Acute contraction-induced strength loss, muscle inexcitability and recovery after exercise in males with Duchenne muscular dystrophy

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Dedication

In memory of Dr. Stephen Smith, a great educator who first introduced me to boys with Duchenne.

In memory of my parents, Rita and Joe Phelps, and my brother Stevan Phelps who encouraged me in my academic pursuits.

I dedicate this work to our children Emi, Karl, Dan and Aaron; may you realize your unique God-given talents and pursue what He has put on your heart.

Finally, I dedicate this to my husband, Tim, who pledged on our wedding day to walk together with me as we pursued our dreams. Thank you for all you sacrificed to enable me to pursue this dream.

Abstract

This dissertation had three objectives with the overall goal to explore contraction-induced strength loss and recovery in males with Duchenne muscular dystrophy (DMD) specifically to: 1) evaluate a novel protocol, combining voluntary and evoked contractions to measure strength and excitability of wrist extensor muscles, for safety, feasibility, reliability and discriminant validity for males with DMD and aged-matched controls, 2) explore strength loss and muscle fiber inexcitability contribution to strength loss after submaximal isometric contractions in males with DMD, and. 3) determine the recovery of strength and muscle excitability immediately following contraction-induced force loss in males with DMD.

Through the literature review, I discovered a need for a reliable and valid measurement protocol of muscle contractile function that could be used for DMD across all disease levels. Several measures for walking and upper extremity function are currently used to measure outcomes during clinical trials. However, none of the current measurements for DMD incorporate a way to quantitatively measure both voluntary and evoked strength along with muscle excitability over disease progression or in response to an intervention. The key to the assessment and protocol design needed to be feasibility and safety for boys and men with DMD at different stages of the disease process.

I was able to design and evaluate a novel protocol, combining voluntary and evoked contractions to measure strength and excitability of wrist extensor muscles for safety, feasibility, reliability and discriminant validity between males with DMD and controls (Chapter 3). Wrist extensor muscle strength and excitability were

assessed in males with DMD (N=10; mean 15.4 [SD 5.9] years) (Brooke 1-6) and age-matched healthy male controls (N=15; mean 15.5 [SD 5.0] years). Torque and EMG measurements were analyzed under maximum voluntary and stimulated conditions at two visits. I discovered that our protocol of multiple maximal voluntary contractions (MVC) and evoked twitch contractions was feasible and safe, with 96% of the participants able to complete the assessment protocol maintaining >93% strength both for DMD and controls ($P \geq 0.074$). Reliability was excellent for voluntary and evoked measurements. Torque, EMG and timing of twitch onset measurements discriminated between DMD and controls ($P < 0.001$). This first part of the study demonstrated a useful protocol for measuring skeletal muscle function in clinical trials in males with DMD across various ages and disease levels.

The second part of the study addressed in the context of a neuromuscular disease the concern of injury to dystrophic deficient skeletal muscle during and after repeated contractions (exercise) that result in acute strength loss. I knew that a feature of dystrophin-deficient skeletal muscle in the *mdx* mouse model was a hypersensitivity to strength loss from eccentric exercise due to fibers becoming unexcitable. I wanted to explore how this feature translated to humans lacking dystrophin. We hypothesized that there would be no difference in strength loss during exercise and that males with Duchenne muscular dystrophy (DMD) would have more significant impairment in muscle excitability corresponding with a loss of strength than age-matched controls (Chapter 4). Males with DMD and a group of age-matched controls performed a sub-maximal voluntary isometric wrist extensor exercise protocol until 55% of maximal voluntary contraction (MVC) could no longer

be sustained. Voluntary and evoked force and EMG were accessed before, during, and after exercise. There was a significant interaction (time*group) ($p<0.001$) for MVC torque during exercise, suggesting that the two groups were different in how they reached muscle fatigue. No difference was measured between groups in MVC torque decrement at the time of exercise cessation (by design). Evoked twitch torque decrement was 34% for DMD and 36% for control with no group*time interaction observed ($p=0.834$). Muscle excitability contributed to the evoked torque variance in DMD (76%) and control (59%). The groups were not different in RMS EMG decrement ($P=0.986$) or M-wave decrement ($P=0.911$) during exercise, which does not support our hypothesis that the DMD groups had a more considerable decline in muscle excitability with fatigue during exercise.

Lastly, the recovery of both strength and muscle excitability was explored and compared between groups. The DMD group recovered MVC baseline strength by 10 minutes post-exercise ($P=0.530$) and evoked torque by 5 minutes ($P=0.266$). In contrast, controls were still different from MVC and evoked torque baseline at 15 minutes after exercise ($P<0.002$). Strength loss from submaximal intermittent isometric exercise does not result in more significant impairment of muscle excitability is transient, and recovers faster in males with DMD than controls, suggesting a different mechanism of peripheral fatigue between the groups. The work contained in my dissertation begins to address the fear of exercise that has been a common concern for individuals with DMD and will contribute to knowledge of evidence-based exercise prescription in the future.

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List of Abbreviations

ADLs, Activities of daily living

DMD, Duchenne muscular dystrophy

EK2, Egen Klassification Scale Version 2

EMG, Electromyography

ICC, intraclass correlation coefficient

MVC, maximum voluntary contraction

PMNS, peripheral magnetic nerve stimulation

RMS, root mean square

SEM, Standard error of measure

DCG, dystrophin-glycoprotein complex

nNos, nitric oxide synthase

NO, nitric oxide

ECM, extracellular matrix

FVC, forced vital capacity

MMT, manual muscle testing

QMT, quantitative muscle testing

EK, Egen Klassification Scale

ICU, intensive care unit

Mdx, Mouse model of Duchenne muscular dystrophy

Brooke, Brooke Upper Extremity Scale

SD, standard deviation

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Chapter 1: Introduction/Significance

