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Impact of Strength and Range of Motion on Loss of Ambulation and Functional Change Measures in Boys with DMD

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THE IMPACT OF STRENGTH AND RANGE OF MOTION ON LOSS OF AMBULATION AND FUNCTIONAL CHANGE MEASURES IN BOYS WITH DMD

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We hereby certify that this dissertation, submitted by Tina Duong, conforms to acceptable standards and is fully adequate in scope and quality to fulfill the dissertation requirement for the degree of Doctor of Philosophy in Physical Therapy.

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ABSTRACT

Duchenne muscular dystrophy (DMD) is an x-linked lethal progressive disorder affecting boys who are typically diagnosed due to motor delays or weakness. Key features include loss of motor function throughout the lifetime resulting in early mortality from cardiopulmonary complications in the late 2nd and 3rd decades of life. Rates of progression are variable yet sequential due to a typical pattern of motor weakness that occurs from proximal to distal muscle groups. Current standards of care include contracture management and Glucocorticosteroids (GC). Timed function tests have been used to understand and predict loss of functional milestones such as walking, getting off the ground and going upstairs. This study will describe contracture development; associated strength loss and its effect on function for boys with DMD. With differing rates of decline in boys with DMD, this study will also determine minimal clinical important difference (MCID) based on annual progression rates of 3 critical timed function tests (supine to stand, climb 4 stairs and 10meter walk test).

This is a retrospective study of the largest natural history of 440 boys with DMD. Data was collected by physical therapists in a standardized manner across 22 sites in 9 countries. Mixed models with repeated measures were used to understand the relationship between knee strength, ankle range of motion, GC use and timed function tests. Linear regressions were used to assess the effect of ankle range of motion and strength measures on functional ability. Anchor based MCID measures were calculated based on change of the Vignos scale, an 8 point lower extremity functional scale.

Results found that there is great variability in contracture development in boys with DMD and that a 12-month time frame may be too short to assess contracture changes. Knee strength and ankle ROM are good predictors of loss of function. In an anticipatory care model, MCID values for these functional tests are important in clinical management and necessary for design of clinical trials.

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CHAPTER 1

INTRODUCTION

The most common form of pediatric muscular dystrophy is Duchenne muscular dystrophy (DMD), caused by a genetic mutation in the dystrophin gene that leads to progressive muscle weakness and loss of ambulation. The discovery of this gene over 30 years ago led to improved diagnostic capabilities for DMD. Improved physical therapy management strategies and therapeutic interventions have resulted in a longer lifespan and as a result increased variability in rates of disease progression. Individuals with DMD have a chronic progressive loss of strength and function, resulting in loss of ambulation in the teenage years and premature mortality typically in the second or third decade of life.

Currently, standard of care guidelines for DMD emphasize appropriate musculoskeletal management through physical therapy and corticosteroid therapy to maintain strength and function. Obtaining a better understanding of how assessments used to measure impairments, such as strength and range of motion (ROM), can be used in the clinic to predict disease progression is important to achieve optimal, individualized rehabilitation management. Predictive factors such as walking speed are associated with the loss of major functional milestones including the ability to get off the ground, climb stairs and walk. This information is vital to anticipatory care for rehabilitation specialists so that treatment recommendations can be appropriately modified for contracture management. This information can also help rehab professionals recommend equipment to ease the transition from losing such crucial functional

milestones like walking. In addition, understanding loss of functional milestones can be crucial when designing a clinical trial in order to obtain the appropriate efficacy endpoint, study population, and timeline.

Physical therapy is an essential component of care for individuals with DMD because of the direct involvement of skeletal muscles in the disease process, and the secondary effects associated with loss of joint ROM. The purpose of this research proposal is to better understand the contribution of impairments, such as strength and ROM, to losses of functional abilities including the ability to walk, get off the floor, and climb stairs.

For this project, the researcher performed a retrospective review of the largest longitudinal natural history study of individuals with DMD. This study was performed by Cooperative International Neuromuscular Research Group (CINRG), a consortium of 26 clinical sites around the world with a primary goal of characterizing and understanding the pathophysiology and clinical presentation of neuromuscular diseases and contributing to improved clinical outcomes that are relevant to daily functional activities and quality of life (www.Cinrgresearch.org). Data analysis determined the relationship between strength and ankle range of motion and ability to perform functional activities including walk, climb stairs and get up off of the floor in individuals with DMD. Minimal Clinically Important Differences (MCID) was evaluated using an anchor-based approach that was anchored to an ordinal scale that describes lower extremity functional changes, Vignos Scale.[1] MCID for walking, climbing stairs, and getting up off the floor was calculated by evaluating 12 month change scores of 3 timed functional tests anchored to a change of levels on the Vignos scale.

The results of this study evaluated the relationship between musculoskeletal joint and strength changes and performance on functional tests for individuals with DMD. Determining MCID provided clinical thresholds for loss of function. This information will help physical therapists better understand the potential effects of impairments on activity limitations and may help to drive clinical decision making in selecting procedural interventions in boys with DMD. In addition, this information will assist individuals and families in preparing for changes that occur throughout the progression of the disease. As therapeutic trials have drastically increased in the last decade, this predictive information will also help the researchers design better clinical trials focused on a targeted population of individuals with DMD.

Background and Problem Statement

Duchenne muscular dystrophy (DMD) is a progressive genetic neuromuscular disease with a prevalence of in every 7250 males from ages 5-24 years.[2, 3] It is an X -linked developmental disorder that causes progressive muscle weakness, usually leading to death by early adulthood. Individuals with DMD battle many musculoskeletal challenges, including progressive muscle weakness and development of contractures. Dystrophin deficiency is the primary defect in DMD, resulting in a fragile muscle membrane that permits an abnormal permeability to electrolytes, especially Ca^{++} [4]. The increase in intracellular calcium triggers a pathological cascade of events that results in downstream effects of muscle necrosis and fibrosis, which impede normal muscle regeneration. Disease severity has not been directly related to amount of dystrophin in the muscle. This dystrophin deficiency leads to downstream effects of muscle fibrosis and weakness that lead to loss of function and development of contractures.[5, 6]

Due to the intrinsic instability in the muscle membrane, individuals with DMD are at high risk for formation of contractures.[7-10] Contracture is defined as a lack of full passive range of motion due to soft tissue, joint, or muscle strength imbalances in opposing muscle groups.[9, 11] Fibrosis in combination with weakness result in loss of sarcomeres, which causes shortening of muscle fibers and may contribute to development of contractures at the hip, knee, ankle, and fingers.[8] Additionally, this combination of progressive weakness and continued fibrosis results in changes in muscle length that alter biomechanics of movement, especially during ambulation.[12-14] These changes in musculoskeletal alignment affect muscle force demands and result in decreased gait velocity and associated loss of ambulation in boys with DMD.[15-18]

CLINICAL PRESENTATION

Many children show early signs of motor delays before the actual diagnosis of DMD. Due to early pelvic girdle weakness, individuals typically have difficulty rising from the floor and will use a classic compensatory movement strategy called Gower's maneuver to get up from the ground..[19]This maneuver is comprised of children using their hands to crawl up their legs in order to get up from the ground. Children will also have difficulty obtaining some gross motor milestones such as jumping or reciprocal stair climbing and may experience frequent falls. Over the years, muscle imbalances lead to the development of contractures, scoliosis, and progressive impairments of respiratory and cardiac capacity.[5, 8] Phenotypical[20] progression is variable in DMD, but occurs in a predictable sequential manner. Individuals with DMD first lose the ability to get off of the floor, then climb stairs, walk, feed oneself, and eventually a premature death typically due to cardiorespiratory insufficiency.[5, 6, 8, 13, 14, 21, 22]

CURRENT THERAPIES

Corticosteroids, aggressive stretching/bracing, and physical therapy have effectively changed the clinical course of the disease for most individuals with DMD.[3, 6, 14, 21-36] Of all neuromuscular disorders, DMD results in the greatest annual per capita cost for outpatient rehabilitative treatment.[20] Therapy goals focus on prevention of contractures and countering the effects of deconditioning, along with maintenance of musculoskeletal health with range of motion and weight bearing activities. For best utilization of rehabilitation interventions, an evidence-based plan of care for DMD is necessary to understand the interplay between body systems and disease progression.

With progression of the disease, therapy goals focus on addressing muscle imbalances around joints to maintain optimal biomechanics for movement. Muscle weakness is initially seen in the hip flexor, hip extensor, and knee extensor muscles resulting in functional changes. However, contractures typically develop first at ankle plantarflexors, followed by hip flexors, then iliotibial bands, and then forearm/finger contractures[8]. Significant contractures are rare in children younger than 9 years and tend to develop more aggressively as individuals lose the ability to stand or walk.[8] This supports the theory that contracture development is multifactorial; and has physiological and environmental components. Due to the high rate of fibrosis in DMD, muscles with poor anti-gravity strength which persist in a shortened position will develop contractures.[5, 8-11]The muscle imbalance between hip extensor weakness and hip flexor contractures causes a pelvic anterior tilt resulting in a compensatory lumbar hyperlordosis. Increased plantarflexion along with increased lumbar lordosis are a stabilizing mechanism to shift the line of gravity behind the greater trochanter (posterior to the hip) and anterior to the

knee, in an effort to sustain a lower extremity extension moment. These compensations actually lead to greater gait deficits including excessive plantarflexion, increased lateral trunk flexion, circumduction, and a wide base of support.

With corticosteroid use, the time to when young boys lose ambulation has increased by 1-2 years, however, loss is still inevitable between the ages 9-15.[37] Having at least a neutral (0 degrees) ankle range of motion (ROM) allows for better vertical alignment of the body's center of gravity in standing as well as foot clearance through the swing phase of ambulation. Older studies representing a steroid naïve population have provided insight on associations with functional abilities and ankle contractures/lower extremity strength deficits. A combination of strong knee extensors and approximately normal ankle ROM has been shown to prolong ambulation and positively affect the ability to get off the ground and climb stairs. [5, 12] Walking speed in boys with DMD is directly correlated with prolonging ambulation, with individuals who walked faster maintaining ambulation longer.[8] There have been numerous studies linking ambulation to improved quality of life and prevention of the development of scoliosis and joint contractures/deformities.[7, 8, 13] Once an individual becomes non-ambulatory, secondary issues occur such as development of scoliosis, osteoporosis, obesity, disuse atrophy, increased rate of contracture development, and psychosocial issues which impact the perception of disease progression. The psychological effects of loss of a function are similar to the fears and anxiety that is associated with loss felt at the time of diagnosis and death for families affected by DMD.[38]

Standards of care play a role in some of the variability in clinical progression. A study investigating how DMD genetic factors were associated with loss of ambulation, found that SOC

were a significant factor in differences in clinical progression.[37] Additionally, findings from the 2005 Centers for Disease Control (CDC) expert panel indicate that SOC greatly varied based on geographical location. Identifying and optimally managing secondary conditions in a comprehensive and consistent manner is key for quality care.[21, 25] Some of the discrepancies in care include use of corticosteroid steroids and contracture management. The CDC determined that stretching is the standard of care for management of contractures in boys with DMD, with further recommendations for future research to validate expert consensus statements on bracing and orthosis.[25] However, there is inconsistency in the application of night time braces for management of ankle contractures. Some of the inconsistencies in contracture management may also be attributed to the lack of quality evidence to promote stretching or bracing as effective means to manage contractures in individuals with neuromuscular disease (NMD). More recently, a 2010 Cochrane review of interventions to increase ankle range of motion in neuromuscular diseases concluded that the quality of evidence for interventions to increase ankle range of motion was low and further research is required.[10]

SIGNIFICANCE

At a 2006 National Institute on Disability and Rehabilitation Research (NIDRR) meeting to discuss challenges in muscle disease, the United States Food and Drug Administration (FDA) specified that clinical trials conducted in support of new drug approvals or revised indications for already approved drugs must incorporate a primary endpoint that objectively measures a clinically meaningful "life-changing" milestone that has a significant impact on a patient's perceived overall health and well-being.[31] In DMD, these milestones are likely to include events such as loss of ability to rise from the floor and loss of ambulation. McDonald et al first showed

significant correlations of the Pediatric Outcomes Data Collection Instrument (PODCI) and the Pediatric Quality of Life Inventory (PEDSQL) with age and timed functional tests in boys with DMD.[39]This study was pivotal in proving construct validity of a parent reported health related quality of life measure.[39] The study team found that the PODCI domains of mobility/transfers and sports/physical function best correlated to age, knee strength and timed tests.[39] Even though not explored in the McDonald[40] study, contractures may play a significant role in the ability to accomplish these "life changing" milestones, as the interactions of muscle force production and the biomechanical demands may change based on the alignment of the musculoskeletal system and progressive strength loss.

PURPOSE

The objective of this retrospective study is to explore the relationship of joint contractures and muscle weakness to 3 timed functional test scores: supine to stand,10MWT, 4 stair climb. To achieve this objective, the researcher will explore relationship between knee strength and ankle range of motion on timed function tests (10MWT, Supine to stand, 4 stair climb) in ambulatory boys. In addition, the MCID and minimal detectable change (MDC) on the 10-meter walk test, supine to floor test, and stair climb test will be estimated for boys with DMD.

This CINRG study followed 440 boys aged 1-28 years for up to 10 years from the ages of 2-28 for 8 years. Data collected in this study includes a long list of clinical outcomes developed based on the World Health Organization's (WHO) International Classification of Function and Disability (ICF) model.[41] Data collected in this study ranges from basic anthropometric measurements (height, weight, range of motion, limb measures) to functional assessments

(strength, gait velocity, stand from floor, stairs, Northstar Ambulatory Assessment (NSAA)), quality of life PODCI, PedsQL, Short Form Health Survey (SF36), Neuro Quality of Life Measure (NeuroQOL) measures, and surveys on access and use of interventions by the DMD community.

Published work during the pre-steroid era by McDonald et al (1995) associated age ranges with 4 distinct disease stages of DMD (< 6 years; 6 to <9 years; 9 to <12 years; > 12 years). Since that time, the CDC care guidelines have described DMD in 5 stages based on function. These include 1) diagnosis; 2) early Ambulatory; 3) late ambulatory; 4) early non- ambulatory; 5) late non-ambulatory. Of the 5 groups, major changes in clinical care recommendations for cardiac, respiratory and gastrointestinal care were considered in 3 primary groups: early (ambulatory); middle (early non-ambulatory); and late (late stage ambulatory). Because this project is exploring rehabilitation, physical therapy, and orthopedic clinical care considerations with recommendations based on the 5 age groupings,[5, 21, 22, 42, 43] the researcher performed data analysis based on 5 distinct functional groups based on age. These groups were defined as diagnosis (0-4 years); early Ambulatory (5-8.9 years); late ambulatory (9-12.9 years); early non-ambulatory (13-16 years); and late non-ambulatory (>17years). This 5-tier stratification based on loss of function describes distinct phases where significant changes in care may occur. Estimates of MCID of functional timed measures were based on validated functional categories from the Vignos Lower Extremity Scale,[44] which aligns with the National Heart Blood Lung Institute's (NHBLI) recommendations on estimating MCID values for diseases that have a wide

heterogeneity and also shown to be better applicability in clinical interpretation and efficacy trials.[45-47]

RESEARCH QUESTIONS:

As boys with DMD get older and weaker, contractures, strength, and function are expected decline. However, differences in rates of decline based on functional groups is not known. Due to the variability in rates of functional decline in boys with DMD, there are expected differences in these 5 functional groups. With the different musculoskeletal, respiratory and equipment needs for boys in these 5 groups, this information will be helpful in anticipatory planning for clinical decision making. Additionally, understanding these changes based on age and function will help clinical trials group boys with DMD into more homogenous groups to decrease phenotypical variability thus requiring smaller sample sizes. This study aimed to characterize the relationship of contracture, strength, and timed function tests based on the 5 functional stages (Diagnosis; Early, Middle, Late Ambulatory; Early, Late Non-Ambulatory) and estimate MCID in boys with DMD in the corticosteroid era. The research questions that were addressed in this study:

1. How does ankle ROM, knee strength, age and GC use affect loss of functional abilities such as ability to walk, get off the floor and climb stairs for boys with DMD.
2. What is the MDC and MCID values for 3 validated and commonly used timed function tests (10MWT, Supine to Stand, and 4 Stair Climb) to assess disease progression for individuals with DMD.

Rationale

Since the 3 timed test velocity scores have been determined as a good clinical predictor of loss of ambulation in individuals with DMD,[5, 10] the researcher determined the degree that knee strength and ankle range of motion impact changes in timed function velocities. Previously published research regarding contractures and function in DMD have analyzed the entire population of boys with DMD. This analysis focused on the 5 functional groups to better characterize strength, function and contracture declines in a more homogenous fashion. With the appropriate stratification of disease stages in DMD, the results of the research study explored the impact of strength and ROM on function for individuals with DMD, in order to improve anticipatory care management.

Since there is no consensus in the literature on determining responsiveness of these clinical measures, an anchor based approach was used.[45] Most published studies in DMD which have looked at determining clinical differences have used both MCID and MDC. There are advantages to each of these techniques, but based on review of literature in other progressive disease groups,[45, 48, 49] a longitudinal anchor-based approach was sued as it is most relevant to the progressive nature and variability of DMD.[49] Estimates of MCID for functional velocity measures (10mwt, supine to stand, 4 Stair climb) were anchored to the Vignos Lower Extremity Scale, a validated 8-point ordinal scale in DMD categorizing lower extremity function.[44] This approach aligns with the National Heart Blood Lung Institute's (NHBLI) recommendations on estimating MCID values for diseases with a wide heterogeneity and also shown to be better applicability in clinical interpretation and efficacy trials.[45-47]

To understand the impact of changes in function, MCID in timed velocity scores were anchored the Vignos Scale. Most studies using MCID scores focus on determining the MCID required to detect changes in treatment.[50, 51] Despite the paucity of research[45, 52] using natural history studies to determine MCID scores, this work used DMD natural history to determine MCID scores associated with prediction of loss of function, instead of treatment effectiveness. This is a logical choice given the progressive nature of DMD. In a study of Chronic Obstructive Pulmonary Disease, a retrospective study assessed MCID values to determine 6MWT MCID values that may be good predictors of mortality or hospitalization, and found that observational studies were more robust to answer these questions because of the lack of confounding treatment interventions.[45] In this study, the researcher examined MCID scores as possible predictors of loss of ambulation.

OBJECTIVES

A. Determine the relationship between knee extensor muscle strength (measured by Quantitative Muscle Testing (QMT)) and 3 functional timed tests: 10-meter walk test, 4 stair climb and supine to stand in boys with DMD.
B. Determine the relationship between ankle dorsiflexion joint range of motion (measured by goniometry) and 3 functional timed tests: 10-meter walk test, 4 stair climb and supine to stand in boys with DMD.
C. Determine if strength and ROM are significant predictors of 3 timed function tests (10MWT, Stairs, Supine to Stand)
D. Estimate MCID and MDC values of 3 timed functional assessments for individuals with DMD

a. for 10-meter walk/run (10MWT)

b. 4 stair climb (4SC)

c. supine to stand (STS)

IMPACT

With the alteration in the disease course due to standard use of corticosteroids for disease management and the recent developments in drug therapies, there has been great interest in understanding the downstream effects of contractures on strength and function across the spectrum of disease progression. Improvements in standards of care including management of primary and secondary effects of DMD with GC use, respiratory care, coronary care, and contracture management have been linked to longer lifespans.[53] The economic impact of longer survival is associated with much higher medical costs, especially in the later stages of the disease. Medical costs are nearly six times higher for those who are non-ambulatory compared to those who are ambulatory.[54] Thus, making it more important to better understand the reasons that contribute to loss of ambulation. When it comes to direct medical costs compared to indirect costs linked to loss of work time or copays, Ryder et al[53] found that for those who are non-ambulatory, the indirect costs are more than 2.5 times the amount of direct medical costs, resulting in increased time and financial burden on the family.

Understanding the impairment-based changes that affect the biomechanics of gait and related loss of functional activities will influence proactive physical therapy clinical decision-making through prophylactic range of motion management through stretching, bracing, assisted active range of motion and exercise.[4] Boys with DMD have increased bone fragility and fractures due

to prolong use of GC, progressive muscle weakness on bone health.[55] Management of contractures may allow longer time standing and walking thus reducing osteoporosis risk and medical costs associated with being non-ambulatory. By understanding these changes, clinicians may also anticipate loss of ambulation and risk of falls due to weakness and lack of ROM as these boys are also at a higher risk of fat embolisms long bone fractures thus adding to medical costs.[56]

Due to the multi-organ effects of DMD and multitude of needs that go alongside changes in the 5 major stages of DMD progression (Figure 1),[42] understanding and determining MCID values in DMD may greatly impact the interpretation of functional changes commonly used in the clinic, such as the 10MWT , supine to stand, and stair climb. Better understanding of the prediction of loss of function will contribute to anticipatory care planning for physical therapy in contracture management and improve planning to obtain appropriate assistive devices that promote independence and decrease caregiver burden. Additionally, MCID values will help in designing clinical trials that use study endpoints that show clinical relevance.[48]

MCID values have typically been reported in intervention-based clinical trials, however, a few studies have looked at the MCID values on progressive diseases such as COPD, Huntington's, cancer, disease, cancer, multiple sclerosis, and Pompe disease.[45, 48, 49, 57-59] Determining MCID values in the context of disease deterioration is important to understand the true impact of future therapeutic interventions. Applying MCID values to commonly used clinical assessments helps appropriately power clinical trials. MCID is assessed based on rate of disease progression on an untreated population. Understanding the etiology, symptomology, and

natural history of degenerative diseases is essential in determining MCID values that are relevant to the prediction of loss of functional milestones and its impact on the health-related quality of life measures.[45]

Results from this study may greatly impact clinical decisions, outcomes, insurance reimbursements, and design of clinical trials in DMD. They will also aid in better understanding of ankle contracture development and conservative approaches to management of these contractures, which will ultimately contribute to improved standardization of physical therapy care in DMD. There are currently no studies that assess the frequency of use of braces and stretching on contracture development, and its associated impact on gait velocity and timed tests. Since gait velocity has been shown to be a good predictor of loss of ambulation,[35] it is important to understand the impact of contractures on gait velocity, given that contracture management and corticosteroid use are the few published interventions that have changed the natural history of the disease.[3, 5, 6, 8, 13, 14, 32, 34-36, 60-62]

Additionally, the determination of MCID values functional timed tests will aid in clinical research design by establishing clinically relevant endpoints that are associated with meaningful change for individuals with DMD. Koynova et al[63] performed a literature review of MCID measures used in clinic research and found that MCID measures must be based on reliable outcome measures and based on a specific disease group and functional level. Values determined in this manner may show better responsiveness to changes in disease progression compared to a more heterogeneous group that encompasses both the ambulatory and non-ambulatory populations.[49] The NIH Heart, Lung and Blood Institute (NHLBI) supports the stratification of

progressive clinically heterogeneous diseases by taking into account genotype, phenotype, symptoms, and functional measures to ensure better applicability of MCID values and validation.[45]

This study determined MCID values for rate of decline in timed tests over a 12 month period based on the natural history of the disease. This will impact power analyses used in large multi-center clinical trials to determine sample size and contribute to better validation of the appropriate measures used for ambulatory boys with DMD to reduce the amount of assessments required in clinical trials. Not only is DMD a rare disease, but recent DMD therapeutic discoveries [64, 65] have been based on specific genetic mutations, making clinical trials recruitment even more difficult. Therefore, having accurate MCID values that reflect rate of decline that is associated with loss of clinical milestones is important to understand possible impact of new treatments on the disease progression. Additionally, it may help reduce patient burden by reducing the number of outcomes required in clinical trials, decrease costliness of large clinical trials with more accuracy in calculating sample size, and contribute to better clinical interpretation and standardization of clinical care in DMD.

ABBREVIATIONS:

10MWT	10-meter walk/run test
6MWT	6-minute walk test
CDC	Centers for Disease Control
CE	Clinical Evaluator
CINRG	Clinical International Neuromuscular Research Group
ClinRo	Clinician-Reported Outcome Measure

COA	Clinical Outcome Assessment
DMD	Duchenne Muscular Dystrophy
FDA	Food and Drug Administration
EMA	European Medicines Agency
GCP	Good Clinical Practice
HHM	Hand Held Myometry
ICF	International Classification of Function and Disability
IM	Investigators Meeting
LOA	Loss of Ambulation
MCID	Minimal Clinically Important Difference
MDC	Minimal Detectable Change
MMT	Manual Muscle Testing
MRC	Medical Research Council
MVICT	Maximal Voluntary Isometric Contraction Testing
NIDRR	National Institute on Disability and Rehabilitation Research
NIH	National Institutes of Health
NMD	Neuromuscular Disorder
NSAA	North Star Ambulatory Assessment
PODCI	Pediatric Outcomes Data Collection Instrument
PRO	Patient Reported Outcome Measure
QC	Quality Control
QMT	Quantitative Muscle Testing
ROM	Range of Motion

SOC	Standards of Care
SOP	Standard Operating Procedure
TFT	Timed function tests
WHO	World Health Organization

DEFINITIONS:

Clinical Evaluator (CE): Individual, often a physical therapist, who will perform clinical research assessment of outcomes that will be implemented in clinical trials or in the clinic. This individual will have training in implementation of clinical research assessment of outcomes in a standardized manner following good clinical practice[66, 67].

