential use of imaging as a new biomarker for future clinical trials.

**Functional Tests**

Functional tests, which are commonly used by a physical therapist for evaluating function of lower limbs and upper limbs, have been used in clinical trials. These functional tests are low-cost, reproducible, and easy to use in clinical trials\textsuperscript{88}. These tests can give a quick assessment of how the disease is progressing in boys with DMD. Different tests are used for assessing lower limb and upper limb function as described below.

**Lower Limb Function Tests**

Most clinical trials in DMD focus on changes in lower limb muscle function since they target the ambulatory population. Due to this, many functional tests have been developed by physical therapists and clinicians over the years to assess the function of lower limbs. These tests have been extensively used in DMD population, both clinically
and in research studies, so clinical trials are using them for testing the efficacy of their drugs. Below are some of the functional tests that have been used to assess the function of lower limbs:

**Timed function tests.** Different timed function tests (TFTs) are used for assessing lower limb function in DMD population. Some of the recently used TFTs measure the time taken: to walk/run 10 meters, to get up from the floor (supine to stand test), and to climb four stairs\(^9\)\(^8\)\(^9\)\(^1\). These tests have been used to illustrate the disease progression in DMD. For example, it has been reported that if the time taken to walk/run 10 meters is more than 12 seconds or the time taken to climb four stairs is greater than 8 seconds, then the subject has a higher chance of losing ambulation within 12 months\(^9\)\(^1\), \(^9\)\(^2\)\(^9\). TFTs have also demonstrated to be reliable outcome measures for use in multi-center clinical trials\(^9\)\(^1\), \(^9\)\(^3\). They have been used to test the efficacy of corticosteroids in DMD\(^9\)\(^4\)-\(^9\)\(^6\). However, these tests are limited to ambulatory DMD patients\(^9\)\(^1\). Moreover, the performance on these tests is dependent upon the motivation and co-operation of the patients, which makes them unsuitable for use in younger age groups.

**Six minute walk test.** The Six minute walk test (6 MWT) is another functional test commonly used in the DMD population, which has shown its reliability, validity, and reproducibility in assessing DMD patients\(^5\), \(^9\)\(^7\)-\(^9\)\(^0\). It is presently a primary endpoint for clinical trials in ambulatory DMD patients\(^9\)\(^2\), \(^9\)\(^7\)-\(^9\)\(^9\). This test can demonstrate disease progression in DMD. For example, it has been reported that a baseline 6 MWT distance of 330 meters and above decreases the chances of ambulation loss within 2 years in DMD patients\(^1\)\(^0\).
Though 6 MWT is commonly used in clinical trials, it is dependent on motivation and age of the patient. Alfano et al. demonstrated a performance improvement of 44 m in the 6 MWT distance, with a financial reward of $50 in boys with DMD. This distance exceeds the clinical meaningful threshold targeted in several clinical trials. Moreover, over a one year period, DMD patients less than 7 years old show improvement in their 6 MWT distance, due to maturational effects, compared to those who are 7 years or older. So, both these age groups would require different clinical outcome measures for clinical trials. This shows that the 6 MWT is not a good test for use in younger DMD patients. Like TFTs, the 6 MWT can only be used in ambulatory DMD patients and cannot be used in clinical trials for non-ambulatory patients.

**Upper Limb Function Tests**

Since most of the literature concerning DMD has focused on assessing the function and strength of lower limbs, very little is known about the rate of loss of upper limb functions. Researchers and the DMD community have recently started focusing on evaluating upper limb functions. This is necessary so that non-ambulatory DMD patients can also participate in the clinical trials. Upper limb functions are necessary for performing daily living activities like eating, brushing teeth, and bathing. These functions are maintained for a period of time after a DMD patient loses his ambulation. Below is summary of some of the upper limb function tests used:

**Performance of upper limb.** Performance of Upper Limb (PUL) has recently been developed to evaluate the function of upper extremities in the DMD population. This is a reliable and valid scale that can be used for both ambulatory and non-ambulatory DMD patients. It has the ability to assess the extent and the severity of the
disease. It consists of 22 items, divided into 3 level dimensions – shoulder, elbow, and wrist, which can help track in tracking impairments in upper limb function\textsuperscript{105, 109, 110}. PUL has shown good intra-observer and inter-observer reliability in a multi-center study. This test is suitable for use in DMD patients of age 5 years and older\textsuperscript{110}. PUL results have shown that impairment in upper extremities starts even while DMD patients are still ambulatory\textsuperscript{111}.

**Jebsen hand function test.** Jebsen Hand Function Test (JHFT) is a timed test, that has shown its sensitivity in evaluating hand function in DMD patients\textsuperscript{107, 112}. In this test, the subject performs 7 tasks that include activities that are commonly used in daily living\textsuperscript{107, 112-114}. However, it does not include tasks that measure the function of proximal muscles of the upper extremity which are important for activities such as bathing, grooming, etc. The writing task in JHFT is also not very reliable, since its results may be indicative of learning disabilities rather than poor hand function in DMD patients\textsuperscript{106}.

**Microsoft Kinect gaming.** Microsoft Kinect gaming is a newly developed outcome measure for assessing upper extremities in DMD\textsuperscript{115, 116}. It is a reliable and valid test that has the ability to evaluate upper extremity impairments in both ambulatory and non-ambulatory patients with DMD or BMD\textsuperscript{105, 115-117}. Kinect gaming can be used to determine the reachable workspace\textsuperscript{115} or functional reaching volume, velocity of movement, and fatigue rate while playing video games\textsuperscript{116}. However, the major disadvantages of this outcome measure are the cost and the complexity of the setup. The complexity of the setup makes it harder for physical therapists and clinicians to easily use this tool in clinics for assessing upper limb function in the DMD population.
**Functional Scales**

Functional scales are used to determine the quality of movements\textsuperscript{118}. They are very easy to use in clinics by physical therapists and give a quick assessment of the quality of movements of both upper and lower extremities. Below are some of the functional scales used in DMD:

**Brooke scales.** Brooke Scales are used for grading both upper extremity and lower extremity functions. These scales are commonly used outcome measures in DMD\textsuperscript{94,107}. They are very easy to use and are a quick way to evaluate the quality of movement to determine whether patient is performing compensatory movements. However, these scales are very subjective and do not provide much information about the ability to perform various activities of daily living.

**North star ambulatory assessment.** The North Star Ambulatory Assessment (NSAA) is a relatively new scale, developed in Europe over the last decade\textsuperscript{119}. The purpose for designing this scale was to include tasks that are focused more on activities of daily living in DMD population. This clinical outcome measure contains 17 activities involving lower limbs, varying from easy tasks such as standing to difficult tasks like running a distance of 10 meters\textsuperscript{5,120}. It is a valid and reliable scale, and is used in ambulatory DMD patients to evaluate functional performance\textsuperscript{5,119,121-124}. For example, it has been reported that a baseline NSAA score of 18 or above indicates a reduced risk of losing ambulation within a 2 year period in DMD patients\textsuperscript{5,101}. This scale has also been frequently used in new clinical trials\textsuperscript{86}. This scale has the disadvantage of being age-dependent. It has been shown that NSAA scores tend to increase for DMD patients who are less than 7 years old and decrease for those who are of 7 years of age or older within one year\textsuperscript{104}. So, this scale might not be useful for younger DMD patients.
**Motor function measure.** The Motor Function Measure (MFM) scale was developed for objectively evaluating motor function in neuromuscular diseases\textsuperscript{125, 126}. This scale was developed so that it can be easily used by physical therapists and clinicians for determining function of patients with neuromuscular diseases. This scale has the advantage that it can be used for both ambulatory and non-ambulatory patients\textsuperscript{5, 87, 126}. In patients with DMD, MFM is valuable in indicating the disease stage and assessing whether the patient’s motor function is improving, stabilizing, or declining. MFM can be used to assess the effect of steroids or other therapies on DMD patients\textsuperscript{127}. However, since physical therapists determine grades in this scale, its results can be subjective and are prone to inter-rater variability. Moreover, this test is time consuming, and maybe physically tiring for patients who are weak and mentally exhausting for those who have attention deficit disorder\textsuperscript{128}.

Other commonly used functional scales in DMD population are Egen-Klassification scale\textsuperscript{5, 91, 96, 107, 108, 118, 129}, the Muscular Dystrophy Functional Rating Scale\textsuperscript{130}, the Hammersmith functional motor Scale\textsuperscript{131}, the Barthel index\textsuperscript{132}.

**Strength Testing**

Muscle strength can be measured either manually or by using quantitative devices. Manual muscle testing (MMT) has been used to measure muscle strength manually\textsuperscript{133} for a long time. MMT is used to evaluate the muscle strength of patients with neuromuscular diseases clinically\textsuperscript{134}. It has been used to evaluate muscle strength for both upper and lower limb muscles\textsuperscript{51, 112, 134-138} and subsequently, to assess changes in muscle strength in patients with DMD over the period of time\textsuperscript{134, 139}. It was used as one of the outcome measures to determine the efficacy of therapeutic drugs in DMD\textsuperscript{134}, like in the earlier clinical trials evaluating the efficacy of prednisone in treating DMD\textsuperscript{94, 95}.  

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High intra-rater reliability is found in MMT grades\textsuperscript{134}. The major disadvantage of using MMT is that it is too subjective in nature\textsuperscript{93, 140, 141}. MMT grades greater than 3 depend heavily on resistance provided by individual physical therapist to the patient. Thus, there is lot of inter-rater variability, thereby making MMT a less accurate and a less sensitive method\textsuperscript{142, 143}. Extensive training and experience is required to decrease variability in using MMT method\textsuperscript{93, 141, 144}. Moreover, it is difficult to apply MMT in young boys with DMD as they are unable to cooperate\textsuperscript{145}.

Quantitative muscle testing is an alternative to traditional MMT for evaluating muscle strength\textsuperscript{93}. It is a validated outcome measure for measuring muscle strength in patients with DMD\textsuperscript{60}. Being a sensitive outcome measure, it has the capability of recording even small changes in muscle strength and is thereby able to test specific muscles\textsuperscript{146}. It has been demonstrated that this is a reliable and accurate tool for assessing muscle strength and can be used for multi-center clinical trials\textsuperscript{141}. The hand held dynamometer can be objectively used to measure muscle strength\textsuperscript{108, 144, 147-149}. Its ease of use and limited space requirement make it a desirable option\textsuperscript{147}. It has shown intra-rater reliability\textsuperscript{147} and can thus be used in measuring isometric muscle strength in patients with DMD\textsuperscript{64, 121, 147, 150}. But, it is not sensitive enough to detect measurements in weak patients\textsuperscript{128}. Myometer can quantitatively measure muscle strength for various upper and lower limb muscles\textsuperscript{60, 92, 95, 100, 151, 152}. Being lightweight and small in size\textsuperscript{153}, it can be easily used anywhere. However, it is not a very sensitive tool for measuring strength in weak DMD patients. Isokinetic dynamometer (Biodex system) has been used to measure isometric peak torque of hip flexors\textsuperscript{154}, hip extensors\textsuperscript{154}, knee flexors\textsuperscript{154}, knee extensors\textsuperscript{29, 154, 155}, plantarflexors, and dorsiflexors\textsuperscript{90, 142, 154}. For this
test, the patient is asked to push or pull as hard as they can for a few seconds while simultaneously viewing their performance on a computer screen. This visual feedback motivates the patients to give their best effort. Though it objectively measures the strength of different muscles, it's set up is a major disadvantage. The set up requires lots of space so it cannot be easily used by physical therapists in clinics.

All the above described quantitative tools have been used by physical therapists for a long time for assessing muscle strength of upper and lower extremities. However, there was a need to develop easy to use tools that do not require a lot of space, and can be used even in very weak non-ambulatory DMD patients. To meet these requirements, new tools have recently been developed for assessing upper limb strength in non-ambulatory patients with DMD. They measure isometric grip strength using myogrip and key pinch force using myopinch. These tools have been proposed as outcome measures in non-ambulatory patients with DMD because of their sensitivity and reliability. They are easy to use and do not require a lot of space. They have the ability to detect strength in weak non-ambulatory patients with DMD. The only disadvantage of these tools is their cost. Being very costly, they may not be easily available for physical therapists to use in clinics.

**Magnetic Resonance Measures**

Quantitative magnetic resonance (MR) measures are emerging as objective and sensitive outcome measures in detecting disease progression in DMD patients. Though function and strength tests are still used by physical therapists to evaluate disease progression, MR has the unique advantage of being quantitative, objective, non-invasive, reliable, and independent of patient motivation. MR measures are starting to
be used as primary or secondary outcome measures in clinical trials. Below is a summary of some of MR strategies used:

**Magnetic resonance imaging.** Magnetic resonance imaging (MRI) is a non-invasive imaging technique that forms images of the inside of the body. MRI works on the principle of nuclear magnetic resonance (NMR), a phenomenon where nuclei of atoms get excited in the magnetic field by electromagnetic waves and emit signals. MRI is a promising “surrogate outcome measure” that, compared to muscle biopsy, has the advantage of being non-invasive, yet sensitive to muscle changes. MRI has shown that different muscles are affected to variable extents in various muscle diseases. In DMD patients, MRI can be used for tracking disease progression and testing the efficacy of drugs. MRI has also shown its reproducibility in a multi-center study in boys with DMD.

MRI T₂ and Dixon MRI are used for determining disease progression in individuals with DMD. MRI T₂ (transverse relaxation time) is sensitive to muscle damage, inflammation, and fatty infiltration in muscles. Thus, MRI T₂ has the potential of tracking disease progression across different ages in individuals with DMD. Dixon MRI is used to determine fatty infiltration in muscles, thereby determining the disease progression in DMD.

MRI T₂ has been used to evaluate disease progression in both mice and humans. It has been shown to detect age-related muscle changes in mdx mice, a mouse model for DMD. Thus, MRI T₂ has the potential to assess future therapeutic drugs in preclinical phases in mdx mice. Similarly, MRI T₂ has also been used in DMD patients for evaluating muscle changes. MRI T₂ changes have been able to
identify affected muscles even in the initial stages of the disease\textsuperscript{174}. This could help in differentiating muscles of boys with DMD from their control peers in all age groups, including in the young age groups\textsuperscript{171, 172, 174}. Different MRI T\textsubscript{2} values in different muscles would also provide insight into the heterogeneity of the disease\textsuperscript{174}. Longitudinal changes in MRI T\textsubscript{2} in muscles of boys with DMD could be used to track the disease progression\textsuperscript{171, 175, 176}. Moreover, MRI T\textsubscript{2} changes had been shown to correlate with timed functional tests\textsuperscript{174, 176, 177}, which is important for fulfilling the criteria of a biomarker. MRI T\textsubscript{2} has the potential for testing the efficacy of therapeutic drugs\textsuperscript{178}. Apart from being used in assessing lower extremity muscles, MRI T\textsubscript{2} has recently been used in evaluating disease progression in upper extremity muscles in boys with DMD. Recently, MRI T\textsubscript{2} values had been found to be elevated in shoulder\textsuperscript{159} and upper arm muscles of boys with DMD as compared to their control counterparts\textsuperscript{159, 179}.