"Families and boys/men with Duchenne muscular dystrophy (DMD), as well as physical therapists and educators, often inquire as to how much and what types of exercise are appropriate to help alleviate signs of the disease and possibly improve function. For all of us, life is a continuous balancing act filled with cautious modifications and adjustments. We look to health care professionals and researchers for better guidelines and treatments and remain hopeful that evidence-based exercise prescriptions will be developed for the Duchenne community." (Pat Furlong, President and CEO of Parent Project Muscular Dystrophy-Paraphrased) [6]. Boys with Duchenne muscular dystrophy (DMD) experience muscle weakness starting around two years of age due to the lack of the protein dystrophin.[7] Primary muscle pathology, necrosis, increased fat mass, atrophy, and fibrosis contribute to functional losses and difficulty moving for males with DMD as the disease progresses.[8] However, due to the fear of muscle damage or injury further exacerbating the disease progression from exercise or physical activity, males with DMD are increasingly sedentary with age contributing to co-morbidities of progressive disuse: muscle weakness[9], obesity, type II diabetes, joint contractures, and deformity, osteoporosis, and cardiovascular disease.[10] Physical activity that causes a transient strength loss that recovers quickly may counter these comorbidities since, in healthy individuals, exercise produces positive muscle adaptations. Fear of the accelerating muscle pathology caused by contraction-induced strength loss during exercise has prevented physical activity from being prescribed as a standard of care for individuals with DMD [6,8,11,12]. This barrier

continues to exist despite studies that report the benefits of prescribed exercise with no deleterious consequences.[13-16] The use of exercise to counteract the deleterious effects of DMD has been a topic of high interest over the past decade in review articles and at workshops [6,8,11,12,17-21]. More information is needed to know how to design personalized exercise programs that do not cause muscle injury (strength loss that takes weeks to recover) yet allow fatigue (transient strength loss) appropriate for positive muscle adaptations. Understanding how to prescribe and monitor exercise to meet the above criteria is imperative if physical activity is to become a regular part of comprehensive treatment for males with DMD.[22] Continued pre-clinical research into the mechanisms of contraction-induced strength loss [23-26] and incorporating exercise into experimental designs for males with DMD is critical to inform personalized exercise prescription for standard of care [6,8] and as an adjuvant to new therapeutics.[12]

A study with three aims was designed to explore contraction-induced strength loss and recovery in males with DMD. Aim 1 of this work evaluated a novel protocol, combining voluntary and evoked contractions to measure strength and excitability of wrist extensor muscles for safety, feasibility, reliability and discriminant validity in males with DMD and aged-matched controls. Previously this type of assessment has been completed in adults [27-30] but not in males with DMD. Aim 2 explored strength loss and muscle fiber inexcitability contribution to strength loss after submaximal isometric contractions in males with DMD. Based on key *mdx* mouse model studies, we hypothesized that males with DMD would not be different in strength loss. However, they would have a more significant loss of muscle

excitability corresponding to a loss of strength from repeated muscle contractions than age-matched controls during exercise[1,6,24]. Aim 3 of this work explored recovery immediately following contraction-induced strength loss in boys with DMD as compared to controls. Through addressing these three aims, I plan to propose a protocol for measurement of strength loss, which may improve inclusion into clinical trials for all males with DMD regardless of disease state or functional ability. Furthermore, this work will advance knowledge about strength loss and recovery and contribute to knowledge regarding evidence-based exercise prescription for males with DMD.

Chapter 2: Literature Review

2.1 Duchenne Muscular Dystrophy

Pathology

Muscular dystrophies are a heterogeneous group of genetic disorders characterized by the progressive loss of muscle strength and integrity due to the lack of the protein dystrophin. The dystrophin gene, the largest known human gene, provides instructions for dystrophin production and includes a coding sequence of 79 exons. The types of deviations that can occur include partial deletions, duplications and point mutations. Twenty-nine different gene mutation locations give rise to 34 distinct disorders of muscular dystrophy. [31] The most common form, Duchenne muscular dystrophy (DMD), is an inherited neuromuscular X-linked disorder that affects one in 3600-6000 live male births.[7] The pathology of DMD is a frame-shift mutation in the dystrophin gene Xp21leading to failure of dystrophin production.[32] A reading frame or in-frame mutation hypothesis has been proposed to explain the abnormal translation of the dystrophin gene.[33] Mutations that disrupt the reading frame result in premature termination and loss of dystrophin and lead to the more severe phenotype DMD. Mutations that retain the reading frame and generate a shortened protein lead to a milder phenotype, Becker muscular dystrophy (Becker). Dystrophin levels in Becker are generally 30-80% of normal, in contrast to DMD, where they are 0-5% of normal.[34]

The resulting deficiency of dystrophin in skeletal muscle has been linked with recurring muscle damage, degeneration, regeneration, and subsequent muscle

weakness.[20] Muscle biopsies characteristically demonstrate necrosis and degeneration of muscle fibers.[35] Necrotic fiber clusters are surrounded by macrophages, lymphocytes and small immature centrally nucleated fibers reflecting muscle regeneration, resulting in a balance between degeneration and regeneration early in the disease.[36] Over time, muscle regeneration does not keep up, and muscle fibers are gradually replaced by connective and adipose tissue.[35-37]

Progressive muscle wasting resulting from an imbalance between muscle fiber necrosis and myoblast regeneration starts with the proximal muscles as early as two years of age. Between two and five years of age, boys show signs of toe walking, clumsiness, falling, and a waddling gait, as well as difficulty on stairs. [38] Quantitative strength testing showed greater than 40–50% loss of strength by six years of age compared to peers.[39,40] Pseudohypertrophy may be visible in the calf muscles, contractures may develop, and an exaggerated lordosis of the spine may be seen.[7,20] To try to keep their balance, boys may walk with an anterior pelvic tilt, pull back their shoulders, and increase their base of support.[38] Boys have difficulty getting up from the floor and use a compensatory mechanism to get up, a positive Gowers' sign. The inability to walk can begin between eight to twelve years of age, with most boys using a wheelchair sometime during the day by ten years of age.[39] Multiple factors contribute to the ongoing reduction in motor ability across the lifespan and the need to exert more effort to accomplish a given task (often perceived and reported as fatigue). Primary reasons could be the microlesions in the muscle fiber that lead to massive calcium entry, loss of calcium homeostasis, loss of the mechanical, electrical and signaling ability of the sarcolemma that

eventually leads to muscle cell death.[35] Secondary reasons could be altered regeneration, inflammation, apoptosis, fatty infiltration and impaired vascular adaptation.[41] Tertiary reasons could be fear of increased muscle damage leading to increased sedentary behavior[35], biomechanical problems including ankle contractures, poor balance, foot and knee deformities, and increased body fat mass induced by inactivity and muscle atrophy. [7,20,42,43] Very little has been studied in males with DMD related to fatigue either perceived (subjective) or physiological (transient muscle strength loss or impaired performance).[44,45]