Neuromuscular Disorders (NMD): Disorders that impair functioning of muscle (directly or indirectly) and originate from: anterior horn cells, nerves, neuromuscular junction, muscle and peripheral nervous system pathology[5, 8, 67-70].

Cooperative International Neuromuscular Research Group (CINRG): Consortium of 26 national and international sites who participated in the Duchenne Muscular Dystrophy Natural History Study[29, 30]

Contracture: Shortening or hardening of muscles, tendons, or ligaments that may lead to loss of range of motion at joints[7, 11, 38]

Duchenne Muscular Dystrophy: A genetic disorder characterized by progressive muscle degeneration and weakness due to lack of dystrophin[4].

Gower's Maneuver: A maneuver that describes how a patient must use their hands to "walk" up their body to get up from the ground due to proximal weakness[6].

Minimal Clinically Important Difference (MCID)/ Minimal Important Difference (MID): the smallest difference in a clinical outcome that a patient would identify as important[46]

Minimal Detectable Change: Statistical estimate of smallest amount of change that may be detected by a clinical outcome [71]

Dystrophin-Associated Protein Complex: Structural protein that links the actin cytoskeleton to the extracellular matrix acting to stabilize the sarcolemma.

***MDX* Mouse**: Mouse model with a point mutation in the dystrophin gene, typically used for early animal research.

CHAPTER 2

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) was first described in the medical literature in the 1850s, yet the molecular basis for the disease was not identified until the 1980s.[4] The gene responsible for DMD, dystrophin, is one of the largest in the body with 79 exons.[4] Since this discovery, scientists have been trying to understand the pathophysiology of the disease by studying the MDX dystrophin-deficient mouse model. The *MDX* mouse has provided the scientific community with a better understanding of dystrophin's function in skeletal, smooth and cardiac muscles.[72]

As basic scientists worked to understand the pathophysiology of DMD, clinicians were also working on better characterizing the clinical features of this x-linked disorder. Individuals were first diagnosed due to motor delays, elevated creatinine kinase levels, a positive Gower's sign, and pseudo hypertrophic calf muscles.[73, 74] Currently, molecular advances have led to improved diagnostic abilities, where individuals who show the clinical signs of DMD may be confirmed to have DMD through genetic testing instead of the previously used method of confirmation by muscle biopsy. Clinical care has focused on managing the symptoms of the disease in absence of a cure. Pharmacologic treatment with the corticosteroid prednisone has changed the natural progression of DMD by alleviating the severity of muscle decline and prolonging ambulation by as much as 2 years.[61] Because of the progressive nature of this disease, rehabilitation to return to a previous functional level is not a realistic goal for individuals with DMD. Therefore, physical therapy interventions aim to address the chronic progressive nature of symptoms of DMD such as decreased activity from progressive strength loss, contractures, and

pain. Management includes adaptive exercise, stretching, bracing, and positioning to improve quality of life for individuals with DMD.[7, 14, 75-77]

The DMD community has worked diligently towards a cure for this disease by learning and better understanding 1) the pathophysiology behind dystrophin deficiency; 2) clinical features and progression rates in boys with DMD; and 3) development and use of clinical outcomes to better understand and predict the variability of DMD for improved care management and clinical trial design. The clinical endpoints (10MWT, supine to stand, 4-stair climb) described in this research project are used in the clinical setting but were developed in natural history studies to better understand the variations seen in the phenotypical presentation of DMD. With better understanding of the impact of these clinical endpoints on disease progression, clinicians started to use these as assessment tools to manage patient care. This represents a great example of translational science where academically developed tools are used to affect clinical care. However, these tools need further development to understand variables that affect change in functional status as well as contribute to clinical trials design.

DMD Pathophysiology

Dystrophin is the gene associated with DMD. It was fully cloned and sequenced in 1988 and led to the identification of large complex proteins that play an important role in muscle physiology.[4, 78, 79] At a cellular level, the lack of the structural protein dystrophin leads to progressive muscle weakness associated with disruption in the degeneration and regeneration cycle of repair, in which the muscle fibers undergo irreversible degradation with resultant muscle fibers being replaced by adipose and connective tissue.

In skeletal muscle, dystrophin is found on the plasma membrane as a part of the cytoskeleton that may play a role in protecting the sarcolemma from mechanical stress from stretch-induced contractions.[80] It helps link the cytoskeleton and the extracellular matrix as part of the Dystrophin-Glycoprotein Complex (DGC).[72] The DGC is a "shock absorber" that maintains sarcolemma integrity during muscle contractions.[81] Disruption of the DGC pathway leads to destabilization of the sarcolemma, disturbed cellular defense mechanisms, and impaired calcium homeostasis resulting in mechanical damage to muscle fibers, loss of cell integrity, and necrosis.[82] Costameres and other proteins form a lattice that anchors to the sarcolemma and acts as a mechanical coupler to distribute contractile forces along the length of the muscle fiber leads by maintaining uniform sarcolemma length. The absence of dystrophin contributes to muscle membrane fragility, so its dysfunction also causes increased membrane permeability and accumulation of excessive proteins not normally present in muscle membranes. Calcium homeostasis is essential to muscle function, and increased accumulation of calcium leads to gradual reduced regenerative capacities and increased necrosis. Ultimately this results in fatty tissue infiltration with subsequent muscle weakness and decreased functional contractile myofibers.[72, 83]

Since dystrophin provides mechanical stability to the sarcolemma, force transmission may be impaired between the intracellular and extracellular matrix that is enveloped by the muscle fiber and connected to the muscle tendon.[84] Therefore, the combination of compositional changes and structural instability leads to muscle weakness, starting proximally with larger muscle groups and followed by distal weakness. In addition to the fatty infiltration and

connective tissue found between muscle fibers in DMD, muscle biopsies showed progressive degeneration/necrotic muscle fibers that are generally surrounded by lymphocytes and macrophages with variations in muscle fiber size and centralized nuclei. The immature central nuclei represent regeneration of myoblasts. This results in an imbalance between degenerating and regenerating fibers.[73, 85] As the disease progresses, the capacity for muscle fibers to regenerate diminishes resulting in replacement of the muscle tissues with connective or adipose tissue. This pathophysiological imbalance between myoblast regeneration and muscle fiber necrosis leads to progressive muscle weakness in DMD.[82]

Animal models have typically been used to understand the pathogenic mechanisms behind DMD and therapeutic targets. The best way to fully understand the complexities of DMD would ideally be to perform serial muscle biopsies, but due to the ethical implications and invasiveness of these procedures, dystrophin-deficient animal models are used to better understand the mechanism of disease. The *MDX* mouse has been used for over 30 years and is the most studied of all the animal models. The *MDX* is genotypically identical to DMD, but phenotypically less severe. Another model, such as the golden retriever muscular dystrophy dog (GRMD), has also been used in preclinical trials as it is phenotypically much more similar to DMD than the *MDX* mouse. However, ethical and financial issues resulted in the *GRMD* models being primarily used for promising therapeutic agents, whereas the *MDX* mouse model has remained the primary preclinical model for studying DMD. An exercise regimen implemented in *MDX* mice has been shown to exacerbate the imbalance between muscle degeneration and regeneration seen in DMD, therefore this model has been suggested to this regiment better

represent the DMD clinical presentation.[86] Similar to DMD clinical trials, preclinical outcomes have established standardized techniques to ensure consistency across *MDX* trials.[87] Additionally, reliability and sensitivity of measures were established to determine best preclinical outcomes to assess change in disease progression.[88] Despite the challenges and differences seen in the *MDX* mouse, it is still the most utilized preclinical model to study disease. However, researchers must understand the limitations and interpretation of data that come from preclinical *MDX* trials due to the phenotypical differences. That being said, without this model, the DMD community would not have the pathophysiological understanding of the disease and there would be fewer therapeutic treatment options for clinical trials.

Since the discovery of the dystrophin gene, the DMD diagnostic process has greatly improved. Diagnosis was originally obtained through a muscle biopsy where a southern blot analysis was performed to identify large deletions. The problem with this method is that only 65% of DMD are due to deletions detectable though southern blot analysis; the other 30% have point mutations or small deletions, while 5% have duplications.

The lack of dystrophin production due to a truncated or deleted dystrophin gene is the reason for variations in phenotypic manifestations of the disease. The differences in clinical and biochemical presentation depend on the mutation. Dystrophin that is expressed in an open reading frame results in a milder form of DMD called Becker muscular dystrophy (BMD), in which dystrophin is partially present. Patients diagnosed with DMD have an in-frame deletion resulting in complete loss of dystrophin.[89] Dystrophin has many functions in both the cardiac,

skeletal and smooth muscles of the body. In this research study, we will focus on the mechanical effect of lack of dystrophin in skeletal muscle resulting in skeletal muscle weakness.

In DMD, some of the primary consequence of dystrophin deficiency relate to sarcolemma instability, impaired calcium homeostasis and glycosylation between the Dystrophin-Associated Protein Complex (DAPC) and the extracellular matrix.[90, 91] The DAPC is made up of 3 subgroups with a focus in dystroglycans of which dystrophin is the most relevant to DMD. Some theories on secondary effects associated with disease severity are related to tissue remodeling and chronic inflammatory changes.

Some of the physiologically based theories on the variability in disease progression are related to the effects of tissue remodeling and inflammation. In vivo studies of macrophages show that reduced levels of CD4+, CD8+ or macrophages significantly reduce the pathology in *MDX* mouse models.[82] Activation of inflammatory pathways occurs as early as 8-10 months, but then remains relatively stable throughout the disease course despite the muscle degeneration that occurs later in the disease progression. Damaged or stressed cells trigger an immune-response leading to overactivation of the TGFB2 pathway resulting in chronic inflammation.[92]

The lack of dystrophin leads to a cascade of pathophysiological decline resulting in muscle fiber degeneration. The degenerative process incapacitates the muscle's regenerative capabilities, resulting and in muscle fibers being replaced with fat, connective tissue and fibroblasts decreasing with a decrease in muscle contractility and ultimately strength. Alongside is this loss of muscle strength there is a process of chronic inflammation that further triggers muscle degeneration and contributes to strength declines.

DMD Clinical Presentation

DMD is the most prevalent of childhood neuromuscular diseases with an occurrence of 1 in every 7250 males.[2, 3] DMD is an x-linked inherited degenerative disorder, however, approximately 30% of cases arise from spontaneous mutations.[93-95] Duchenne was initially described as a severe muscle wasting disease with muscle weakness and pseudohypertrophy.[96]

DMD primarily affects skeletal and heart muscle with premature death from cardio-respiratory complications.[40, 97] The onset of weakness occurs between 3 to 5 years of age and is characterized by motor delays, increased falling, inability to run or climb stairs, muscle cramping and fatigue. Complications include a combination of impairment-based weakness and a decreased range of motion with impact on functional mobility.

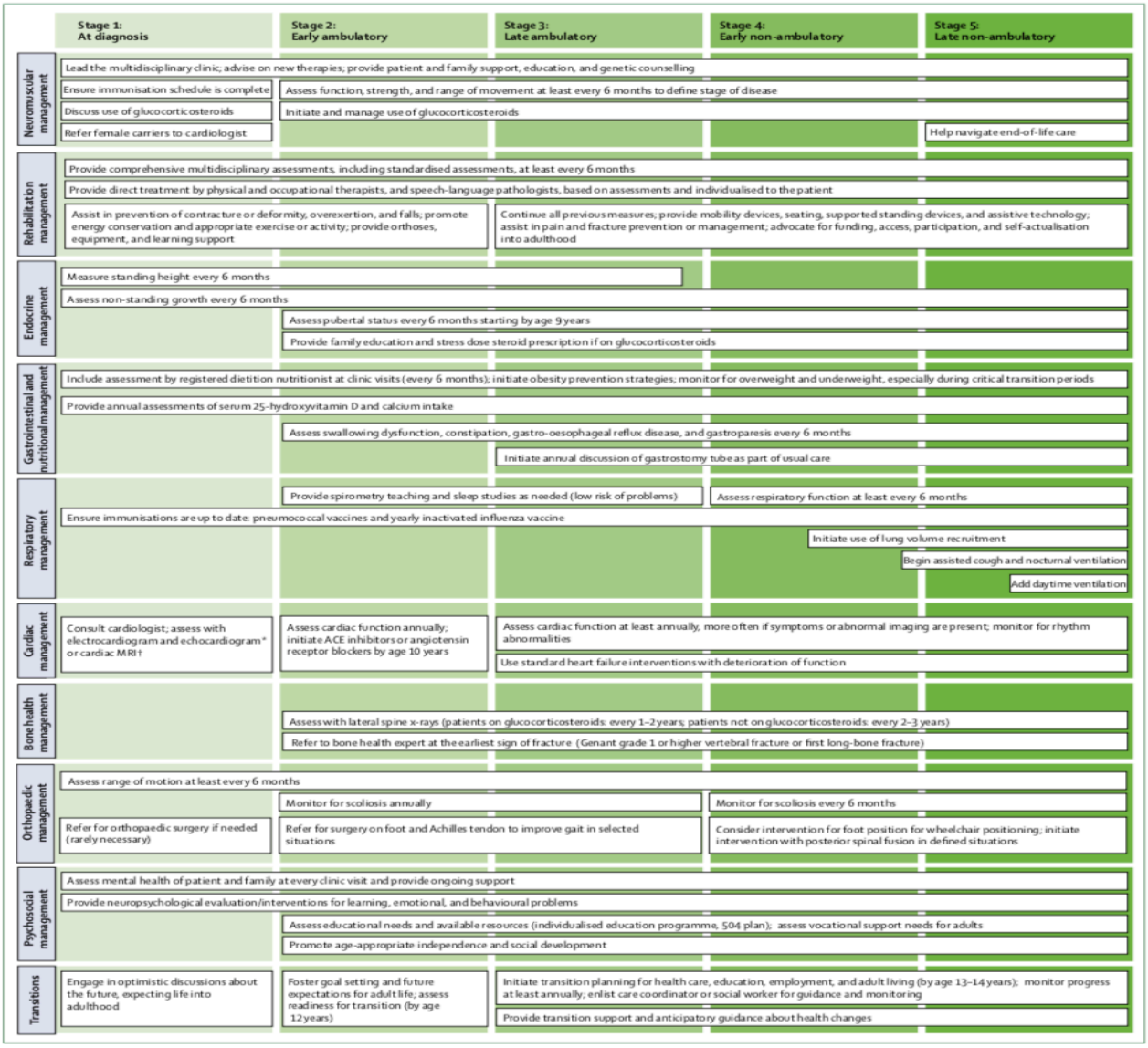
Throughout development, muscle imbalances lead to the development of contractures, scoliosis, and progressive impairments of respiratory and cardiac capacity.[5, 8] Many children who are diagnosed with DMD show early signs of functional motor delay. Proximal weakness and contracture development lead to progressive difficulty performing functional activities. Difficulty rising from the floor and running, along with and frequent falling are usually the first signs that lead to the initial investigation of the possible causes for these motor deficits. Boys with DMD are generally diagnosed around 5 years of age, when teachers and family observe their inability to keep up with their peers, difficulty getting up from the floor, or a waddling gait pattern. The progressive decline in strength results in sequential loss of functional mobility from the inability to stand up from the floor, to later inability to stand up from a chair and eventual loss of independent ambulation.

Confirmation of diagnosis is usually made from a combination of clinical observations and genetic testing. Clinical assessments include observations of pseudohypertrophy of the calves, a positive Gower's maneuver, and proximal muscle weakness. Laboratory; tests may confirm extremely high creatine kinase values, as well as elevated transaminases (aspartate aminotransferase and alanine aminotransferase produced by muscle and liver cells).[21]

Along with an adaptive Gower's sign, children with DMD have difficulty obtaining some gross motor milestones such as jumping, hopping, reciprocal stair climbing, and experience frequent falls. [9] Clinical progression rates are variable, but occurs in a predictable, sequential manner[20] starting with loss of the ability to get up from the floor, climb stairs, walk, feed oneself, and early mortality from cardiac and respiratory complications.[5, 6, 8, 13, 14, 21, 22] Birnkrant[42] et al Care Considerations defined stages of DMD based on the functional loss of ambulation as (Figure 1) Pre-symptomatic; 2) Early Ambulatory; 3) Late Ambulatory; 4) Early Non-Ambulatory; and 5) Late non-ambulatory. In the pre-symptomatic stage of the disease, boys may gain motor skills up to 7 years of age, but continue to fall behind their peers.[98] After 7 years, there continues to be a progressive decline in muscle strength and function throughout the disease course. Significant clinical milestones are lost in a sequential manner throughout the ambulatory and non-ambulatory stages of the disease. Individuals who are still ambulatory first show a Gower's sign, then progressively lose the ability to get up from the floor, to climb stairs, and to walk. After loss of ambulation, individuals lose the ability to get hands to the head for grooming, bed mobility, hands to mouth for eating, coughing, ability to get hands on the table, ability to use the computer/phone, and the ability to drive a wheelchair even with adapted micro

joysticks.[5, 6, 8, 13, 14, 21, 22] DMD is technically a muscle disease, but it affects multiple organs, therefore disease management is based on a multi-disciplinary approach (Figure 1). For the purposes of this project, the focus will be on the progression of contractures and strength declines in boys with DMD.

FIGURE 1 CARE CONSIDERATIONS FOR DMD



Muscle Weakness

Pathophysiological muscle changes typically occur first in proximal large muscles such as pelvic and shoulder girdle muscle, and progress to distal muscles.[99-103] Lower extremity muscles that are affected first include the gluteus maximus, adductor magnus, then quadriceps and bicep femoris. [102-107]. Strength has been assessed in DMD by manual muscle testing using the Medical Research Council (MRC) ordinal grades, quantitative muscle testing, and more recently magnetic resonance imaging (MRI). Manual muscle testing data has shown that strength loss is linear starting at 6 years of age.[5, 108] Young boys between ages 4-6 years with a manual muscle test (MMT) grade of 4 (moderate resistance against gravity) showed a 40-60% difference in isometric strength compared to age-matched healthy controls.[5] Decreased isometric quantitative muscle strength of nearly 50% was seen in knee and elbow extensors by the age of 6 years compared to children without DMD.[5] Loss in function for both upper and lower extremities correlated with the ability to contract muscles against gravity (MRC grade 3).[5] Similar to many neuromuscular diseases, the rate of change during the teenage years is greater and appears to coincide with the loss of ambulation. [5]

Magnetic Resonance Imaging and spectroscopy (MRI/MRS) testing has provided further understanding of muscle degradation in DMD. MRI studies have shown that during the transitional ambulatory stage of DMD (9-13 years) there was a faster rate of fatty infiltration of the muscle.[109] Distal lower limbs show significant fatty infiltration in the soleus and tibialis anterior, with the peroneus muscle having the most lipid infiltration.[101, 106, 110] Muscles that appear to show sparing are the hamstrings and adductor muscles.[106, 111] These publications

appear to explain some of the possible contracture development and gait abnormalities seen in individuals with DMD.

CONTRACTURES

Contracture is a term used to describe the abnormal shortening of muscles, skin or connective tissue causing restrictions in active and passive ROM.[8, 112] Contractures are prevalent and inevitably develop in many pediatric neuromuscular disorders.[113] Joint contractures can result in a number of disabling complications such as orthopedic deformity, pain, and decreased function and mobility. These complications have direct impacts on activities of daily living (ADL) and quality of life. The underlying pathology of dystrophic muscles contributes to the nature of muscle weakness, contracture development, and progression.

Contractures are typically categorized into 2 primary groups (physiological or myostatic) based on the etiology of their development. Most physiologically based contractures result from upper motor neuron defects, physiological processes, or electrolyte imbalances. Myostatic contractures, which are most common in NMD, result from immobilization or muscle weakness/imbalances.

Contractures in DMD are myopathic in origin. Contractures are more prevalent in myopathic disease as patients typically have proximal weakness, compared with neuropathic diseases which have more distal weakness.[113] Loss of joint ROM typically occurs due to fibrosis and fatty tissue infiltration of the muscle, and develops alongside progressive muscle weakness and imbalance. This causes increased passive resistance and non-contractility in the endomysium, perimysium and epimysium, contributing to shortened muscle fibers.[114] A study of

dystrophic muscle found that the non-contractile component highly correlated with muscle strength and function.[115] Increased stiffness within the muscle fibers reduced contractile force, whereas stiffness in passive structures such as tendons and connective tissues was associated with severity of contracture.[114, 116, 117] The reduction in sarcomeres as a result of the pathological process changes the force tension curve, causing shortened muscles and contracture formation.

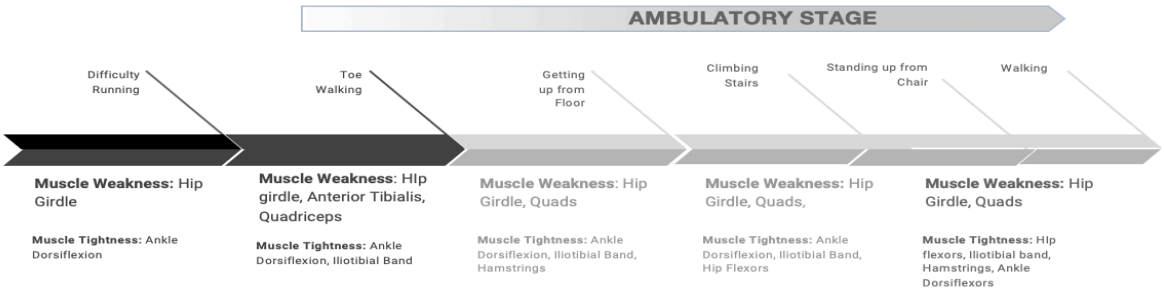
Contractures can be a critical clinical marker for DMD, with significant value in diagnosing and tracking progression. With DMD, contractures appear to develop alongside weakness patterns. Contractures in weight-bearing joints such as knees and ankles appear to be most debilitating as they affect walking. The inability to actively move a joint through full available ROM is one of the leading causes for fixed contractures because it results in static positioning of limbs, shortening of muscle fibers, and decreased sarcomeres.

Muscles that cross multiple joints are at higher risk of contractures compared to those that only cross one joint, since multiple-joint muscles must have sufficient flexibility to allow both joints to move through full range. Bi-articular muscles have origins and insertions crossing two different joints and have greater risk of change within the length-tension curve for muscle force production when they develop contractures. Biarticular muscles serve multiple roles in movement, especially in closed-chain activities which constitute most functional mobility tasks. With isometric contractions, bi-articular muscles assist in controlling movement through the range. Examples of muscles that fall into this category are pectoralis major, biceps brachii, pronator teres, rectus femoris, tensor fascia latae (IT band), and gastrocnemius. As a result of

muscle weakness in DMD, these muscles are not stretched to their full functional range across both joints, inhibiting appropriate biomechanics for movement and tissue shortening.

To better understand contracture development in boys with DMD, it is important to understand the patterns of weakness and imbalance unique to the disease. Muscle weakness is initially seen in proximal muscles including the hip flexors/extensors and knee extensor muscles.[8] However, contractures typically develop first in ankle plantarflexors, followed by hip flexors, hip abductors and knee flexors.[8] This is largely caused by the number of biomechanical adaptations made by patients to maintain standing and ambulation. When looking at torque production and association with contractile versus non-contractile tissue, a study by Akima et al[115] noted that there was not an age-related decrease in specific force production compared to controls. In boys with DMD, torque was more closely related to functional assessments such as supine to stand, timed up and go and walk/run assessments.[115] Recently, studies on cross-sectional area and torque production in boys with DMD validated this observation, showing a decline in specific torque compared to controls, specifically in the gastroc-soleus complex and tibialis anterior groups.[118] Armand et al[119] found that plantarflexion strength is the greatest predictor for loss of ambulation. Quadriceps and plantarflexor strength? showed the highest correlation with functional activities such as getting out of a chair and walking (Figure 2).[118]

FIGURE 2 DISEASE PROGRESSION AND ASSOCIATED TIGHTNESS



The unique combination of contracture development and weakness leads to a standing posture of increased plantarflexion, hyper-lordosis, and an anterior pelvic tilt. This alignment creates a biomechanical advantage that causes an extensor moment arm that allows for maintenance of upright standing, despite dorsiflexion range deficits and quadriceps weakness. With progressive plantarflexion contractures, this moment arm becomes smaller, producing less of an extensor moment at the knee to maintain upright posture. Additionally, as the hip extensors become weaker, a larger compensation becomes necessary in order to support the overall extensor moment needed to stand upright. The muscle imbalance between hip extensor weakness and hip flexor tightness causes a pelvic anterior tilt and contracture of the hip flexors resulting in a compensatory lumbar hyperlordosis resulting in a shift of the line of gravity posteriorly to produce an extensor moment.[15] Additionally, the line of gravity then falls anterior to the knee causing an extensor moment to remain upright.