Dixon MRI is used in measuring fat fractions in muscles\textsuperscript{29, 170, 180}. In DMD patients, it can precisely measure the severity of the disease\textsuperscript{180}. Boys with DMD had been shown to have elevated fat fraction as measured by Dixon MRI, when compared to controls\textsuperscript{181}. It has been reported that muscle fat fraction determined by Dixon MRI correlated well with functional tests so it has the potential of being used as a surrogate outcome measure and in clinical trials\textsuperscript{87, 180}. It may also be a predictor of ambulatory loss in DMD patients\textsuperscript{182}. Recently, Dixon MRI was used to evaluate muscles of upper extremity in boys with DMD. It was found that the upper arm\textsuperscript{179} and forearm\textsuperscript{152} muscles of boys with DMD had higher fat fractions than controls.

**Magnetic Resonance Spectroscopy.** Magnetic Resonance Spectroscopy (MRS), specifically, proton magnetic resonance spectroscopy (\textsuperscript{1H-MRS}), a non-invasive
technique, is used for quantification of fats and metabolites in muscles\textsuperscript{170}. \textsuperscript{1}H-MRS also helps in locating the presence of lipids in muscles. It is valuable in distinguishing between intramyocellular lipids that are present inside skeletal muscles, and extramyocellular lipids which are present outside the muscle cells. In \textsuperscript{1}H-MRS, fat and water concentrations in the muscle can be determined by area integration. Fat fraction is obtained as fat / (fat + water)\textsuperscript{183}. Increased fat fractions were seen in DMD patients as compared to the controls\textsuperscript{172, 181, 183, 184}. Fat fractions elevated with an increase in age in DMD patients\textsuperscript{172, 183}. \textsuperscript{1}H-MRS also has potential to determine the efficacy of therapeutic drugs in DMD. DMD patients exhibited a lower fat fraction in their muscles after being treated with corticosteroids\textsuperscript{178}.

**Summary**

Though DMD is currently incurable, many therapeutic drugs are in clinical trials. All these clinical trials use outcome measures to assess efficacy of the therapeutic drugs. This chapter reviewed several commonly used outcome measures. There is a desperate need for sensitive, non-invasive outcome measures that can be used in clinical trials. Most clinical trials have been using strength and functional tests; however, strength and functional tests are highly reliant on the behavior and motivation of the subject performing the tests, are subject to inter-tester variability, and are dependent on the age of the subjects. There are chances of variability in the results, depending upon the mood of the patient on the testing day. Compliance can also vary between ambulatory and non-ambulatory subjects\textsuperscript{87}. Moreover, younger subjects or subjects with some cognitive deficits may have trouble understanding the instructions provided to them for performing the functional tests. The testers conducting the tests need to provide constant encouragement to subjects undergoing the testing. This results in an
element of variability being introduced in the process, depending on the encouragement that was provided to the subject. Moreover, grades in functional scales are qualitative and thus, subjective in nature. In timed tests, measuring time manually on a stopwatch introduces the possibility of human error. In multi-center studies, there are multiple testers involved, which could cause unintended variability. Not all functional tests are suitable for all age groups in DMD, so different age groups require different functional tests. Developing such tests is time-consuming. With so many limitations of functional tests in clinical trials, there is a requirement for a more sensitive and reliable outcome measure. Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) are promising “surrogate outcome measures” because of their sensitivity in detecting changes in muscles in DMD as well as being non-invasive and highly reproducible\textsuperscript{87, 172, 185}. Moreover, they are comparatively less dependent on a subject's cooperation and less affected by an examiner’s biases or errors\textsuperscript{185}. MRI has shown correlation with timed function tests\textsuperscript{155, 174, 176}. Moreover, both MR measures can detect muscle changes in the initial stages of the disease and also in the very young DMD population\textsuperscript{171, 174}. MRI and MRS also have the potential for testing the efficacy of therapeutic drugs\textsuperscript{178}. Thus, MRI and MRS seem more promising than other clinical outcome measures for use in clinical trials. They have significant importance in the design of future clinical trials for detecting the effectiveness of therapeutic drugs in a shorter time span\textsuperscript{171}. 
CHAPTER 3
OUTLINE OF EXPERIMENTS

Overview

The overall objective of this dissertation was to examine disease progression in both the upper and lower extremity muscles in boys with Duchenne muscular dystrophy (DMD) using functional outcome measures and magnetic resonance imaging (MRI). Boys with DMD have progressive muscle weakness, which causes functional impairment in their lower and upper extremity muscles. Different functional tests are used to evaluate the function of lower and upper extremity muscles. MRI T$_2$ is able to non-invasively detect physiological changes in muscle, which the skeletal muscles of boys with DMD are very prone to, such as muscle damage, regeneration, inflammation, and subsequent fatty infiltration. This can be used to determine disease progression, as well as measure response to an intervention.

The first experiment was designed to examine the natural history of disease progression in ambulatory boys with DMD and compared them with age-matched controls across age groups, using lower limb functional tests, which are commonly used in clinical trials. In the second experiment, the decline in upper extremity function and strength was evaluated using upper limb function and strength tests in both ambulatory and non-ambulatory boys with DMD and compared to age-matched controls across different age groups. The last experiment examined disease progression in boys with DMD, using MRI T$_2$, specifically, MRI T$_2$ was utilized to evaluate disease progression in important upper extremity muscles of boys with DMD and then compared them with age-matched controls across age groups.
Primary Objective

To examine the natural history of disease progression in upper and lower limb muscles in boys with Duchenne muscular dystrophy (ambulatory and non-ambulatory) using functional outcome measures and magnetic resonance imaging.

Significance, Scope, and Applicability

The results of these experiments will provide valuable natural history data that can be used to facilitate the design of clinical trials. Since we included both ambulatory and non-ambulatory boys with DMD, results of our research will be widely applicable across a wide range of boys. The MRI T₂ technique used in this dissertation will help increase our knowledge of disease progression in specific upper extremity muscles in boys with DMD.

Experiment One

To examine the natural history of disease progression in ambulatory boys with DMD using established clinical endpoints.

Specific Aims

1. Evaluate lower extremity function in ambulatory boys with DMD, ages 5-12 years, compared to age-matched controls.

2. Evaluate the disease progression over 1 year in boys with DMD across age groups.

Hypotheses

1. Boys with DMD will take more time to complete all timed function tests, compared to controls, and will cover less distance in the six minute walk test than controls at all ages tested.

2. Boys with DMD older than 7 years of age will experience decline in performance on functional tests over 1 year.
Experiment Two

To examine the natural decline in upper extremity function and strength in boys with DMD ages 5-18 years, compared to age-matched controls.

Specific Aims

1. Perform a pilot exploratory study comparing the sensitivity of different outcome measures assessing upper extremity function.

2. Measure upper extremity function and strength in a large cohort of boys with DMD (ambulatory and non-ambulatory) at varying disease stages, compared to age-matched controls.

Hypotheses

1. Performance of upper limb test (PUL) will be sensitive in assessing differences between boys with DMD and controls than other upper extremity functional tests.

2. Boys with DMD will have lower performance than age-matched controls, in the upper extremity functional and strength tests across all age groups, including in the youngest age group.

3. Boys with DMD of age 9 years and above will have lower performance on upper extremity functional tests than less than 9 year old boys with DMD. Upper extremity strength will not significantly decline across age groups in boys with DMD.

Experiment Three

To examine the natural history of disease progression in upper extremity in boys with DMD (ambulatory and non-ambulatory) using T$_2$ magnetic resonance imaging biomarkers.

Specific Aims

1. Evaluate MRI T$_2$ in boys with DMD, ages 5-18 years, compared to age-matched controls in different muscles of upper extremity.

2. To evaluate the relationship between upper extremity MRI T$_2$ biomarkers and performance on upper limb functional and strength tests.

3. To compare disease progression in upper extremity muscles with disease progression in lower extremity muscles using MRI T$_2$. 
Hypotheses

1. MRI T₂ values will be elevated in boys with DMD, compared to controls across different age groups, including in the youngest age group for all the upper extremity muscles.

2. MRI T₂ will show significantly strong correlation with functional tests than with strength tests for all the upper extremity muscles.

3. MRI T₂ will show that proximal upper extremity muscles are less advanced in disease progression than proximal lower extremity muscles.
CHAPTER 4
EXAMINATION OF THE NATURAL HISTORY OF DISEASE PROGRESSION IN AMBULATORY BOYS WITH DMD USING ESTABLISHED CLINICAL ENDPOINTS

Background

Duchenne muscular dystrophy (DMD) is the most common X-linked muscular dystrophy, caused by mutations in the dystrophin gene\textsuperscript{16, 186}, with an incidence of 1 in 3500 - 5000 male births\textsuperscript{21, 22}. Progressive loss of muscle strength, initially in the proximal muscles, leads to significant functional impairment and loss of ambulation in the second decade of life\textsuperscript{27, 30}. Management of DMD focuses on a multi-disciplinary approach targeting the maintenance of quality of life and function\textsuperscript{53, 54}.

Glucocorticosteroid treatment, which delays loss of ambulation by 1 - 2 years\textsuperscript{60, 61}, is currently the only effective treatment available to all boys with DMD.

While there is currently no cure for DMD, significant investment and partnerships between industry, advocacy groups, and funding agencies have led to a surge in promising therapeutic strategies which are rapidly moving into clinical trials\textsuperscript{89, 103}. However, the design of clinical trials has proven challenging, with few double blinded studies and narrow inclusion criteria targeting specific age groups. The recent controversy around the FDA approval of Eteplirsen highlights the complexity of designing clinical trials in DMD. Additionally, there are a limited number of natural history studies\textsuperscript{92, 101, 104, 187} available in large patient cohorts characterizing disease progression and permitting a comparison with experimental treatment groups. There is a pressing need for additional natural history data, with granularity in regard to both age and ambulatory function.

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Well designed clinical trials also demand sensitive and reliable outcome measures that can detect disease progression and the potential efficacy of therapeutic drugs. Unfortunately, there is a scarcity of such clinical endpoints in DMD. Tests of ambulatory function, including the six minute walk test (6 MWT) and timed functional tests (TFTs: timed supine to stand (STS), 10 meter walk/run, time to climb four stairs (4 stairs) are often used as clinical endpoints. In fact, while the utility of the 6 MWT has been questioned, especially in younger boys, it is the most commonly used primary endpoint for current clinical trials in DMD.

The primary aim of this study was to collect natural history data in a large cohort of ambulatory boys with DMD across multiple centers, and to examine 1 year longitudinal changes in performance on the ambulatory functional tests. A secondary aim was to evaluate the ability of each test to predict loss of function in the following year and to examine the relationship between baseline 6 MWT performance and functional decline.

**Materials and Methods**

**Study Design**

Subjects were recruited at three sites (University of Florida (Gainesville, Florida), Children's Hospital of Philadelphia (Philadelphia, Pennsylvania), and Oregon Health and Science University (Portland, Oregon) to participate in a multi-center natural history study, referred to as the ImagingDMD. The study was approved by each site’s institutional review board. This sub study summarizes data from 125 boys with DMD and 45 control subjects, aged 5 – 12.9 at the time of enrollment. Subjects were divided into 4 age groups: 5 - 6.9 years, 7 - 8.9 years, 9 - 10.9 years, and 11 – 12.9 years. 1 year follow up data are presented for 106 boys with DMD. Additional annual follow-up
data are presented for up to 4 years in a subset of the subjects with DMD (around 400 data points for each test). Inclusion criteria for boys with DMD were: 1) age 5 to 12.9 years, 2) previously diagnosed with DMD with clinical symptoms before 5 years of age, 3) elevated serum creatine kinase level, or absence of dystrophin expression, and 4) ability to walk at least 100 m and climb four stairs. Exclusion criteria for recruitment of boys with DMD were: 1) unstable medical problems, 2) behavioral or cognitive issues during testing that prevent valid data collection, and 3) a medical condition which could affect muscle function or functional performance. Control subjects were included if they: 1) were between 5 and 12.9 years old, 2) had normal developmental milestones, and 3) had no injury to the lower limb which required immobilization within the last year. Control subjects were excluded if they participated in sport specific training for more than 8 hours per week. Parents and/or guardians of subjects provided informed consent, and subjects provided written assent.

**Functional Measures**

Tests were performed in the following order: 10 m walk/run, climbing four stairs, STS, and 6 MWT. The 6 MWT was performed as described by McDonald et al. (2010). Subjects were also graded using the Modified Brooke Lower Extremity Scale, which ranges from 1 (walks and climbs stairs without assistance) to 10 (confined to bed). Subjects wore comfortable walking shoes while performing the tests. Parents/guardians of subjects were not present during testing. Each functional test, except 6 MWT, was performed three times and verbal encouragement was provided. The fastest time for each TFT was recorded in seconds to two decimal places. The maximum time allowed to complete each TFT was 45 seconds. If the subject took more than 45 seconds, he was considered to have lost the ability to perform the test. The time
started when the evaluator said “Ready, set, go.” If the subject fell, assistance was provided to help the subject back up, if needed and the test was continued. The stopwatch was not stopped during the fall but the fall was recorded.

To ensure high quality data, all tests were performed by trained evaluators according to a standard manual of operating procedures (MOP) using simple, standardized instructions to the subjects. Comments recorded by the functional evaluator at the time of testing as well as the time or distance were inspected by two independent reviewers to confirm the validity of the data. A third adjudicating reviewer made a final decision if needed. 1% of tests (7/500) in boys with DMD and 0.6% of tests (1/180) in controls had missing data, typically because the parent or subject chose not to participate in that test or because of logistical issues such as time constraints. 2% of tests (8/500) were invalid due to compliance or behavioral issues and were not included in analysis. Compliance was most commonly an issue in young boys performing the 6 MWT. 97% of tests (485/500 tests) in boys with DMD and 99.4% of tests (179/180 tests) in controls were considered valid.

Data Analysis

Non-parametric tests were used to compare controls and boys with DMD for each age group, (Mann Whitney Test), and to examine longitudinal changes (Wilcoxon signed rank test). Non-parametric spearman correlation test was used to find the relationship between 10 m walk/run test and 6 MWT. A distance of 0 m was recorded for subjects who lost the ability to perform the 6 MWT. Percentage predicted 6 MWT distance was calculated based on the Geiger equation\textsuperscript{190}. An additional set of analyses was conducted on all available annual follow up data points. To investigate loss of function these data were binned into 1 year age groups. To investigate prediction of loss
of function in the following year, a receiver operating characteristic curve (ROC) was estimated. We estimated sensitivity and specificity as the point on the ROC curve closest to (0, 100%). To properly account for uncertainty, given multiple (correlated) time points from the same subject, we used the bootstrap (resampling subjects, not individual data points for a subject) to compute 95% confidence intervals for the area under the curve (AUC), the optimal cutoff, and the sensitivity and specificity. Data are reported as mean ± standard deviation (SD), unless otherwise stated. Significance was set at 0.05 with Bonferroni corrections for multiple comparisons as needed.