Treatment

During their lifespan, males with DMD undergo many clinical treatments, including extensive use of corticosteroids, which aim to maintain or improve muscle strength and function for a period of time; however, the mechanism of how corticosteroids work is not well understood, and there are many side effects.[34,46-48] Other pharmaceuticals and nutritional supplements are used for cardiac, respiratory and general health benefits.[7,49] Orthopaedic surgery, allied health services, and complementary therapies are also incorporated into treatment plans to address and attempt to slow the decline of function, improve health, and preserve quality of life.[7,20,38,42,49,50] Rehabilitation, including submaximal exercise with gentle stretching, may be prescribed to attenuate further functional loss[15,16,18]. In contrast, other care teams emphasize avoidance of exertion and exercise due to concern of further exacerbating the disease.[9,18,19,21,51]

As therapeutics are being developed to address dystrophin gene expression[52], promote muscle growth, and up-regulate utrophin, there is a renewed

interest and hope in a more active lifestyle for males with DMD.[11,12,32] The role rehabilitation and exercise prescription will play in the ongoing treatment with new pharmaceuticals will need to be evaluated.[6,8] New treatments can open up new opportunities for activity and participation, which will need to be realized through prescribed evidence-based rehabilitation and exercise. There is a lack of research investigating the outcomes of physical activity for individuals with DMD.[17,19,49,53,54] Up to this point, little is known about prescribing exercise programs or monitoring the outcomes throughout the lifespan. It would make sense that because exercise has positive effects in healthy muscle, if prescribed correctly, it could augment other treatments in males with DMD to optimize their impact as well as improve patient well-being.[12,17,22]

Mdx mouse model of Duchenne muscular dystrophy

There are many challenges in exercise research in males with DMD, including the inability to control participants' heterogeneity, the environment, food, timing of tests, and assessment techniques. Several animal models have been developed to meet these needs.[55] Animal research can also be conducted in vitro and in vivo, allowing for exploration of strength loss mechanisms in dystrophic deficient muscles. The most universally used animal for research of dystrophic muscle is the *mdx* mouse.[55] The *mdx* mouse has been accepted as a genetic model of DMD since the 1980s.[56] Although useful as a model of DMD, a weakness is that the *mdx* mouse model does not fully replicate the phenotype seen in human patients.[6]

The X-linked recessive mutation in the dystrophin gene of the *mdx* mouse, a nonsense mutation in exon 23, resembles that seen in boys with DMD; however, the disease phenotype is relatively mild, producing mice with a slight reduction in life span, increased capacity for muscle repair, and more mild locomotor deficits.[18,55,57] This is evidence that the dystrophin protein is less critical to muscle function in mice than in humans or is moderated by an upregulation of the protein utrophin, which can partially compensate for the function of dystrophin.[35] Despite their mild phenotype, mouse models have been used to understand the disease's basic mechanisms, respond to exercise and recovery, and develop and test the safety and efficacy of treatments in a controlled environment using both in vivo and in vitro techniques.[18,58] Physical similarities between the *mdx* mice and boys with DMD include pseudohypertrophy of certain muscles, fibrosis, fatty infiltrates, and increased susceptibility to muscle strength loss with excessive and strenuous exercise.

Mdx mice live about two years and are much smaller than humans with a faster growth rate, allowing researchers to produce findings more quickly and efficiently than studying similar processes in humans.[57] Size has implications on the biomechanical stresses experienced by bone, connective tissue and muscles in both species. Humans and mice have different ratios of fibers type I and II muscle fibers and in sub-types IIA, IIX and IIB, and therefore they may respond differently to disease, treatment and research findings. Size, function and variation in electrical activity of the nerve innervation and other biochemical differences influence the muscle fiber types that are the most prevalent in each species. Differences in

isoforms, contractile speed, and primary energy usage (oxidative vs. glycolytic) may also cause the human muscles to respond differently to stresses and recovery than mouse muscle. These differences can affect the translation of the current research on muscle inexcitability with force loss in *mdx* mice to research with boys with DMD. The milder phenotype of the mice allows maximal, high force eccentric contractions to be done and recovery to be observed within several days.[1,25,59] In fact, some researchers will use intense exercise to worsen the *mdx* phenotype intentionally and exacerbate muscle damage for other research efforts. Pre-clinical studies using *mdx* mice have contributed extensively to the knowledge of skeletal muscle and mechanisms of force loss for dystrophic deficient muscle. Translation of this work to males with DMD is key to improving care and treatments. This dissertation successfully took that step as I designed a study to translate work on the acute failure of action potential conduction in *mdx* muscle into a study that explored the relationship of muscle excitability and force loss and recovery after exercise in males with DMD.

2.2 Skeletal Muscle Structure and Function

Healthy skeletal muscle function

Skeletal muscle is the largest internal organ in the human body, comprising, on average, 40% of total body weight. It is multifunctional, helping to support position, move our bodies and provide homeostasis through temperature regulation and nutrient stores. Skeletal muscle is a contractile organ directly or indirectly attached to bone through tendons. It converts chemical energy to mechanical energy to generate force and power, maintains posture, produces movement allowing for social and occupational endeavors, and contributes to functional independence. Muscle actions and the ability to produce force, power and movement are influenced by muscle structure and architecture, fiber types, excitation-contraction coupling, and energy release.[60] Skeletal muscles are controlled through the somatic nervous system through efferent nerves (α motor neurons), sending out commands for movement, and afferent nerves (γ motor neurons) relaying sensation/position sense back to the central nervous system.

The fundamental unit of skeletal muscle is the muscle fiber, a multi-nucleated cell, varying in length, grouped into bundles called fascicles by a connective tissue network to make up the whole muscle (Figure 2.1). The individual muscle is a group of these muscle fibers surrounded by a layer of connective tissue (epimysium) (Figure 2.1). The perimysium binds together groups of fibers within the muscle. The plasmalemma and basal lamina make up the sarcolemma, the muscle fiber cell membrane. I will use the term sarcolemma to refer to the muscle-fiber membrane within this work.