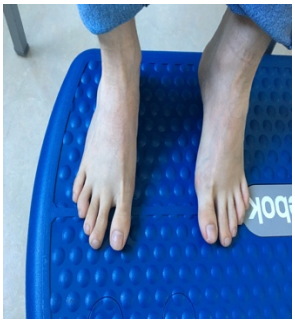
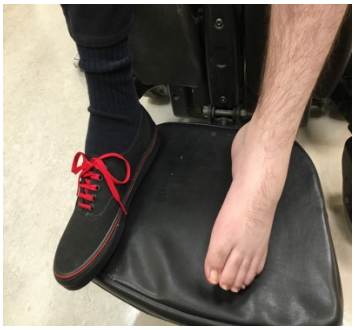
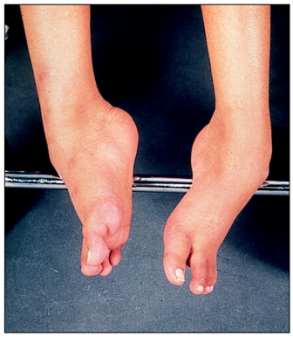
While individuals are still ambulatory, lower extremity contractures are less prevalent and severe. Biomechanical changes allows boys with DMD to maintain the ability to walk for a longer period of time despite the lack in muscle strength, but result in a less efficient gait pattern with increased energy demands.[120] Progression of gait compensations follow the course of

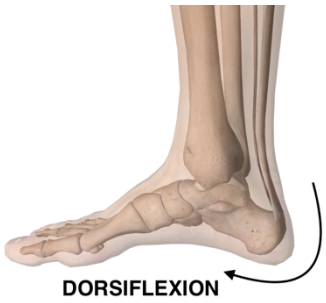
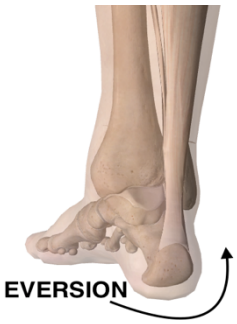
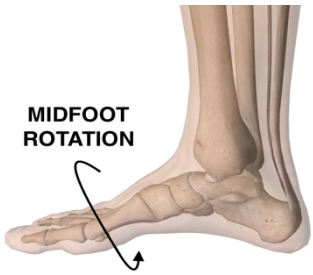
muscle weakness and contracture development with a sequential pattern of toe walking, increased base of support, lumbar hyperlordosis, circumduction of the lower extremities with no midrange knee flexion, no push off through swing phase, and increased use of upper arms and trunk to provide forward propulsion momentum.[15, 121-125] Boys with DMD develop plantarflexion contractures, which along with an increased lumbar lordosis help shift their line of gravity posteriorly at the hip and anteriorly at the knee. This leads to the adoption of compensatory strategies with a more inefficient but stable gait pattern, equinus posture and excessive lumbar lordosis. Postural compensations allow boys with DMD to stay upright, but result in decreased gait velocity and contribute to eventual loss of ambulation.[15-18] Electromyographic (EMG) studies have found increased coactivation of rectus femoris and hamstrings as well as tibialis anterior and gastroc-soleus complex in DMD to maintain gait stability.[120] During stance phase, due to hip and knee extensor weakness, there is a shift in the loading response and decreased vertical ground reaction force which displaces the body anterior to the center of gravity (COG) resulting in abnormal knee flexion contributions to lower limb stability.[18, 123, 126-128] During swing phase, boys with DMD tend to hike their hips with a lateral trunk lean due to weakness of the hip flexors, specifically iliopsoas, and abductor muscles.[16, 128]

Declines in muscle strength along with the development of contractures lead to adopted compensatory postures to preserve function such as Trendelenburg gait and excessive plantarflexion throughout the gait cycle. Although initially a functional adaptation, with time muscle weakness and contractures become so severe that patients with DMD are unable to rely on these compensations to maintain upright standing. Ankle/foot contractures progress from loss

of dorsiflexion range to loss of mid to forefoot range into eversion, due to a combination of weakness and positioning of the feet while sitting in a wheelchair (Table 1). In the later stages, the IT bands become tighter, pulling the hips into external rotation and leading to primary weight-bearing on the lateral border of the foot. This pattern contributes to positional shortening of the inversion muscles of the foot, leading to more severe equino-varus with pain and difficulty wearing shoes/socks.

TABLE 1: ANKLE CONTRACTURE PROGRESSION

Ankle Contracture Progression			
Contracture Description	Loss of range in dorsiflexion	Loss of range in eversion	Loss of range in midfoot rotation & eversion
Example Image			
Functional Loss	Loss of ambulation	Loss of weight-bearing in transfer	Increased pain and difficulty in positioning

Stretch Direction		 • Dorsiflexion	 + Dorsiflexion and Eversion

Images by Kate Witherspoon

Time required to complete a task has been used to predict functional milestone loss in DMD, such as loss of ambulation. Because the loss of clinical milestones occurs in a variable yet sequential manner, the time it takes to complete functional tasks (supine to stand, climb 4 stairs and walk/run 10 meters) has been used to predict loss of function. These predictive values are important in clinical trials as well as care management. For example, individuals who take > 12 seconds to ambulate 10 meters will likely require a wheelchair for mobility within a year.[5] This allows clinicians to speak to the family about ordering a wheelchair, home modifications, planning for school with modifications in the Individual Education Plan (IEP), and numerous other life changes that come along with losing ambulation. In clinical trials, it is important for trials to be able to predict loss of ambulation so that they define the study inclusion criteria to mitigate the risk of individuals losing the ambulation during a clinical trial. Even though work has been done on the prediction of these losses, minimal work has been done on understanding the impairments leading to decreased walking velocity, which is an observable variable precluding the ultimate loss of ambulation. Recently, a study by Ropars, et. al.[129] examined muscle activation using EMG during gait among boys with DMD, and found that there is

increased co-activation at the knees and ankles to maintain stability during gait. Due to the profound proximal weakness at the hips, individuals with DMD used agonist/antagonist coactivation of the rectus femoris and hamstrings, and tibialis anterior and gastric-soleus complex, to provide stability during gait. Additionally, the authors found a positive relationship between gait velocity, Vignos score, and co-activation of these muscle groups.[129] Due to the progressive nature of DMD, approaches to physical therapy are to provide adaptations to preserve function instead of normalizing movement patterns. Findings from the Ropars[120] study have a clinical impact on physical therapy interventions which indicate that increasing co-activation exercises and possibly providing better ankle support by using orthotics for individuals with fixed ankle contractures may be beneficial to support a stable, energy efficient gait for individuals with DMD.

Current Therapies

Therapies in DMD have evolved since the discovery of the dystrophin gene along with an improved understanding of the physiological roles of dystrophin in muscle and other organs, however, there are still limited treatments that delay the progression of the disease. Treatments that have changed the landscape of DMD include corticosteroid medications (GC), improved respiratory management with non-invasive ventilation, and physical therapy interventions for management of contractures to promote functional mobility throughout the lifespan. These therapies have impacted disease progression, however, boys with DMD continue to lose function throughout the lifespan with early mortality. Academicians and pharmaceutical companies have focused on DMD-specific drug development including antisense oligonucleotide therapy (AON)

mediated exon skipping, small molecule compounds, stem cell therapy, gene transfer, and muscle growth promotion (utrophin, myostatin inhibitors, GC equivalents).

CORTICOSTEROID TREATMENT (GC)

Multiple preclinical and clinical studies have shown that GC treatment decreases the progression of DMD.[6, 26, 28, 36, 130-136]. GC treatments include the use of prednisone, prednisolone and deflazacort. These may be described as "corticosteroids" or "steroids." Dosages for GC therapy range from 0.3mg/kg/day to 1.5 mg/kg/day with a variety of regimens from daily or alternate days to higher weekend only doses.[32, 35, 61, 137, 138] The most commonly recommended dose of GC is 75 mg/kg/day however these other regimens have been studied to try to mediate some of the side effects associated with GC use. Until the first approval of an anti-inflammatory drug deflazacort, *Emflaza,* in February of 2017, prednisone or prednisolone were has been recommended and prescribed but never an official FDA approved treatment for boys with DMD.[139] Despite all of the studies indicating the efficacy of GC for individuals with DMD, the mechanism of action is still unknown. Multiple studies have hypothesized possible benefits ranging from myoblast proliferation, stabilization of the muscle membrane, anti-inflammatory effects, decreased cytosolic calcium, upregulation of utrophin, or improved regulation of muscle fiber genes to an increase myogenic repair.[33, 34, 140-143] The reported short-term benefits of GC come with undesirable long-term consequences, although studies have not researched GC treatment for more than 2 years to adequately report on the effects of long-term use.[130] Reported side effects vary but include behavioral changes and irritability, hyperactivity, Cushingoid features, osteoporosis, weight gain, short stature, cataracts, hypertension, hyperglycemia, and delayed puberty.[30, 36, 40, 54, 61, 62, 135, 144-146]

Based on the 2018 SOC[21, 42, 43] boys with DMD should be treated with GC. At around the time of the initial SOC recommendations in 2010, a population-based study found that >50% of boys diagnosed with DMD are treated with GC.[147] A more recent study by the Muscular Dystrophy Surveillance Tracking and Research Network (MDSTARNET) published in 2015 reported that 54% of individuals with DMD are not treated with GC.[148] With the rapidly growing clinical trials for DMD in the pipeline where being on a stable dose of GC is a study inclusion criteria, many more boys with DMD will likely be following this GC recommendation in the near future. Untreated individuals with DMD typically lose ambulation by the age of 9.7-10 years, whereas, those treated with GC maintain ambulation to approximately 13.4 years of age.[37, 40]

The benefits of GC therapy in DMD were initially reported in the 1970s[149] and confirmed by the Clinical Investigation in Duchenne Dystrophy (CIDD) study group in a randomized control study (RCT) in the late 1980's.[6, 149] In this placebo-controlled study, they found increases in manual muscle strength, timed function tests, and respiratory function.[6, 32] Recently, a Cochrane review of 1RCTs in GC therapy found "moderate quality evidence from RCTs indicates that corticosteroid therapy in DMD improves muscle strength and function in the short term (12 months) and strength up to 2 years".[130] The outcomes used in these studies included time to loss of ambulation, mean change in muscle score, pulmonary function, walking velocity, supine to stand, 4-stair climb, Brooke and Vignos scales, as well as composite functional scales such as the NorthStar Ambulatory Assessment (NSAA).

When comparing GC treatment versus placebo, most studies, show a significant improvement with GC therapy, even with varying doses and regimens. Studies comparing average MRC scores in a 6-month trial showed mean differences in total muscle score between 0.34[150] to and 0.45.[32] When investigating strength differences between individuals treated with deflazacort versus prednisone, Griggs et al [151] found that in a 52-week trial there was a 0.38 mean change in average MRC score in the deflazacort treated group compared to a mean change of 0.23 in the prednisone group.

Most of these studies examined timed function tests as a secondary measure of efficacy. This includes supine to stand, 10-meter walk test (10MWT), and 4 stair climb. A recent study from the ACT trial using the 6MWT as the primary efficacy endpoint found a difference in their 48-week trial between deflazacort (-39.0 meters) and prednisone (-70.6 meters) treated patients with DMD. Two studies used the 10-point Vignos Lower Extremity score and found improvements of a 0.39 point[152] and a 0.49 point[32] in prednisone treated groups. For timed tests, all studies found significant differences between the treatment and placebo groups. For the supine to stand tests, there was an average minimum difference of 2.22 seconds between placebo and GC-treated. [54, 130] For the 4-stair climb test, there was a pooled difference of 3.09 seconds.[130] The 10MWT test also showed significant differences which ranged from 1.18 seconds[152] to 2.64 seconds.[32] These studies show the beneficial impact of GC treatment on strength and function for up to 12 months in boys with DMD. Currently, only CINRG, has prospectively studied the long-term effects of GC therapy in boys with DMD. Retrospective studies had already correlated declines in function to disease progression and found that timed function tests were the best

predictor of disease progression.[5] In a review of data collected in the pre-GC era, McDonald et al found that boys with DMD who took ≥ 9 seconds to walk 10 meters lost ambulation in 2 years, while those who took ≥ 12 seconds lost ambulation in one year[5], linking walking velocity to functional decline. The CINRG study published in 2018 found that the time required to accomplish the 3 timed function tests (supine to stand, 4-stair climb, 10MWT) predicted loss of function. They found that age of loss of ambulation predicted respiratory progression of a Forced Vital Capacity(FVC) < 1 liter, which is highly correlated to mortality.[40] In DMD, loss of clinical milestones occurs in a sequential manner first impacting the ability to get up from the ground, then climbing stairs, and then walking. In a prospective post GC study by CINRG, the researchers found that loss of the ability to stand resulted in a 70% chance of losing ambulation within one year, and the inability to climb stairs resulted in a 50% chance of ambulation loss within the same time frame. This same group used time to event analysis to determine if GC treatment resulted in faster walking velocity with ultimate delays in loss of ambulation.[40] This data showed that GC treatment indirectly contributed to improved overall survival by affecting predictive trajectories of the decline of clinically meaningful milestones.

STRETCHING/BRACING/PHYSICAL THERAPY

Stretching interventions refer to techniques or modalities that apply tension to soft tissues.[153] Physical therapists use a number of techniques that elicit short (active and passive stretches) and long duration (positioning, night splinting, and serial casting) stretches to maintain or improve joint flexibility.

Stretching interventions are thought to increase soft tissue extensibility via two different mechanisms which include initial viscous deformation of the muscle (dynamic phase) and structural adaptations within the muscle and surrounding soft tissues (static phase).[154] Viscous deformation refers to the initial mechanical response of soft tissue, such as muscle, ligament and tendon, to sustained stretching that may initiate the "stretch reflex." The "stretch reflex" is a transient response that is thought to last as long as the actual stretch time, followed by the static phase that allows for viscoelastic stress relaxation.[155] If a muscle is immobilized in a lengthened position, longer lasting changes in muscle length occur as a result of structural adaptations taking place within the muscle.[154] Via this mechanism, increases in muscle length occur as sarcomeres are added in series, and can occur within 48 hours of a muscle being immobilized in a lengthened position.[156-159] This suggests that the duration of the stretch is an important factor in achieving molecular changes in muscle length. The intensity of the stretch appears to be another important factor in achieving increases in muscle length. Studies of muscular adaptations following limb lengthening surgery, in which up to 10 cm in bone length can be gained in a matter of months, provided evidence that large increases in muscle length are possible with an intense stretch.[160]

The actual physiology of increased flexibility is unclear, which has led to many different types of stretch interventions falling under either static (isometric, active and passive) or active; and more dynamic approaches (proprioceptive neuromuscular (PNF) contract relax, or ballistic stretching). PNF employs isometric agonist contraction/relaxation techniques based on the theory of reciprocal inhibition to facilitate a stretch. An individual's strength will determine whether they are able to perform some of the active or PNF techniques. Stretching for NMD

should never include ballistic stretches because the approach uses the body's momentum to force a stretch beyond its available range. Many have theorized that a possible mechanism behind stretching is autogenic inhibition where a muscle relaxes with time, which dampens the stretch reflexive or other neuro-reflexive mechanism.[161, 162] Many people report pain or discomfort associated with stretching, but many studies have shown that improved stretch tolerance with a daily stretch routine is the primary factor in improved ROM.[117, 153, 163-165] Structures associated with stretch tolerance are unknown, but it has been hypothesized that tolerance may be related to nociceptive nerve endings in the muscle. This supports the rationale behind active stretch techniques, activating an agonist muscle to stretch the antagonist, which appear to have improved short-term range of motion from enhanced tolerance to stretch rather than muscle extensibility.[117, 153, 165, 166]

Management of contractures requires a multifaceted approach with focus on prevention/delay of contracture development. Due to time constraints, clinicians should prioritize muscle groups most prone to contractures by first understanding the phenotypical presentation of the neuromuscular disease. Secondly, a thorough strength exam identifying muscles that lack full antigravity strength would provide insight on muscles prone to contracture development.

The key to contraction management is prevention with both daily short-duration and long-duration stretches. Stretch studies in NMD have yielded inconclusive results[10] regarding recommendations for effectiveness of short- or long-duration stretching. A possible explanation could be the method of measurement of stretch effectiveness. Most studies use ROM as an indicator for improvement. Multiple studies have shown that ROM is a surrogate marker for

stretch tolerance, instead of structural or morphological changes or mechanical changes in viscoelastic properties.[117, 161-163] Physiologic factors associated with stretching may compromise effective stretching in DMD. Changes to molecular and stretch pathways, especially those that monitor the sensitivity of stretch responses (such as the stretch reflex, Golgi tendon apparatus and muscle spindle) may hinder normal responses to stretch therapy.

There is a need for standardization of stretching recommendations in DMD. Many recommendations are from anecdotal reports, rather than randomized controlled trials. These trials had conflicting results due to lack of standardized interventions and appropriate endpoints to measure actual tissue extensibility, versus stretch tolerance, which is extrapolated from joint ROM. Stretch studies address short-duration stretching defined as manual stretches of 15 seconds up to 30 minutes,[165] and long-duration stretch managed by bracing or positioning. Despite the lack of concrete evidence in the types of stretching modalities that should be used for management, smaller studies and case reports indicate the extreme benefits of daily passive range on overall muscular and joint health. For individuals who have less active movement, the significance of stretching becomes greater.

Stretching: Short Duration

Passive stretching involves an external force to passively lengthen muscles, however, active-assisted force by the agonist muscle is required to maintain range of motion. Stretches that are sustained between 15 sec to 2-minute intervals have been reported[165, 167-171] and devices can be used to assist short-duration stretching. The Kiddy Up Strap Kit™, or similar strap-based systems, are low in cost and complexity, and can contribute to autonomy in stretching and

improve the ability to hold a stretch for an increased duration. However, the individual does not have control over the intensity of the stretch and may require a caregiver to don/doff the device. Additionally, the device is positioned on the forefoot and lower back, which does not target the Achilles tendon, thus risks over stretching the plantar fascia in the arch of the foot and adds stress on the low back.

A study exploring the duration of a static stretch showed that there were no differences between 30 and 90 second holds in improving stretch tolerance.[169] Passive lengthening tended to activate the stretch reflex, therefore, methods to inhibit the stretch reflex would result in a greater stretch.[172, 173] These methods include activation of the agonist to inhibit the antagonist muscle. However, as this may not be possible in late stages of muscular dystrophy, passive lengthening would be the optimal treatment. With passive lengthening, the joint must be slowly moved to the maximum tolerated range and held for a prolonged period of time to desensitize the stretch receptors. Ballistic or rapid maneuvers with the intention of removing adhesions are contraindicated. To affect muscle physiology, viscoelastic accommodation of the muscle has been shown at 90 seconds, to result in a stress relaxation response to stretching.[117] Additionally, in view of the viscoelastic response, early stretching would be beneficial in younger individuals because their muscles are more pliable to maintain extensibility. For maximum impact of change in the muscle physiology and stretch tolerance, a stretch of 90 seconds or more is recommended. Daily passive and active range is recommended to counter complications that may arise in muscles and joints from muscle imbalances and immobilization, along with possible downstream influences on cellular and biomechanical processes of tissue repair.[174] Immobilization may lead to chemical changes consistent with osteoarthritis,[175] dysfunction of extra-articular tissue

alignment[176, 177] and degradation of collagen mass that may be further complicated by inflammation or fibrosis typically seen in many NMD, thus inhibiting normal joint gliding motion.[178]

Two systematic reviews have provided evidence that manual stretching of the hamstrings[153] and gastrocnemius/soleus [179] increase joint range of motion, while other studies support evidence of improved range with stretching but no evidence of long-term changes in muscle extensibility.[164, 165, 180, 181] The studies reviewed included otherwise healthy individuals with limitations in joint range of motion. While these results are promising, individuals with pathological changes in the structure and function of their muscle, such as those with neuromuscular disease, are unlikely to respond to stretch in a similar way.

Two further systematic reviews have investigated stretching interventions for individuals with neuromuscular[10] and other neurological disorders.[153] One systematic review specifically investigated interventions for increasing ankle range of motion in patients with neuromuscular disease.[10] Although stretching interventions aiming to improve ankle dorsiflexion range of motion are widely employed for patients with neuromuscular disease, only four studies involving 149 patients met inclusion criteria for the review. This review included three studies investigating stretching interventions for patients with neuromuscular disorders, and it is highly likely that stretching interventions of greater intensity and duration would be required to achieve results similar to those seen in the otherwise healthy individuals with restricted joint range.

Stretching: Long Duration

Long-duration stretching consists of wearing custom-made ankle foot orthoses (AFOs), also referred to as orthoses. These are prescribed to be worn for 6+ hours.[7, 14]

At initial diagnosis, AFOs are generally prescribed for night wear. AFOs worn at night are not well tolerated by some individuals, with complaints of the device being heavy and resulting in the inability to move in bed or walk to the bathroom. Some individuals wear AFOs while awake but participating in seated activities. With increased use of a wheelchair, AFOs can be worn during the day or night. Self-reported wear duration varies widely. Some biomechanical limitations to AFOs include, 1) AFOs do not address foot inversion contracture; 2) AFOs do not adequately target Achilles for stretch; and 3) Pressure sores commonly arise with growth and contracture progression.

Animal studies support management of contractures with long duration stretching.[178, 182, 183] These studies have shown that, although both dystrophic and denervated muscle fibers are diseased, they are capable of adding sarcomeres in series when immobilized in a lengthened position. The major difference between diseased muscle and unaffected muscle is that addition of sarcomeres occurs at a slower rate.[184] It is also likely that response to stretching interventions differs depending on the type of neuromuscular disorder. While similarities in muscle morphology are seen in some disorders, the response to stretching interventions may be better in some disorders than others. For example, dystrophic muscle, as seen in DMD, is characterized by a high percentage of fat and scar tissue that increases with disease progression.[185] Collagen, which comprises a large portion of scar tissue, has a greater modulus of stiffness than muscle, so that muscle with scar tissue has reduced extensibility, requiring greater force to stretch it.[154] By comparison, muscle in Charcot-Marie-Tooth (CMT) disease is characterized by fatty infiltration

of selectively denervated muscles.[185, 186] While some scar tissue is present, it is not as abundant as seen in DMD, which might indicate that individuals with CMT may respond better to stretch and require a stretch of a lesser intensity and duration than patients with DMD. A systematic review of neurological conditions including traumatic brain injury, cerebral palsy, and stroke noted moderate evidence of short term effect of joint mobility and no effect on functional activities.[187]

Long-duration stretches are defined as anything greater than 30 minutes. There are numerous ways to perform a long-duration stretch: Positioning, hand splints, standing frames, serial casting, Ankle-Foot Orthoses (AFO) or Knee-Ankle-Foot Orthoses (KAFO). There are 2 general types of AFOs: 1. Static (hold ankle in one position) 2. Dynamic (hinged joint which provides added stretch). The static AFO maintains the current ROM, while the dynamic AFO has the ability to provide added force for a more intensive stretch. Most studies have assessed static nighttime splints. Two studies investigated night splinting for patients with CMT[188] and the other investigated serial casting at night[189] for patients with CMT. A small, randomized comparative study of patients with DMD found that strength was a significant variable in determining the degree of contractures and concluded that individuals who both performed manual stretches and wore night-time splints had 23% less chance of developing contractures compared to stretching alone.[190] Hyde et al[190] and Seeger et al[191] found a decrease in plantarflexion contractures with the use of night time orthoses, while Brooke, et. al[192] did not find any differences with night time orthoses. Possible explanations for the differences would be small sample sizes and methodology in assessing contractures. Most of these studies measured ROM with a goniometer, without

standard force applied to the measurement. As elastic deformation of soft tissue is directly proportional to stretch velocity and force of application. Long-duration stretches are defined as anything greater than 30 minutes. There are numerous ways to perform a long-duration stretch: Positioning, hand splints, standing frames, serial casting, Ankle-Foot Orthoses (AFO) or Knee-Ankle-Foot Orthoses (KAFO). There are 2 general types of AFOs: 1) static (hold ankle in one position) and 2) dynamic (hinged joint which provides added stretch). The static AFO maintains the current ROM, while the dynamic AFO has the ability to provide added force for a more intensive stretch. Most studies have assessed static nighttime orthosis. Two studies investigated night splinting for patients with CMT[188] and another investigated serial night casting[189] for patients with CMT. A small, randomized comparative study of patients with DMD found that strength was a significant variable in determining the degree of contractures and concluded that individuals who both performed manual stretches and wore night-time orthosis had 23% less chance of developing contractures compared to stretching alone.[190] Hyde, et. al[190] and Seeger, et. al.[191] found a decrease in plantarflexion contractures with the use of night time orthoses while Brooke, et. al[192] did not find any differences with night time orthoses Possible explanations for the differences would be small sample sizes and methodology in assessing contractures. Most of these studies measured ROM with a goniometer, without standard force applied to the measurement. As elastic deformation of soft tissue is directly proportional to stretch velocity and force of application,[193, 194] the amount of force placed on the limb by the evaluator could vary in each of these studies.