Results

Cross-Sectional Comparison in Functional Measures Across Age Groups

Table 4-1 shows the demographics of the DMD and age-matched control groups at baseline. Boys with DMD required more time than controls to complete the TFTs and traversed a shorter distance during the 6 MWT in all age groups (Figure 4-1). The gap between control and DMD increased with age for all functional tests. The youngest boys with DMD (5 – 6.9 year olds) took more than twice as long as controls, while the oldest boys (11 – 12.9 year olds) took over four times as long as controls on the climbing four stairs and STS tests. More modest differences were noted on the 6 MWT; age-matched controls walked about 1.3 times farther than boys with DMD in the youngest age group and 1.8 times farther in the oldest age group. Controls in our study walked 464 m – 720 m during the 6 MWT. Percentage predicted 6 MWT distance attempts to account for maturational changes in unaffected boys\textsuperscript{191}, and also showed significant differences between boys with DMD (62.6 ± 12.8%) and controls (95.3 ± 10.6%) at baseline (Figure 4-2a), in all of the age groups.
Examination of the relationship between the two walking tests, the 10 m walk/run and 6 MWT, shows a strong relationship between both measures. However, there was considerable variability in 6 MWT distance among boys who were able to complete the 10 m walk/run test in less than 5 sec (274 m – 508 m) (Figure 4-3).

One Year Longitudinal Change in Functional Tests in Boys With DMD

Loss of the ability to complete TFTs presents a considerable challenge in describing group changes in DMD cohorts, as traditional statistical descriptors are not appropriate. Here, data are presented as frequency histograms, with performance on each test categorized into 4 bins based on the time taken or distance walked. For TFTs, the bins were defined as < 5 s, 5 – 10 s, > 10 s, and unable to perform. Figure 4-4 shows the distribution of times across all boys with DMD at baseline for each TFT, in reference to the bin markers. For the 6 MWT, the bins were defined as: ≤ 300 m, 301 m – 375 m, 376 m – 450 m, and ≥ 451 m. Over 1 year, there was an overall shift to slower times/shorter distance in all age groups, except in the youngest age group (Figure 4-5). The shift was most pronounced in STS and climbing four stairs test.

Time to persistent 10% worsening is gaining popularity as a secondary analysis in clinical trials. Although we did not examine the persistence of the decline, we calculated the proportion of subjects who declined >10% over 1 year for each age group on each test. The proportion of subjects who declined >10% over 1 year was highest for STS (67%), which reflects both increases in time and loss of the ability to perform the test, and lowest for the 6 MWT (35%) (Figure 4-6).

Because of the importance of the 6 MWT in many clinical trials in DMD, we performed several additional analyses of these data. First, we examined individual 1 year trajectories (Figure 4-7a). Substantial inter-subject heterogeneity was noted in the
decline in 6 MWT distances from baseline to 1 year. However, group mean data showed significant declines in 6 MWT performance (as well as percentage predicted 6 MWT (Figure 4-2b) in the two oldest age groups (9 - 10.9 and 11 - 12.9 year old boys) (Figure 4-7b), with a trend toward increased distance in the 5 – 6.9 year old group and a trend toward decreased distance in the 7 – 8.9 age group.

**Relationship between Baseline 6 MWT Distance and Clinically Meaningful Change in Functional Tests Over 1 Year**

Previous studies have reported greater functional decline over 48 weeks in subjects with a baseline 6 MWT distance of < 350 m. Consistent with these reports, in this cohort there was a greater percentage of boys in the < 350 m group who experienced clinically important decline on each TFT (27% for 10 m walk/run, 35% for 4 stairs, and 11% for STS) or loss of function (5% for 10 m walk/run, 14% for 4 stairs, and 37% for STS) over 1 year, compared to boys with baseline distance ≥ 350 m (12% for 10 m walk/run, 10% for 4 stairs, and 8% for STS for clinically important decline and 2% for 10 m walk/run, 2% for 4 stairs, and 10% for STS for loss of function). However, for the 6 MWT, the two groups did not demonstrate a substantial difference in progression (Figure 4-8).

To further explore the association between baseline 6 MWT and clinically meaningful changes in functional tests, we subdivided the cohort into four groups based on baseline 6 MWT: ≤ 300 m, 301 - 375 m, 376 - 450 m, and ≥ 451 m. One year changes in functional tests performance for each subject were classified using previously published values for minimal clinically important difference (Figure 4-9). Overall, subjects with a baseline 6 MWT of ≤ 375 m showed a higher incidence of clinical meaningful decline over 1 year on all functional tests. Most subjects (~90%) with
baseline 6 MWT of ≥ 376 m were stable over 12 months TFTs. However, for the 6 MWT, 73% of subjects who walked more than 451 m at baseline had a clinically meaningful decline over 1 year.

**48 Months Data in a Subgroup of DMD Subjects**

Figure 4-10 displays the loss of functional ability in the subgroup of boys with longitudinal data over up to 48 months for each functional test. Characteristic of DMD, STS was the earliest ability lost; 50% of subjects were unable to perform STS by age 12 years, while 80% of 12 year old boys could still perform the 10 m walk/run, 6 MWT and climbing four stairs test. After 12 years, loss of function was rapid, with only 50% of subjects able to walk or climb stairs at 14 years of age. Individual trajectories of performance on the 6 MWT test over 48 months are displayed in annual increments in Figure 4-11, with the data displayed in reference to the subject’s baseline 6 MWT.

Finally, to examine the value of each test in predicting loss of ability within the following year, all annual follow up data were combined to estimate an ROC curve. Results for the ROC curves for the functional tests can be found in Table 4-2. All functional tests significantly predicted loss of function, but the 6 MWT tended to have lower sensitivity and specificity.

**Discussion**

With many ongoing clinical trials and several on the horizon, it is crucial to have comprehensive natural history data describing disease progression in DMD. The overall aim of this large multi-center sub study was to collect natural history data using common clinical endpoints in ambulatory boys with DMD. The study also examined the prediction of loss of function in the following year for each functional test. The primary findings of this study were: 1) functional test performance in boys with DMD was worse than
controls across all age groups, including 5 – 6.9 year olds; 2) boys with DMD older than 7 years experienced declines in performance on TFTs over 12 months; 3) 6 MWT did not show a significant decline over 1 year in DMD subjects less than 9 years old; 4) ~80% of enrolled DMD subjects could perform all functional tests until at least 9 years of age; 5) all functional tests were able to predict loss of function in the following year though TFTs had better sensitivity and specificity than the 6 MWT.

The mean baseline 6 MWT distance in the iDMD cohort was 365 m. Previously, McDonald et al. (2013) reported a mean baseline 6 MWT distance of 360 m in 5-15 year olds, and Pane et al. (2014) reported a mean baseline 6 MWT of 378 m in 3-17 year olds. The 12 month decline in 6 MWT performance in the iDMD cohort (-23 m) was similar to that reported by Pane (-10 m), but slower than the cohort described by McDonald et al. (-44 m).

Unlike other recent natural history studies, this study included an unaffected age-matched control group for the TFTs, allowing us to quantify the increasing gap in performance between boys with DMD and their peers with age. Subjects with DMD performed all functional tests more slowly than controls even at 5 – 6.9 years old, and the difference was larger in older boys. Specifically, boys in the youngest age group took between 130% and 200% of the time taken by controls to complete the tests, and boys in the eldest age group took 180-400% of the time taken by controls. Controls took about 3 seconds to walk/run 10 m and between 1 and 2 seconds for climbing four stairs and STS. These normative data may be helpful in understanding alterations in disease progression with treatment in DMD.
Over 12 months, performance on TFTs, especially 4 stairs and STS, slowed whereas the group distribution of distances for the 6 MWT did not change substantially. However, in the youngest boys (5 – 6.9 year olds), performance on both 10 m walk/run and 6 MWT tests appeared to improve over 1 year. Supporting this, most of the older boys had at least 10% worsening over 12 months on all tests, but that was not true for the youngest boys (Figure 4-6).

Though commonly used in clinical trials, the 6 MWT has been criticized\cite{86, 188}, and is particularly limited by its inability to detect disease progression in younger boys. In this study, there was substantial heterogeneity in 6 MWT performance in both older and younger boys (Figure 4-7a). In addition, there were a wide range of 6 MWT distances achieved by boys who were considered fast walkers based on the 10 m walk/run test (<5 s) (Figure 4-3). No significant change over 1 year could be detected in the 6 MWT in the two youngest age groups (5 - 6.9 and 7 - 8.9) (Figure 4-7b). This is in contrast to previous studies, where a decline in 6 MWT performance was reported starting at ≥ 7 years of age\cite{92, 101, 104}. Some of the variability in 6 MWT distance may be due to the impact of motivation, which is known to have a strong effect on 6 MWT distance. Alfano et al.\cite{102} showed on average a 44 m increase in 6 MWT distance in boys with DMD with the promise of a financial reward of $50, exceeding the clinical meaningful threshold targeted in several clinical trials\cite{89, 103}. While motivation is hard to assess, in this study there were few boys that were not able to reliably perform the 6 MWT, even in the youngest age group. In fact, across all functional tests only 2% (8/500) of the test were scored as invalid due to compliance or behavioral issues.
Boys with low baseline 6 MWT distance were more likely to experience declines in performance over the next 12 months for the TFTs, as previously reported\textsuperscript{92}. Interestingly, the relationship between baseline 6 MWT distance and future change in 6 MWT itself was limited. It is noteworthy that no boys with DMD experienced clinically important improvement in TFT performance, however, there was clinically important improvement seen over 12 months for the 6 MWT in a number of boys. This variability in progression, along with the lack of significant decline in boys < 9 years of age in 6 MWT, and its reported dependence on motivation suggests other outcome measures should be given more consideration as primary outcomes for DMD.

Individuals with DMD typically first lose the ability to complete the STS test, followed by a loss of ability to climb four stairs, and then a loss of ability to complete the 10 m walk/run test and the 6 MWT\textsuperscript{91}. In pooled data from all time points collected in this longitudinal study, most boys under 9 years of age were able to perform all functional tests. At 12 years of age, more than 80% of the subjects could perform all tests other than STS, and by age 14, about half could still perform the 10 m walk/run and 6 MWT. The 9 - 14 year age range is when many boys lost the ability to perform tests of ambulatory function, and, thus, caution must be used in test selection for trials in boys aged 9 and older. Clinical trials aim to include boys whose performance is declining, in order to detect a stabilization with treatment, but not to include boys who will lose function. Thus, the selection of functional endpoints for clinical trials in ambulatory boys may be age-specific, to balance these desired cohort characteristics.

As previously described, baseline function was predictive of loss of ability over time, and while results of the three natural history studies are not directly comparable
due to differences in study design, the thresholds predicting an increased risk of loss of function are broadly congruent across studies. The most similarity was found with the 6 MWT, where 6 MWT distances less than 319 m (current study) and 325 m (McDonald et al.) predicted an increased risk of loss of ambulation within one year, and distances less than 330 m predicted an increased risk of loss of ambulation within 2 years (Mazzone et al.). More discrepancy was seen when the 10 m walk/run test was used as a predictor. For prediction of loss of ambulation within the next 12 months, the threshold identified in this study using ROC curves, 7.6 seconds, was similar to that identified for prediction of loss of ambulation within 2 years by Mazzone et al. (7 seconds), though faster than the 10-12 second cut-off identified by McDonald et al.

Increased risk of loss of stair climbing ability was related to the inability to climb stairs faster than 5.6 seconds based on ROC curves in the current study, and increased risk of loss of STS ability was related to baseline performance slower than 6.6 seconds. Both of these threshold values are similar to previous reports. This information is valuable to parents and clinicians as well as researchers, allowing families to prepare for transitions in functional ability in a timely manner and allowing research studies to identify cohorts of boys who are at lower risk of losing the ability to complete a primary endpoint.

This large multi-center natural history study provides important information to help guide clinical trial design, suggesting that TFTs may be more sensitive than the 6 MWT in DMD. We conjecture that the 6 MWT may not be well suited to assess muscle performance, as it does not rely on muscle power, but rather assesses cardiopulmonary function and is subject to compensatory strategies, in addition to motivation. We
propose that the 10 m walk/run test in particular offers a valuable alternative, providing high sensitivity, a strong predictive value, and can be performed for an extended period of time. In contrast, the STS test has good sensitivity to change in boys with DMD > 7 years, but can only be performed in a narrow age window. The 4 stairs climbing test is similarly appealing, however, in performing this TFT, subjects often utilize compensatory movement strategies, such as reliance on the upper body via handrail use to complete the task, reducing its specificity to ambulatory decline.

There are some limitations in this study. Our DMD cohort contains both steroid treated and steroid naive subjects. A comparison of these groups has been published previously\textsuperscript{178}, but a thorough investigation of the effect of steroids on functional tests was not possible in this manuscript because very few longitudinal data were available in steroid-naive subjects. We did not examine differences in functional test performance based on the genetic mutation in the dystrophin gene. Additionally, the study was not able to investigate the presence or impact of differences in clinical care, such as age at steroid initiation, use of dietary supplements and medications, or physical therapy treatment. Moreover, the inclusion criteria in this study targeted subjects who were able to walk >100 m and climb four stairs at baseline, while other natural history studies and clinical trials may have utilized a lower functional threshold for inclusion\textsuperscript{92, 101, 104, 187}.