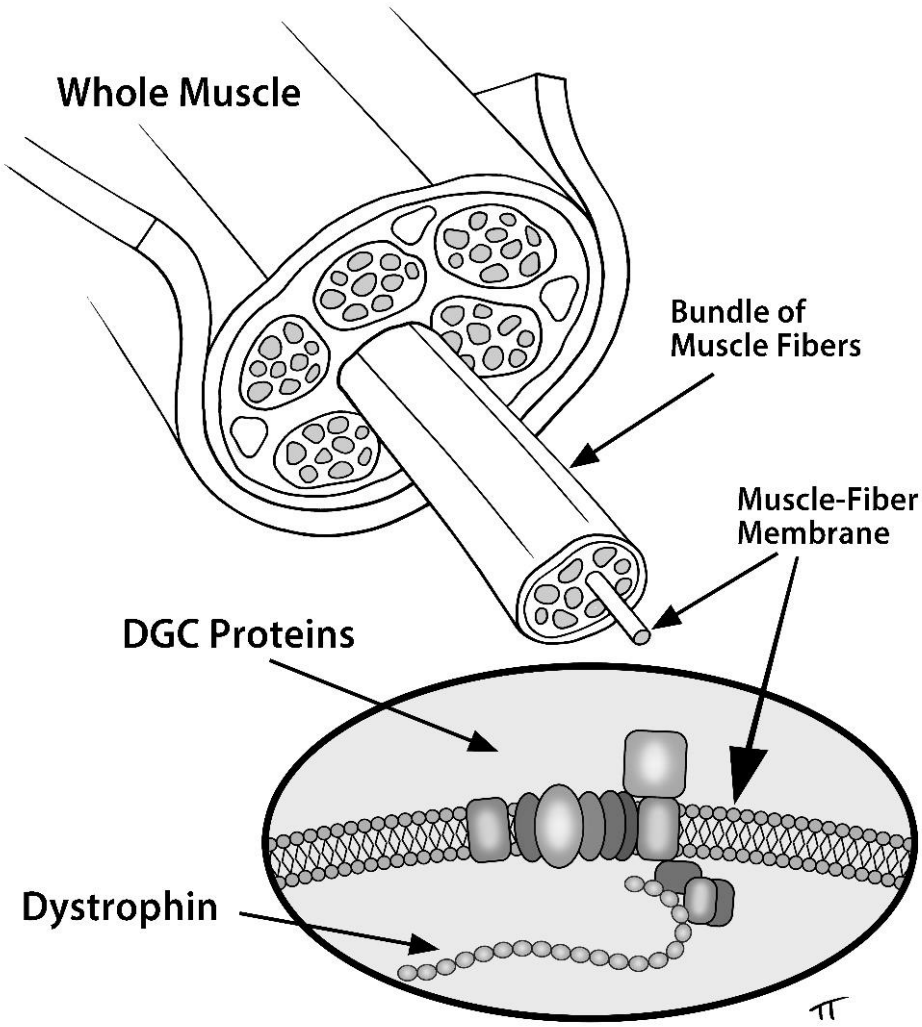

Figure 2.1 Muscle fiber schematic; DGC=Dystrophin-associated Glycoprotein Complex

Within the muscle fiber, running longitudinally, are thousands of myofibrils and billions of myofilaments. The two most abundant myofilaments are the proteins actin and myosin. When put together in an orderly repeating manner, the myofilaments form the sarcomere, the basic contractile unit of skeletal muscle. In addition to myofibrils, the sarcoplasm of a muscle fiber contains nuclei, mitochondria, the T-tubule system, and sarcoplasmic reticulum that work together in synchronization to allow muscle contraction.[60]

The function of the muscle fiber membrane, the sarcolemma, is to provide a barrier between the intra- and extra-cellular compartments, maintain membrane potential, transport action potentials, and add structural support to the muscle. Embedded within the sarcolemma is a complex of proteins, dystrophin-glycoprotein complex (DGC), that physically connects the sarcolemma to the internal myofilament structure through the cytoskeletal costameres (Figure 2.1 and 2.2).[61] The DGC also anchors the nitric oxide synthase (nNOS) that functions to regulate blood flow through production of nitric oxide (NO). Satellite cells contributing to muscle growth and regeneration are found within the sarcolemma between the plasmalemma and the basal lamina.[43,60] The cytoskeleton is the cell's scaffolding that provides mechanical support enabling the cell to carry out essential functions. This complex network of proteins within the muscle fiber functions structurally to transmit force during contraction. The cytoskeleton is comprised primarily of costameres and intermediate filaments that connect the sarcolemma to the z-disk of the contractile unit to ensure stability and coordinated movements. Costameres function to distribute contractile forces generated in the sarcomere laterally through the

sarcolemma to the basal lamina through mechanical coupling and therefore maintain uniform sarcomere length along the fiber.[35] Dystrophin is the largest human gene known and contains 79 exons and provides instructions for making the protein dystrophin. The dystrophin protein is a rod-shaped 427 kDa cytoplasmic protein, a vital component of the DGC that connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix (ECM) through the cell membrane (Figure 2.2).[62] The DGC through dystrophin provides a mechanical linkage between the extracellular matrix (laminin) and the intracellular cytoskeleton (F-actin) (Figure 2.2). The structural and functional integrity of the DGC is crucial to stabilize the sarcolemma during contractions.[43] Although dystrophin itself is not a signaling molecule, it anchors signaling proteins through the DGC. The DGC has signaling and regulation roles due to its interaction with nNOS, which is anchored at the sarcolemma and forms NO, a messenger molecule that regulates development and contractility in addition to blood flow. It may also have a role in sensing mechanical perturbations and converting this signal into a biochemical response such as alterations in phosphorylation and changes in the expression levels of specific proteins.[35,63]