Other orthoses that can be used for stretching and weight-bearing are KAFOs. KAFOs have been used in DMD[195] studies for upright stretching of the lower extremities and assisted gait. Considerations for KAFOs should focus on lightweight polypropylene material, drop-locked knee joints, solid neutral ankle and ischial weight-bearing bearing. Ischial height is important for appropriate trunk support and upright positioning. Additionally, patients must have some truncal strength in order to use KAFOs. Many of these studies did not focus on ROM and only noted minimal to poor evidence for the use of KAFOs[196] in DMD, but a few case studies in SMA have found that early fitting of KAFOs proved beneficial for gait.[197] KAFOs are not normally recommended for ambulation due to safety concerns and the increased energy cost associated with walking in heavy KAFOs.

Serial casting is another method that may improve range of motion. This is typically done for ankle contractures. Considerations include the negative effect of the weight of the cast on function, atrophy due to immobilization, and tolerance. Serial casting has been studied in DMD,[187, 198, 199] but a serial casting , with a mean of 12 degrees improvement in range of motion, but without effects on timed function tests.[187] Main[198] also found that a serial casting procedure showed reduction in contractures but associated with mental distress. A study of serial casting in CMT showed a mean improvement of 4 degrees with improvement on time tests.[189] Serial casting may be considered for patients with DMD with careful consideration of biomechanics, strength, and functional status of the patient. A study of serial casting in CMT showed a mean improvement of 4 degrees with improvement on time tests.[189] Serial casting may be considered

for patients with DMD with careful consideration of biomechanics, strength and the functional status of the patient.

STANDARDS OF CARE

Management of individuals with DMD greatly varies based on geographical location (CDC expert panel, 2005). Medical care largely varies around the world with most variations associated with medical equipment, frequency of visits, physical/occupational therapy, and psychological care. [53] Identifying and optimally managing secondary conditions in a comprehensive and consistent manner is key for the provision of quality care.[21, 25] With the promising therapeutic development pipeline in DMD, there needs to be consistency in SOC. The development of the SOC guideline was an international initiative led by the CDC of key stakeholders including patients, clinicians, government agencies, scientists, health agencies, and patient advocacy groups including the Parent Project Muscular dystrophy, Muscular Dystrophy Association and Treat-NMD.[21] Optimizing SOC is now a necessary initiative to support clinical trials readiness in DMD.

In 2010, the CDC published a 2-part series on DMD Care guidelines and management.[21, 25] which highlighted the need for an interdisciplinary approach and coordination of care among subspecialties to provide appropriate management of this multi-systemic disease. This document involved over 80 clinicians and patient advocates and highlighted primary and secondary manifestations of DMD that require management, with sections on neurology, steroids, cardiology, pulmonology, physical therapy/physical medicine and rehabilitation, orthopedics, surgical considerations and psychosocial care (Figure1). Care recommendations were based on

the 5 stages of DMD: pre-symptomatic, early ambulatory, late ambulatory, early non-ambulatory and late non-ambulatory.[21, 25, 42, 43] With the changing landscape of DMD and a paradigm shift to a more anticipatory and prevention-based model of practice, the original CDC Care Guidelines were updated in 2018.[42, 43] A steering committee of clinicians published a 3-part care considerations document with the goal to increase survival and guide care interventions with consideration of emerging molecular and genetic treatments in DMD. Three additional topics were added from the original 8 in the 2010 guidelines including primary care and emergency management, endocrine, and transitional care across the lifespan.[42, 43]

As part of the guidelines' recommendations, GC therapy should be assessed on the course of motor abilities, (ie: progress, plateau, or decline phase). The decision to start GC should be based on the family's report and using a team approach to care management. After start of GC, the physician must closely monitor for side effects, and motor skills should be assessed by physical therapist.[21, 43]

Standards of care suggest that "assessment and anticipatory management must be provided across all domains of the International Classification of Functioning, Disability and Healthy (ICF) model" starting from the time of diagnosis.[43] Contracture management was noted as a primary focus of treatment in DMD with emphasis on short duration stretches 4-6x/week,[25, 43] and on long-duration stretching with Ankle Foot Orthoses (AFO) for night time or day time wear in those who are non-ambulant.[25, 43] Musculoskeletal assessments should be performed by an experienced clinician who understands the DMD disease course and how to monitor disease state through strength testing, range of motion measurements, observational gait analysis, and

established timed function tests.[42]

A 2010 Cochrane review of interventions to increase ankle range of motion in neuromuscular diseases concluded that the quality of evidence for interventions to increase ankle range of motion, such as stretching and bracing, was low and further research is required.[10] Currently, in DMD there is lack of evidence on the effects of short or long duration stretching on dystrophic muscle, however, this does not negate the benefits for individuals who have muscle imbalances or weakness that prevents active joint movement, as stretching can result in enhanced joint lubrication, enhanced circulation, and overall decreased tightness. Expert consensus among physical therapists continues to support the use of stretching and night time braces for management of contractures.[25, 43, 200]

DRUG DEVELOPMENT IN DMD

As we better understand the pathophysiology of dystrophin deficiency in different body organs and gain a better understanding of the natural history of DMD, the treatment options appear to be multifaceted. While there has not been a cure for this disease, treatment has been focused on supportive therapies of impairment-based difficulties, such as the weakness and contractures associated with disease manifestation.

With the changing SOC to improve the quality of life for boys with DMD, there has been an evolution in structured natural history studies to better understand this changing phenotype and its impact on function (Figure 3) Corticosteroids as a standard of care recommendation in DMD resulted in a slowing of disease progression as evidenced by boys maintaining the ability to ambulate longer than in the pre-steroid era. One of the fundamental barriers in DMD research that

has been identified is the lack of detailed understanding of the characteristics and natural history of DMD. To address this gap, CINRG developed one the longest natural history studies performed in neuromuscular disease. CINRG developed the first multicenter international clinical trial system for DMD since the dissolution of the MDA-funded Clinical Investigation of Duchenne Dystrophy (CIDD) over 15 years ago. The design of the CINRG DMD natural history study is based on previous developments in clinical outcomes and an ICF-based evaluation model to better understand the DMD phenotype in the age of steroids. Clinical outcome measures used in this natural history study are now currently used as primary and/or secondary endpoints in large therapeutic clinical trials from the pharmaceutical industry. From a clinical perspective, the strength and functional assessments used in this study are now providing insight on disease progression to help with care management and planning. Ideal clinical endpoints for future clinical trials must be sensitive to show the magnitude of disease-related changes, and clinically meaningful in regards to their impact on boys with DMD and key milestones such as loss of ambulation.

FIGURE 3 EVOLUTION OF DMD NATURAL HISTORY STUDIES

Since dystrophin is not only located in skeletal muscle, there are many potential targets in the drug development pipeline for DMD. Compared to 30 years ago, when there were only natural history studies; there are currently 41 drugs being developed or tested for DMD.[201] As is typical in drug development, many drugs have shown great promise in early-stage pre-clinical development, but then failed to show efficacy in clinical trials. The research presented in this dissertation are relevant to drug development as many of the clinical outcomes used in trials for boys with DMD include strength measures and timed function tests. Additionally, ROM and velocity measures of the timed function tests have also been used as inclusion/exclusion criteria to ensure homogenous populations in studies and decrease the risk of attrition from an individual with DMD losing ambulation during a clinical trial. Because of the extensive scope of discussing potential therapies for DMD, this section will focus on later-stage therapeutic targets associated with current novel gene therapies of exon skipping for DMD and downstream targets affecting skeletal muscle strength and function and fibrosis.

Exon Skipping Therapies

The most novel of the therapeutic approaches is restoration of dystrophin, a mutation-specific gene therapy approach. The severity of the disease is based on mutations on the DMD gene, which codes for the dystrophin protein. To understand exon skipping, we will broadly address the genetic defect of dystrophin deficiency in DMD. There are over 7,000 different mutations in the dystrophin gene.[202] These mutations tend to be out of the reading frame, and may be small or large deletions that result in a non-dysfunctional dystrophin protein. As discussed earlier, dystrophin attaches the cytoskeleton to the extracellular matrix and is essential

in sarcolemma stability. Some in-frame mutations that maintain a partial reading frame will result in a dysfunctional or partially truncated dystrophin, protein meaning that some dystrophin is produced. In-frame deletions or duplications result in a less severe form of DMD called Becker muscular dystrophy (BMD). Currently eteplirsen, a treatment specifically targeting exon 51 which applies to approximately 13% of the population, has received accelerated approval from the FDA in 2016.[203] By skipping exon 51 which contains the mutation, the drug produces a truncated dystrophin protein that may result in some clinical benefit. Ataluren is another treatment targeting non-sense mutations in the dystrophin gene with the purpose of increasing expression of full-length dystrophin and was one of the first trials in DMD. It currently has approval by the EMA based on sufficient clinical data based on a biomarker outcome of increase in dystrophin production yet did not meet the primary study endpoint of significant increase in the 6MWT[204] resulting in the FDA declining its approval in 2016.

Corticosteroids and Nuclear Factor the NF-Kappa B Pathway

Currently, an anti-inflammatory GC is the only widely accepted therapeutic intervention to maintain strength and function for boys with DMD. Even though the exact mechanism of action of GCs in DMD is not fully known, these drugs are thought to inhibit the NF-Kappa B pathway. [32, 205] Due to the enormous problems with long-term use of GCs, a GC analog, vamorolone, has been developed by ReveraGen BioPharma. Pre-clinical trials in *MDX* mice have shown decreased side effects from this drug, such as a lack of stunted growth and immunotoxicities that are traditionally associated with standard GCs.[206] In the drug development process has ReverGen has successfully completed a phase 1 safety and tolerability study, and is

currently enrolling in a phase 2b pivotal trial, placebo controlled with a prednisone arm. Other pharmacological approaches to inhibiting the NF-Kappa B pathway, have also been explored by Catabasis. Their compound CAT-1004 (edasalonexent) showed a reduction of muscle degeneration and improved regeneration in both skeletal and cardiac muscle in *MDX* mice. They have successfully completed phase 1 studies showing significant decreases in NF-Kappa B activity[207] and phase 1/2 clinical trial in young boys ages 4-7 with DMD. (https://clinicaltrials.gov/ct2/show/NCT02439216)).

Increasing Muscle Mass

There are a few different approaches to increasing muscle mass in DMD, which have been studied in *MDX* mouse models and are slowly moving into the clinical space. Due to the loss of muscle mass as a result of fiber degeneration and decreased regeneration capabilities, increasing muscle mass seems to be a logical approach.

Myostatin Inhibitors

Myostatin inhibition is the furthest developed strategy for increasing muscle mass. Myostatin is part of the transforming growth factor-beta (TGF-Beta) family that regulates skeletal muscle growth. The exact mechanism of action is not completely understood, but it is believed to suppress precursor muscle differentiation cells through the *Mstn* regulatory gene.[208] Animals who have the *Mstn* gene knocked down have been found to show extremely hypertrophied muscles. Studies specific to dystrophic muscles have been performed in *MDX* mice indicating that anti-myostatin may increase muscle mass by ≥35% from muscle fiber

enlargement with decreased creatine kinase levels. When studied in larger animal models, such as the dystrophin-deficient golden retriever dog, this approach they showed negative effects of unequal muscle growth and increased development of joint contractures.[209] However, clinical trials have been initiated due to the promising results from the *MDX* trials. Recently, Pfizer ended a 2 year study of domagrozumab in boys with DMD early due to the inability to demonstrate efficacy with their primary endpoint of stair climb after one year of treatment.[210] Another study involving the drug MYO-029 was also terminated due to the inability to show increases in muscle strength or function for adults with Becker Muscular Dystrophy (BMD), Limb Girdle Muscular Dystrophy (LGMD), and for Fascioscapulo Humeral Dystrophy (FSHD). [211] More recently, the myostatin inhibitor that was developed by Bristol-Meyers Squibb and licensed to Roche had a phase 2/3 clinical trial to evaluate the safety, efficacy and tolerability of RO7239361 in ambulatory boys ages 6-11 years but terminated due to the study not meeting its clinical endpoint.[212]

Anti-Fibrotics to address Fibrosis

In a disease such as DMD where muscle degeneration is replaced by connective tissue and fibrosis, a primary therapeutic target would be to address fibrosis. The extracellular matrix is a crucial component to healthy muscle by providing the necessary scaffolding for tissue repair and growth. The downstream effects of dystrophin loss result in fragility of the sarcolemma, muscle fiber degeneration, and increased activation of profibrotic signaling pathways so that muscle tissue is replaced by adipose and connective tissue. Muscle tissue is much more pliable and has more extensibility compared to the more rigid fibrotic tissue. As previously mentioned,

TGF-Beta is important in muscle growth but also in regenerating fibers after tissue injury with the expression of profibrogenic factors.[213, 214] Tissue damage initiates a transcription of profibrotic genes such as fibronectin and collagen, resulting in degradation of the extracellular matrix. Studies have found that TGF-Beta levels correlate to muscle fibrosis in boys with DMD.[215] The hypothesis is that targeting the TGF-Beta pathway may increase muscle regeneration, thus reducing fibrosis in DMD.

A well-known TGF-Beta inhibitor, Losartan, has been studied in *MDX* mice and in a small clinical trial. It blocks the angiotensin-II receptor that is required for appropriate TGF-Beta signaling.[216]Mice studies showed increased grip strength and decreased fibrosis of the diaphragm and cardiac muscle.[217, 218] Human trials showed that it improved cardiac ejection fraction with a good safety profile.[219]

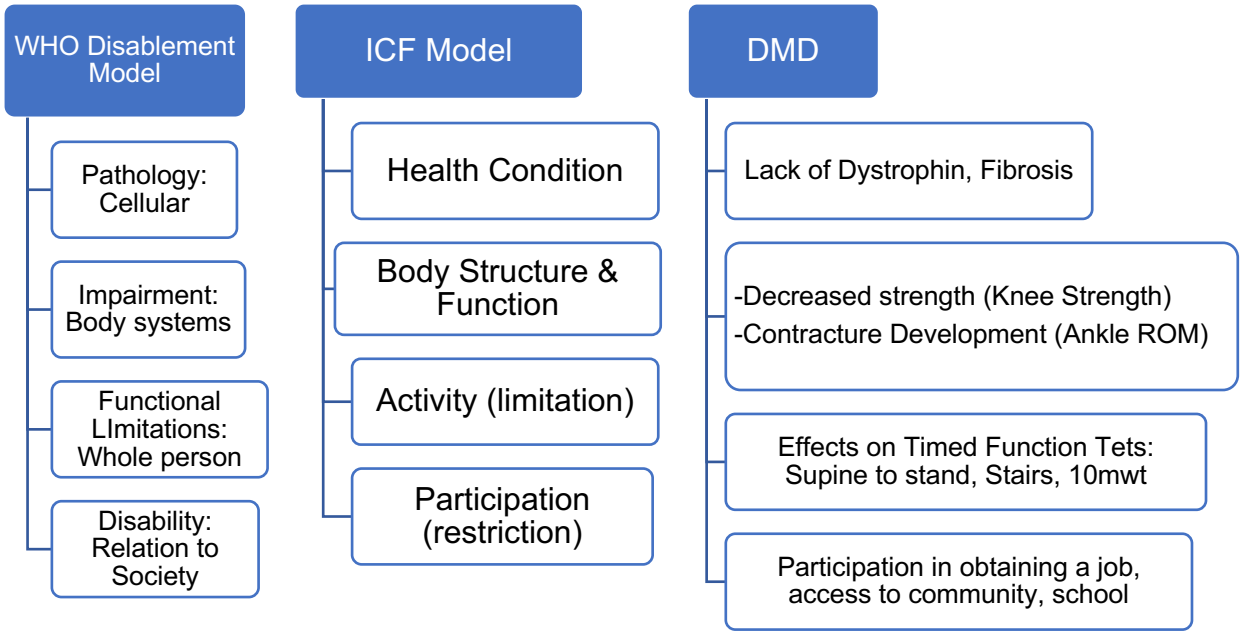
Other more recent trials of anti TGF-Beta drugs include Halofuginone (HT-100), an anti-fibrotic that inhibits collagen synthesis by preventing SMAD-3 from binding to DNA to control the activity of specific genes.[220] The results for this study were promising, with more than 10% increase in muscle strength and signs of increased collagen degradation, however the FDA put the study on hold after a patient died. Currently, the FDA has provided clearance for Akashi to resume enrollment in the study.[221]

Clinical Endpoints used in Clinical Trials

Clinical Endpoints used in Clinical Trials Based on the framework of the WHO ICF,[222] there are 4 domains that impact activity including body function and structure, participation, and environmental factors. Data collection for clinical outcomes in the natural history of DMD has

been based on this framework to promote patient center care and clinical outcomes assessment.[223] The ICF model and disablement models facilitates a common language towards clinical research that is patient centered and disease specific. This approach promotes alignment of clinical outcomes to disease related outcomes where the dimensions of the model coincides with the level of disablement related to DMD (Figure 4).[41, 224, 225]

FIGURE 4: DISABLEMENT DIMENSIONS AND LEVELS



Physical therapy evaluations have focused on understanding the effect of body functions/structures (strength and range of motion impairments) on activity (functional limitations), and their association with mobility and participation. These functional limitations have been reported by clinicians and families as important but limited published data relating it to patient reported outcomes (PROs). Additionally, there continues to be minimal information on losses related to socio-economic costs and quality of life.

In August 2005, the NIH-led Muscular Dystrophy Coordinating Committee (MDCC) established by congressional mandate identified a number of research priorities for muscular dystrophies which included 1) natural history studies in specific conditions; 2) determination of the sensitivity of clinical endpoints to changes in disease severity; 3) determination of the magnitude of changes in endpoints which are clinically meaningful to patients; 4) study of the interrelationship of clinical endpoints for specific muscular dystrophies; 5) development of standardized data collection and a minimum data set for multicenter data gathering; and 6) identification and development of standardized instruments to measure quality of life.

In 2006, at a National Institute on Disability and Rehabilitation Research (NIDRR) meeting to discuss challenges in muscle disease, the FDA specified that clinical trials conducted in support of new drug approvals or revised indications for already approved drugs must incorporate a primary endpoint that objectively measures a clinically meaningful "life-changing" milestone that has a significant impact on a patient's perceived overall health and well-being.[31]

In DMD, these functional milestones have been used to stage or describe 5 different significant stages of DMD (Figure 1).[5, 21, 22] With better understanding of the sequential loss of function, clinicians were able to define stages of the disease based on functional level. As previously described, supine to stand, 10MWT test, and 4-stair climb tests have frequently been used in both the clinic and research to understand and predict disease progression. These clinical outcomes are used in clinic for a proactive approach to clinical care utilizing predictive modeling for losses of function, while clinical trials utilize it in a similar manner to reduce attrition rates in trials from patients losing the ability to perform a clinical measure. For example, as part of

inclusion criteria, studies may assess ankle range of motion and some timed function tests to evaluate function, as timed measures have been good predictors of loss of function. This information helps manage possible attrition in clinical trials from patients losing function that may impact a primary endpoint chosen for the study. Ankle range of motion is thought to play a factor in the ability to perform these timed tests, but no studies have looked into its contribution.

Researchers have also evaluated timed tests and their association with patient-reported outcome measures to show impact on quality of life and perception of abilities. McDonald et al (2010)[39] first showed significant correlations in boys with DMD of the Pediatric Outcomes Data Collection Instrument (PODCI) and the Pediatric Quality of Life Inventory (PEDSQL) with age and timed functional tests such as 10-meter run/walk, supine to stand, and stair climb. This study was pivotal in proving construct validity with a parent-reported health-related quality of life measure and functional measures.[39] The study team found that the PODCI domains mobility/transfers and sports/physical function best correlated with age, knee strength, and timed tests;[39] linking these activity-based measurements to significant changes in the perception of function. Additionally, clinicians have debated over the role of contractures in the ability to perform some of these "life-changing" milestones. Biomechanically, we know that muscle force production and biomechanical demands change based on the alignment of the musculoskeletal structures and progressive strength loss.

Following the FDA's request for better understanding of the relationship between timed function tests and patient-reported outcomes, in 2009 the European Medicines Agency (EMA) provided a directive for the international DMD community to create consensus and guidance for

age-appropriate clinical outcome measures that may be used in multi-site international trials.[226] In response to this request, an international clinical outcomes working group was assembled in 2010 consisting of 25 leading experts across Europe and the United States, with the aim to discuss current available natural history data that may help determine consensus based on the robust and valid measures being used around the world. In order to come to a consensus, the group reviewed large datasets and determined gaps in the understanding of the longitudinal clinical picture of boys with DMD. The international group found that the functional outcomes used to assess boys who are ambulatory were psychometrically sound and valid to be used in large multi-site clinical trials. Despite this they found a huge gap in the amount of quality outcome measures that encompass the spectrum of the disease, particularly for boys who are non-ambulatory.[22] Most treatments for DMD focus on boys who have more preserved muscle function, meaning that they will most likely be ambulatory. The meeting results included 1) identification of gaps in the current literature for individuals with DMD, including a need for an upper limb measure that may be assessed throughout the spectrum of the disease; 2) a significant understanding of the steroid-era natural history of the disease; 3) psychometric properties required for clinical outcomes that may be used in clinical trials; and 4) establishment of focused working groups to address needs that arose from this meeting. A subgroup of international physical therapists and a few physicians later developed an upper limb tool called the Performance of Upper Limb Scale for DMD [227] and established a framework for inclusion of clinical outcomes in clinical trials (Table 2). This table also outlines how well DMD clinical outcome measures meet the necessary psychometric criteria to be a valid assessment in DMD.

TABLE2: INCLUSION OF CLINICAL OUTCOMES CRITERIA

Properties for Clinical Outcomes Evaluation	**Timed Function Tests** (10MWT, supine to stand, 4 Stair Climb)	**Strength (Quantitative)**	**PROs-PROM**
Clinical subgroups	4 years until non-ambulatory	Lower Extremity=5-12 years Upper Extremity=5-20+ years	7-20+ years
Supports mechanism of action of Drug being studied	✓	✓ ✓	
Conceptual framework **fits DMD**	✓	✓	✓
Reliability	✓	✓	✓
Validation with other measures	✓	✓	In progress
Normative ranges	✓	In progress	In progress
Ongoing natural history studies	✓	✓	✓
Multicenter studies	✓	✓	✓
Responsiveness to treatment	✓	✓ (more research required)	✓
Clinical meaningfulness	✓ (more research for MDC/MCID)	x	✓

These discussions brought forth a gap in the knowledge regarding the understanding of minimal clinically important differences of these measures, so it was difficult to understand the meaning behind raw scores of these timed tests, and their relevance to clinical change. Functional losses or delays in losses have historically been equated to clinical meaningful change in boys with DMD, which also contributed to disease staging.[5, 21, 22] For diseases that are chronic, progressive and variable in clinical presentation, a deep understanding of the variables that contribute to inconsistency is crucial for appropriate medical management[71]. Parents have reported that loss of ambulation, the ability to get up from the ground, or to climb stairs re significant factors impacting family quality of life and reported perceptions of disability (PPMD, Washington DC, Annual Connect Meeting, 2015). This may be due to parents being well-versed in the science of the disease and understanding that prolonged time needed to complete these measures would be an indicator of disease progression.