**Summary**

This study describes the natural history of disease progression in a large cohort of school aged, ambulatory boys with DMD enrolled in the ImagingDMD. Four commonly used clinical trial endpoints were measured in over 100 boys with DMD at baseline and 1 year follow-up. Annual longitudinal data are also provided for up to 4 years in a subset of subjects. In general, the functional decline observed in the
55

ImagingDMD cohort is equivalent to previous reports in other cohorts\textsuperscript{192}, but slower than the 44 m decline on the 6 MWT reported by the CINRG group. Loss of ambulatory function was observed starting after the age of 9 years, with only 50\% of subjects having the ability to walk or climb stairs at 14 years of age. Subjects with a baseline 6 MWT < 375 m showed a higher incidence of clinically meaningful decline on all TFTs over the next year. The 10 m walk/run, stair climbing and STS tests were more sensitive to 1 year changes and predictive of loss of function than the 6 MWT. An optimal predictive cut-off value of 7.6 s was identified for the 10 m walk/run test and 5.6 s for stair climbing. The variability in 6 MWT was high, even in boys that were considered fast walkers on the 10 m walk/run test (times < 5s). In addition to providing natural history data in boys with DMD, this study also includes normative data for controls, ages 5 - 12 years old. This study provides important information for families, clinicians and scientists involved in clinical trial design. The natural history data may be particularly valuable for clinical trial planning, both to appropriately power studies and to select the most appropriate cohort or outcome measure, as well as provide context for comparison with experimental treatment groups.
<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Total</th>
<th>5 – 6.9</th>
<th>7 – 8.9</th>
<th>9 – 10.9</th>
<th>11 – 12.9</th>
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<td>Age (years)</td>
<td></td>
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<tr>
<td>DMD</td>
<td>8.2 ± 2.1</td>
<td>6.1 ± 0.6</td>
<td>8.0 ± 0.6</td>
<td>9.9 ± 0.6</td>
<td>11.6 ± 0.6</td>
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<td>(n = 125)</td>
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<tr>
<td>Control</td>
<td>8.9 ± 2.0</td>
<td>6.1 ± 0.6</td>
<td>8.1 ± 0.6</td>
<td>10.2 ± 0.5</td>
<td>11.7 ± 0.7</td>
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<td>(n = 45)</td>
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<td>Weight (kg)</td>
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<td></td>
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<tr>
<td>DMD</td>
<td>27.8 ± 9.6(^a)</td>
<td>20.9 ± 3.5</td>
<td>26.7 ± 5.4</td>
<td>30.2 ± 6.8</td>
<td>42.8 ± 10.7</td>
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<td>Control</td>
<td>32.3 ± 11.2</td>
<td>23.2 ± 5.4</td>
<td>29.1 ± 6.7</td>
<td>36.0 ± 11.7</td>
<td>44.6 ± 12.1</td>
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<td>DMD</td>
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<td>1.11 ± 0.05</td>
<td>1.21 ± 0.06</td>
<td>1.26 ± 0.09</td>
<td>1.32 ± 0.07</td>
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<td>Control</td>
<td>1.36 ± 0.13</td>
<td>1.20 ± 0.08</td>
<td>1.32 ± 0.06</td>
<td>1.44 ± 0.08</td>
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<tr>
<td>DMD</td>
<td>18.8 ± 4.0(^a)</td>
<td>16.9 ± 2.0</td>
<td>18.1 ± 3.1</td>
<td>19.0 ± 3.2</td>
<td>24.3 ± 5.2</td>
</tr>
<tr>
<td>Control</td>
<td>16.9 ± 3.2</td>
<td>15.8 ± 1.4</td>
<td>16.7 ± 3.2</td>
<td>17.1 ± 3.9</td>
<td>18.6 ± 3.1</td>
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<td>Brooke Score</td>
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<td></td>
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<td>1 – 3</td>
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<tr>
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<td>1</td>
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<td>Steroids</td>
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<tr>
<td>DMD</td>
<td>74%</td>
<td>51%</td>
<td>75%</td>
<td>96%</td>
<td>95%</td>
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<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

Data are means ± standard deviations with a range given for Brooke Score. BMI: Body Mass Index; \(^a\) indicates data collected in n = 124. None of the controls were on steroids.
Table 4-2. Predicting loss of function over 1 year using ROC curves for each timed function test.

<table>
<thead>
<tr>
<th>Functional tests</th>
<th>Area Under ROC Curve</th>
<th>Cut-off Time or Distance</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 meter walk/run</td>
<td>0.93</td>
<td>&gt;7.6s</td>
<td>94%</td>
<td>81%</td>
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<td></td>
<td>(0.89-0.97)</td>
<td>(6.5s-9.2s)</td>
<td>(78%-100%)</td>
<td>(73%-95%)</td>
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<td>4 Stairs</td>
<td>0.94</td>
<td>&gt;5.6s</td>
<td>92%</td>
<td>85%</td>
</tr>
<tr>
<td></td>
<td>(0.90-0.97)</td>
<td>(5.6s-6.7s)</td>
<td>(81%-100%)</td>
<td>(79%-93%)</td>
</tr>
<tr>
<td>STS</td>
<td>0.94</td>
<td>&gt;6.6s</td>
<td>95%</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td>(0.90-0.97)</td>
<td>(6.3s-7.8s)</td>
<td>(87%-100%)</td>
<td>(83%-93%)</td>
</tr>
<tr>
<td>6 MWT</td>
<td>0.86</td>
<td>&lt;319m</td>
<td>88%</td>
<td>73%</td>
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<tr>
<td></td>
<td>(0.78-0.92)</td>
<td>(277m-334m)</td>
<td>(71%-100%)</td>
<td>(65%-90%)</td>
</tr>
</tbody>
</table>

All the TFTs have the ability to predict loss of function within the following year. 95% CI range given in parentheses.
Figure 4-1. Baseline functional tests in DMD and Controls at Different Ages for functional tests - (a) 10 meter walk/run test, (b) 4 stairs, (c) STS, and (d) 6 MWT. Bars represent mean with standard error. There were significant differences between boys with DMD and controls for all functional tests (p < 0.001). Data were missing or invalid for 6 MWT in 11 boys with DMD and 1 control, for climbing four stairs in 1 boy with DMD, and for STS in 3 boys with DMD.
Figure 4-2. Percentage predicted 6 MWT distance (a) at baseline for boys with DMD and controls, and (b) at 1 year for boys with DMD. Bars represent standard error of the mean. At all age groups, boys with DMD performed significantly worse than controls at baseline. Over 1 year, significant decline was seen in the 9 – 10.9 and 11 – 12.9 year age groups (*: p < 0.05, **: p < 0.01, ***: p < 0.001).

Figure 4-3. Relationship between 6 MWT distance and 10 m walk/run time. Boys who performed the 10 m walk/run test in less than < 5 seconds showed considerable variability in 6 MWT distance.
Figure 4-4. Histograms of all TFTs at baseline for different age groups in DMD for (a) 10 meter walk/run test, (b) 4 stairs, and (c) STS. Most of the subjects with DMD took ≥ 4 s to complete the 10 m walk/run, ≥ 2 to complete the climbing four stairs test, and ≥ 3 s to perform the STS.
Figure 4-5. Functional decline over 1 year for all functional tests - (a) 10 meter walk/run test, (b) 4 stairs, (c) STS, and (d) 6 MWT, for all age groups in boys with DMD. BL = Baseline. Overall there was a shift to slower times/shorter distance in all age groups, over the period of 1 year, although this was not true in the youngest age group for the 6 MWT and 10 m walk/run. None of the 5 – 6.9 year old boys lost ability to perform the test over 1 year except in STS. Data represent 35 boys aged 5 – 6.9, 30 boys aged 7 – 8.9, 24 boys aged 9 – 10.9, and 17 boys aged 11 – 12.9. Missing data or invalid data did not exceed 10% for any test in any age group.
Figure 4-6. Percentage of subjects with >10% worsening on functional tests over 1 year. The lowest percentage of subjects with >10% worsening was found in the 6 MWT. For the TFTs, at least 50% of participants 7 years old or older had 10% worsening. Subjects who lost the ability to perform a test were considered to have >10% worsening.

Figure 4-7. Heterogeneity in 6 MWT progression over 1 year across ages. (a) Both increases and decreases in 6 MWT were seen over 12 months across age groups and baseline 6 MWT performance. 3 individuals lost ambulation over 12 months; all had baseline 6 MWT distances < 376 m. (b) A significant decline in 6 MWT over 1 year was found for boys > 9 years of age.
Figure 4-8. Change over 1 year in 6 MWT distance groupings used in previous publications for all functional tests - (a) 10 meter walk/run test, (b) 4 stairs, (c) STS, and (d) 6 MWT. A greater percentage of boys in the < 350 m group experienced clinically important decline over 1 year, as compared to boys with baseline distance ≥ 350 m for the TFTs but not 6 MWT.
Figure 4-9. Clinically meaningful change in all functional tests - (a) 10 meter walk/run test, (b) 4 stairs, (c) STS, and (d) 6 MWT over 1 year by baseline 6 MWT distance. Subjects with lower baseline 6 MWT distances were more likely to experience declines in functional performance or loss of functional ability over 1 year on TFTs but not in 6 MWT.
Figure 4-10. Number of subjects who are still able to perform each functional test by age (compiled from more than 12 months of longitudinal data). After ~7 years of age, individuals began to lose functional ability in greater numbers. At 12 years of age, more than 80% subjects could perform all TFTs except the STS, which only 40% could perform.
Figure 4-11. Heterogeneity in 6 MWT performance over 48 months. Data are presented as (a) baseline to 1 year, (b) baseline to 2 years, (c) baseline to 3 years, and (d) baseline to 4 years. As the study progressed, an increasing number of boys, from all baseline 6 MWT distance groups, lost ambulation. However, some boys were able to maintain performance over 4 years.
CHAPTER 5
EXAMINATION OF THE NATURAL DECLINE IN UPPER EXTREMITY FUNCTION AND STRENGTH IN BOYS WITH DMD AGES 5-18 YEARS, COMPARED TO AGE-MATCHED CONTROLS

Background

Duchenne muscular dystrophy (DMD), an X-linked recessive disorder, is a progressive muscular disease. Over time, individuals with DMD can no longer ambulate, rely on wheelchairs for mobility, and become increasingly dependent on the use of their arms to perform activities of daily living. Boys with DMD spend over half of their lives in a wheelchair. Their upper limb function is preserved for an extended period, but subsequently deteriorates as the disease progresses. Unlike lower limb function, which in part can be compensated by using wheelchairs, upper limb function is not as easily substituted with the use of assistive devices. Despite the importance of upper limb function for quality of life, there have only been a few studies describing the upper limb impairments in DMD subjects, especially in the non-ambulatory DMD population.

Clinical trials primarily focus on ambulatory subjects with DMD due to the limited availability of reliable clinical endpoints for upper extremity muscles. Therefore, non-ambulatory individuals, who comprise over half of the DMD population, are unable to participate in the vast majority of clinical trials. This has both ethical concerns and practical implications for clinical trial recruitment given the limited pool of patients available to enroll in the rising number of clinical trials. To address these concerns, the DMD community has started developing upper extremity clinical endpoints, suitable for the non-ambulatory population.
NCT02310763, NCT02606136) have already started implementing upper extremity clinical endpoints.

There is a paucity of data demonstrating the reliability of functional tests for upper extremity in the literature. Some studies conducted in the late 70s and 80s utilized upper extremity tests, such as the Jebsen Hand Function test (JHFT)\textsuperscript{114} and the Brooke Upper Extremity Scale\textsuperscript{189}. However, few studies compared the utility of these tests\textsuperscript{113}. More recently, a new test, referred to as the Performance of Upper Limb (PUL)\textsuperscript{109}, was developed with input of a large number of international stakeholders. The PUL has been validated and implemented in a large multi-center natural history study in Europe\textsuperscript{110}. However, no systematic comparison of upper extremity outcome measures has been conducted to determine their feasibility and sensitivity in patients with DMD. Further investigation of these tests is warranted to determine the most appropriate upper extremity clinical outcome measures for future use in both natural history studies and clinical trials.

Upper extremity muscles have largely been ignored in natural history studies of DMD, even though functionally they are very important for activities of daily living like eating, grooming etc. There have been very few natural history studies\textsuperscript{110, 158, 195} that describe DMD disease progression in the upper extremities. Natural history data are very important to clinicians and parents of DMD patients, as it allows them to make well-informed clinical decisions about upper extremity impairments. Additionally natural history data acquired in a large cohort of individuals with DMD are important to help make critical decisions in the design of clinical trials, such as for example, inclusion criteria, sample size, and the time window required to detect therapeutic efficacy.

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The primary objectives of this study were: 1) to perform a pilot exploratory study comparing the sensitivity of different outcome measures assessing upper extremity function and 2) to measure upper extremity function and strength in a large cohort of boys with DMD (ambulatory and non-ambulatory) at varying disease stages, compared to age-matched controls.

Methods

Study Design for Pilot Study

The pilot study conducted at the University of Florida was approved by the institutional review board. 10 boys with DMD (8 ambulatory, 2 non-ambulatory), aged 7 - 15 years (9.7 ± 2.9 years) and 5 age-matched controls, aged 8 - 14 years (10.8 ± 2.6 years) participated in this study. The feasibility and sensitivity of four tests was compared: Brooke Upper Extremity Scale\(^{189}\), Jebsen Hand Function Test\(^{114}\), Motor Function Measure\(^{125}\), and Performance of Upper Limb Test, version 1.2\(^{109}\).

Study Design for Multi-Center Study

Subjects participated at three different sites in this cross-sectional multi-center natural history study. The three participating sites were University of Florida (Gainesville, Florida), Children's Hospital of Philadelphia (Philadelphia, Pennsylvania), and Oregon Health and Science University (Portland, Oregon), collectively known as the ImagingDMD group. Each site received approval from its respective institutional review board for conducting this study. 96 subjects with DMD (12.1 ± 3.1 years), 69 ambulatory and 27 non-ambulatory, and 27 age-matched control subjects (12.1 ± 3.4 years), between the ages 5 – 18.9 years participated in this study. We only selected those subjects who were on steroids because we did not have enough steroid naïve subjects for a statistical comparison with subjects on steroids. Subjects were divided
into three age groups: 5 - 8.9 years, 9 - 12.9 years, and 13 - 18.9 years. The demographics are shown in Table 5-1. The inclusion criteria for boys with DMD were: 1) between the ages of 5 and 18.9, 2) ambulatory or non-ambulatory, 3) previously diagnosed with DMD, with the onset of clinical symptoms before the age of 5, and 4) increased serum creatine kinase level or lack of dystrophin expression. The exclusion criteria for boys with DMD were: 1) unstable medical problems, 2) cognitive problems, that make it difficult to participate in testing, and 3) secondary conditions that could affect muscle metabolism or muscle function. The inclusion criteria for control subjects were: 1) between the ages of 5 and 18.9, 2) having normal developmental milestones, and 3) no history of fracture or injury to either the upper or the lower extremity that caused immobilization in the preceding year. Control subjects were excluded if they had: 1) unstable medical problems, 2) cognitive deficits preventing them from cooperating during the testing, or 3) conditions that impact muscle metabolism or muscle function. Once the purpose of the study was well-explained, parents and/or guardians provided written consent. Subjects provided written assent if they were less than 18 years old.

**Functional Measures**

**Pilot study.** Subjects performed the following functional tests: Brooke Upper Extremity Scale\(^{189}\), Jebsen Hand Function Test\(^{114}\), Motor Function Measure\(^{125}\), and Performance of Upper Limb Test, version 1.2\(^{109}\). The Brooke Upper Extremity Scale was used to determine a qualitative grade, as a point of reference, grade 1 indicated that subjects could fully abduct both arms until they touched above their heads and grade 6 indicating that subjects were unable to raise their hands to their mouths and had no useful function of their hands\(^{189}\). Jebsen Hand Function test (JHFT), a timed
test, was used for assessing 7 daily living activities such as writing, turning cards, lifting small objects, simulated feeding, stacking checkers, and lifting large light and heavy objects\textsuperscript{114}. Motor Function Measure (MFM) test was used to evaluate motor function with grades ranging from 0 to 3\textsuperscript{125}. This test consists of 32 items, which are further divided into three dimensions: dimension 1 (13 items) for evaluating standing position and transfers, dimension 2 (12 items) for examining axial and proximal motor function, and dimension 3 (7 items) for assessing distal motor function\textsuperscript{87, 91, 125, 126}. This tool has proven useful in the DMD population for evaluating the stage of the disease\textsuperscript{127}. Performance of Upper Limb (PUL) test was specifically designed by the international community to assess upper limb function in subjects with DMD. It consists of items at 3 levels – shoulder, upper arm, and forearm\textsuperscript{109}. PUL version 1.2 was used for the pilot study.