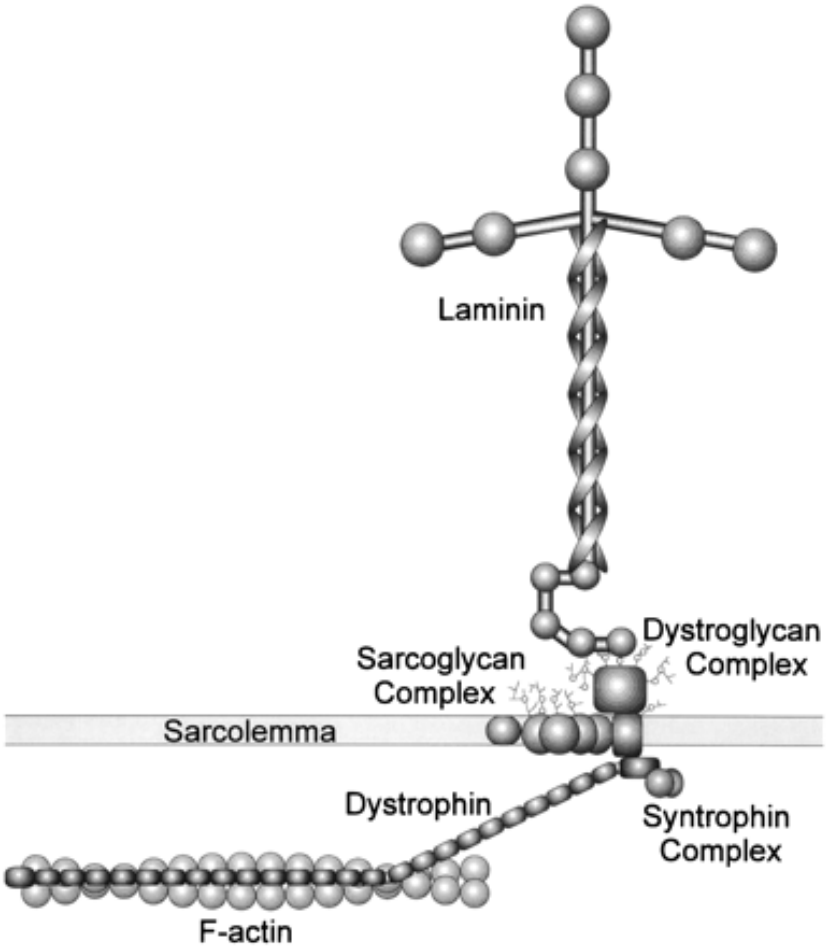

Figure 2.2 Schematic of the role of dystrophin in sarcolemma stability and integrity.[5]

Transverse tubules, essential structures for excitation-contraction coupling, are deep periodic invaginations along the sarcolemma. They provide a pathway for rapid transmission of the electrical signal received at the neuromuscular junction to penetrate the muscle fiber. The tubules contain calcium channels that serve as pathways that transport the electrical impulse or action potential to activate the sarcoplasmic reticulum. The T-tubule lumen is continuous with the extracellular fluid,

and the membrane depolarization during an action potential occurs across the T-tubule membrane. The sarcoplasmic reticulum is a reservoir of intracellular Ca^{2+} and provides a balance of calcium release and reuptake in the sarcoplasm skeletal muscle. This system plays an essential role in muscle contraction by the release of calcium ions.[60]

An impulse from a neuron initiates a muscle contraction. The nervous system communicates with the muscle via the neuromuscular junction. The nerve impulse arrives at the synapse; chemical transmitters are released and diffuse across the junction. If the potential is reached, an impulse travels along the sarcolemma and down the t-tubules to the sarcoplasmic reticulum. Located in the T-tubule membrane is the T-tubule voltage sensor. The voltage sensor changes conformation in response to depolarization from the action potential. This conformational change is transmitted to the base of the sarcoplasmic reticulum Ca^{2+} channel, causing it to open, allowing Ca^{2+} release and calcium concentration at the myofilaments to increase and bind to the troponin. The myosin head can contact actin, myofilaments slide past one another, and a contraction occurs. This process is known as excitation-contraction coupling. Suppose any part of this process does not work correctly, such as the action potential not traveling along the sarcolemma. In that case, the excitation-contraction process's cascade reaction is interrupted, and no contraction will occur within that muscle fiber, decreasing force production of the overall muscle.[60,64]

Dystrophin-deficient skeletal muscle function

The primary abnormality in DMD is the lack of or abnormality of dystrophin. Dystrophin, integral to the DGC, plays a vital role in linking the cytoskeleton to the sarcolemma contributing to lateral force transmission as described in detail above.[6] Dystrophin acts to stabilize the muscle membrane against forces caused by contraction, acting as a shock absorber or force dampener. Disruption of the link between the cytoskeleton and the extracellular matrix also causes a loss of sarcolemma integrity.[36,62] Excessive fragility of the sarcolemma in the dystrophin-associated protein complex scaffolding can compromise the cell membrane as a barrier to maintain homeostasis.[31] Maintenance of this barrier is crucial for the survival of the muscle cells.[36] A compromised sarcolemma leads to Ca^{2+} influx, which leads to inappropriate cell signaling and down-regulation of nNOS, leading to ischemia, causing secondary changes of necrosis, apoptosis, and inflammation and fibrosis.[65] Repeated rounds of degradation and regeneration happen from repeated bouts of contraction-induced injury and impaired homeostasis. Eventually, the regeneration cannot keep pace, and the cells undergo complete necrosis and are replaced by fibroblasts, myofibroblasts, immune cells, and sometimes, adipose cells. Along with the sarcolemma serving as a barrier between intra- and extra-cellular spaces, it serves the critical function of action potential propagation to initiate muscle contraction.[1] Damage to the sarcolemma will affect excitation-contraction coupling due to mechanical, chemical and electrical sequelae.[6,66]

2.3 Skeletal Muscle Strength Loss and Fatigue

Muscle fatigue and force loss models

A common definition of fatigue is, “An exercise-related decrease in the maximal voluntary force or power output“.[67,68] Fatigue is also defined as a decline in the maximal contractile force of a muscle or contraction-induced force loss which may or may not be reflected in performance or task failure.[3,4,69,70] Another definition of fatigue is “a reduction in the maximal capacity to generate force and a slowed ability to produce a contraction that can be revealed through different mechanisms and processes and is often associated with a given task”.[4,71] Multiple definitions and methods for assessing fatigue in the literature attest to the complexity and the challenge in defining such a phenomenon.[4,43] A mechanistic model of fatigue first introduced by Edwards (1983) categorizes fatigue into two broad areas: central fatigue and peripheral fatigue (Figure 2.3).[3,4] This model accounts for losses in muscle force production due to transmission, excitation/activation, energy, and structural components.[3,4,43] Central fatigue includes the central nervous system (brain and spinal cord) with mechanisms of impaired motivation, neural drive and motor unit recruitment. Peripheral fatigue includes impairments or imbalances in neuromuscular junction transmission, action potential propagation, excitation-contraction coupling, blood flow, the contractile apparatus (DGC), energy supply (ATP), vasodilation by nitric oxide, and K^+, Na^+, H_20, Ca^2 (Figure 2.3).[3,4,43,72] Contraction-induced force loss after exercise or activity can be attributed to either or both central or peripheral components of the neuromuscular process.[73] Other models add additional variables such as the type of task or the fitness level of the

individual, emphasizing the complex nature and interactions that contribute to fatigue (Figure 2.4).[71]