MINIMAL CLINICALLY IMPORTANT DIFFERENCE

With the growing number of clinical trials in DMD and rising healthcare costs for treatments, there is a need for clinicians to use clinical measures to predict a patient's response to treatment in order to help guide clinical decision-making for anticipatory care management. With the physical therapy profession moving towards evidence-based practice, there's a need for outcome measures to help inform clinical planning, decision-making, and care management, especially in chronic and progressive disease. In parallel to treatment trials for DMD, academicians are also studying the possible genetic associations with single nucleotide polymorphisms (SNPS) that may predict functional decline[146, 228] or response to treatment. The timed function tests that were analyzed in this study have been reported to have sound

psychometric properties of reliability and validity, as well as good predictive qualities. These properties give assurance that changes in these clinical endpoints are less likely to be due to measurement error. However, there needs to be a better understanding of the impact of change in the timed function tests to patients' quality of life. This is where MCID measures become important to provide the patient/clinical perspective to these outcome measures. MCID is typically defined as a minimal change that is significant to a patient.[46] MCID measures are not only important to patients, but they provide clinicians with a critical value that corresponds to a measurable change helping determine clinical goals and disease monitoring to improve anticipatory care management. It is also increasingly important to payers, as they are the ones defining approval or payment for therapies based on published MCID outcome reports making it essential for researchers to accurately report measures that are specific to the particular patient population.

Anchor-based and distribution-based methods to provide meaningful estimates of MCID for outcome measures are increasingly employed in disease-based treatment development research.[229-232] In the anchor-based approach, the meaningfulness of the change in a study measurement is determined based on the ability of a change score to predict the occurrence of a clinically meaningful milestone in the natural history of the disease.[232, 233] MCID is the minimal amount of change to a clinical outcome that may make a difference in clinical care or quality of life to a patient.[63, 234] MDC also has been used to describe as true change that is not attributed to error or variability. MDC is a statistical approach based on distribution-based methods and not anchored to clinical significance.[63] MDC is calculated based on the standard error of the mean

(SEM) of the clinical outcome being used to determine minimal change. Statistical change does not necessarily translate to clinical benefits.

Researchers have used both distribution-based and anchor-based methods to determine MCID. Distribution-based methods depend on statistical approaches based on psychometric soundness of a clinical outcome, without reference to a clinical or patient perspective of change. Anchor-based approaches use clinical or patient interpretation to understand the magnitude of change and is typically are compared with other clinical measures.[71] A lack of consensus on determining and defining MCID measures has caused confusion in interpretations of their meaning. MCID approaches consist of a variety of methods including clinician consensus approaches, psychometric-based, distribution, and anchor-based approaches.[63] On a document about patient-reported outcomes, the FDA attempted to provide guidance on interpretation and implications of MCID measures.[235] However, due to lack of consensus, MCID was defined as the smallest difference in outcomes that may be proxies of perceived importance to patients.[235] With little guidance, clinical trials continue to report MDC measures of change, which may under or overestimate the clinically important differences or significance of results. This becomes a problem as payers tend to require clinical outcomes used in trials as a minimal criterion for payment. Non-anchored based MDC may not be the best method to determine importance of a clinical outcome measure, as a whole.

In describing change, there are multiple stakeholders indicated by the range in methods of determining MCID. These stakeholders include clinicians, patients, researchers, pharmaceutical industry, and payers. Determining MCID in clinical trials is crucial as it determines the minimal

criteria that will be required for payers and used by clinicians to assess change. In describing change, literature looks at functional status, health status, and health-related quality of life (HRQOL). Patient's perception of change is usually described as health status or HRQOL, while functional status change is based on performance of a physical measure.[71]

To improve the applicability of MCID measures to a specific disease or patient population, the method of recruitment of patients used in determining MCID measures must be considered.[236] Another factor to consider is the use of cross-sectional or longitudinal data to determine MCID. Cross-sectional approaches compare different groups based on a disease-related criteria, such as ambulatory status, at a given single time point, whereas longitudinal methods use an anchor-based approach to establish a meaningful change over time.[71] Longitudinal methods are commonly used in clinical trials, including DMD studies. This highlights the many different definitions of MCID used to describe changes in a clinical outcome. Accurate interpretation of MCID for ambulatory boys with DMD must use the same methodologies from clinical trial recruitment, such as the definitions for inclusion/exclusion criteria. With the variability in phenotype in boys with DMD, this study focused on a range of MCID scores for more of a population-based approach to determining MCID rather than a single value that is more patient-focused.[237] In order to do this, construct validity was established between the 3 timed function tests, strength, and range of motion. Understanding the relationship between these measures leads to better interpretability of the MCID values presented for ambulatory boys with DMD.

Understanding and determining MCID values for these commonly used assessments in

DMD may greatly impact the interpretation of functional changes commonly used in the clinic, such as the 10MWT, supine to stand and stair climb. This provides a better understanding of the current natural history of DMD and better predict loss of function for improved planning to obtain appropriate assistive devices that promote independence and decrease caregiver burden. Additionally, MCID values assist in determining sample sizes when designing clinical trials for better interpretation of clinical outcomes and relevance.[48]

CHAPTER 3: METHODOLOGY

RESEARCH DESIGN AND METHODOLOGY STUDY DESIGN

Data was collected retrospectively from the largest longitudinal natural history study of boys with DMD. This study was performed by CINRG, a consortium of 26 clinical sites around the world with the primary goal of better characterizing and understanding the pathophysiology and clinical presentation of neuromuscular diseases, and of contributing to improved clinical outcomes that are relevant to daily functional activities and quality of life (www. Cinrgresearch.org). The DMD Natural History study has evolved over a 10-year period. Data collected in this study included a long list of clinical outcomes developed based on the WHOICF model[41]. Data was collected for basic anthropometric measurements (height, weight, goniometric range of motion, ulna and tibial length), functional assessments (MMT and QMT, 10MWT, Supine to stand time, 4 stair climb time, Northstar Ambulatory Assessment), quality of life PODCI, PedsQL, Short Form Health Survey (SF36), Neuro Quality of Life Measure (NeuroQOL) measures, and surveys on access and use of interventions used by the DMD community.

The relationship between contractures, strength, and functional mobility was evaluated using data from this study. Gait speed has been reported to be a good clinical predictor of loss of ambulation in individuals with DMD.[5, 10] Data from this study was used to estimate MCID values for 10MWT, supine to stand, and 4 stair climb anchored to the Vignos lower extremity scale. The 3 timed tests are commonly used to measure function in boys with DMD. The Vignos Lower extremity scale (Figure 5) is an ordinal scale used to describe lower extremity functional ability in boys with DMD. Any changes in the Vignos scale would reflect clinically meaningful

functional loss of either walking, getting out of a chair, or the ability to climb stairs. These changes in the Vignos typically triggers clinical needs for assistive devices and accommodations at school or home. Rate of change was analyzed per timed function tests based on a change in the Vignos scale.

FIGURE 5: VIGNOS LOWER EXTREMITY SCALE

1. Walks and climbs stairs without assistance
2. Walks and climbs stairs with aid of railing
3. Walks and climbs stairs slowly with aid of railing (over 12 seconds for 4 standard stairs)
4. Walks unassisted and rises from chair but cannot climb stairs.
5. Walks unassisted but cannot rise from chair or climb stairs.
6. Walks only with assistance.
7. Participant is dependent on wheelchair for all mobility.
8. Participant is in bed at all times.

INCLUSION/EXCLUSION CRITERIA

Since this was a natural history study, it included anyone who was genetically and/or phenotypically diagnosed with DMD. The inclusion and exclusion criteria were meant to ensure a homogenous population of only individuals who have DMD and not the milder type of BMD. Table 2 lists the inclusion/exclusion criteria for the study. Inclusion criteria for this analysis included all inclusion criteria from the original CINRG natural history study and patients who were able to complete all clinical outcomes that are part of the analysis including: QMT knee strength, Ankle ROM, 10MWT, Supine to stand, 4 stair climb and Vignos LE scale.

TABLE 2: CINRG INCLUSION/EXCLUSION CRITERIA

Inclusion Criteria

• Patients must be male between the ages of 2 and 30
• Affected patients between the ages of 2 and 4 must have a diagnosis of DMD confirmed by at least one the following OR have an older male sibling that meet at least one of the following criteria:
  o Dystrophin immunofluorescence and/or immunoblot showing complete dystrophin deficiency, and clinical picture consistent with typical Duchenne dystrophy OR
  o Gene deletion test positive (missing one or more exons) in the central rod domain (exons 25-60) of dystrophin, where reading frame can be predicted as 'out-of-frame', and clinical picture consistent with typical Duchenne dystrophy.
  o Complete dystrophin gene sequencing showing an alteration (point mutation, duplication, other) that is expected to preclude production of the dystrophin protein (i.e. nonsense mutation, deletion/duplication leading to a downstream stop codon), with a typical clinical picture of DMD.
• Affected patients between the ages of 5 and 30 must either fulfill the above criteria OR show evidence of a dystrophinopathy and clinical picture consistent with Duchenne Muscular Dystrophy
  o Patients who have documented clinical symptoms referable to a dystrophinopathy and direct support of the diagnosis by either (1) a positive DNA analysis for dystrophin mutation, (2) a muscle biopsy demonstrating abnormal dystrophin, or (3) an elevated CK (>5X normal), and X-linked

pedigree and an affected family member who meets either criterion (1) or (2) as described above.

NOTE: Determination of the appropriate clinical symptoms consistent with DMD will generally be the responsibility of the clinician. At a minimum this will include progressive loss of function, with additional consideration for other clinical features such as a characteristic gait, a positive Gower's sign and calf pseudohypertrophy. When immunostaining of muscle biopsy is used to determining case definition, the clinical reviewer (site PI) should confirm that appropriate testing has ruled out a secondary deficiency of dystrophin. Affected patients that do not exhibit the above symptoms consistent with DMD should be excluded.

- Muscle weakness prevalent by 5 years of age

- Non-affected adult patients must be Parent(s) or legal guardian(s) of an eligible affected subject.

Exclusion Criteria

For those patients that confirm DMD diagnosis through a clinical picture consistent with DMD

- Steroid-naïve patients ambulating past the 13th birthday
- Steroid users ambulating past the 16th birthday
- Patients/families who are unwilling or unable to comply with the protocol study procedures or visits

Recruiting Procedures

Individuals participating in this DMD natural history study were a sample of convenience who were identified at the 26 international and national clinical sites by the neurologist or individuals from the clinical or research team at each institution. Methods of recruitment were by word of mouth, the website www.clinicaltrials.gov., and internet or other means of IRB-approved advertisement from their respective institutions.

Specific Procedures

DATA COLLECTION AND MANAGEMENT PROCEDURES

Clinical endpoints used in this study were all collected by licensed physical therapists who received annual certification from CINRG to ensure standardization of procedures (Table 3). Details of study procedures for the study are included as part of the CQMS User's Manual Appendix. Clinical assessments were performed most frequently in the first year with 5 visits; 2 visits in the second year followed by annual visits (Figure 6)

FIGURE 6: STUDY VISITS

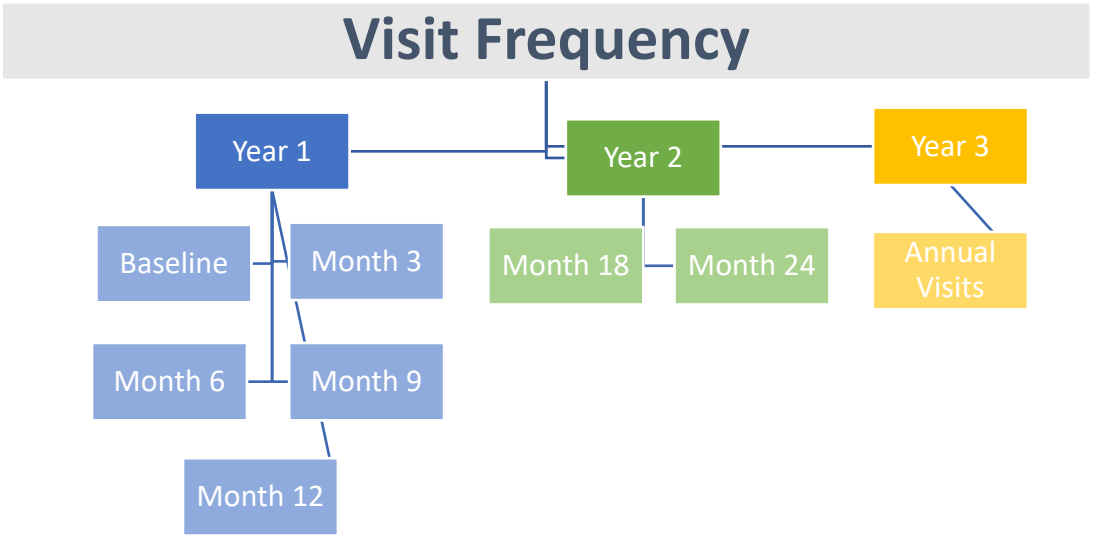


TABLE 3: STUDY OUTCOMES

Outcomes	**Data Collected by**	**Method of data collection**
Patient demographics	Site Clinical Coordinator	Data entered into EDC
Antropometrics: Height & Weight, goniometry	Physical Therapist	Paper source & Data entry into EDC
Vignos Scale	Physical Therapist	Paper source & Data entry into EDC
10MWT	Physical Therapist	Paper source & Data entry into EDC
Supine to Stand	Physical Therapist	Paper source & Data entry into EDC
4 Stair Climb	Physical Therapist	Paper source & Data entry into EDC
Quantitative Muscle Strength from CQMS (QMT)	Physical Therapist	Direct transfer from CQMS system to EDC

Description of study outcomes

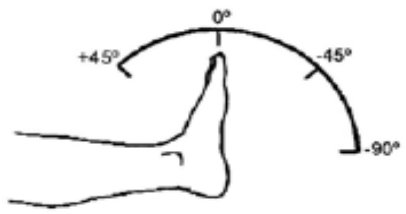
Patient demographics and medical history was collected in the study. Data used for this analysis included race, ethnicity, age, height, weight, and steroid usage history.

GONIOMETRY

Clinically significant contractures are defined as a reduction in ROM by 20 degrees. Joint ROM measurements followed the protocol used by Brooke and colleagues[238] using standard goniometry as previously reported by Johnson[113]. Measurements for contractures included elbow and knee extension, wrist extension, and ankle dorsiflexion. For patients who are unable to transfer to the exam table, ROM was be measured while participant was seated in the wheelchair**.**

Passive Range of Motion Testing Procedures: All measured bilaterally

1.) **Ankle Dorsiflexion**



- Measured in supine with the knee extended as much as possible.
- The goniometer center is placed over the lateral malleolus, arms are aligned with lateral midline of the fibula using head of fibula for reference, and parallel to the lateral aspect of the fifth metatarsal.
- Full Normal Range= 20 degrees

2.) **Knee Extension**

- Measured in supine with hip in **neutral,** ankle relaxed.
- Goniometer center is placed lateral to knee joint (lateral epicondyle of femur), arms are aligned with midline of the femur using greater trochanter for reference, and lateral midline of the fibula using lateral malleolus for reference.
- Full Normal Range= 0 degrees

VIGNOS LOWER EXTREMITY SCALE

The functional classification used by CINRG utilizes a scale modified from and the lower extremity scales used by Vignos et al.[1] This scale was administered and rated by the physical therapists who were evaluating the participants in the study. The scale includes eight levels to describe the ability to get hands over head, hand to mouth to ability use his hands (Figure 5).

Timed Function Testing

Timed motor performance (e.g. 10MWT, time to climb 4 stairs and time to stand from the floor) tests followed the protocol reported by CINRG group. If the patient was unable to perform the test, the test was skipped and re-introduced at the next visit.

Timed Test Procedures

10MWT

The participant stood at the beginning of a measured 10-meter length of hallway or walkway. He was then asked to traverse the 10 meters as quickly and safely as possible. This test was performed without shoes.

Equipment: Stopwatch, 12-meter hallway.

Instructions: The participant stood with his hands by his side. On the word "go" he was asked to go as fast as he can past the 10-meter mark. The evaluator stopped the time when the first foot passes the 10-meter mark. (cold start, flying finish)

4 Stair Climb

The participant stood at the foot and center of the stairs, without holding the rails. He was asked to climb the 4 stairs and stop on the top platform without turning around. When he reached the top, the participant stood still and returned his hands to his sides to end the test. Use of the railing was allowed if preferred by the participant. The participant was not to skip any stair.

Equipment: Stopwatch, Standard 4 steps with railing

Instructions: The participant was asked to start on "go" and climbed the stairs as quickly and safely as possible. The evaluator stopped the timer when the participant was standing with both feet on the top stairs, feet within shoulder width apart and hands by his side. (start in soldier position; end in soldier position)

Supine to Stand

The participant laid on the floor with arms at sides if able. If unable participant was able to sit in a chair with his hands folded in his lap or across his chest. The chair height allowed the participant's hips and knees to be at 90 degree angles with feet resting on floor. The participant was told to stand up as quickly as he can while maintaining arms crossed across chest. After standing the participant returned his arms to his sides to end the test.

If the participant was unable to perform the sit to stand without the use of his upper body, then the participant was able to sue the arm of the chair or push up from the floor. This is noted in the study worksheet.

Equipment: Stopwatch, Low pile carpet or linoleum floor

Instructions: The participant was positioned with arms by his side and legs hip width apart or as close as possible due to IT band tightness. The participant was asked to get up from the floor as quickly and safe as he possible on the word "go". The evaluator stopped the timer when the participant was standing with both feet within shoulder width apart and hands by his side.

Timed function test data (10MWT, Supine to stand, 4 stair climb) was collected in seconds using a standard stopwatch. Statistical analysis utilized the data expressed as speed (Table 4) with imputed zeros for those who cannot perform the test due to disease progression.

TABLE 4: VELOCITY CALCULATIONS FOR 3 TIMED TESTS

	Speed Calculation	**Example**
10MWT	10/seconds to run/walk Units = meters walked/second	If it takes a boy 5 seconds to perform test 10MWT=5 sec Velocity=10/5=2m/sec
Supine to Stand	1/seconds to stand Units = rise/second	If it takes a boy 3 seconds to stand up, his velocity is being able to accomplish a third or 0.33 of the task per second If it takes him 5 seconds, his velocity is 0.2 of the task per second, or he accomplished one fifth of the task in one second.
4 Stair Climb	1/time to climb Units = 4 stairs/second	The entire task of climbing the 4 stairs combined is used, rather than a per step unit, which would not reflect a meaningful entity If it takes a boy 9 seconds to climb 4 stairs 1/9=.11/sec

QUANTITATIVE MUSCLE TESTING

QMT of the knee and elbow flexors and extensors was performed by the methods described by Escolar (Table 5).[67] QMT has been shown to be reliable for these muscle groups, with an intraclass correlation coefficient (ICC) greater than 0.90.

TABLE 5: QUANTITATIVE MUSCLE TESTING PROCEDURES

Muscle Group	Patient Position	Limb Position	Stabilization	Limb Strap Position
Knee Extensors	Sitting with back support, facing away from the wall	-Hip and knee flexed at 90 degrees-Towel roll under tested knee	Seat belt; Chest strap if needed; Examiner stabilizing proximal thigh	Proximal to malleoli
Knee Flexors	Sitting with back support, facing toward the wall	-Hip and knee flexed at 90 degrees-Towel roll under tested knee	Seat belt; Chest strap if needed; Examiner stabilizing distal thigh	Proximal to malleoli

CQMS SYSTEM

Data from the CQMS system is a stand-alone software and hardware package which facilitates collection of data from quantitative muscle testing (QMT), manual muscle testing (MMT), timed function tests (TFTs), functional evaluation (FEs) and pulmonary function testing (PFTs). Design and validation of this system has been previously reported.[67] This system leads the physical therapists at each study site step by step through a standardized set of assessments and at the conclusion of the battery of tests resulting data is backed up locally to compact disc and then transmitted via a remote connection to a central directory on one of the OmniComm

TrialMaster's servers. All data is copied by the OmniComm TrialMaster system to a secure backup server at another site several times per day.

The study manager, data monitor, biostatisticians and DSMB had access to the entire study dataset throughout the entire study. This was for monitoring of specific site data, for performing quality control, for periodic data analysis, or for study-wide monitoring. These individuals only had read-only access to the study data via the TrialMaster CQMS web interface. Where the content or integrity of a specific data point was in question, site specific individuals were responsible for answering the query for comment/revision. Any data revised or altered in any way was captured by the system's audit trail mechanism.

Pursuant to 21 CFR Part 11, the data management system for the CQMS and the OmniComm Trialmaster system complied with all federal regulations regarding electronic signatures, audit trails, and date and time stamps; collection, inspection and review of data, retrieval of data, and reconstruction of the study; physical and logical security; system documentation, validation and change control; software version control, system failure contingency plans, backup and recovery of electronic records; personnel qualifications, training and documentation.

Data Analyses

General considerations for clinical data used in this study

Clinical outcomes in this study included knee strength, 10MWT, Supine to stand, 4stair climb measures. Most of the measures are continuous and quantitative. Descriptive analyses were grouped based on the distribution of data.

For continuous measures, histograms, box and whisker plots and Q-Q plots were used to assess normality, need for transformation of data and to identify outliers. Normality was assessed using a combination of the Shapiro-Wilk normality test and visual inspection of histograms. Measurements were summarized for each visit and the 12-month change from baseline. The operational definition for a 12-month change is considered 305-426 days). If distribution was approximately normal, mean and standard deviation were reported. For non-normal distributions, median, minimum-maximum and interquartile ranges were reported. For categorical data, frequencies and percentages were reported.

To get a true representation of the cohort, patients who lost the ability to perform the time function tests during the study were included in analyses by imputation of velocity. If the participant lost the ability to perform a task since the previous visit due to progression of disease, a velocity value of 0 was imputed for the first visit where they could not accomplish the task. The participant was then excluded for that test in subsequent visits.

Due to differences in definition for ambulation and steroid use, we operationally defined these descriptions in this study. Patients were considered non-ambulatory if they reported full time wheelchair use at the time of the visit or if they reported the age at which a wheelchair was used full-time. All other patients were categorized as ambulatory which encompass both household and community ambulators. Steroid use was defined for each visit and based on patient report of steroid use during time of the visit. Descriptive analysis also summarized the total duration of steroid use up to each visit.

THE RELATIONSHIP BETWEEN STRENGTH, ROM, AND FUNCTION (FOR OBJECTIVES A-C)

Objectives A-C include describing the relationship between knee extensor strength, range of motion, and 3 timed function tests. Descriptive statistics to describe the data, then the ability of knee strength and ankle ROM to predict walking, climbing, or standing ability was analyzed using mixed effects linear models. All statistical tests were performed with STATA V15 (College Station, TX) and a p-value of ≤ 0.05 was considered statistically significant.

Descriptive summary statistics such as age, gender, ambulatory status data were presented as mean and standard deviation and median, minimum, and maximum values. Summary statistics of both absolute values of knee extensor and ankle dorsiflexion and 12-month changes in knee extensor and ankle dorsiflexion were calculated and reported with several different categorizations. Summaries were calculated for each of the above-named outcomes stratified by 1-year age groups and either glucocorticoid use or ambulatory status. In these summaries, all available data points were used for each participant, therefore many participants contribute more than one observation per stratified group. The number of data points and the number of unique participants were reported for each stratified group. For summaries of 12-month changes, the glucocorticoid and ambulation status at the beginning of the 12-month period were used for purposes of stratification. For this analysis participants were divided into the 5 stages based on age. For example, if a participant was 4.5 years old at the start of the 12-month period, they were included in the 0 to 4.9 year age group even though they were older than 4.9 years at the end of the interval.

To assess the relationship between knee extensor strength, knee ROM, and ankle dorsiflexion ROM and functional ability, mixed effects linear models were used. The goal was

to assess how useful these strength and ROM assessments are at predicting functional abilities (i.e. the ability to walk, climb stairs, or stand from the floor). In each model the functional ability was considered the dependent variable and strength, or range of motion was the independent predictor. Age and glucocorticoid use (yes/no dichotomous variable describing use at the time of the visit) were included as covariates and each model included a random coefficient for participant. This model allowed assessment of the relationship between the predictor and the dependent variable while allowing for multiple related assessments per participant. Each model provided an estimate of the amount of change in functional outcome that can be attributed to the predictor while accounting for covariates and repeated measurements taken on the same participants. In addition, the model provided a significance test for each predictor. Several mixed effect models were performed. These included models where 1) walking velocity, climbing velocity or standing velocity was the dependent variable and the absolute value of knee extensor strength, knee ROM, or ankle ROM was the independent predictor and 2) the 12-month change in walking velocity, climbing velocity or standing velocity was the dependent variable and the 12-month change in knee extensor strength, knee ROM, or ankle ROM was the independent predictor.