**Multi-center study.** After evaluating the sensitivity of functional tests in the pilot study, we chose to implement the Brooke Upper Extremity Scale and PUL version 2.0 for the multi-center study. The PUL was updated to version 2.0 after psychometric analysis of the scale. It has a simplified scoring system as compared to PUL 1.2. Moreover, certain items from PUL 1.2 were removed from PUL 2.0 as it was determined that they measured the same level of ability as other items. In the multicenter study, strength was also measured using – myogrip\textsuperscript{128} and myopinch\textsuperscript{128}, which have proven to be sensitive and reliable tools\textsuperscript{128}. Subjects were asked to squeeze the tool as hard as possible. Three trials were performed, with a rest of 30 seconds in between, and the largest strength measure obtained was used for analysis. All tests were performed by trained evaluators using a standard operating procedure manual. All subjects were
given simple, standardized instructions for each test. Parents/guardians of subjects were not present during testing for either the pilot or the multi-center studies.

**Data Analysis**

The non-parametric Mann-Whitney test was used to compare between controls and DMD subjects for each age group and also between ambulatory and non-ambulatory DMD subjects. The Kruskal-Wallis Test was used to compare across three different age groups in boys with DMD. The Spearman correlation test was performed for observing the relationship between age and PUL score and also the relationship between total PUL score and strength measures (myogrip and myopinch). Missing data were excluded from analysis. Missing data were due to lack of availability of equipment, or due to time constraints. The data are reported as mean ± standard deviation (SD), unless otherwise stated. The significance value was set at 0.05.

**Results**

**Pilot Study to Evaluate Upper Extremity Endpoints**

The goal of the pilot study was to determine the feasibility and sensitivity of each upper extremity functional test. We compared 4 commonly used upper extremity functional tests in DMD and control subjects. Using the Brooke Upper Extremity Scale, no significant difference could be detected between DMD and control subjects. All control subjects scored grade 1, whereas subjects with DMD obtained grades ranging from 1 – 4 (Figure 5-1). Using the JHFT, lifting small and large heavy objects were the only activities out of a total of 7, where DMD subjects took a significantly longer time than the controls (Figure 5-2). On the writing task, no significant difference could be found, even though DMD subjects took longer than controls to complete the task. This is likely due to a large amount of variability in this task across the different ages. Using the
MFM, a significant difference was found between DMD and controls. All controls obtained a total score of 27 except one subject, who received a total score of 26. Subjects with DMD scored in the 20 – 27 range (Figure 5-3). Similarly, using the PUL 1.2, a significant difference was seen between DMD and controls. All controls obtained a total score of 74, whereas DMD subjects’ total scores ranged from 46 – 73 (Figure 5-4).

In summary, 2 out of the 4 functional tests performed, specifically MFM and PUL 1.2, showed a significant difference between DMD and control subjects. While the MFM was sensitive, only a limited number of tasks focused on the upper extremity muscles. In contrast, the PUL was specifically designed for upper extremity function in patients with DMD and appeared to be sensitive and a wide range of scores across our DMD population. Therefore the PUL was selected for inclusion in the multi-center natural history study. In addition, we selected the Brooke Upper Extremity Scale, despite not showing a significant difference between DMD and control subjects, because it was a very quick test and is commonly used clinically.

**Comparison Between DMD and Controls in a Multi-Center Study**

Based upon the pilot study results, the Brooke Upper Extremity Scale and PUL were included as upper extremity functional tests in the multi-center study. As shown in Table 5-1, a significant difference was found on the Brooke Upper Extremity Scale between controls and DMD enrolled in the multi-center study. Overall, all control subjects scored grade 1, whereas subjects with DMD scored grades ranging from 1 – 5 (Table 5-1). Similarly, a significant difference was observed on the total PUL between controls and subjects with DMD. All controls scored the highest possible score of 42 except one subject (in the 5 – 8.9 year age group), who found it difficult to complete the
tearing paper task. Subjects with DMD had a wide range of scores, ranging from 15 – 42 for total PUL score (Table 5-1). When we compared the total PUL score of DMD subjects across all the age groups, we found significant functional decline in each age group, including in the youngest (5 – 8.9 years) as compared to controls. However, the performance of DMD subjects on the Brooke Scale was significantly different from controls in the two oldest age groups (9 – 12.9 and 13 – 18.9 years) and not in the youngest age group (5 – 8.9 years).

Both myogrip (Figure 5-5a) and myopinch (Figure 5-5b), showed a significant difference between controls and DMD. Controls had higher myogrip strength than their DMD counterparts across all age groups (1.6 times greater in 5 – 8.9 years, 2.2 times greater in 9 – 12.9 years, and 3.7 times greater in 13 – 18.9 years). Similarly, controls had higher myopinch strength than subjects with DMD across all age groups (1.8 times greater in 5 – 8.9 years, 2.1 times greater in 9 – 12.9 years, and 3.1 times greater in 13 – 18.9 years).

Upper Extremity Function and Strength in Young Versus Old Boys with DMD

For Brooke Upper Extremity Scale, the oldest age group showed a significant decline in function compared to the two other age groups (Figure 5-6). Increase of age revealed a decline in upper limb function. For PUL, there was a significant decline in upper limb function between the oldest and youngest age groups for total PUL score (Figure 5-7a), shoulder dimension scores (Figure 5-7b), and upper arm dimension scores (Figure 5-7c). However, no significant difference was found in the forearm dimension across all age groups in subjects with DMD (Figure 5-7d). Similarly, no significant difference was found in myogrip (Figure 5-8a) and myopinch strength tests (Figure 5-8b), across all age groups in DMD.
Upper Extremity Function and Strength in Ambulatory Versus Non-Ambulatory Subjects with DMD

Significant difference was observed between ambulatory and non-ambulatory patients with DMD on the Brooke Upper Extremity Scale. Subjects in both groups scored grades ranging from 1 – 5 (Figure 5-9). Similarly, a significant difference was observed between both groups on the total PUL score (Figure 5-10a) and shoulder and upper arm dimensions of PUL (Figure 5-10b, c). However, the forearm dimension of PUL did not show a significant difference between ambulatory and non-ambulatory subjects with DMD (Figure 5-10d).

To evaluate the PUL functional test more closely, we correlated the total PUL score and all three PUL dimensions with age and separated the cohort according to ambulatory status. A significant mild negative correlation was observed between age and total PUL score (Figure 5-11a), and shoulder and upper arm dimensions (Figure 5-11b-c). However, there was no significant correlation between age and forearm dimension (Figure 5-11d). On the myogrip test, ambulatory subjects with DMD scored 1.4 times greater than non-ambulatory subjects with DMD (Figure 5-12a). Similarly, on the myopinch test, ambulatory subjects with DMD scored 1.3 times greater than non-ambulatory subjects with DMD (Figure 5-12b).

Relationship between Function and Strength Measures

There was a significant moderate positive correlation between total PUL score and strength measured by the myogrip and myopinch tests. However, there was a lot of variability in strength measures for subjects with high total PUL scores. For subjects whose total PUL scores fell between 40 - 42, myogrip strength varied from 4.79 kg –
20.14 kg (Figure 5-13a). Similarly, in myopinch, strength varied from 1.33 kg – 4.36 kg for subjects with a total PUL score of 40 - 42 (Figure 5-13b).

**Discussion**

The primary aim of the pilot study was to critically compare the 4 commonly used upper limb functional tests – Brooke Upper Extremity Scale, Performance of Upper Limb test, Jebsen Hand Function Test, and Motor Function Measure test and to determine which would be best suited for the subsequent large multi-center study in the DMD population. Primarily, we found that PUL and MFM were more sensitive amongst all the tests we performed. However, the MFM test had a limited number of upper extremity tasks and did not include shoulder height activities. JHFT, on the other hand, did not show sensitivity and did not have many tasks related to activities of daily living. Based on the pilot study results, we chose to implement PUL, which was sensitive, in our multi-center study. In addition, Brooke Scale, though not sensitive but clinically used, was also selected in our multi-center study. The primary findings of our large natural history study of boys of age range 5 – 18.9 years: 1) On comparing across age groups, significant functional impairment was seen in boys with DMD, even in the youngest age group of 5 - 8.9 years as compared to the controls in total PUL score and in both strength tests (myogrip and myopinch). However, for Brooke Scale, no significant difference was found between subjects with DMD and controls in the 5 – 8.9 year old age group; 2) Non-ambulatory boys with DMD performed significantly worse than ambulatory boys with DMD on both Brooke Scale and total PUL. Ambulatory boys were about 1.3 times stronger than non-ambulatory boys with DMD as examined by strength tests (myogrip and myopinch); 3) The oldest boys with DMD (13 – 18.9 years) performed significantly worse than the youngest boys (5 – 8.9 years) in total PUL.
However, these differences were driven by significant differences in shoulder and upper arm dimensions of PUL and not by the forearm dimension of PUL; 4) Boys with DMD did not differ significantly across age groups in strength tests (myogrip and myopinch).

Functional tests (Brooke Scale and total PUL) were more sensitive than strength tests (myogrip and myopinch) in observing disease progression in DMD subjects across different age groups. Brooke Scale was able to show significant changes across different age groups in subjects with DMD. PUL could also significantly differentiate between the youngest and the oldest age group for total score, shoulder, and upper arm dimensions, but not for the forearm dimension in the DMD cohort. This may mean that PUL is not sensitive enough to reliably detect progressive decline in functional impairments in distal muscles in DMD subjects. However, both myogrip and myopinch tools, used for strength testing, did not show significant differences across age groups in DMD subjects, in contrast to the previous study\textsuperscript{157}, where they found that strength decreased with increasing age. This difference in findings could be due to different inclusion criteria. The study conducted by Hogrel et al. (2016) was limited to subjects who could be treated by exon 53 skipping\textsuperscript{157}, which may not be representative of the whole DMD population. Our study, on the other hand, included DMD subjects regardless of genetic mutation. According to Hogrel et al. (2016), subjects who could be treated by exon 53 skipping might be weaker with more severe phenotype and might have more rapid loss of strength\textsuperscript{157}. Unfortunately, we only had 3 subjects who could be treated by exon 53 skipping, so we could not perform sub-analysis on those subjects to compare our results with their study. Moreover, Hogrel et al. (2016) included both steroid and steroid naïve subjects\textsuperscript{157}, whereas, our study, examined only subjects taking
steroids. It has been reported that steroids tend to have a positive effect on upper extremity function$^{195}$.

Proximal muscles of the upper extremity exhibited functional decline with age around 9 years of age. Both shoulder and upper arm muscles showed that there was a functional decline with age, as assessed by the PUL. We observed declining performance in total PUL score in subjects around 9 years of age. For the shoulder and upper arm dimensions of PUL, subjects exhibited functional decline after 9 and 10 years of age respectively. This was similar to result reported by Pane et al. (2014), where the decline was observed after age of 8 years, 9 – 10 years, and 8 – 9 years for total PUL score, shoulder dimension, and upper arm dimension respectively$^{110}$. This showed that functional decline in upper extremity muscles happened earlier than anticipated by the DMD community. This information may help clinicians to also focus on maintaining function of upper extremity along with lower extremity at an early age, considering the important role of these proximal upper extremity muscles in activities of daily living.

Both functional (Brooke Scale and total PUL) and strength (myogrip and myopinch) tests could significantly differentiate between ambulatory and non-ambulatory subjects with DMD. Similar to Hogrel et al. (2016)$^{157}$, our study found significant differences between ambulatory and non-ambulatory subjects with DMD on the Brooke Upper Extremity Scale. Non-ambulatory subjects with DMD showed a wide range of variability in their scores, ranging from 1 – 5 on the Brooke Scale in our study, which is similar to other studies where scores range from 2 – 6$^{158}$ and 3 – 5$^{157}$ on the Brooke Scale for non-ambulatory subjects. Moreover, total PUL score and proximal PUL dimensions (shoulder and upper arm) were also able to distinguish significantly between
ambulatory and non-ambulatory boys with DMD. We also found significant differences between ambulatory and non-ambulatory boys with DMD in strength measurement, similar to what was found by Hogrel et al. (2016)\textsuperscript{157}. So, these function and strength tests have the ability to be used across a wide spectrum of the disease, including both ambulatory and non-ambulatory boys. Thus, clinical trials and natural history studies could continue to include boys with DMD, even after they lose ambulation to track disease progression by using these clinical endpoints.

Functional tests were practically more useful than strength tests for clinical use in DMD population. Both upper limb function tests (Brooke Upper Extremity Scale and PUL) took approximately 30 minutes to administer to the subjects. The main advantages of using these tests were that they were easy to use and clinically relevant. Although myotools are sensitive, quantitative tools for measuring subject’s strength, they are more expensive so they cannot be used commonly by clinicians. We included these tools in our study to observe strength in our population and compare our findings to previous studies in which these tools were used\textsuperscript{157}. Moreover, these tools could only evaluate strength of distal muscles\textsuperscript{158}, and are not useful to measure strength of more proximal muscles like the shoulder and upper arm. So, functional tests might be clinically more useful to evaluate functional impairment in proximal muscles of the upper extremity in DMD patients.

There are some limitations in our study design. First, we were unable to see the decline in upper extremity function and strength in DMD population over a period of time because this was a cross-sectional study and not a longitudinal study. Second, this study only included subjects on steroids because we had a very limited sample size of
steroid naïve subjects, since steroids are used as a standard of care in DMD. Third, we examined a limited age range of DMD cohort (5 – 18.9 years), and did not consider DMD subjects outside this age range.