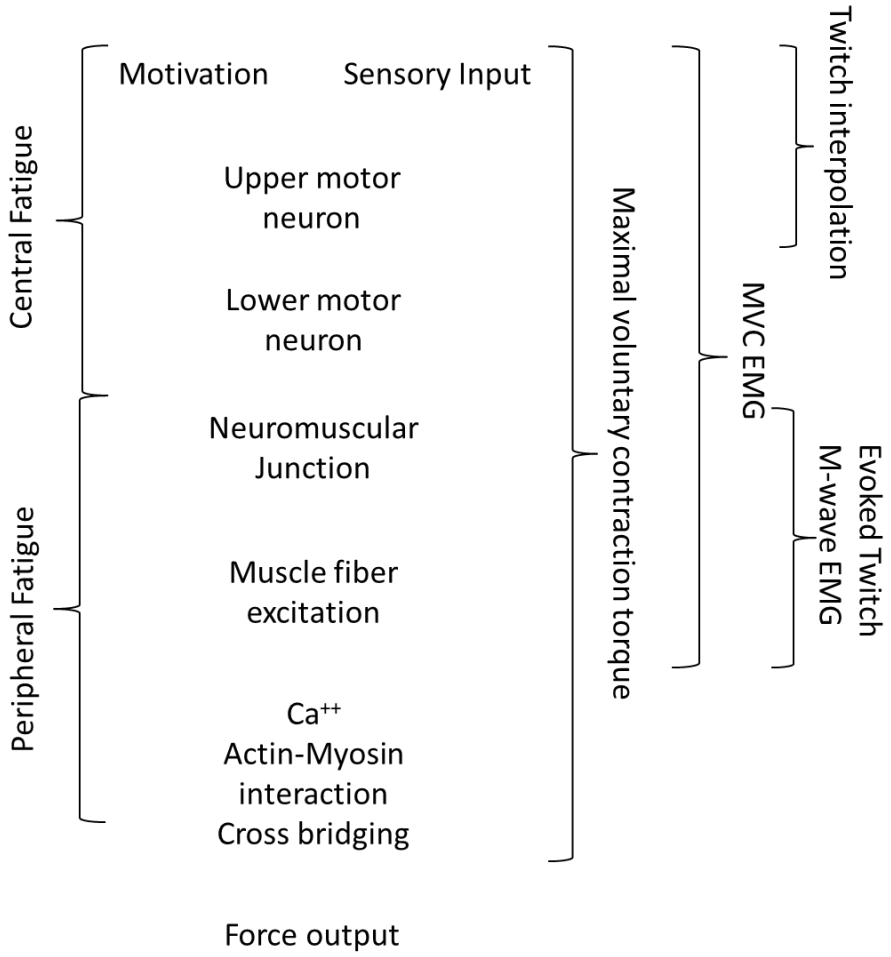

Figure 2.3 A mechanistic model of fatigue; Adapted from "Chain of Command" Edwards (1983) and Lou (2012) outlining contributions at various levels of the system from the brain to the actin-myosin cross bridges which impact force loss during exercise.[2,3]

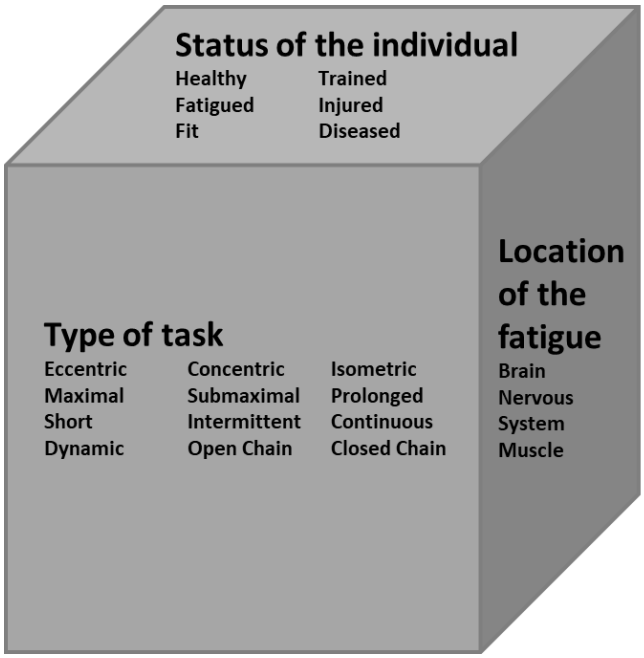

Figure 2.4 Multifactorial perspective of fatigue (adapted from Williams, and Ratel 2009)[4]

The evidence suggests that fatigue is exceptionally complex and should not be attributed to any single mechanism. It has been difficult to assign the fatigue under different conditions to a set of mechanisms even in healthy individuals,.[68] The fatigue literature focuses on the impairment of physiological processes distal to the muscle fiber action potential. It supports adjustments in the motor unit activity that is influenced by load, duration and contraction type. These adjustments occur in all individuals but may not be consistent between healthy and diseased populations.[74] For example, motor unit twitch force and contraction velocity can impact voluntary muscle force without observed changes in electromyography signal (EMG).

Amplitude of the EMG signal can be altered by propagation velocity and the motor unit action potentials' shape without a concurrent change in muscle force.

What I will focus on in this work is peripheral fatigue, the contributing mechanisms and the recovery from force loss caused by peripheral fatigue as it relates to DMD and exercise. This dissertation focuses on measuring and describing acute skeletal muscle force loss and recovery after intermittent submaximal isometric contractions through analysis of EMG and torque output in males with DMD compared to an age-matched control group.

Measurement of contraction-induced force loss and muscle fatigue

The measurement of transient muscle fatigue after repeated contractions was first recorded by Merton (1954) when force produced through voluntary efforts were compared to the force of electrically stimulated contractions of the adductor pollicis. [73] Since then, the field of fatigue measurement has continued to evolve. Measurement methods of contraction-induced force loss can be classified into two different models.[4] The first is to measure the power output during a real exercise such as cycling, running or swimming. The second is to measure maximal isometric muscle force before, during and after exercise. This section will focus on the second method and describe how muscle force and simultaneous EMG have been used to investigate peripheral mechanisms of fatigue in healthy controls.