Lastly, to assess the combined effect of knee extension strength and ankle ROM on functional ability, three additional mixed effects linear regression models were performed. In these models, both knee extension strength and ankle ROM were included as independent predictors and walking velocity, climbing velocity or standing velocity was the dependent

variable along. Models again included covariates for age and glucocorticoid use and a random coefficient for participant.

This cohort represents a wide range of ages and the full spectrum of functional abilities. DMD progression rate is variable at different stages of the disease. This analysis described range of motion and strength based on the 5 stages of function, as previously described. The breakdown provided a more granular understanding of differences in ROM and strength within the different stages. It also allowed evaluation of the relationship between knee strength/ROM in the lower limbs and functional ability at different stages of the disease.

Minimally Clinically Importance Difference Analysis

An anchor-based method was used to define the MCID in the ability to walk, stand, or climb stairs. Clinical meaningfulness of walking, standing, and climbing were assessed by anchoring them to changes in the Vignos functional scales. Descriptive statistics were used to summarize participants' age, current usage of steroids and 12 month change of clinical endpoints grouped by Vignos decline status decline status.

Logistical regression models, adjusted for age, were used to test 12 month changes in timed function tests associated with a 12 month decline in the Vignos score. Participant IDs were clustered to account for multiple occurrences of more than one 12 month interval measurements. This clustered sandwich estimator specified that the standard errors allowed for intragroup correlation, and also affected the variance-covariance matrix of the estimators. The model was

as follows: Vignos decline status= 12 month function change of each time function outcome + age, stratified for ambulation with clustering on subjects.

In order to identify the anchor based MCID, the timed function tests were associated with a Vignos decline of p values less than 0.05. Candidate MCID values were developed with use of receiver operating characteristic curve analysis following the ROC sensitivity to change method by Stratford et al.[239] To obtain anchor based MCID, a new binary variable for each of the 3 timed function tests was developed, namely, the new binary variable of 1 or 0 based on whether the 12 month change of time function tests were $\leq$ the cutoff score. The optimal cutoff point was estimated by the point with maximum likelihood ratio obtained by dividing sensitivity (true positive rate) by 1 minus specificity (false positive rate).[239] A logistic regression model was then fit to the new binary variable.

Instruments, Measures and Design Threats

With increases in the numbers of experimental therapies and clinical trials in DMD, it is important to define reliable and sensitive endpoints that fulfill FDA requirements for relevance to quality of life. The key to a successful clinical trial is the utilization of reliable outcome measures.[31] The primary outcome measure should have demonstrated relevance to patient quality of life, but also be sensitive and reliable.[31, 240] Both sensitivity and reliability ensure that clinical trials can be appropriately powered with a reasonable number of patients, and thus at a reasonable cost. Reliability is particularly important for multi-site clinical trials, with demonstrated inter-class correlation coefficients sufficient for conducting the trials with a reasonable number of patients.

Two more recent methods for assessing muscle strength use mechanical (analogue or digital) measures of force generated by the DMD patient; hand-held myometry (HHM), and the CINRG Quantitative Measurement System (CQMS). Quantitative muscle testing (QMT), where isometric strength is monitored by patient maximal voluntary contraction against a force transducer, has become a common method in neuromuscular research to best assess isometric strength in adults. To increase reliability and sensitivity in children (e.g. DMD), a QMT system interfaced with audiovisual feedback was developed by the CINRG group and called CQMS (CINRG quantitative measurement system). In essence, the audiovisual feedback turns the strength measurement process into a 'video game', leading to increased compliance and effort (reliability) in DMD children.[67, 241] A reliability study produced by CINRG found the CQMS to have high sensitivity and reliability for large multi-center trials in children with Duchenne Muscular Dystrophy.[241]

The MD-CARE Act passed in 2001 brought to the forefront a large initiative to allocate federal funding that encompasses these goals.[31] One of the key issues from the FDA focused on clinical trial endpoints that have proof of principle data to incorporate a functional component.[31]This has led to the need to find surrogate outcomes. In order to reach this point, we must identify a sensitive and reliable measure of strength related to functional outcomes, such as walking, that may correlate to quality of life. If a primary clinical endpoint shows poor reliability and/or sensitivity, then the required statistical powering of the study requires many more patients. In fact, even relatively small decreases in reliability and sensitivity can significantly increase the number of DMD patients needed for the trial, to the point of becoming exceedingly

expensive and problematic for adequate recruitment. A second important issue, particularly for pharmaceutical companies, is acceptance of a trial endpoint by the FDA as relevant to the 'quality of life'. While it may seem intuitive that improved muscle strength should improve the quality of life of DMD patients, the FDA argues that this cannot be assumed to be true unless clearly demonstrated. For example, statistically significant (but small) increases in strength could be associated with worsening of contractures or other side effects that would not lead to improved quality of life.

Timed function tests are increasingly used as clinical trial endpoints in neuromuscular disease, as these are readily accepted by FDA as relevant to quality of life. Timed function tests have been found to correlate with quantitative muscle strength in different stages of the disease process in DMD; a small reduction in muscle forces showed a large decrease in functional ability.[242] Due to some subjectivity in timed function test secondary to confounding variables such as fatigue and motivation, many clinical trials also employ a quantitative measure to assess functional status. Any confounding variable serves to reduce the sensitivity and reliability of the test, decreasing statistical power, and increasing the number of patients to detect change. This can lead to 'failure' of a trial, even though there is other evidence of strength improvement and improvements in quality of life.

In order to show the relationship between body systems, function and quality of life, the International Classification of Functioning, Disability and Health (ICFDH) was developed by the WHO. It aimed to progress DMD research by providing a common framework and language for clinicians and scientists across all domains toward an anticipated clinical endpoint. This model

standardized the definition of body functions and measures to make comparable observations and develop appropriate design tools throughout each phase of clinical development[31]. The ICFDH improved the communication between basic scientists and clinical researchers to parallel endpoints relevant to proposed anticipated outcomes of a clinical trial. A common tool to assess strength measures that correlate with "life-altering" outcomes would be important in order to find surrogate measures. Without these measures, it would be difficult to find intrinsic meaning in strength data and measures of functional outcomes. A valid and sensitive strength measure will propel correlations in surrogate measures to improve the ability to predict clinically meaningful outcomes such as ambulation and quality of life. These factors from the ICF create a good framework to base the correlation of health parameters to endpoint assessment in translational research.

Direct measurements of strength, either of isolated muscle groups or combined scores of multiple muscles, have long been used for clinical trials in DMD and other neuromuscular disorders. The most heavily utilized approach has been 'manual muscle testing' (MMT), where a trained evaluator manually assesses the strength of a patient, typically on a 1-10 scale. MMT was used extensively by the CIDD group, and shown to be sensitive and reliable with highly trained evaluators.[32, 98] Clearly, MMT is entirely based upon subjective assessments of clinical evaluators, and the experience of the evaluator becomes a strong potential confounder, decreasing sensitivity and reliability. To date, there has been data to support quantitative muscle testing as a sensitive and reliable measure to assess muscle weakness.[67, 240-246] However, there fails to be a study that investigates and directly compares reliability and sensitivity of the two

most widely used isometric quantitative muscle testing devices: hand held dynamometry and quantitative muscle testing. Both these strength measures have been demonstrated to be more reliable and sensitive than manual muscle testing.[67, 240-246]

Strength and functional assessments performed in this retrospective analysis included strength assessments using the CINRG Quantitative Measurement System (CQMS). CQMS requires a strain gauge to determine maximal isometric contraction. The advantage of a strain gauge system such as the ability for it to assess a range of very strong to very weak muscles. This is important as DMD is a slow progressing disease, and it is essential to have a sensitive method to measure strength progression throughout the course of the disease.

The CQMS incorporates a standard sequence of assessments, audiovisual feedback, and real time data checks designed to improve effort-dependent strength measures and test performance. This system has the capability of recording pulmonary function tests, timed function tests, functional tests, range of motion, and manual muscle testing in a standardized fashion. For the purposes of this study, we will only use the strength measures function. Variability appears to be less in QMT versus manual muscle test measures.[247] A study by Mayhew et al (2007)[241] showed a high level of agreement between "expert" evaluators and newly trained evaluators (Table 6).

TABLE 6: STRENGTH RELIABILTY

Measurement	**Expert Evaluators**		**Novice Evaluators**	
	ICC	Confidence Interval (2 SD)	ICC	Confidence Interval (2 SD)

Total Manual Muscle Test Scores	0.83	0.32-.94	0.61	0.3-0.80
Total Quantitative Muscle Test Score	0.97	0.93-0.98	0.96	.092-0.98

Comparison of expert versus novice evaluator differences in muscle testing showed a much larger confidence interval with manual muscle testing compared to quantitative muscle testing for both experts and novice evaluators, indicating much more variability in the manual muscle test data. CQMS measures appear to be more consistent with a higher ICC and much narrower confidence intervals, indicating less variability in the data for CQMS testing.[241] Other assessments used in this study include functional measures of timed functional tests (10-meter walk test, supine to stand and stairs) as well as an ordinal scale of lower extremity function, the Vignos Lower Extremity Scale.

Anthropometric measurements relevant in the analysis included standing height measured by a stadiometer with standard standing posture requirements, weight measured in kg, and joint ROM measured by a standard goniometer following procedures by Norkin.[248] Lower extremity ROM measurements used in this retrospective analysis included knee extension and ankle dorsiflexion. It is important to note the quality and reliability of joint range measurement relies largely on the physical therapist's knowledge of anatomy and physiology for proper alignment of the axis of the goniometer to the fulcrum of the joint and positioning. Additional factors affecting measurement error include the force applied to the end range, end range feel, test position, and instrumental differences.[249-251] We recognize that there are other methods to measure ROM but

chose the standard goniometer as most of these methods have an equitable reliability to goniometry with intraclass correlations between 0.85 and 0.99.[249]

STRENGTHS AND WEAKNESSES OF STUDY DESIGN

STRENGTHS:

Understanding the natural history of DMD is essential to building clinical understanding for care management, and a requirement to understand the impact of interventions. This natural history study has furthered the development of psychometric soundness of strength and timed function tests used in DMD. With this large dataset, methodology, feasibility, reliability, sensitivity and magnitude of these clinical outcome measurements have been developed and validated. The advantage of this study design allowed for all individuals with DMD regardless of functional status to learn and participate in a clinical trial. This broad inclusion criteria is expected for natural history studies as it better generalizes a representation of patients around the world.

This study design consisted of a rigorous methodology in training and administration of clinical evaluations, which is not always the case for earlier natural history studies. Patients enrolled in the study came into a separate clinic visit from their normal clinic visit which allowed for focused time with the evaluators. Additionally, CINRG has an established infrastructure to provide training and consistency with administration of physical therapy evaluations to ensure standardization and reduce drift of knowledge. All data used in this analysis was evaluated by a licensed physical therapist who met the minimal annual training criteria. This is crucial in reducing variability in data. A physical therapy training plan was developed to ensure consistency of the evaluations throughout the length of the study which consisted of initial face

to face training, reliability assessment, and annual refreshers throughout the study. Other methods to ensure quality data included a rigorous data cleaning plan that included range checks for data entry into the electronic database, and clinical sensibility checks performed throughout the entirety of the study. Despite the heterogeneity in the disease progression of DMD, the CINRG training and data management plan helped reduce the usual variability in clinical outcomes that can be a problem in natural history studies.

Weaknesses:

The greatest weakness in the study may also be considered a strength of the study. Despite the advantage of the inclusive nature of this study, it lends to increased heterogeneity in the data for a complex disease with well-known variability in disease progression rates. Additionally, patients in this study were recruited from different parts of the world. Because this study recruited internationally, the different SOC around the world contributed to added variability in patient presentation. It is well documented that the 2 primary factors impacting changes in clinical presentation for boys with DMD are the use of steroids and management of contractures. Steroid use varies in regard to dosing, frequency, and type; while stretching and brace management have even greater variability. All of these traits make it difficult to determine clinical meaningfulness in these measures with a small sample size. Here, given the inclusive nature of the study, we were able to recruit a large cohort for a rare disease giving us larger numbers than typically available in smaller studies.

ETHICAL CONSIDERATIONS AND REVIEW

All individuals participating in the CINRG DMD natural history study consented to participation in the study using standard forms provided by CINRG and approved by each local institutions Institutional Review Board (IRB) or Ethics Committee (EC). All 26 sites submitted to their local IRB and received approval prior to initiation of any study related activities.

Informed consent/assent was documented for each patient in any study. The date and time of the consent/assent was made prior to the initiation of any study-related tests or procedures, including diagnostics that might be required to confirm a patient's study eligibility.

Consent included dialogue with the investigator, parents and patient. All informed consent forms were written at the 8th grade reading level without technical language, and including an explanation of study procedures, risks and benefits of performing the assessments, and participation in a natural history study. For international locations, all consents/assents were translated to the primary language of the patient and family. Translation of the consent/assent document was certified in writing by a certified translator. All patients were provided with a copy of the informed consent/assent.

Confidentiality and Health Information Portability and Accountability Act (HIPAA)

Confidentiality was maintained throughout the study. A unique code rather than a name was used to identify test results and study data. No personally identifiable information was released beyond the Study Coordinating Center and data center without prior written consent of the patients. All records were kept in a HIPAA-approved secure facility. Medical history, questionnaire response data, and information collected from medical records is all considered personal health information (PHI) as per guidelines set forth by HIPAA. Data was entered by site staff into a large, registry-style secure database that was under the control of the CINRG Coordinating Center and was stored in a de-identified coded manner using unique study ID numbers. Any information collected following cessation of a patients being in the study was destroyed, but information collected during their participation remained as the part of the overall study data set.

The case report form collection electronic data capture systems were HIPAA-compliant, and the computers were password protected and accessible only to study personnel. All CINRG

Coordinating center personnel who had access to the database performed the necessary Human Subject Protection training.

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Parent Project Muscular Dystrophy PPMD

Summary

This is a retrospective review of data collected prospectively from a longitudinal natural history study for boys with DMD ages 2-28 years in 28 centers throughout the world. The objective of the study was to better understand the disease progression for boys with DMD in the era of corticosteroid therapies. This analysis included 440 individuals who completed at least one year of data collection between the years of 2006-2009 and 2012-2016. Inclusion and exclusion criteria were set to ensure that all patients enrolled in the study had DMD and not the milder phenotype, BMD. The protocol was approved by each institutions' ethical review board with appropriate consents/assents prior to enrollment into the study.

Clinical outcome measures included multi-organ assessments, but this analysis focused on musculoskeletal related outcomes of ankle ROM, knee strength and timed function tests that are associated with loss of ambulation. Individuals were assessed at 3, 6, 9 and 12 months during the first year followed by 18 and 24 months and annually thereafter. Standardization of study procedures was ensured through rigorous hands on training, reliability testing and quality control review of data throughout the trial.

Data analysis in this study aims to better understand variables that contribute to loss of function in individuals with DMD who are still ambulatory. Correlation of strength, range of motion and function will provide better understanding of factors that may predict loss of ambulation. Anchor-based methods to provide MCID values were calculated to help better understand the clinical meaningfulness of these changes. Conclusions from this study will contribute to proactive care management and to improve design of clinical trials for a rare disease where limited recruitment numbers are a limiting factor to the success of trials, thus making the determination of the appropriate patient and endpoint selection crucial in the success or failure of a study.

CHAPTER 4: MANUSCRIPT 1: CHARACTERIZATION OF CONTRACTURE DEVELOPMENT AND EFFECTS ON FUNCTION IN DUCHENNE MUSCULAR DYSTROPHY: IN THE ERA OF GLUCOCORTICOIDS

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Abstract

Duchenne Muscular Dystrophy (DMD) is a neuromuscular disorder that presents in childhood characterized by slowly progressive proximal weakness and lower extremity contractures that limit ambulatory ability.[1,2] Contractures develop in the ankles, knees, and hips due to muscle imbalances, fibrotic changes, loss of strength, and static positioning.[2,5] Currently, standards of care (SOC) guidelines emphasize the importance of maintaining good musculoskeletal alignment through stretching, bracing, and glucocorticoid (GC) therapy to maintain strength and function.

A collection of timed function tests (TFT) supine to stand, 10 meter walk test (10MWT), and timed stair climbing, have been used to monitor disease progression and are predictive of loss of ambulation in these patients.[4] A better understanding of how other assessments used in clinic, such as strength and range of motion (ROM), predict loss of these functions is important in more individualized rehabilitation management.

The objectives of this analysis are to understand the progression of contractures for individuals with DMD and to investigate the relationship between progressive lower limb contractures, strength, and TFTs.

Keywords:
Duchenne muscular dystrophy, gait, contracture, strength

Introduction:

Duchenne muscular dystrophy (DMD) is a progressive genetic neuromuscular disease affecting 1 in every 7250 males from ages 5-24 years.[2, 3] This X -linked developmental disorder causes progressive muscle weakness, usually leading to death by early adulthood. Individuals with DMD have a mutation in the dystrophin gene which leads to musculoskeletal impairments including progressive muscle weakness, imbalances, and contracture development which eventually result in loss of ambulation.[4] Discovery of the dystrophin gene over 30 years ago has led to improved diagnostic and treatment capabilities in people with DMD. Improved physical therapy (PT) management strategies and therapeutic interventions result in a longer lifespan for individuals with DMD and contribute to the increased variability in rates of disease progression. Phenotypical progression varies in DMD but occurs in a predictable, sequential manner that includes the loss of functional abilities (e.g. ability to rise from the floor, climb stairs, walk, and self-feed) with eventual premature death due to cardiorespiratory insufficiency.[5, 6, 8, 13, 14, 21, 22, 40]

Physical therapy goals focus on addressing muscle imbalances around joints to maintain optimal biomechanics for movement and energy conservation. Proximal hip flexor, hip extensor, and knee extensor muscle weakness is seen initially. Contractures typically first develop distally in the ankles followed by the hip flexors, iliotibial bands, and forearm/finger musculature.[8] Significant contractures are rare in children younger than 9 and tend to develop more aggressively as they lose the ability to stand or walk.[8] This supports the theory that contracture development is multifactorial with physiological and environmental components. Due to the high rate of fibrosis in DMD, muscles with poor anti-gravity strength positioned in a shortened position will develop

contractures.[5, 8-11] Progressive muscle weakness and the development of contractures change musculoskeletal alignment and affect muscle force demands, which translates functionally in decreased gait speed and eventual loss of ambulation.[15-18]

Currently, standards of care guidelines for DMD emphasize an anticipatory care approach by maintaining appropriate musculoskeletal management through physical therapy and Glucocorticosteroids (GC) to maintain strength and function.[42, 43] GCs are frequently used for individuals with DMD and have beneficial effects on TFTs as reported by multiple studies.[6, 36, 40, 61, 151, 152, 252, 253] However, few studies have studied the possible effect of GCs on the onset and progression of ankle contractures nor their impact on TFTs in DMD.

There is a consensus on a collection of clinical assessments, including TFTs, that should be used both in DMD-focused multidisciplinary clinics and clinical trials due to their ability to track disease progression and prognostic capabilities.[254] Since gait speed has been shown to be a good predictor of loss of ambulation,[35] it is important to understand the implications of contractures on gait speed. Contracture management and GC use are the few published interventions that have changed the natural history of the disease;[40 3, 5, 6, 8, 13, 14, 32, 34-36, 60-62] therefore, understanding the effects of shortening in specific muscle groups can be clinically meaningful. Muscle imbalances and associated ankle contractures change the biomechanics of gait and may contribute to loss of ambulation.[12-14] For example, a minimum of neutral ankle range of motion (0 degrees) allows for better vertical alignment of the body's center of gravity in standing and assists in accomplishing foot clearance through the swing phase of ambulation.[16, 18]

The 10MWT has been typically used to assess gait speed in DMD and is an important predictor

in loss of ambulation.[35] Understanding the effects of impairment-based changes on the biomechanics of gait and the resultant loss of functional activities will influence proactive clinical decision-making. For clinicians, understanding how TFTs, strength assessments, and ankle range of motion are associated with the loss of ambulation will help make proactive care management decisions, including timing of equipment or orthotic recommendations. In clinical trials, this information is beneficial for trial design to determine the appropriate efficacy endpoint, study population, and timeline.

Objective

Currently, there is minimal evidence to help physical therapists provide quality anticipatory plan of care for contracture management. The objective of this study is 1. Understand contracture onset and progression in boys with DMD 2. Explore the impact of contributing factors such as ankle ROM, knee strength, age, and GC use on losses of functional abilities such as the ability to walk, transition off the floor, and climb stairs.

Method:

Data was collected through the CINRG Duchenne Natural history study, a consortium of 22 sites in over 9 countries. Design and methodology was based on previously published study design and methodology.[29, 255] All clinical sites had institutional or ethics review board approvals prior to data collection. Informed consent/assent was obtained for each participant and/or caregiver prior to study procedures. This analysis included 440 individuals who had at least one range of motion assessment during the study collected between 2006 and 2016. Assessments were

performed at baseline and months 3, 6, 9, 12, 18, 24 (ambulatory) or months 6, 12, 18, 24 (non-ambulatory) followed by annual visits after year 3. Historical and current GC use was obtained throughout the study.

Standardized methods and training were observed throughout the trial for all physical therapy assessments to ensure consistency of measurements. Data extracted for analysis included TFTs, specifically supine to stand, climb 4 stairs, and 10 Meter walk/run (10MWT); fixed quantitative strength measures (QMT) of knee extension/flexion; and passive range of motion (PROM) measurements which included knee extension and ankle dorsiflexion. TFT variables were assessed in seconds but expressed as speeds for analysis and reporting. PROM was measured in degrees, and QMT was measured in pounds (lb.) of force.

Equipment and training: Timed tests were measured using a standard stopwatch and Sammons Preston 4 stairs with bilateral handrails. Instructions included standard start and stop positions. All timed tests started with hands by side and feet within base of support. End position for supine to stand and stairs were the same as the start position. The 10MWT end position included when the patient's first leg passed the 10-meter line (cold start, flying finish). Quantitative muscle testing was assessed using the CINRG Quantitative Measurement System (CQMS) developed and validated as a reliable measure for strength in boys with DMD.[67, 241] Passive range of motion (ROM) measurements were assessed using a standard goniometer. Knee and ankle ROM were measured in supine (Figure 1). Ankle ROM was measured in the best obtainable subtalar neutral position. Passive measurement into dorsiflexion was measured with positive values indicating more dorsiflexion, while negative values indicated loss of dorsiflexion past neutral (0 degrees).

Methodology for all assessments followed a detailed manual and worksheets with standard test positions throughout the trial.

All physical therapists participating in this study followed CINRG standards for certification including central training and support provided by the Central Evaluations Manager (CEM). Certification encompassed initial hands-on training and reliability testing, annual webinar to review data quality and reduce drift in administration of items, and bi-annual face to face hands-on meetings.

Statistical analysis was performed using STATA V15 (College Station, TX). Clinical outcomes in this study included range of motion assessments, knee strength, 10MWT, supine to stand, and 4-stair climb measures which were continuous and quantitative variables. Descriptive analyses were grouped based on the distribution of data.

For continuous measures, histograms, box and whisker plots and Q-Q plots were used to assess normality and the need for transformation of data. Normality was assessed using a combination of the Shapiro-Wilk normality test and visual inspection of histograms. Measurements were summarized for each visit including the 12-month change from the baseline assessment. The operational definition for a 12-month change is considered 305-426 days. If the outcome's distribution was approximately normal, mean and standard deviation were reported. For non-normal distributions, median, minimum-maximum, and interquartile ranges were reported. For categorical data, frequencies and percentages were reported.

TFTs were collected using a standard stopwatch. Statistical analysis utilized the data expressed as speed: (1) 10MWT = m/s, (2) supine to stand = rise/second, and (3) 4 stair climb = 4 stairs/second. To get a true representation of the cohort, patients who lost the ability to perform the TFTs during the study were included in analyses by unit imputation of zero for the respective test. If the participant lost the ability to perform a task since the previous visit due to progression of disease, a value of 0 was entered for the first visit where they could not accomplish the task. The participant was then excluded for that test in subsequent visits. The three TFTs were treated independently.

Due to differences in definition for ambulation and steroid use, we operationally defined these descriptions in this study. Patients were considered non-ambulatory if they reported full-time wheelchair use at the time of the visit, or if they reported the age at which a wheelchair was used full-time. All other patients were categorized as ambulatory, encompassing both household and community ambulators. GC use was defined for each visit and based on patient report of steroid use at time of the visit. Descriptive analysis also summarized the total duration of GC use up to each visit.