**Summary**

In conclusion, it could be said that out of different upper extremity outcome measures evaluated in the pilot study, PUL and MFM were more sensitive measures. However, MFM did not include shoulder height activities and had very few upper extremity tasks. Based upon pilot study results, PUL was selected for our large cohort multi-center study because of its sensitivity. Brooke Scale was also selected because of its clinical use, even though it did not show sensitivity. In a large cohort natural history study, we found a presence of upper extremity function and strength decline in individuals with DMD compared to healthy controls. Non-ambulatory boys with DMD performed significantly worse than ambulatory boys on both functional tests (total PUL and Brooke Scale) and strength tests (myogrip and myopinch). However, functional measures were more sensitive to disease progression with age compared to strength tools. This natural history information may be important for the design of clinical trials and to inform parents and clinicians. Future studies could investigate how these upper limb function and strength measures change over time in individuals with DMD in a longitudinal study design.
Table 5-1. Demographics of enrolled subjects (DMD and controls) at baseline in a multi-center study.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Total</th>
<th>5 – 8.9</th>
<th>9 – 12.9</th>
<th>13 – 18.9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SD</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><strong>Age (years)</strong></td>
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</tr>
<tr>
<td>DMD</td>
<td>12.1 ± 3.1</td>
<td>7.2 ± 1.0</td>
<td>11.1 ± 1.1</td>
<td>15.3 ± 1.5</td>
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<td>(n = 96)</td>
<td>(n = 14)</td>
<td>(n = 47)</td>
<td>(n = 35)</td>
<td></td>
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<tr>
<td>Control</td>
<td>12.1 ± 3.4</td>
<td>6.7 ± 0.5</td>
<td>11.1 ± 1.5</td>
<td>15.3 ± 1.6</td>
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<tr>
<td>(n = 27)</td>
<td>(n = 4)</td>
<td>(n = 12)</td>
<td>(n = 11)</td>
<td></td>
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<tr>
<td><strong>Weight (kg)</strong></td>
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<td></td>
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<tr>
<td>DMD</td>
<td>40.0 ± 13.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.5 ± 6.1</td>
<td>37.6 ± 9.3</td>
<td>50.1 ± 12.5</td>
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<tr>
<td>Control</td>
<td>47.6 ± 20.5</td>
<td>23.7 ± 7.5</td>
<td>39.8 ± 8.7</td>
<td>64.7 ± 19.2</td>
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<td><strong>Height (m)</strong></td>
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<tr>
<td>DMD</td>
<td>1.29 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.15 ± 0.09</td>
<td>1.29 ± 0.06</td>
<td>1.35 ± 0.09</td>
</tr>
<tr>
<td>Control</td>
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<td>1.23 ± 0.09</td>
<td>1.49 ± 0.11</td>
<td>1.72 ± 0.11</td>
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<td><strong>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</strong></td>
<td></td>
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<tr>
<td>DMD</td>
<td>23.0 ± 6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.8 ± 2.4</td>
<td>22.2 ± 5.1</td>
<td>26.9 ± 6.1</td>
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<tr>
<td>Control</td>
<td>18.9 ± 4.0</td>
<td>15.3 ± 2.4</td>
<td>17.7 ± 1.8</td>
<td>21.4 ± 4.7</td>
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<td><strong>Brooke Score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMD</td>
<td>1-5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1-3</td>
<td>1-5</td>
<td>1-5</td>
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<tr>
<td>Control</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total PUL Score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMD</td>
<td>15-42&lt;sup&gt;d&lt;/sup&gt;,&lt;sup&gt;*&lt;/sup&gt;</td>
<td>25-41</td>
<td>16-42</td>
<td>15-42</td>
</tr>
<tr>
<td>Control</td>
<td>41-42</td>
<td>41-42</td>
<td>42</td>
<td>42</td>
</tr>
</tbody>
</table>

Data are means ± standard deviations. BMI: Body Mass Index; <sup>a</sup> indicates data collected in n = 94; <sup>b</sup> indicates data collected in n = 89; <sup>c</sup> indicates data collected in n = 92; <sup>d</sup> indicates data collected in n = 90. All DMD subjects were on steroids. <sup>*</sup> indicates DMD boys were significantly different than controls on Brooke Score and total PUL Score.
Figure 5-1. Cross sectional comparison between DMD and control subjects for Brooke Upper Extremity Scale in the pilot study. No significant difference was found between DMD and control subjects, p < 0.05. Range of data is shown.

Figure 5-2. Cross sectional comparison between DMD and control subjects for Jebsen Hand Function Test in the pilot study. DMD subjects took longer time to lift small and large heavy objects than controls, p < 0.05.
Figure 5-3. Cross sectional comparison between DMD and control subjects for Motor Function Measure Test in the pilot study. Total MFM score was significantly different between controls and DMD subjects, p < 0.05. Range of data is shown.

Figure 5-4. Cross sectional comparison between DMD and control subjects for Performance of Upper Limb Test in the pilot study. Total PUL score was significantly different between controls and DMD subjects, p < 0.05. Range of data is shown.
Figure 5-5. Cross sectional comparison between DMD and control subjects for myogrip and myopinch across all age groups in a multi-center study. Significant difference was found between DMD and control subjects for all age groups for both a) myogrip and b) myopinch. Data with standard error of mean is shown, p < 0.05.

Figure 5-6. Comparison across different age groups in subjects with DMD for Brooke Upper Extremity Scale in a multi-center study. The oldest age group scored significantly worse than the other two youngest age groups, p < 0.05. Range of data is shown.
Figure 5-7. Comparison across different age groups in subjects with DMD for Performance of Upper Limb test in a multi-center study. The oldest age group of 13 – 18.9 years performed significantly worse than the youngest age group of 5 – 8.9 years in a) Total PUL score, b) Total High PUL score, and c) Total Mid PUL score. However, there was no significant difference for d) Total Distal PUL score across all age groups, p < 0.05. Range of data is shown.
Figure 5-8. Comparison across different age groups in subjects with DMD for myogrip and myopinch in a multi-center study. No significant difference was found across age groups for both a) myogrip and b) myopinch. Data with standard error of mean is shown, p < 0.05.

Figure 5-9. Comparison between ambulatory and non-ambulatory boys with DMD for Brooke Upper Extremity Scale in a multi-center study. Significant difference was found between both the groups, p < 0.05. Range of data is shown.
Figure 5-10. Comparison between ambulatory and non-ambulatory subjects with DMD for Performance of Upper Limb test in a multi-center study. Significant difference was found between both the groups for a) Total PUL score, b) Total High PUL score, c) Total Mid PUL score. However, no significant difference was found between ambulatory and non-ambulatory boys with DMD for d) Total Distal PUL, p < 0.05. Range of data is shown.
Figure 5-11. Relationship of Performance of Upper Limb test with age in subjects with DMD in a multi-center study. Mild negative correlation was found between age and a) Total PUL score, b) Total High PUL score, and c) Total Mid PUL score. However, there was a negligible correlation between age and d) Total Distal PUL.
Figure 5-12. Comparison between ambulatory and non-ambulatory subjects with DMD for a) myogrip and b) myopinch in a multi-center study. Significant difference was found between both the groups, p < 0.05. Data with standard error of mean is shown, p < 0.05.

Figure 5-13. Relationship between Total PUL Score and a) myogrip and b) myopinch in a multi-center study. There was a moderate positive correlation between PUL score and strength measures.
CHAPTER 6
EXAMINATION OF THE NATURAL HISTORY OF DISEASE PROGRESSION IN
UPPER EXTREMITY IN BOYS WITH DMD (AMBULATORY AND NON-
AMBULATORY) USING $T_2$ MAGNETIC RESONANCE IMAGING BIOMARKERS

Background

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder, characterized by progressive muscle weakness, and ultimately leading to death of patients due to respiratory and cardiac muscle involvement\textsuperscript{30}. Individuals with DMD typically lose ambulation around 12 years of age\textsuperscript{39}. Declines in upper extremity function typically follow, with loss of self-feeding in the mid-teens. The loss of upper extremity function compromises quality of life and independence, and increases the burden on caregivers\textsuperscript{197}. Despite the importance of the upper extremities in DMD, there have been relatively few studies aimed at characterizing the upper extremity muscles in DMD.

Most clinical trials have prioritized the inclusion of ambulatory patients, even though the non-ambulatory patients account for roughly two-thirds of the DMD population\textsuperscript{197}. However, it is potentially problematic from an ethical standpoint to exclude these non-ambulatory boys from participation in clinical trials that maybe beneficial to them. It is also a concern from a practical standpoint due to limited pool of ambulatory subjects available for recruitment. In view of these concerns, upper extremity endpoints are being developed which can be used in the non-ambulatory DMD population\textsuperscript{110, 128}.

Some clinical trials have failed to demonstrate efficacy, which may be partly due to a lack of sensitive outcome measures. Unfortunately, functional tests used in clinical trials are motivation and age-dependent and may not be sensitive enough to track disease progression. Further, muscle biopsy is invasive in nature, and due to
considerable muscle heterogeneity, does not necessarily provide a complete picture of the muscle’s condition. There is an urgent need to develop non-invasive, sensitive outcome measures that can detect changes in the whole muscle. Magnetic resonance imaging (MRI) is a non-invasive technique that provides a sensitive measure of the condition of the muscle. MRI can be used in both ambulatory and non-ambulatory patients. MRI has been able to demonstrably track disease progression in the lower extremity\textsuperscript{171, 174, 176} and is currently being used as a measure in several clinical trials (NCT02439216, NCT03179631).

There is a pressing need to develop upper extremity biomarkers of disease progression in DMD. Because of this need, investigators and the DMD community have showed increased interest in examining the upper extremity in individuals with DMD using MRI. Recent studies have focused on forearm muscles\textsuperscript{152, 198} or upper arm muscles\textsuperscript{199} and were implemented in a relatively small cohort. To our knowledge, no one has evaluated proximal shoulder muscles, despite their functional importance. Our group published a pilot study focused on 3 segments of the upper extremity: shoulder, upper arm, and forearm\textsuperscript{197}. This present study builds on our previous pilot study and expands to a larger cohort over multiple centers. Specifically, this study focuses on MRI T\textsubscript{2}, which is sensitive to inflammation, damage, and fatty infiltration, and has proven to be a valuable marker of disease progression in the lower extremity\textsuperscript{174, 176}.

The primary objectives for this large, multi-center study were: 1) To evaluate MRI T\textsubscript{2} in boys with DMD, ages 5 - 18 years, compared to age-matched controls in different muscles of upper extremity; 2) To evaluate the relationship between upper extremity MR T\textsubscript{2} biomarkers and performance on upper limb functional tests and strength.
measures; and 3) To compare disease progression in upper extremity muscles with disease progression in lower extremity muscles using MRI $T_2$.

**Materials and Methods**

**Study Design**

100 boys with DMD (71 ambulatory, 29 non-ambulatory) and 28 controls aged 5 - 18 years participated in this cross-sectional multi-center study. Table 6-1 illustrates the demographics of subjects with DMD and controls in each of the age groups. The sites participating in this study were University of Florida in Gainesville, Florida; Children’s Hospital of Philadelphia (CHOP) in Philadelphia, Pennsylvania, and Oregon Health and Science University (OHSU) in Portland, Oregon, together known as the ImagingDMD group. The study was approved by the Institutional Review Boards at each site. Written informed consent was obtained from 18 year old subjects, and from parents/guardians of subjects under the age of 18, and written assent was obtained from subjects under the age of 18. The inclusion criteria for boys with DMD were: 1) between the ages of 5 - 18 years, 2) previously diagnosed with DMD, with the onset of clinical symptoms before 5 years of age, 3) increased serum creatine kinase level or lack of dystrophin expression. The exclusion criteria for boys with DMD were: 1) with a contraindication to undergoing MRI examination, 2) unstable medical problems, 3) cognitive problems, that make it difficult to participate in testing, and 4) secondary conditions that could affect muscle metabolism or muscle function. The inclusion criteria for including healthy control subjects were: 1) between the ages of 5 - 18 years, 2) having achieved normal developmental milestones, 3) no history of fracture or injury to either upper or lower extremity, causing immobilization over the last 1 year. Control subjects were excluded if they had: 1) contraindications to MR examination, 2) unstable medical problems, 3)
cognitive deficits preventing them from cooperating during the testing, 4) conditions that impact muscle metabolism or muscle function. The study consisted of MRI testing and was followed by functional testing.

**MRI Data Acquisition and Analysis**

MRI measurements were obtained using a 3T MR whole body scanner, available at all 3 sites (University of Florida: Philips Achieva Quasar dual system [Philips, Amsterdam, The Netherlands]; Oregon Health and Science University: Siemens Magnetom TIM Trio system [Siemens, Munich, Germany]; Children’s Hospital of Philadelphia: Siemens Magnetom Verio system), with data transmitted to and stored securely at the University of Florida site. All the MR operators at each site used standard operating procedures (SOPs) to ensure consistency across sites. All MR operators at each site were trained and then certified to acquire MR data per SOPs.

**MRI of upper extremity.** MRI of upper extremity was acquired in one session. This session took about 60-90 minutes. Before the subjects entered the MR room, they were screened to make sure that they had no metallic objects with them and had no other contraindications to MR. Parents/guardians were permitted to sit inside the MR room with the subject after appropriate screening procedures. A research staff member was always present inside the MR room with the subject for safety and compliance purposes. For the MR data acquisition, subjects were positioned in supine inside the MR bore. The dominant arm was scanned. At the University of Florida, a SENSE eight channel flex coil was used for acquiring upper extremity MR data. At Children’s Hospital of Philadelphia, the coil used for upper extremity was a Siemens 6 channel body Array Flex coil, and at Oregon Health and Science University, an 18-channel Siemens Body Matrix coil was used. Custom-built thermoplastic splints were used to position the upper
extremity of the subjects and minimize motion artifact. Rice pads were used to prevent motion of the extremities during scanning and subjects typically viewed a movie during the MR examination.

$T_2$-weighted images were obtained by using a spin echo sequence (repetition time - TR of 3000 ms; echo times - TEs from 20 ms to 320 ms, with 20 ms spacing), and $T_2$ maps were generated by fitting a mono-exponential model to the 40-100 ms TEs. The $T_2$ maps were automatically processed within 24 hours of data acquisition. MRI $T_2$ was determined for upper extremity muscles by manually generating regions of interest (ROI) on $T_2$-weighted images (TE 20 ms) by trained and certified analyzers. Analyzers followed standard operating procedures (SOPs) to analyze MRI $T_2$ data. For each segment (shoulder, upper arm, and forearm), analyzers selected slices based on an internal anatomic landmark to ensure consistency across subjects of different sizes. Anatomic landmarks were chosen to allow the regions of interest (ROIs) to be drawn within the belly of the muscles of interest. Three slices were included for each segment. ROIs were traced close to the border of each muscle or muscle group of interest: subscapularis (SUB), infraspinatus (INFRA), deltoid (DEL), biceps brachii (BB), brachialis (BRA), triceps brachii (TB), anterior forearm compartment (AF), and posterior forearm compartment (PF). Analyzers made sure not to include intramuscular fascia while drawing ROIs. Mean $T_2$ for each muscle was obtained by averaging all pixels across the three ROIs.

Following analysis, data were examined by two independent reviewers to ensure that ROIs were accurate and that the image quality was sufficient for analysis. If the two reviewers disagreed, an adjudicating expert reviewer made a final decision. By
implementing this protocol, there was an average success rate of 90% for the upper extremity, out of all the data we collected. The primary reason for the unusable data was related to motion artifacts and image inhomogeneity. The average number of pixels were 1846 for DEL, 334 for BB, 787 for TB, 962 for AF, and 603 for PF. The slice to slice variability was 4% for DEL, 5% for BB, 4% for TB, 4% for AF, and 4% for PF.