Maximal voluntary contraction (MVC) force is the most direct assessment of fatigue that includes the entire chain of central and peripheral neuromuscular events.[69] MVC is typically collected during a 3-5 sec contraction with a ramp-up period and a plateau.[4] Because muscle strength measurement requires the full co-

operation and motivation of the subject, electrical stimulation during MVC has been used to confirm that the MVCs are truly maximal. The interpolation twitch technique (ITT) is electrical or magnetic stimuli superimposed on the MVC plateau to assess voluntary muscle activation failure.[27,29,30,75-81] During a twitch interpolation, an additional force produced by the stimulation would indicate incomplete motor unit recruitment. Supramaximality of the stimulation to the motor axons innervating the muscle is ensured by comparing voluntary and evoked outputs. This technique, however, has been controversial for assessing muscle activation because of methodological and physiological concerns.[82-87] The use of magnetic stimulation rather than electrical stimulation for ITT has also added complexity to the discussion with mixed results.[75,78,88-90]

Peripheral fatigue can be measured through electrically or magnetically evoked twitch contractions.[2,27-30,54,75,87,90-92] Force can be assessed by a single, pair, or trains of stimuli under potentiated and non-potentiated conditions to measure transmission or contractile impairments while the muscle is at rest.[29,54,75,87,90] Potentiated twitches should be used to measure fatigue because they are more sensitive for detecting contractile fatigue than non-potentiated responses.[93] Simultaneous EMG measurement of M-wave can help discern electrical transmission versus muscle contractile contributions to fatigue. M-wave amplitude is a measure of electrical transmission across the neuromuscular junction and sarcolemma excitability. With transmission failure, a decrease in force would be in the same direction and the same magnitude as the M-wave amplitude decrease.

When contraction failure is predominant, there would be little to no M-wave amplitude decrement but significant force decrement.

In this study, peripheral magnetic stimulation was chosen over electrical stimulation to produce the evoked muscle twitches. Electrical stimulation stimulates the skin nociceptors which can be an uncomfortable or painful feeling.[27,78,88] Magnetic stimulation non-invasively and with minimal to no pain or discomfort activates localized regions of the human central and peripheral nervous systems. Pulsed, electric currents flow through a coil, resulting in a magnetic field around the coil. This rapidly changing magnetic field then penetrates the body's soft tissues inducing a voltage difference.[27,94] The induced currents penetrate the membranes of the neurons and axons. If the induced current is of sufficient amplitude and duration to depolarize nerve cell membranes, action potentials are generated similar to conventional electrical stimulation.[95] No severe adverse events have been reported despite the long history of peripheral magnetic stimulation.[96]

MVC and magnetically evoked twitches were used in this dissertation to measure force decrement during a series of submaximal contractions and force recovery after the contractions. Simultaneous EMG was collected during both MVC and evoked twitches to measure muscle excitability and give insight into the mechanism of fatigue in males with DMD.

2.4 Contraction-induced Force Loss in Dystrophin-Deficient Skeletal Muscle

Measurement of muscle strength and function in DMD

Clinical trials are being designed to evaluate therapeutics to improve muscle strength and endurance, precision-based medicine to address muscle pathology, gene therapy to improve dystrophin production and exercise prescription to mitigate the effects of deconditioning. These exciting developments create a pressing need for broad inclusion and standardization in clinical trial protocols across the DMD population regardless of disease state, ambulatory ability, cardiopulmonary function, or cognitive level. [11,27,97-101]

The most comprehensive method to assess intervention efficacy in a clinical trial in a patient population is to include key outcome measures that cover body function and structure, activity and participation as outlined by the International Classification of Functioning, Disability and Health.[102] Clinical trials in DMD typically use outcome measures such as respiratory function, volitional skeletal muscle strength assessment, upper limb and ambulatory timed activity and performance-based outcome measures, or patient-reported outcomes.[103,104]

The most commonly used measure of strength in DMD has been manual muscle testing (MMT) [105] and, more recently, quantitative muscle testing (QMT). [106] Experienced physical therapists have reliably measured strength in a small group of boys with DMD using the modified Medical Research Council scale.[105] Concerns regarding the extensive experience required to perform MMT accurately have led to

the development of assessment techniques and devices for QMT.[107] Muscle strength and endurance has been indirectly measured through timed activities and performance-based assessments such as 6-minute walk test [108,109], North Star Ambulatory Assessment [110,111], Motor Function Measure [112], Performance of the Upper Limb [113-115], and Brooke Upper Extremity Scale.[116] All of these measures have been evaluated for feasibility and reliability, with good results. They are practical and important for tracking natural history and longitudinal functional changes in individuals with DMD.[100,117] Patient-reported scales such as the Egen Klassifikation (EK) Scale[118], EK version 2 (EK2) [119], and a newly created DMD Upper Limb PROM [120] have been developed to obtain the participant's perspective on their function. Functional outcome measures have positive and negative attributes. Many require volitional effort, are not useful longitudinally because they are specific to ambulatory or non-ambulatory participants[98,121], may not be sensitive to small changes[97,100] and do not give insight into the underlying physiology change in muscle strength and performance after treatment.[100,121,122] As primary outcome measures, they may bias results and limit participation because of a boy's age, ambulatory ability, motivation, cardiopulmonary status, or cognition.[104,122] One or more pulmonary function tests is typically used in all clinical trials to assess respiratory function. These include forced vital capacity (FVC), forced expiratory volume in 1 s, peak expiratory flow rate, and maximal inspiratory and expiratory pressures.[104] The rate of decline in FVC is predictive of life expectancy.[123] 3-D motion analysis can be added as an outcome measure[124] and muscle composition through magnetic resonance imaging and magnetic resonance spectroscopy can be added to quantify muscle response to interventions.[125]

Man et al. (2004) described the importance of incorporating a non-volitional assessment of muscle strength when assessing patients in the intensive care unit (ICU), children, patients with cognitive difficulties and those prevented from performing a true MVC by pain [27]. He advocates the use of peripheral magnetic stimulation in these patient populations rather than electrical stimulation to evoke muscle contraction because of the ability to ensure complete nerve trunk stimulation without inducing high painful currents in the skin [27,90]. Magnetic stimulation has been used to assess muscle strength in previous studies with healthy adults, adults in the ICU, and adults with various neuromuscular conditions. [28,29,54,75,79,87,90,126,127] Peripheral magnetic stimulation has not been used in clinical trials for individuals with DMD. Magnetic stimulation to evoke muscle contraction should be assessed as part of a complete protocol for muscle strength measurement for DMD to tease out mechanisms underlying loss of torque and EMG changes during exercise and recovery.[97] In chapter 3 of this dissertation, I will describe the safety, feasibility, reliability and validity of peripheral magnetic stimulation to evoke contractions in males with DMD. I will demonstrate the use of peripheral magnetic stimulation to measure muscle strength before and strength loss during and after an exercise protocol designed to induce acute and transient strength loss, i.e., fatigue.