Summary statistics of both absolute values of knee extensor and ankle dorsiflexion and 12-month changes in knee extensor and ankle dorsiflexion were calculated and reported with several different categorizations. Summaries were calculated for each of the above-named outcomes, stratified by 1-year age groups and either glucocorticoid use or ambulatory status. In these observational summaries, all available data points were used for each participant; therefore many participants contribute more than one observation per stratified group. The number of data points

and the number of unique participants is reported for each stratified group. For summaries of 12-month changes, the GC and ambulation status at the beginning of the 12-month period were used for purposes of stratification. For this analysis participants were divided into the 5 stages based on age. For example, if a participant was 4.5 years old at the start of the 12-month period, they were included in the 0 to 4.9 year age group, even though they were older than 4.9 years at the end of the interval.

To assess the change in knee and ankle ROM over time and the effect of GC use we performed mixed models with repeated measures. Each model included the ROM outcome as the dependent variable, age (representing time) as the independent variable, and a random coefficient for each participant. Models were run separately for those currently using GCs and those who were not. A third model assessed an interaction between age and GC use.

To assess the relationship between knee extensor strength, knee PROM, and ankle dorsiflexion PROM and functional ability, we again used mixed models with repeated measures. The goal was to assess how useful these strength and PROM assessments are at predicting functional abilities (i.e. the ability to walk, climb stairs, or stand from the floor). In each model, the functional ability was considered the dependent variable, and strength or PROM was the independent predictor. Age and GC use (yes/no dichotomous variable describing use at the time of the visit) were included as covariates. Each model included a random coefficient for each participant. Models included only data on participants while ambulatory. The models allowed us to assess the relationship between the predictor and the dependent variable while allowing for multiple related assessments per participant. Each model provided an estimate of the amount of

change in functional outcome that can be attributed to the predictor, while accounting for covariates and repeated measurements taken on the same participants. In addition, the model provided a significance test for each predictor. Several models were built. These included models where 1) walking speed, climbing speed or standing speed was defined as the dependent variable and the absolute value of knee extensor strength, knee PROM, or ankle PROM was the independent predictor and 2) the 12-month change in walking speed, climbing speed or standing speed was the dependent variable and the 12-month change in knee extensor strength, knee PROM, or ankle PROM was the independent predictor.

Lastly, to assess the combined effect of knee extension strength and ankle PROM on functional ability, three additional mixed effects linear regression models were performed. Variables used were based on known clinical observations and published research significant predictors of functional decline in DMD.[5, 29, 40] In these models, both knee extension strength and ankle PROM were included as independent predictors and walking speed, climbing speed or standing speed was the dependent variable. Models again included covariates for age and glucocorticoid use and a random coefficient for each participant.

Results:

A cohort of 440 participants and 1321 observations were used for analysis. Patient demographics are in table 1. GC use was stratified based on duration on drug with 18.7% with <6 months or naïve; 4.3% <1 year; 58.0% 1≤ 10 years; and 19.3% between 10-25 years of GC use.

GC's appear to have an effect on ankle ROM in boys beginning at age 9, those taking GC's have a greater observed ankle ROM than those not taking GC's.(Figure 2 and Table 2). Ankle and knee contractures were observed to increase after ambulation was lost based on boys typical age at loss of ambulation. Progression of contractures over a 12-month period for both knee and ankle PROM showed very minimal changes for either GC or naïve groups. On average, for knee PROM, there was a -1 degree change over a year, whereas ankle PROM had a mean change of 3.6 degrees over one year with the greatest observed change being -5 degrees (Figure 3). Ankle PROM are very similar in early ages however shows a distinct difference in rate of decline based on GC use. When estimating the change in ankle and knee ROM over time (table 4), those not on GCs exhibited a greater decline than those who were on GCs. Ankle ROM declined an average of -3.28 degrees per year in those not taking steroids, but only -1.86 degrees per year in those taking steroids. Figure 4 shows this sharper decline clearly.

Assessing longitudinal data over the life span indicated that QMT, PROM, age, and GC use are all key variables that are significantly related to a decrease in functional ability. Observation of ambulatory and non-ambulatory ankle PROM median values show ankle PROM is more negative in the non-ambulatory versus ambulatory participants at all ages. For those who are ambulatory, the median value for any participant who was still ambulatory was -10 degrees (Table 3). This study found that once accounting for age and GC status, TFT values were significantly predicted by knee strength and ankle PROM. The results of these mixed effect linear models for the 10MWT are shown in table 5. This demonstrates that an increase in knee strength or PROM predicted a statistically significant increase in walking speed. When evaluating the

combined predictive effect of knee extensor strength and knee PROM (Table 6), knee extensor strength predicted the greatest change in speed. A single pound increase in knee extensor strength predicted a 0.042 m/s increase in walking speed whereas a single degree of improvement in PROM predicted only a 0.009 m/s increase in walking speed. In all cases, speed significantly decreased with age and those who were not currently on GCs. We did assess changes in ROM over a 12-month period, the typical length of an interventional clinical trial, although due to small changes in PROM over a 12-month period of this analysis, there were no significant relationships with change in PROM with TFT performance nor was there a significant effect of GC use in this short time frame. However, 12-month changes in both knee extensor and flexor strength showed a significant relationship with declines in TFT performance (data not shown).

Discussion:

This study describes the onset and progression of contractures in the largest longitudinal natural history study in boys with DMD. With the alteration in the disease course from the standard use of GC for disease management and the recent developments in drug therapies, there has been great interest in understanding the downstream effects of contractures on strength and function across the spectrum of disease progression. Improvements in standards of care including management of primary and secondary effects of DMD with physical therapy, GC use, respiratory care, coronary care, and contracture management have led to longer ambulation status and lifespans.[53] Once an individual becomes non-ambulatory, there is increased economic burden on the family and increased risk for secondary issues such as the development of scoliosis,

osteoporosis, obesity, disuse atrophy, increased rate of contracture development, and psychosocial issues related to the loss of function.

The economic impact of longer survival is associated with much higher medical costs, especially in the later stages of the disease. Medical costs are nearly 6x higher for those who are non-ambulatory compared to those who are ambulatory, [54] thus making it critical to better understand the reasons that contribute to loss of ambulation. Understanding the variability in contractures and strength allow for more individualized anticipatory care and justification for rehabilitation-related interventions that have been associated with reducing the effects of contractures, such as night orthosis[256], serial casting[187, 198, 199], frequency of stretching[7, 190], and timing of ordering equipment.

Our study findings validated previous studies on the prevalence of ankle contractures in ambulatory DMD boys.[5, 257, 258] These study results are consistent with other published studies showing that ankle dorsiflexion contractures are associated with decreased function. Kiefer et al[258] found that increased ankle PROM contractures resulted in lower Northstar Ambulatory Assessment scores, a 17-item functional assessment of gross motor skills such as walking, standing and jumping validated in boys with DMD. Akkurt et al[259] assessed timed function tests including the 6MWT and found a moderate significant correlation of ankle PROM on TFTs. Mendell et al[32] initially found positive effects on strength and function and no changes on contractures; however based on our results, we believe 6 months may not be long enough to understand impacts on contracture. However, none of these studies evaluated progression of contractures and its possible impact on disease progression. Our study results did not show a significant relationship with a 12-

month change of PROM and TFT. This may be due to the very small ROM changes over a 12-month period and suggests that 12 months may be too short of a timeframe to see changes in contracture progression even though strength does show significant declines during this period. Our results showed, for the first time, possible impact of GC use on progression of ankle contractures. Ankle ROM was similar among boys in the early ages of the disease but those who were GC naïve appeared to have a faster rate of ankle contractures indicating that GC treatment may affect the rate of contracture development which may directly impact care management especially for those who are not using GC as part of their SOC.

Previously published studies have associated time required to complete the 3 timed tests (supine to stand, 4-stair climb, and 10MWT) with loss of function in DMD.[40] Gait speed is determined by the power generated within the gait cycle in both the stance and swing phases. This power is dependent on both pelvic girdle muscles and the gastroc-soleus complex affecting both phases of the gait cycle. There is significant decrease in power generation and absorption in both of these phases for individuals with DMD due to the early weakness of the hip flexors and extensor muscles and tightness of the plantarflexors. This does not allow for peak hip extensor force during terminal stance phase. There is also decreased hip flexor propulsion for pre-swing and reduced push off during terminal swing due to plantarflexion contractures.[18, 125] In the Cohen et al[260] study of 15 boys with DMD and 336 healthy controls found that boys with DMD had a reduction in their walking rate beginning at ages 4-6. In a study looking at EMG activity during gait, Ropars et al[120] found that rectus femoris and hamstrings were active throughout most of the gait cycle in boys with DMD, indicating the need for stability with muscle weakness.

Gait studies have shown that ankle contractures contribute to decreased gait speed at the initial stance phase.[128] In boys with DMD, it serves a different purpose because it acts as a biomechanical advantage by providing an extension moment during the stance phase of gait to oppose knee flexion moments, allowing the center of gravity line to fall in front of the knee.[123, 128] However, with disease progression, the excessive ankle plantarflexion contractures and increased hip and knee weakness lead to added instability during stance[17, 18] and result in a tipping point where the biomechanical advantage does not override the muscle weakness. Houx et al[261] found that -10 degrees of dorsiflexion was the threshold at which gait kinematics changed for typically developing children. For children with muscle weakness, these gait changes could be detrimental in maintaining ambulation. The results of our study validate the kinematic findings from Houx et al[261] indicating that that -10 degrees appears to be a critical value of dorsiflexion loss that puts boys at greater risk of losing ambulation.

One of the primary limitations in this study is that it is a natural history study. Therefore, factors such as GC and contracture management were not controlled. Interventions that may impact contractures such as stretching and orthotics were not assessed and therefore not accounted for in the analysis, which may have an impact on progression. Additionally, based on Heberer's[125] study showing decreases in peak hip extensor moments in boys with DMD, it would have been informative to understand the contribution of hip extensors in gait speed. However, this study did not measure pelvic girdle muscles which may be a significant factor in gait speed, as knee flexion/extension may act more as a method of stability rather than power generation. Future

studies should investigate the contribution of typically weakened muscle groups, such as hip and pelvic musculature, on gait in individuals with DMD.

Conclusion

In conclusion, there is a great degree of variability in contracture development in boys with DMD, especially in the GC era. Our study found that ankle and knee PROM was better for those on GC and effected long-term progression rates. However, the minimal progression of contractures in our study when limited to 12-month may be indicative that the 1-year period may be too short to assess changes in contractures.

Significant factors affecting functional speed include knee extension, PROM, GC use, and age, with knee extensor strength having more of an impact than ankle ROM. Loss of ankle dorsiflexion of >10 degrees affects the kinematics of gait[261] and is detrimental. This combined with muscle weakness may put boys with DMD at risk for losing ambulation. Results from this long-term follow up of DMD boys show that knee strength along with management of contractures are important to maintain the ability to walk, climb stairs, and stand. These results contribute to improved understanding of the progression of strength and ROM on the DMD disease trajectory thus an important piece of the puzzle that is needed to understand impacts of SOC for clinical management and how it may be incorporated in clinical trials design.

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Abbreviations:

10MWT: 10 Meter Walk/run Test

CINRG: Cooperative International Neuromuscular Research Group

DMD: Duchenne muscular dystrophy

DMD-NHS: CINRG Duchenne Natural History Study

PROM: Passive Range of Motion

QMT: Quantitative Isometric Muscle Strength Testing

ROM: Range of Motion

TFT: Timed Function Tests

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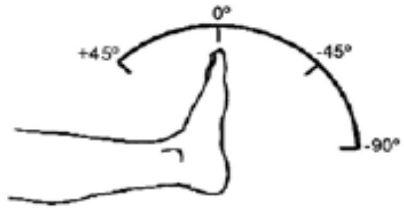
Figures and Tables

MANUSCRIPT 1: FIGURE 1: PROM PROCEDURES

1.) **Ankle Dorsiflexion**

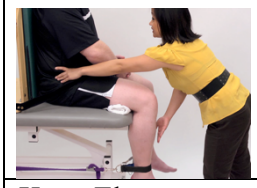
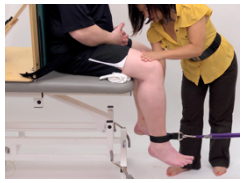


- Measured in supine with the knee extended as much as possible.
- The goniometer center is placed over the lateral malleolus, arms are aligned with lateral midline of the fibula using head of fibula for reference, and parallel to the lateral aspect of the fifth metatarsal.
- Full Normal Range= 20 degrees

2.) **Knee Extension**

- Measured in supine with hip in **neutral,** ankle relaxed.
- Goniometer center is placed lateral to knee joint (lateral epicondyle of femur), arms are aligned with midline of the femur using greater trochanter for reference, and lateral midline of the fibula using lateral malleolus for reference.
- Full Normal Range= 0 degrees

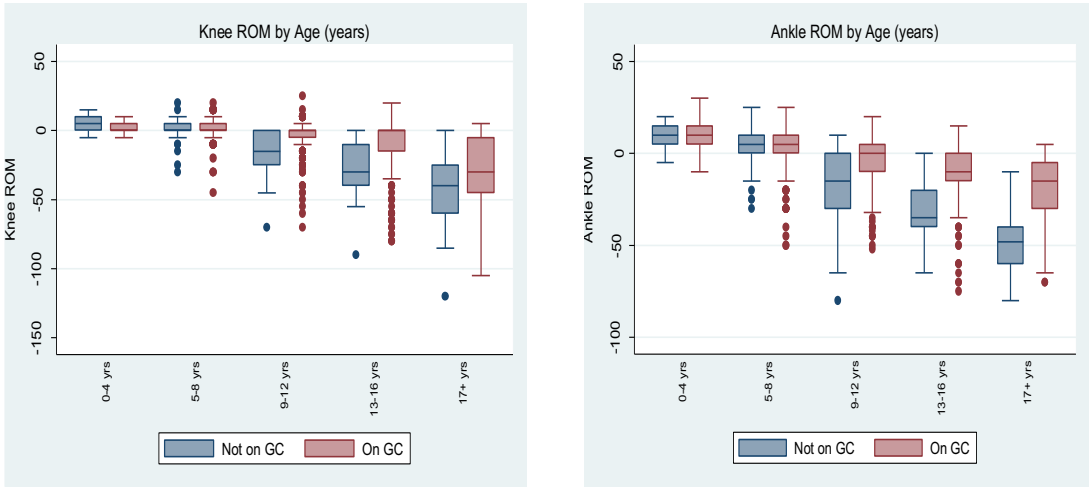
MANUSCRIPT 1 TABLE 1: QMT PROCEDURES

Muscle Group	**Patient Position**	**Limb Position**	**Stabilization**	**Limb Strap Position**
Knee Extensors 	Sitting with back support, facing away from the wall	• Hip and knee flexed at 90 degrees • Towel roll under tested knee	• Seat belt, • chest strap if needed • Examiner stabilizing proximal thigh	Proximal to malleoli
Knee Flexors 	Sitting with back support, facing toward the wall	• Hip and knee flexed at 90 degrees • Towel roll under tested knee	• Seat belt, • Chest strap if needed • Examiner stabilizing distal thigh	Proximal to malleoli

MANUSCRIPT 1 TABLE 2: STUDY DEMOGRAPHICS

Characteristic	N (%)	Median (min, max)
Age (years) – At baseline visit	440	8.9 (2.1, 28.0)
Age (years) – At last study visit	440	14.6 (4.5, 33.9)
Ambulatory status – At last baseline		
Ambulatory	292 (66.4%)	
Non-ambulatory	148 (33.6%)	
Ambulatory status – At last study visit		
Ambulatory	192 (43.6%)	
Non-ambulatory	248 (56.4%)	

MANUSCRIPT 1: FIGURE 2 ROM STRATIFID BY GC USE

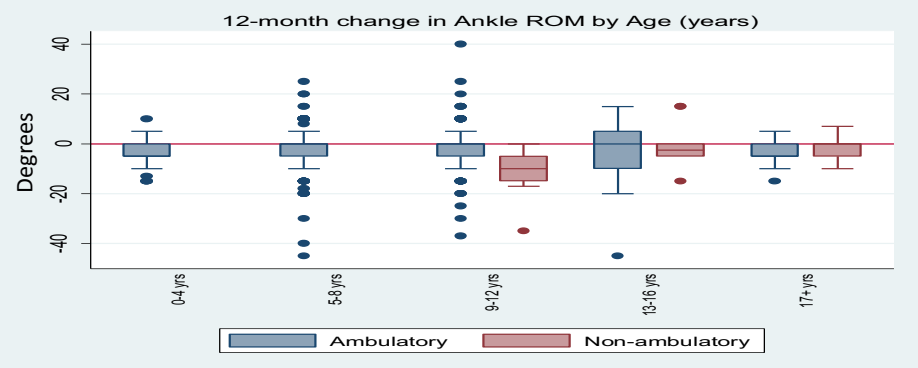


Abbreviations: GC=Glucocorticoid, ROM=Range of Motion

MANUSCRIPT 1 TABLE 3: ANKLE DORSIFLEXION MEASUREMENTS

	Degrees of Ankle Dorsiflexion (Ambulatory)					Degrees of Ankle Dorsiflexion (Non-ambulatory)				
Age interval (years)	**N (Obs)**	**N (Ind.)**	**Median** (Degrees)	**Min**	**Max**	**N (Obs)**	**N (Ind.)**	**Median** (Degrees)	**Min**	**Max**
<4.9	100	51	10	-10	30	0				
5.0 to 8.9	685	216	5	-50	25	3	2	-7	-30	-3
9.0 to 12.9	365	155	0	-52	20	42	35	-20	-80	10
13.0 to 16.9	137	67	0	-70	15	61	39	-25	-75	10
17+	29	15	-10	-35	5	87	46	-30	-80	0

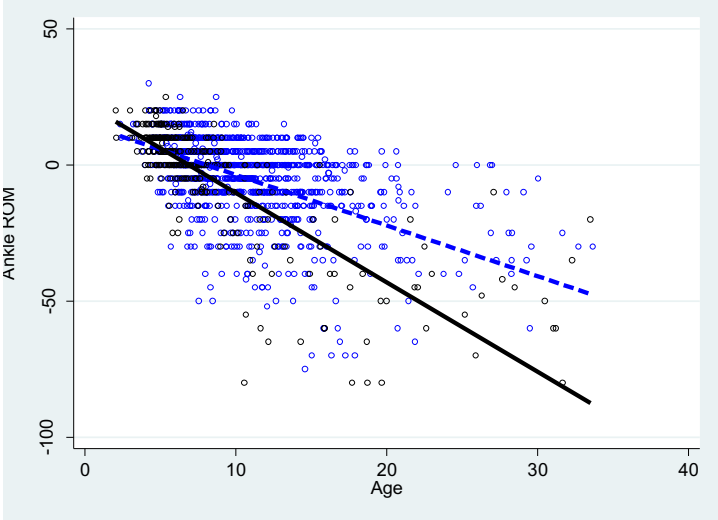
MANUSCRIPT 1: FIGURE 3 12-MONTH CHANGES OF ANKLE ROM STRATIFIED BY AMBULATORY STATUS



MANUSCRIPT 1 TABLE 4: CHANGE IN ROM OVER TIME

ROM assessment	GC use status	N (obs) N (indiv.)	Coefficient (p-value)	Presence of significant interaction
Knee ROM	Not on GC	321 145	-2.78 (<0.001)	Yes (p<0.001)
	On GC	1270 285	-2.05 (<0.001)	
Ankle ROM	Not on GC	281 126	-3.28 (<0.001)	Yes (p<0.001)
	On GC	1228 277	-1.86 (<0.001)	

MANUSCRIPT 1: FIGURE 4: CHANGE IN ANKLE ROM OVER THE STUDY STRATIFIED BY GC USE



Blue: participants taking GCs at the time of the study visit

Black: participants not taking GCs at the time of the study visit

MANUSCRIPT 1 TABLE 5: STRENGTH AND ROM AS INDEPENDENT PREDICTORS OF 10M RUN/WALK

Predictor of 10MWT	N (obs) N (indiv.)	Predictor	Age	Steroid use
		Coefficient (p-value)	Coefficient (p-value)	Coefficient (p-value)
Knee flexor QMT	1555 263	0.031 (p<0.001)	-0.131 (p<0.001)	0.222 (p<0.001)
Knee extensor QMT	1570 265	0.039 (p<0.001)	-0.104 (p<0.001)	0.195 (p<0.001)
Knee ROM	1305 283	0.014 (p<0.001)	-0.108 (p<0.001)	0.340 (p<0.001)
Ankle ROM	1300 284	0.009 (p<0.001)	-0.101 (p<0.001)	0.348 (p<0.001)

MANUSCRIPT 1 TABLE 6: THE COMBINED EFFECT OF KNEE STRENGTH AND ROM ON TFT

Outcome	N (obs) N (indiv.)	Knee extensor (lb)	Ankle ROM (degrees)	Age (years)	Steroid use
		Coefficient (p-value)	Coefficient (p-value)	Coefficient (p-value)	Coefficient (p-value)
Run/walk velocity	1073 265	0.042 (p<0.001)	0.009 (p<0.001)	-0.086 (p<0.001)	0.193 (p<0.001)
Climb velocity	1033 265	0.012 (p<0.001)	0.002 (p<0.001)	-0.017 (p<0.001)	0.039 (p<0.001)
Stand velocity	954 264	0.007 (p<0.001)	0.002 (p<0.001)	-0.020 (p<0.001)	0.036 (p<0.001)

CHAPTER 5: MANUSCRIPT 2: THE EFFECT OF CHANGE IN TIMED FUNCTION TESTS AND ASSOCIATED MINIMAL CLINICAL IMPORTANT DIFFERENCE IN BOYS WITH DMD

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Contributions: Conceived and implemented study design; Analyzed data. Wrote first draft of manuscript

Co-author: Jennifer Canbek[3]
Contributions: Provided feedback on statistical analysis. Provided edits on early manuscript drafts

Coauthor: Alicia Fernandez-Fernandez[3]
Contributions: Provided feedback on statistical analysis. Provided edits on early manuscript drafts

Coauthor: Catherine Siener[4]
Contributions: Implemented study design. Provided early manuscript draft edits. Provided field expertise

Coauthor: Craig McDonald[5]
Coauthor: Conceived study design. Provided statistical input. Provided feedback on statistical analysis.

Coauthor: Heather Gordish-Dressman[6]
Contributions: Conceived study design. Provided field expertise and performed statistical analysis. Provided manuscript draft edits.

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Abstract:

Duchenne muscular Dystrophy (DMD) is a rare x-linked recessive genetic disorder affecting 1 in every 5000-10000.[53, 262] This disease leads to a variable but progressive sequential pattern of muscle weakness that eventual leads to loss of important functional milestones such as the ability to walk. With promising drugs in development to ameliorate the effects of muscle weakness, these treatments must be associated clinically meaningful functional change. The objective of this analysis is to determine both distribution (minimal detectable change (MDC)) and anchor-based (minimal important clinical difference (MCID)) of 12-month change values in standardized time function tests (TFT) used to monitor disease progression in DMD.

Method: This is a multi-center prospective natural history study with the Cooperative International Neuromuscular Research Group (CINRG). This study calculated MID and MCID values for 3 commonly used timed function tests typically used to monitor disease progression; supine to stand (STS), 10-meter walk(10MWT), 4 stair climb(4SC). MID used standard error of measurement (SEM) while MCID measurements used the Vignos scale as an anchor to determine clinical change in functional status.

Results: All 3 TFT were significantly important clinical endpoints to detect MDC and MCID changes. MDC and MCID 12 month changes were significant in 10MWT (-0.138, .-0.212), Supine to Stand -0.026, -0.023) and 4 stair climb(-0.034, -.035) with an effect size greater or close to 0.2.

Conclusion: The 3 TFTs are clinically meaningful endpoints used to establish change in DMD. MDC values were higher than MCID values indicating that an anchor-based approach using Vignos as an anchor for functional loss of lower extremity abilities is appropriate to assess change in boys with DMD.