**MRI of lower extremity.** MRI of lower extremity was acquired in another session, usually a day prior to acquiring MRI of the upper extremity. This session also took about 60-90 minutes. Screening of the subject was done similar to MRI of upper extremity. Subjects were positioned in supine inside the MR bore for their lower extremity scan. The right leg was scanned unless it had a previous fracture, in which case the left leg was used. Lower extremity data were collected as described previously.\(^{172}\)

MRI \(T_2\) for lower extremity muscles was determined using the same protocol described above for the upper extremity muscles. For both upper and lower leg muscles, analyzers drew ROIs on three slices based on anatomic landmarks described in the SOPs. ROIs were traced on these muscles of interest: biceps femoris long head (BFLH), vastus lateralis (VL), gracilis (GRA), soleus (SOL), medial gastrocnemius (MG), peroneals (PER), tibialis anterior (TA), and tibialis posterior (TP). Intramuscular fascia was not included while manually drawing ROIs. Out of all the data we collected for our study, we had an average success rate of 90% for the lower extremity muscles.

**Function and Strength Evaluation**

After the MR examination was complete, each subject performed upper extremity functional evaluation tests including – Performance of Upper Limb (PUL), version 2.0,\(^{109, 110}\), the Brooke Upper Extremity Scale\(^{189}\), and strength testing using myogrip and
myopinch\textsuperscript{128}. The tests were administered by trained evaluators, who followed standard operating procedures and gave simple, standardized instructions to the subjects. The PUL test was specifically designed for the Duchenne population by an international workgroup\textsuperscript{109}, and was developed for both ambulatory and non-ambulatory subjects. The Brooke Upper Extremity Scale consists of grades 1 - 6 with grade 1 indicating a subject could fully abduct his arms until his hands touched above his head and grade 6 indicated a subject was unable to raise his hands to his mouth and had no functional use of his hands\textsuperscript{189}. Both myogrip and myopinch are sensitive tools that have been designed to measure strength in the DMD population\textsuperscript{128}. Myogrip measures grip strength and myopinch measures key pinch strength. Subjects were asked to squeeze the tool as hard as possible. Both of these strength tests were performed 3 times with a 30 second break after each trial. The largest values were used for analysis.

**Data Analysis**

Non-parametric Mann Whitney test was performed to compare MRI T\textsubscript{2} between DMD and control subjects across all age groups and also to compare MRI T\textsubscript{2} between ambulatory and non-ambulatory subjects with DMD. The Kruskal Wallis test with Dunn’s multiple comparisons test was performed to compare across all age groups for controls and DMD subjects. The Spearman correlation test was used to determine the correlation coefficient between MRI T\textsubscript{2} and functional and strength measures as well as between composite upper extremity MRI T\textsubscript{2} and composite lower extremity MRI T\textsubscript{2}. Composite upper extremity MR T\textsubscript{2} for each subject was determined by taking the average of MR T\textsubscript{2} of all upper extremity muscles analyzed for that subject. We used the average of MR T\textsubscript{2} of all muscles to evaluate the overall condition of the upper extremity.
Composite lower extremity MR $T_2$ was similarly determined. The statistical significance was set at $p<0.05$.

**Results**

**Cross Sectional Comparison Between Controls and DMD Subjects**

We analyzed 8 upper extremity muscles in both DMD and control subjects at all 3 segments of the upper extremity - shoulder, upper arm, and forearm. MRI $T_2$ images for all the upper extremity muscles analyzed are shown in a control subject (Figure 6-1). Comparing images of boys with DMD and controls, fatty infiltration could be visually observed in older boys with DMD (Figure 6-2a-c). This was also observed quantitatively, as displayed in a histogram (Figure 6-2d). $T_2$ values were shifted to the right in both the younger and older DMD subjects as compared to the control subject. All upper extremity muscles showed significantly higher $T_2$ values in subjects with DMD compared to controls across all age groups, including in the youngest age group of 5 – 8.9 years (Figure 6-3).

**MRI $T_2$ in Old Versus Young Boys with DMD**

Significant differences were found between age groups of participants with DMD. The 13 – 18.9 year old subjects had greater $T_2$ values than the youngest age group of 5 – 8.9 years for all the upper extremity muscles examined in subjects with DMD (Figure 6-4).

When dividing the DMD cohort according to ambulatory status, non-ambulatory subjects had significantly higher $T_2$ values than the ambulatory subjects for all the upper extremity muscles examined (Figure 6-5).
Correlation Between MRI T<sub>2</sub> and Function and Strength Measures

MRI T<sub>2</sub> of all upper extremity muscles were significantly correlated with all function and strength measures except distal PUL for a few muscles (Table 6-2). Overall, the functional tests were more strongly correlated with MRI T<sub>2</sub> than the strength tests, and the strongest correlation was found with Brooke Upper Extremity Scale. A significant correlation was observed with each muscle for both high and mid level of the PUL.

When dividing the cohort according to ambulation status, it was observed that ambulatory subjects had overall higher total PUL scores and lower Brooke scores and lower MRI T<sub>2</sub> values than the non-ambulatory subjects with DMD, though considerable heterogeneity was observed among the subjects (Figure 6-6a, b). Similarly, ambulatory subjects had overall higher grip and pinch strength and lower MRI T<sub>2</sub> values than the non-ambulatory subjects with DMD, with heterogeneity present across the cohort (Figure 6-6c, d). Further, when comparing each dimension of the PUL, most of the ambulatory and non-ambulatory subjects with DMD scored high in the distal PUL, whereas there was a wider range of scores observed with the high PUL (Figure 6-7).

Upper Versus Lower Extremity Muscles in Subjects With DMD

There has been considerable interest in the DMD community to know how upper extremity muscles perform in comparison to the lower extremity muscles. We plotted all the upper and lower extremity muscles examined to see the pattern of disease progression (Figure 6-8). Similar to lower extremity muscles, proximal to distal pattern of disease involvement was observed among the upper extremity muscles. When comparing upper and lower extremity muscles, the proximal muscles of the leg (upper leg muscles - BFLH, VL) tended to have the most elevated T<sub>2</sub>, and the proximal muscles
of the upper extremity (shoulder muscles – INFRA, SUB, DEL) had the next highest $T_2$ values as compared to the remaining muscles across all age groups (Figure 6-8a, c, d) except in the youngest age group (Figure 6-8b). Forearm muscles, GRA, TA, and TP were the least affected muscles in all age groups, except in the 5 – 8.9 year old subjects with DMD. In the 5-8.9 year old age group, VL, BFLH, SUB, and INFRA had almost the same $T_2$ values. These results indicate that $T_2$ values in BFLH, VL muscles increase at a faster rate after the age of 9 years.

Also, a composite value for each subject for both upper and lower extremity was derived, and a strong positive correlation between composite upper extremity MRI $T_2$ and composite lower extremity MRI $T_2$ was observed for all subjects (Figure 6-9). The correlation was strongly positive for ambulatory subjects ($r = 0.84$), and moderately positive for non-ambulatory subjects ($r = 0.61$).

**Discussion**

The vast majority of research using MR in the DMD community has focused on lower extremity muscles and ambulatory subjects. The impact of DMD on upper extremity muscles has not received as much attention, and non-ambulatory DMD patients have been largely excluded from clinical trials. Recently there have been a few MR studies of the upper extremity, but they have mostly been limited to forearm muscles$^{152, 157}$, while information in large key upper extremity muscles have not been well explored. The aims of our study were 1) to generate natural history data for upper extremity muscles in both ambulatory and non-ambulatory subjects with DMD using MRI $T_2$ in a large, multi-center study and compare it with age-matched controls; 2) to examine the relationships between MRI $T_2$ and upper extremity functional and strength measures. There has also been a considerable interest in the DMD community to better
understand how lower extremity muscle disease progression compares with the upper extremity. Since we examined MRI T\textsubscript{2} of both upper and lower extremity muscles in our large cohort, this led to an additional aim for our study: to compare disease status of upper extremity muscles to lower extremity muscles using MRI T\textsubscript{2}. The primary findings were: 1) MRI T\textsubscript{2} was significantly elevated in subjects with DMD compared to the controls across all age groups, including in the youngest age group, and in all the upper extremity muscles examined; 2) there was a proximal to distal pattern in disease progression in the upper extremity muscles; 3) all upper extremity muscles showed a significant difference between the youngest and the oldest age groups in subjects with DMD; 4) there was a stronger relationship between MRI T\textsubscript{2} and functional measures than with strength tests; 5) upper leg muscles (BFLH, VL) tended to have higher MRI T\textsubscript{2} values, followed by shoulder muscles (INFRA, SUB, DEL) and lower leg and upper arm muscles. The lowest values were found in the forearm muscles (AF, PF), GRA, TA, and TP.

Upper extremity muscles were affected even in the youngest boys with DMD, as compared to their healthy, age-matched peers. Significant differences were found between subjects with DMD, as compared to the controls across all age groups, for all upper extremity muscles. Similar results have been shown in our previous pilot study\textsuperscript{197} for shoulder and upper arm muscles. Gaur et al. (2016) also observed significant differences between controls and DMD for upper arm muscles, though age-binning was not done in their study\textsuperscript{199}. The upper extremity has been largely ignored for a long time. However, this data shows that all upper extremity muscles are affected at an early age,
as compared to controls. This information is valuable for clinicians and parents, and may be used in making clinical decisions at an early age in subjects with DMD.

MR measurements may also be valuable in clinical trials. MRI T₂ correlated with both function and strength measures of the upper extremity. One of the criteria for using MRI in clinical trials is that it should show a correlation with the current clinical outcome measures. To test this concept, we correlated the MRI T₂ measures in upper extremity with commonly used outcome measures in the upper extremity – PUL, Brooke Scale, myogrip, and myopinch. As with the lower extremity, MRI T₂ correlated with both function and strength measures of the upper extremity, though a stronger correlation was found with the functional measures than with the strength tests. This may not be surprising, since strength changes appear to be less sensitive in the DMD population. Thus, the strong correlation of MRI T₂ with functional tests, may provide further support for the use of MRI T₂ in clinical trials for upper extremity.

Proximal muscles rather than forearm muscles of the upper extremity might be more useful for monitoring disease progression in DMD and for use in clinical trials. MRI T₂ of proximal muscles, showed the strongest correlation with functional tests of upper extremity. SUB, INFRA, and PF did not show a significant correlation with distal PUL. It was expected that shoulder girdle muscles (SUB, INFRA) would not show a strong relationship with distal level activities, such as touching number on a diagram, pick up 10 g weight, because mostly forearm muscles were used for these tasks. Interestingly, the posterior forearm did not show a significant correlation with distal PUL. A potential reason for this might be that distal PUL has not many items to detect disease
progression since most of the subjects scored scores between 10 – 13. Another reason might be that PF is not a sensitive muscle group to detect clinical changes in function. Since most of the studies\(^{152, 157, 198}\) have been focusing on forearm muscles, our results indicate that it would be better to consider other muscles for clinical trials. For example, DEL and BB correlated well with function and were significantly different among age groups in DMD. Therefore, DEL and BB may be the preferred muscles to choose out of all the upper extremity muscles examined for clinical trials.

Disease progression in DMD in proximal muscles (shoulder muscles) of the upper extremity was second only to the progression seen in proximal muscles of the lower extremity, when examined by MRI T\(_2\). It has been observed clinically that proximal lower limb muscles appear to be affected before upper limb and distal muscles\(^{49}\). In our study using MRI T\(_2\) we found that proximal muscles of the lower extremity (BFLH, VL) tended to have the highest T\(_2\) values, followed by the proximal muscles of the upper extremity (shoulder muscles- INFRA, SUB, DEL) across all age groups except in the youngest age group of 5 – 8.9 years. So, it is equally important to examine disease progression in upper extremity muscles, along with lower extremity. The proximal upper extremity muscles play an important role in the activities of daily living such as eating, bathing, etc., thereby, making it important for clinicians to focus on maintaining their function along with the lower extremity functions.

Composite upper extremity MRI T\(_2\) showed a stronger correlation with composite lower extremity MRI T\(_2\) in ambulatory boys than in non-ambulatory boys with DMD. There have been a few studies comparing functional measurements between upper and lower extremity\(^{136}\). In terms of function, a significant relationship between upper and
lower limbs was found in DMD\textsuperscript{136}. The authors of that study recommended that upper and lower extremity function measurements could substitute for each other in clinical trials, in which whole body effects are expected\textsuperscript{136}. However, in a recent study, upper limb involvement was observed in ambulatory boys with DMD, but there was a non-linear correlation between the PUL and the six minute walk test\textsuperscript{111}. When we examined the relationship between upper and lower limb muscles using MRI T\textsubscript{2}, we found that upper extremity MRI T\textsubscript{2} correlated strongly with lower extremity MRI T\textsubscript{2} for ambulatory subjects and moderately for non-ambulatory subjects. The upper and lower extremity muscles of non-ambulatory subjects exhibited greater variability in MRI T\textsubscript{2}. In some subjects, effects on lower extremity did not necessarily manifest corresponding effects on upper extremity, and vice versa. This leads us to tread with caution when collecting data for one extremity but not for the other. Moreover, upper extremity has the advantage of being linear in terms of disease progression and having no sudden dramatic break during the transition period of ambulation loss. Thus, MRI of upper extremity maybe valuable for use in clinical trials, especially in the non-ambulatory DMD population.

Not all muscles have an adequate pixel number for inclusion in the study. Specifically, smaller muscles in the forearm did not meet this criterion. For example, when we attempted to analyze individual forearm muscles, as few as an average of 41 pixels/slice for extensor pollicis longus pixels (posterior forearm muscle) were included, and this resulted in a coefficient of variation of T\textsubscript{2} of 16\%. Similarly, there were an average of 46 pixels/slice for flexor pollicis longus pixels (anterior forearm muscle), and a coefficient of variation of T\textsubscript{2} was 25\%. Because of this variability and lower confidence
in T2 measures, we decided to combine muscles into the anterior and posterior forearm. Despite the limitation of now including muscle fascia (that separates all the individual muscles) in our ROIs, we found this to be a more reliable measure. When the anterior forearm muscles were combined this resulted in a slice to slice variation of 2%, and for posterior forearm, the slice to slice variation was 1%. Overall, we found minimal variation in three slices of the muscle belly (coefficient of variation of each muscle ≤ 5%), nonetheless we used multiple slices to increase confidence, increase coverage, and account for any possible heterogeneity of muscles.

Overall, we had considerable success with the upper extremity measurements. The challenges for scanning upper extremity were that the subjects were further into the magnet than the typical lower extremity MR scans and therefore more likely to feel confined in such a small space. Moreover, the upper extremity measures had a greater offset from center, and therefore the potential for greater inhomogeneity artifacts. In spite of these challenges, the overall success rate was equivalent between upper and lower extremity, after training of MR operators. We implemented various strategies to help overcome the logistical challenges including positioning the subjects more to the center by lying on their side and by putting padding under the torso region and using splint.