Keywords:
Duchenne muscular dystrophy, MCID, time function tests

Introduction:

The most common form of pediatric onset muscular dystrophy is Duchenne muscular dystrophy (DMD), caused by a genetic mutation in the dystrophin gene that leads to progressive muscle weakness and loss of ambulation. DMD affects 1 in every 5000-10000.[2, 53, 262] The discovery of this gene over 30 years ago led to improved diagnostic capabilities for DMD.[4] Standards of care guidelines for DMD emphasize appropriate musculoskeletal management through physical therapy and corticosteroid therapy to maintain strength and function. These interventions have resulted in a longer lifespan yet increased variability in disease progression. DMD progression occurs in a predictable sequential manner with loss of the ability to get off the floor, climb stairs, bring hands to mouth, and early morbidity due to cardiorespiratory insufficiency.[5, 6, 8, 13, 14, 21, 22]

Predictive factors of disease progression are associated with the loss of major functional milestones such as walking ability. With corticosteroids, the timeline when young boys lose ambulation has shifted by 1-2 years however is still inevitable between the ages 9-15.[37] Walking speed in boys with DMD is directly correlated with prolongation of ambulation, indicating that individuals who walk faster maintain ambulation longer. [8] There have been numerous studies linking ambulation to improved quality of life and prevention of the development of scoliosis, and joint contractures/deformities.[7, 8, 13] Once an individual becomes non-ambulatory, secondary issues such as development of scoliosis, osteoporosis, obesity, disuse atrophy, increased rate of contracture development and psychosocial issues impact the perception of disease progression. The psychological effects of loss of a function are similar to the fears and anxiety that is associated with loss felt at the time of diagnosis for boys and families with DMD.[38]

In 2006, at a National Institute on Disability and Rehabilitation Research (NIDRR) meeting to discuss challenges in muscle disease, the FDA specified that clinical trials conducted in support of new drug approvals or revised indications for already approved drugs must incorporate a primary endpoint that objectively measures a clinically meaningful "life-changing" milestone that has a significant impact on a patient's perceived overall health and well-being.[31] Historically, the Vignos scale, an 8 point ordinal scale, has been used to describe lower extremity function in boys with DMD with each level indicating a change in function including significant milestones such as loss of ability to rise from the floor and ambulation.[1, 40] In a study by McDonald et al[39], the authors noted high correlation with the PODCI transfers/basic mobility score, a patient reported outcome

measure, with the Vignos scale and 3 timed function tests including supine to stand, 10 meter run/walk and 4 stair climb.

To determine clinically meaningful difference in clinical assessment measures, there are anchor-based (MCID) and distribution-based (MDC) methods which are distinctly different approaches to provide meaningful estimates of change for outcome measures. They are increasingly employed in disease-based treatment development research.[229-232] In the anchor-based approach, the meaningfulness of the change in a study measurement is determined based on the ability of a change score to predict the occurrence of a clinically meaningful milestone in the natural history of the disease.[232, 233] MCID is the minimal amount of change to a clinical outcome that may make a difference in clinical care or quality of life to a patient.[63, 234]

MDC has been described as true change that is not attributed to error or variability. Distribution-based methods depend on statistical approaches founded on psychometric soundness of a clinical outcome, without reference to a clinical or patient perspective of change. The timed function tests analyzed in this study have been reported to have sound psychometric properties of reliability and validity, as well as good predictive qualities.[40, 67, 241] These properties give assurance that changes in these clinical endpoints are less likely to be due to measurement error. MDC is a statistical approach not anchored to clinical significance.[63] Statistical change does not necessarily translate to clinical benefits. MDC values use direct approaches that compare different groups, whereas MCID methods, use an anchor-based approach to establish a meaningful change over time.[71]

Anchor-based approaches use clinical or patient interpretation to understand the magnitude of change and is typically compared with other clinical measures.[71] On a guidance document about patient-reported outcomes, the FDA attempted to provide guidance on interpretation and implications of MCID measures.[235] However, due to lack of consensus, MCID was defined as the smallest difference in outcomes that may be proxies of perceived importance to patients.[235] With little guidance, clinical trials continue to report MDC measures of change, which may under or overestimate the clinically important differences or significance of results. This becomes a problem as payers tend to require clinical outcomes used in trials as a minimal criterion for payment.

Many studies report velocity measures as predictors of loss of function in DMD.[5, 29, 30, 35, 40, 58, 70, 228, 263, 264] However, there needs to be better understanding of the clinical impact of different change trajectories of timed function tests (TFTs) and its impact on loss of functional milestones. MCID estimates that are based on rate of change can provide a threshold for meaningful change that is important to the patient. MCID measures are not only important to patients[46], but they can provide clinicians with a critical value that corresponds to a measurable change helping determine clinical goals and disease monitoring to improve anticipatory care management. It is also increasingly important to payers, since they often define approval or payment for therapies based on published MCID values. It is essential that researchers accurately report MCID estimates which are specific to a particular patient population to improve the access to care in specific populations. Additionally, MCID values can help determine appropriate sample sizes when designing clinical trials for better interpretation of clinical outcomes and relevance to disease progression.[48] The

objective of this paper is to determine and compare MDC and MCID values of 3 validated and commonly used TFTs (10MWT, STS, 4SC) on disease progression and its impact on function for individuals with DMD.

Methods:

Data was collected as part of the CINRG Duchenne Natural history study, a consortium of 22 sites in over 9 countries. Prior to data collection, all sites had had institutional or ethics review board approvals and informed consent/assent was obtained prior to study procedures. The study population included 391 individuals with DMD with at least 7 years of follow up data. Data was collected between 2006-2016. Assessments were performed at baseline and months 3, 6, 9, 12, 18, 24 (ambulatory) or months 6, 12, 18, 24 (non-ambulatory) followed by annual visits after year 3. Corticosteroid use was also captured at each visit.

Standardized methods and training were observed throughout the trial for all physical therapy assessments to ensure consistency of measurements based on previously published design and methodology.[29, 30, 241, 255]. Data extracted for analysis included TFTs, specifically 10MWT, STS, 4STS and Vignos scale scores (Fig1). The Vignos Lower extremity scale is an ordinal scale used to describe lower extremity functional ability in boys with DMD with higher values representing lower function. Any changes in the Vignos scale would reflect functional loss. TFT variables were assessed in seconds and reported as speed. Example of conversions are below

10MWT speed = 10/seconds to complete the test, resulting in an assessment value in m/s

- 10MWT test=5 seconds

- Velocity=5sec=10/sec=2m/s

4STS speed= 4/seconds to complete the test, resulting in a speed assessment per step

- 4STS= 2 seconds
- Velocity= ½=0.5 of the task per second

STS speed= 1/seconds to complete the test resulting in an assessment in rises/s.

- STS=2 seconds
- Velocity= ½=0.5 of the task per rise

For 12-month changes, we determined the number of participants with at least one 12-month interval of assessments where 12 months was defined as two visits >=304.2 days (47.3 weeks) and <=425.8 days (64.7 weeks) apart. We excluded intervals that overlapped by more than 91.3 days (13 weeks). The final data set for statistical analysis contained 1518 records from 391 individuals. The Vignos scale is organized with higher scores indicating less function while lower scores indicate higher function, thus a decline in score indicates an improvement in function. Vignos was used as a clinical anchor defined as a binary variable: 0=no decline (score remained the same or decreased by 1 or more points over the 12-month interval) or 1=decline (score increased by 1 or more points over the 12-month interval). Independent variables included the 3 TFTs with covariates of age and corticosteroid status (user/non-user) at the start of each 12-month interval.

All statistical tests were performed using STATA V14 (College Station, TX). Statistical tests were conducted using a two-tailed test at an alpha level at 0.05. Descriptive statistics were used to summarize participants' age, current usage of steroids and 12-month change of clinical

endpoints grouped by Vignos decline status. T tests or chi square tests were used to test for significant differences in these variables between Vignos decline status. We also used the sensitivity- and specificity- based approach to compare change of outcome scores from TFTs to the decline status of 12-month changes in the Vignos scale.

Logistic regression models, adjusted for age, were used to assess the 12-month changes in timed function tests associated with a 12 month decline in the Vignos score. Participant IDs were clustered to account for multiple occurrences of more than one 12-month assessment interval. The model specified Vignos decline status as the dependent variable, 12-month change in each time function outcome as the dependent predictor, age and steroid status at baseline as covariates, and was clustered on individual participants. Models were stratified by ambulation status.

In order to define the anchor based MCID as a candidate, the timed function tests must have been associated with a Vignos decline at a p value less than 0.05. Candidate MCID values were then developed with use of receiver operating characteristic curve (ROC) analysis following the ROC sensitivity to change method by Stratford et al.[239] To obtain anchor based MCIDs, a new binary variable for each of the 3 timed function tests were developed, namely, the new binary variable of 1 or 0 based on whether the 12 month change of time function tests were $\leq$ the cutoff score. The optimal cutoff point was estimated by the maximum point reached from the ROC analysis; the largest value defined by dividing sensitivity (true positive rate) by 1 minus specificity (false positive rate).[239] A logistic regression model was then fit to the new binary variable.

In order to calculate the observed distribution-based MDC values for our cohort, we used the effect size approach and observed baseline and 12-month change values. The MDC was estimated as the 12-month change in time function score divided by the standard deviation of each time function outcome at baseline.

Results:

The dataset consisted of 391 participants with at least one 12-month interval for a total of 1518 observations. Of those, 826 observations were used for this analysis based on ambulatory status. Patients were on steroids during 70.8% of their 12-month intervals (82.7% for baseline ambulatory patients). Consistent with published clinical data on loss of ambulation, the average age for ambulatory individuals was 9.1 years (Table 1). Assessment of age and corticosteroid use as potential covariates showed only age to be significantly associated with Vignos scores; therefore, only age at baseline was used as a covariate unignorably in the anchor-based model fitting.

For Vignos model fitting, all three TFTs were associated with Vignos status with an adjusted p value < 0.05 and had acceptable AUC values of ≥ 0.7 within the ROC analysis. AUC is often used as a statistical model fitting indicator where values of 0.7 ~ 0.8 are considered acceptable, that of 0.8 ~ 0.9 to be fairly good, and that of 0.9 ~ 1.0 to be excellent.[265] Table 3 shows the distribution based and anchor based MDC and MCID values. MDC estimates were calculated from the stand deviation of baseline endpoints. MCID measures show significant p-values of <0.05 in the prediction model fitting for all 3 TFTs.

Effect size is a standardized measure of change from baseline over a certain time interval divided by the standard deviation (SD) of the baseline score. The effect size value represents the number of SD by which the scores have changed from baseline. By convention, an effect size of 0.2 is considered small, 0.5 moderate and 0.8 large.[266] The MCIDs anchored to Vignos scores for 2 of the 3 timed tests were slightly larger or near equitable indicating that MDC is not as meaningful a change and there is a need for an anchor based approach for clinical meaningfulness (Table 2); this included 10MWT (MCID=-0.212m/s, MDC=-0.138m/s) and STS (MCID =-0.023 task/sec MDC=-0.026). MCID values show that a small change in velocity measures resulted in a change in Vignos status by at least one level. As DMD is a progressive disease, all individuals showed a decline. For example, in the 10MWT, a -0.212m/s decline in gait speed resulted in a clinically meaningful unit change on 1 in the Vignos scale.

Discussion:

These TFTs are well established and frequently used in trials and the clinic because of their ease of administration.[254] Having a measure that is easy to administer and reliable is important to be used as efficacy endpoints in clinical trials. As noted in more recent approved trials in Spinal Muscular Atrophy, clinical endpoints used in trials are becoming insurance requirements for authorization for access to the treatment.[267, 268]

Most published studies in DMD which have determined clinically meaningful differences have used both MCID and MDC values. MDC measures are a good estimate of the psychometric properties of an instrument but MCID values must be used to link the change to a clinically meaningful functional change in disease milestones. Estimates of MCID of functional speed

measures anchored to a validated scale like the Vignos 8-point ordinal scale in DMD categorizing lower extremity function[44] aligns with the National Heart Blood Lung Institute's (NHBLI) recommendations on estimating MCID values for diseases with a wide heterogeneity and also shown to be better applicability in clinical interpretation and efficacy trials.[45-47] Additionally, the importance of being able to anchor the rate of change in TFTs to the Vignos scale is clinically important as a change in level tends to trigger a change in care needs. For example, the inability to get off of the floor or ambulate may require need for equipment, changes in school individual education plans or home modifications which all require at least 6-9 months to make these modifications. Therefore, having an MCID score that may be used to better predict loss of functional level in the Vignos could be very useful in an anticipatory care approach. Early planning would help clinicians ease the pain of such losses in function for families by providing proper planning and appropriate supports in place with better anticipation of these losses.

Despite the paucity of little research[45, 48, 49, 57-59] using natural history studies to determine MCID scores, this study looked at DMD natural history to determine MCID scores associated with change scores over 12 months that is associated with loss of function, instead of treatment effectiveness. Determining MCID values in the context of disease deterioration is important to understand the true impact of future therapeutic interventions and management compared to basing it on a single time point. SOC has changed the health outcome for boys with DMD despite lack of approved treatments. In this natural history study, calculating MCID based on rate of annual progression takes into account more than one timepoint that may impact prediction of

functional loss thus providing a better understanding of the timeframe and impacts of SOC. In a study of Chronic Obstructive Pulmonary Disease, a retrospective study assessed MCID values to determine 6MWT MCID values that may be good predictors of mortality or hospitalization, and found that observational studies were more robust to answer these questions because of the lack of confounding treatment interventions.[45]

Understanding the etiology, symptomology, and natural history of degenerative diseases is essential in determining MCID values that are relevant to the prediction of loss of functional milestones and its impact on the health-related quality of life measures.[45]. There is also a need to understand the MCID changes for clinical outcomes used throughout the entire spectrum of DMD's disease progression, future analysis will look at strength and pulmonary function measurers that also encompass individuals who are non-ambulatory.

Conclusion

Applying MCID values to commonly used clinical assessments helps appropriately power clinical trials as well as contribute to clinical decision making. MCID values in this study is based on rate of disease progression on an untreated population. Therefore, in a clinical trial, if a treatment improves the rate of change for these TFTs then there may be a clinically meaningful change that is linked to a significant functional milestone loss. MCID values may be instrumental in demonstrating a meaningful objective change that affect clinical management and determination of treatment efficacy. This may be used as a guide for clinicians to adjust treatment plans, anticipate and justify equipment needs.

Results from this study is the first to report minimally important changes in timed function tests that are anchored to crucial loss of functional milestones on the Vignos scale. These tests are frequently used in the clinic and clinical trials and are well validated to be sensitive to disease progression. With variations in disease progression, it is important to understand the impact of the rate of change in these TFT measures for proper anticipatory care planning and clinical trials design.

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Abbreviations:

4 Stair Climb: 4SC

10MWT: 10 Meter Walk/run Test

AUC: Area Under Curve

CINRG: Cooperative International Neuromuscular Research Group

DMD: Duchenne muscular dystrophy

DMD-NHS: CINRG Duchenne Natural History Study

Lower Extremity: LE

Minimal detectable change: MDC

Minimal Clinical Important Difference: MCID

Supine to Stand: STS

TFT: Timed Function Tests

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MANUSCRIPT 2: FIG 1: VIGNOS LOWER EXTREMITY SCALE

1. Walks and climbs stairs without assistance
2. Walks and climbs stairs with aid of railing
3. Walks and climbs stairs slowly with aid of railing (over 12 seconds for 4 standard stairs)
4. Walks unassisted and rises from chair but cannot climb stairs.
5. Walks unassisted but cannot rise from chair or climb stairs.
6. Walks only with assistance.
7. Participant is dependent on wheelchair for all mobility.
8. Participant is in bed at all times.

MANUSCRIPT 2 TABLE 1: STUDY DEMOGRAPHICS

				Vignos		
Variable	Level	N = 1518	N = 826	No Decline N=520	Decline N=256	Adjusted P value
Steroid Status	Non- Steroid User	444 (29.2)	143 (17.3)	103 (81.1)	24 (18.9)	0.307
	Steroid User N(%)	1074 (70.8)	683 (82.7)	417 (64.25)	232 (35.75)	
Age	N	1416	776	520	256	**<.001**
	Mean	13.07	9.117	8.502	10.598	
	Std Err	0.158	0.117	0.14	0.19	

MANUSCRIPT 2 TABLE 2 DESCRIPTIVE STATISTICS OF 12 MONTH CHANGES OF CLINICAL ENDPOINTS BY VIGNOS STATUS

12 month rate of change (m/sec) 10 meters	N	1394	754	508	246	**<.001**
	Mean	-0.085	-0.159	-0.05	-0.398	
	Std Err	0.008	0.015	0.016	0.023	
12 rate of change (rise/sec) of standing from supine	N	1337	697	479	218	**<.001**
	Mean	-0.010	-0.020	-0.012	-0.039	
	Std Err	0.001	0.003	0.003	0.004	
12 month rate of change (step/sec) of 4 steps	N	1360	720	490	230	**<.001**
	Mean	-0.011	-0.022	-0.005	-0.06	
	Std Err	0.0018	0.004	0.005	0.004	

MANUSCRIPT 2 TABLE 3: MDC AND MCID VALUES FOR TFTS ANCHORED WITH VIGNOS

Timed Function Test 12 month speed Change	MDC	MCID
10 MWT	-0.138 m/sec	-0.212*m/sec *#
Supine to Stand	-0.026 rise/sec	-0.023rise/sec *#
4 Stair Climb	-.0.034 steps/sec	-0.035steps/sec*#

Note: * indicates p value <0.05; # indicates AUC >=0.7

CHAPTER 6: SUMMARY

DMD is an x-linked genetic neuromuscular disorders that impair the production of dystrophin resulting in a fragile muscle membrane triggering a cascade of downstream problems with inflammation, necrosis, abnormal regeneration/degeneration of normal muscle function.[2, 53, 262] It is a lethal progressive disease primarily affecting boys showing early loss of motor function and premature mortality from cardiopulmonary complications. Since the discovery of this gene over 30 years ago[4], there has enriched understanding of the pathophysiology and phenotypical manifestations of DMD and its multi-organ impacts for boys affected by this disease. Increasing scientific understanding of DMD has led to an explosion of drug developments in the last decade that addresses the many causes and consequences from the lack of dystrophin.

As a physical therapist working in a multi-disciplinary clinic and research team, it is clear that this disease severely impacts quality of life and function for boys with DMD with significant impact on the entire family unit. Families spend a disproportionate amount of time in the medical arena while juggling to keep a balanced "normal" household. With contracture management being one of the few treatments recommended for musculoskeletal health in DMD, families have struggled with time to perform the daily stretches and guilt associated with the ineveitable development of contractures in their boys. This project was born out of desire to relieve the DMD community and families of the blame associated with contracture development by better understanding how contractures may develop and impact disease progression. To start this journey, I investigated the relationship of strength on function and the use of GC on the most prevalent contractures in boys with DMD, ankle plantarflexors. To understand how this may

impact changes that result in important modifications to clinical care and decision making, I determined MCID values for commonly used timed function tests that have been associated with loss of meaningful functional milestones such as walking. Changes in function for boys with DMD require an anticipatory care approach. Being able to predict care needs is important in adjusting stretching or bracing management for contractures, make recommendations for equipment, home/school modifications, changes to school Individual Education Plans and psychosocially prepare families for a loss of a functional milestone.

In this retrospective analysis of the largest international natural history study in DMD of 440 boys, I first wanted to understand if there were differences in the development and progression of ankle ROM in GC naïve and those on GC by calculating change of ROM over a 12 month period using mixed models with repeated measures approach. The results indicated that those on GC had slower progression of ankle contractures by -3.28 degrees per year compared to GC naïve (-1.86). To better understand the contributions of strength, ankle ROM and GC use on these TFTs, mixed effects linear regression model models were used to better understand the contributions of each of these variables to the speed at which patients with DMD can complete each of the 3 TFTs. We found that all 3 of these variables statistically significantly contributed to TFTs but knee extensor strength and GC were greater contributors and the combination of all 3 of these variables may be better predictors of loss of ambulation than strength or GC alone. In order to understand the clinical implications of TFT as predictors of disease progression, we calculated MCID and MDC scores based on an annual rate of change of the TFT anchored to the Vignos LE scale used to describe changes in lower limb function in

boys with DMD. MCID values showed that a small change in how quickly boys were able to perform the TFTs resulted in a change in Vignos status by at least one level. As a clinician, a decline in one level of the Vignos will trigger changes in my plan of care. In the 10MWT, a -0.212m/s decline in gait speed over one year period resulted in a clinically meaningful change by 1 level on the Vignos scale.

Most published studies have established that TFT are good indicators of disease status and used as predictors for loss of ambulation.[5, 29, 30, 35, 40, 58, 70, 228, 263, 264,37] However, most of these values are based on a single timepoint and does not take into consideration different rates of progression. Boys with DMD have a sequential progression of motor weakness but highly variable in disease progression especially in the GC era. Therefore, having MCID values established on an annual rate of decline provides more accurate prediction of loss in function.

In this past year, over 30 drugs are in the DMD pipeline ranging from restoration of dystrophin, reducing inflammation to providing stability to muscle fibers. Most of the primary or secondary clinical endpoints used in these studies are TFTs. As indicated by the FDA in a recent meeting on DMD platform trials designs, Drs Janet Woodcock and Billy Dunn emphasized the need to better understand clinical outcomes in the context of disease progression and an evolving standard of care to improve disease outcomes. Clinical endpoints must be standardized, easy to administer, have good inter and intra rater reliability, show convergent and construct validity as well as good clinically meaningfulness. In the pre-GC era, TFTs have been used as an efficacy measure in the early studies and its standardization has been consistently administered in neuromuscular clinics, natural history and industry sponsored studies.[254] Having a wealth of data

from TFTs allows for the DMD community to aggregate this data for better understanding of the DMD disease profile. We know that TFTs are good predictors of function but there was still a gap in linking it to something meaningful. The results of this study tries to address this by determining MCID values associated with these tests and lower limb function.

In rare diseases, recruitment of patients become difficult with more therapeutic opportunities. Therefore, we need to have better understanding of commonly used clinical outcomes and its impact on the mechanism of disease allowing scientists to more confidently extrapolate data from smaller studied groups into understanding possible effects in a more general population. It is not possible to predict the outcomes of clinical trials so studies must be designed to detect even the smallest effect size hence the reason for a homogenous study group. Having better understanding of TFTs and its impact on disease trajectories will allow for more precise adaptive or enrichment study designs. In order to do this, we must have sufficient understanding of the how clinical outcomes such as the TFTs respond in the natural progression of the disease.

We have seen in the last few years that FDA approval does not result in access to treatment. Clinical endpoints become an important discussion point as key stakeholders shift roles within drug development, approval and post market access. These gate keepers in treatment access may shift from FDA approval to insurers who will approve/deny payment for treatments based on the seminal study's primary/secondary endpoint. Study trials go through rigorous standardization of training to ensure that tests are administered and assessed in a consistent manner to reduce the variability of the clinical endpoint. We know that an increase in variability

of a clinical outcome leads to decrease sensitivity to detect change. When thinking of clinical endpoints, there are multiple stakeholders that must be considered from the design of the trial to the translation and access into the clinic. Stakeholders include the patients and clinicians who ultimately have to administer these outcomes and justify the need for the treatment. Before approval, researchers play a significant role in the clinical endpoints that are chosen for trials which are mostly influenced by established psychometric soundness of the outcomes and its use in understanding in the natural history of the disease. Fortunately for DMD, there are well established easily administered TFTs that are consistently used in the clinic and trials.[254] Our study data contributes to the confidence of the data and addresses the gap to link clinical significance to these TFTs so that clinicians could make timely recommendations for braces, equipment and supports for families as they navigate the evolving progressive nature of DMD in an era of promising therapeutic trial options.

Future developments needed in this area of contracture management and TFTs include investigation of possible genetic factors that may predispose individuals to contracture development. To better understand the clinical significance of loss of function, we also need to understand patients and caregiver's perception of clinical significance based on a level change on the Vignos scale and its relation to quality of life. Currently, clinicians use this scale to describe losses in function based on ability to negotiate stairs, walk, get up from the chair. These losses allow clinicians to make changes and recommendations to IEPs, bracing and equipment which will ultimately impact families. However, that assumption should not be made and we need to understand from families if these changes are impactful to their lives. Lastly, since the data

regarding stretching and night-time orthosis for contracture management is insufficient, we would need a randomized control trial to determine the effectiveness of these treatments to prevent or slow the progression of contractures in boys with DMD. In doing so, we need better methods to assess not only joint ROM but physiological changes in the muscle or tendon that may occur from stretching. Having good measures to assess possible musculotendinous changes from different stretching regiments maybe key in better understanding the mechanisms impacted by conservative stretch management in DMD.

In summary, the results of this study provided the first insight on contracture development in boys with DMD in the GC era and established better understanding of its contribution to TFTs which provides more confidence in its continued use in clinics and trials to guide decision making.

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