Our study had a few limitations. First, since our study was cross-sectional, we were unable to examine muscle changes in the upper extremity in the DMD population over a period of time. Second, we had a limited sample of steroid naïve subjects, so we could not compare them statistically with subjects on steroids. Third, we only evaluated DMD patients in our age range of 5 – 18.9 years for this study, and did not consider
subjects outside this age range. Fourth, for muscles like SUB and INFRA we did not always capture the mid-belly of the muscles, and the muscles analyzed were relatively more proximal or distal. However, over the regions we acquired the data, we have shown this has minimal effect on the values and would not alter any of the conclusions.

**Summary**

This large, multi-center natural history study showed the disease progression in the upper extremity muscles using MRI $T_2$. The proximal to distal pattern of disease progression was observed in the upper extremity muscles. There was a significant difference between controls and subjects with DMD in MRI $T_2$ for shoulder, upper arm and forearm muscles across all age groups, including in the youngest age group of 5 – 8.9 years. There was a significant correlation between MRI $T_2$ and function (except for distal PUL for a few muscles) and strength measures, supporting the valuable use of MR in clinical trials. Proximal lower extremity muscles (BFLH, VL) tended to have higher $T_2$ values, followed by the proximal muscles of the upper extremity (shoulder muscles-INFRA, SUB, DEL) across all age groups except in the youngest age group of 5 – 6.9 years. It is anticipated this natural history will be helpful in designing clinical trials and assist future studies in selecting the muscles that most effectively illustrate DMD progression and the effect of therapeutic intervention. Future studies should examine longitudinal changes in MRI $T_2$ in upper extremity muscles.
Table 6-1. Demographics of enrolled subjects (DMD and controls) at baseline.

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<td><strong>Mean ± SD</strong></td>
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<td></td>
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<tr>
<td><strong>Age (years)</strong></td>
<td>DMD (n = 100)</td>
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<td>Control (n = 28)</td>
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<td>(n = 11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>DMD</td>
<td>39.4 ± 14.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.9 ± 6.4</td>
<td>38.1 ± 10.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>47.5 ± 18.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.3 ± 10.2</td>
<td>41.2 ± 8.7</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>DMD</td>
<td>1.29 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.13 ± 0.10</td>
<td>1.30 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.55 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.29 ± 0.11</td>
<td>1.56 ± 0.11</td>
</tr>
<tr>
<td><strong>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</strong></td>
<td>DMD</td>
<td>22.7 ± 6.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16.4 ± 1.5</td>
<td>22.7 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>18.8 ± 3.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>16.6 ± 3.1</td>
<td>18.0 ± 1.9</td>
</tr>
<tr>
<td><strong>Brooke Score</strong></td>
<td>DMD</td>
<td>1 – 5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1 – 3</td>
<td>1 – 5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Steroids</strong></td>
<td>DMD</td>
<td>88%</td>
<td>88%</td>
<td>88%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are means ± standard deviations with a range given for Brooke Score. BMI: Body Mass Index; <sup>a</sup> indicates data collected in n = 82; <sup>b</sup> indicates data collected in n = 26; <sup>c</sup> indicates data collected in n = 75; <sup>d</sup> indicates data collected in n = 25; <sup>e</sup> indicates data collected in n = 71; <sup>f</sup> indicates data collected in n = 25; <sup>g</sup> indicates data collected in n = 96; <sup>h</sup> indicates data collected in n = 27. None of the controls were on steroids.
Table 6-2. Correlation between MRI $T_2$ and measures of upper extremity function and strength

<table>
<thead>
<tr>
<th></th>
<th>MRI $T_2$</th>
<th>Shoulder muscles</th>
<th>Upper arm muscles</th>
<th>Forearm muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SUB</td>
<td>INFRA</td>
<td>DEL</td>
<td>BB</td>
</tr>
<tr>
<td>Total PUL Score</td>
<td>-0.61</td>
<td>-0.56</td>
<td>-0.61</td>
<td>-0.66</td>
</tr>
<tr>
<td>High PUL Score</td>
<td>-0.69</td>
<td>-0.64</td>
<td>-0.58</td>
<td>-0.66</td>
</tr>
<tr>
<td>Mid PUL Score</td>
<td>-0.61</td>
<td>-0.52</td>
<td>-0.58</td>
<td>-0.62</td>
</tr>
<tr>
<td>Distal PUL Score</td>
<td>-0.25</td>
<td>-0.21</td>
<td>-0.27</td>
<td>-0.37</td>
</tr>
<tr>
<td>Brooke Upper Extremity Scale</td>
<td>0.72</td>
<td>0.73</td>
<td>0.68</td>
<td>0.74</td>
</tr>
<tr>
<td>Myogrip (kg)</td>
<td>-0.61</td>
<td>-0.48</td>
<td>-0.34</td>
<td>-0.42</td>
</tr>
<tr>
<td>Myopinch (kg)</td>
<td>-0.69</td>
<td>-0.55</td>
<td>-0.39</td>
<td>-0.41</td>
</tr>
</tbody>
</table>

The correlation coefficient, $r$, denotes the correlation between MRI $T_2$ and function and strength measures. All correlations are significant with $p < 0.05$; except the ones where $p$ value is reported.
Figure 6-1. Representative MRI T2 images of a control subject showing all the upper extremity muscles examined. MRI T2 images of (a) Shoulder, (b) Upper arm, and (c) Forearm muscles of 13 year old control subject are shown.
Figure 6-2. MRI $T_2$ weighted trans axial images of the shoulder and $T_2$ histogram of the DEL are shown. MRI $T_2$ images for a) control, b) 6 year old DMD subject, c) 18 year old DMD subject, are shown. (d) Histogram of DEL MRI $T_2$ shows the rightwards shift in $T_2$ values from control to an 18 year old subject with DMD.
Figure 6-3. Comparison between controls and subjects with DMD across all age groups for upper extremity muscles - (a) DEL, (b) BB, (c) TB, (d) AF, and (e) PF. There was a significant difference between subjects with DMD and controls across all the age groups for all the shoulder, upper arm, and forearm muscles. Data with standard error of mean is shown, p < 0.05.
Figure 6-4. Comparison among subjects with DMD across all the age groups for upper extremity muscles - (a) DEL, (b) BB, (c) TB, (d) AF, and (e) PF. More proximal muscles (DEL, BB) detected progressive increases across all age groups, while for more mildly affected (TB) or more distal muscles (PF), differences only emerged over larger age gaps. Data with standard error of mean is shown, p < 0.05.
Figure 6-5. Comparison between ambulatory and non-ambulatory subjects with DMD for MRI $T_2$ of each upper extremity muscle. Non-ambulatory subjects had significantly higher $T_2$ values than the ambulatory subjects for all the upper extremity muscles examined. Note that non-ambulatory boys were significantly older than ambulatory boys. Data with standard error of mean is shown, $p < 0.05$. 
Figure 6-6. Correlation between MRI T2 and function and strength measures. Scatterplots show the correlation between the DEL MRI T2 and (a) total PUL score, (b) Brooke Upper Extremity Scale, (c) myogrip, and (d) myopinch.
Figure 6-7. Correlation between MRI T₂ and dimensions of the PUL. There was a significant correlation between the DEL MRI T₂ with (a) high PUL, (b) mid PUL, and (c) distal PUL.
Figure 6-8. Pattern of disease progression among upper and lower extremity muscles at a) all age groups, (b) 5 – 8.9 years, (c) 9 – 12.9 years, and (d) 13 – 18.9 years. Proximal muscles of the leg (BFLH, VL) tended to have higher T2 values and then proximal muscles of the upper extremity (SUB, INFRA) had next highest T2 values as compared to the rest of the muscles for all age groups except the youngest age group of 5 – 8.9 years. In 5 – 8.9 years, VL, SUB, BFLH, and INFRA had almost similar T2 values. Data with standard error of mean is shown, p < 0.05.
Figure 6-9. Correlation between composite upper extremity MRI T$_2$ and composite lower extremity MRI T$_2$. There was a significant positive correlation between the composite upper extremity MRI T$_2$ and composite lower extremity MRI T$_2$, $p < 0.05$. There was a strong positive correlation between the composite upper extremity MRI T$_2$ and composite lower extremity MRI T$_2$ for ambulatory subjects ($r = 0.84$) and a moderate positive correlation for non-ambulatory subjects ($r = 0.61$). There was more variability observed among non-ambulatory subjects for MRI T$_2$ between the upper and lower extremity.
CHAPTER 7
CONCLUSIONS

Overview

Duchenne muscular dystrophy (DMD) is an incurable, progressive muscular disease, which ultimately leads to death of the patient. Many clinical trials are attempting to find a cure for DMD. To help facilitate the design of clinical trials, there is a need for natural history studies characterizing disease progression in both upper and lower limbs. There is also a need for outcome measures that are sensitive to disease progression. Moreover, about two-thirds of the DMD population is non-ambulatory, so clinical trials should be designed to incorporate both ambulatory and non-ambulatory subjects. In the present study, the natural history of disease progression was examined in upper and lower limb muscles in boys with DMD (ambulatory and non-ambulatory) using functional outcome measures and magnetic resonance imaging.

Summary

Summary of aim 1. In this experiment, we implemented functional outcome measures, which are commonly used in clinical trials to characterize the natural history of disease progression in ambulatory boys with DMD. We evaluated lower extremity function in boys with DMD, ages 5 – 12.9 years, and compared it to age-matched healthy controls across age groups. We also evaluated the disease progression over 1 year in boys with DMD across the different age groups, using clinical endpoints. The results showed that in all age groups, including in the youngest age group of 5 – 6.9 years, boys with DMD performed significantly different than the controls. In general, boys with DMD performed considerably slower than the controls on the climbing four stairs and supine to stand tests, with the youngest age group (5 – 6.9 years) taking

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twice as long as the controls, and the oldest age group (11 – 12.9 years) taking four times as long. Similarly, the controls covered about 1.3 times more distance in the 6 minute walk test when compared to the youngest DMD age group, and 1.8 times more distance than the oldest boys with DMD. Performance on climbing four stairs and supine to stand tests in boys with DMD showed decreased performance over the period of 1 year in all age groups. In contrast, the 6 minute walk test did not show a significant decline over 1 year in DMD subjects younger than 9 years old. Overall, timed functional tests (10 m walk/run, 4 stairs, supine to stand) demonstrated better sensitivity and specificity than the 6 minute walk test for predicting loss of function. At the age of 14 years, more than 70% of subjects had lost the ability to perform the supine to stand test, and more than 60% had lost the ability to perform climbing 4 stairs. However, at this age, only 40% of subjects had lost the ability to perform the 10 m walk/run test. Based on this data, we propose that the 10 m walk/run test might be a better functional outcome to be considered for use in clinical trials.

**Summary of aim 2.** In this experiment, we performed a pilot exploratory study comparing sensitivity of a variety of outcome measures assessing upper extremity function. The results showed that both Performance of Upper Limb (PUL) and Motor Function Measure (MFM) were more sensitive tests than either the Jebsen Hand Function Test or Brooke Scale, for evaluating upper limb function in DMD subjects. However, the MFM test included only a few upper extremity tasks, none of which involved shoulder activities. Based on the pilot study results, we selected the PUL for implementation into our natural history study. The Brooke Scale was also included since it is widely used clinically. The PUL and Brooke Scale were performed in 3 centers in a
large cohort in a multi-center study. The purpose of the multi-center study was to collect the natural history data in DMD patients in upper extremity muscles. We measured upper extremity function and strength in a large cohort of boys with DMD (ambulatory and non-ambulatory) at varying disease stages, and compared them to age-matched controls. Both DMD subjects and controls performed functional tests (PUL and Brooke Scale), and strength tests using myogrip and myopinch. The performance of DMD patients on the total PUL score, and strength tests (myogrip and myopinch) was significantly worse as compared to controls in all age groups, including in the youngest (5 – 8.9 years). Proximal muscles of the upper extremity exhibited functional decline starting at the age of 9 years. There were no differences in the forearm dimension of the PUL across ages groups in subjects with DMD. However, the oldest boys with DMD (age 13 – 18.9 years) had lower performance in the total PUL compared to the youngest age group (age 5 – 8.9 years), which was attributed to differences in the shoulder and upper arm dimensions of the test. There were no significant differences across age groups in muscle strength assessed using myogrip and myopinch in subjects with DMD. Overall, functional tests appeared more sensitive than strength tests using the myotools in monitoring disease progression in DMD patients across ages 5 – 18.9 years.

**Summary of aim 3.** In this experiment, MRI, a non-invasive imaging technique, was used to examine disease progression in DMD in a variety of upper extremity muscles. MRI T2, a non-specific biomarker, sensitive to muscle inflammation, damage, and fatty infiltration, was the focus of this study. We evaluated MRI T2 in upper extremity muscles in individuals with DMD and compared them to controls across age groups. We also examined the relationship between MRI T2 and function and strength measures of
upper extremity. There is much interest in comparing disease progression in upper extremity muscles with the lower extremity. There is a misconception in the DMD community that all muscles of the lower extremity get involved first, followed by the muscles of upper extremity. This leads to the upper extremity muscles being overlooked. Therefore, in our cohort, we collected MR data on both upper and lower extremities, giving us the unique opportunity to compare them. Our study showed that there was a proximal to distal pattern in disease progression in the upper extremity muscles in individuals with DMD. Even in the youngest age group, all upper extremity muscles in boys with DMD had significantly higher T₂ values than controls. MRI T₂ values were significantly correlated with clinical measures of upper limb function. When comparing upper extremity muscles with lower extremity muscles, proximal muscles of the lower extremity (BFLH, VL) tended to have the highest T₂ values, followed by the proximal muscles of the upper extremity (INFRA, SUB, DEL) across all age groups except in the 5 – 8.9 year age group. These initial results provide support for the use of upper extremity MR in clinical trials for DMD in both ambulatory and non-ambulatory subjects. This is the first study to collect MRI T₂ data in all segments of the upper extremity in a large cohort.

Overall, the findings of these experiments provide important natural history data for both upper and lower limbs. They also establish sensitive, objective outcome measures, which can be used in both ambulatory and non-ambulatory subjects with DMD. This may be valuable for parents and clinicians in making good clinical decisions. It may also help in appropriate design of future clinical trials in DMD, which are hoping to find a cure for this disease.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Harneet Arora was born in Punjab, India. She obtained her bachelor’s degree in physical therapy from Guru Nanak Dev University, Amritsar. She worked as a physical therapist in an orthopedics outpatient clinic in India before moving to the United States. Harneet joined the rehabilitation science doctoral program at the University of Florida in 2013, under the mentorship of Dr. Krista Vandenborne. While pursuing her doctoral degree, Harneet has served both as a teaching assistant, and as a research assistant in the Department of Physical Therapy. She received her PhD from the University of Florida in 2017.