Pre-clinical exercise research in dystrophin-deficient muscle (mdx mice)

A round table review of animal and human research of Duchenne muscular dystrophy[8], several review articles,[12,18,128] and key animal papers have evaluated the effect of exercise on dystrophic muscle. Exercise studies in *mdx* mice investigate the effects of isometric, concentric and eccentric muscle contractions and

endurance training on dystrophic muscles. *Mdx* mouse studies (in vivo and in vitro) have contributed valuable information to the debate on the risks and benefits of exercise for males with DMD despite a difference in the human phenotype described above. *Mdx* mouse research to date suggests that low-intensity and low-volume exercise (including some studies involving concentric and isometric contractions) may be beneficial but that high-intensity, high-volume eccentric muscle contractions exacerbate muscle pathology.[11,12]

Many *mdx* mice (the mouse model of DMD) muscle studies have shown advantageous remodeling and functional improvements in response to various exercise paradigms.[24,59,129-131] Endurance types of exercise in *mdx* mice, like voluntary wheel running, have shown positive effects on muscle strength and fatigue resistance with no deleterious effects.[26,129,131,132] Wheel running by *mdx* mice may mimic light to moderate physical activity by boys with DMD as it is a voluntary type of exercise that is controlled by the individual and does not impose excessive resistance. Research has shown that *mdx* mice gained more strength after 12 weeks of voluntary light (no resistance wheel running) or moderate (resistance wheel running) exercise than sedentary mice.[130] There was no increased muscle ability to protect from injury with increased strength after voluntary wheel running.[130] Wheel running has also been shown to elicit hypertrophy of weight-bearing muscles such as the soleus. However, non-weight bearing muscles such as the extensor digitorum longus or tibialis anterior do not hypertrophy with exercise.[129] Improvement in strength, fatigue resistance, and mitochondrial oxidative capacity after 12 weeks of voluntary wheel running without any measured deleterious effects

has also been described.[131] One study showed a dose-effect through increased muscle strength of the tibialis anterior in mice who ran faster than those who ran at a slower speed.[133]

It is unclear if muscle adaptations can be produced by endurance exercise without the deleterious effects of resistance exercise training.[24] The results of a recent study with *mdx* mice completing repeated sessions of stimulated isometric tetanic contractions demonstrated improved skeletal muscle strength and histopathology without deleterious effects.[24] This study suggests efficacy for isometric exercise by males with DMD to increase strength without muscle injury.

Studies have shown that dystrophin-deficient muscle fibers of the *mdx* mouse have increased susceptibility to contraction-induced damage to the contractile and cytoskeletal components of the muscle fibers during lengthening contractions. Contraction-induced damage is directly correlated with the magnitude of mechanical stress placed upon the membrane during the eccentric contractions.[8,51] Investigations have shown evidence of impaired excitability at the sarcolemma in the *mdx* mouse muscle as a consequence of performing eccentric contractions.[1] There is also evidence of greater mechanical disruption of the sarcolemma, increased fiber degeneration, and necrosis after eccentric contractions.[8] While muscles with and without dystrophin demonstrate force loss from repeated contractions, only those in the *mdx* mice have decrements in fiber excitability with eccentric contractions (Figure 2.5 & 2.6).[1,25,58] This is shown through decreased EMG M-wave amplitude in dystrophin-deficient *mdx* mouse

muscle, suggesting a distinct difference in the mechanism of force loss from that in normal, wild type mouse muscle.[1,25,58]

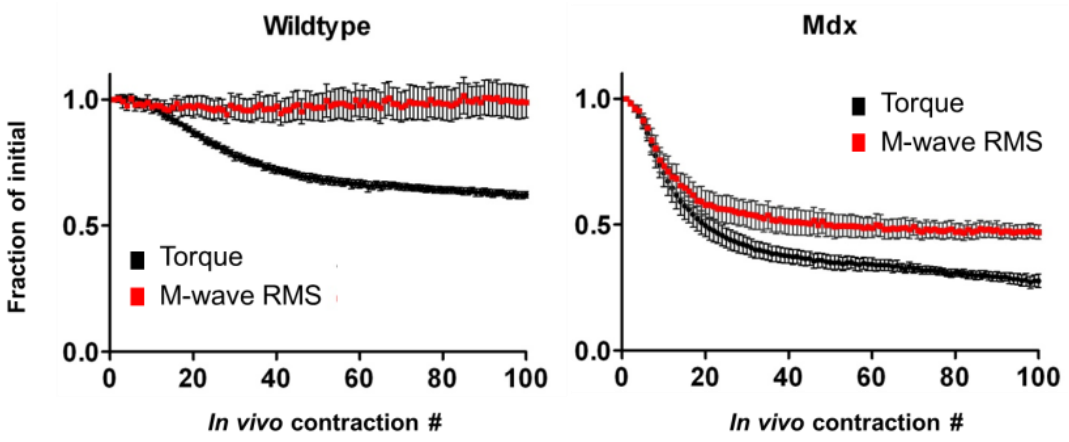

Figure 2.5 Strength (torque) and fiber excitability (M-wave RMS EMG) of tibialis anterior muscles during 100 in vivo eccentric contractions by wildtype and mdx mice.[1]

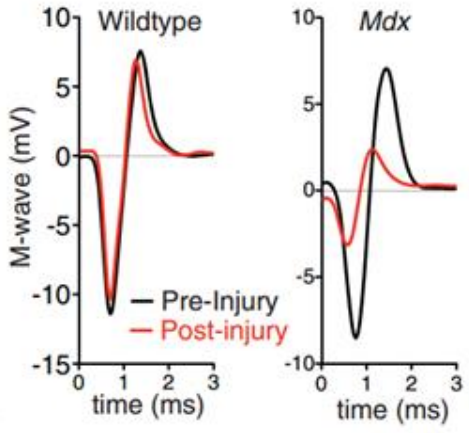

Figure 2.6 Representative M-wave EMG recordings before and after eccentric contractions in wildtype and mdx mice.[1]

Loss of sarcolemma excitability is a transient event associated with eccentric contraction-induced muscle injury in *mdx* mice.[25] Despite dramatic loss in electrophysiological function during and immediately after eccentric contractions, *mdx* mice have the ability to recover sarcolemmal excitability. Full recovery of strength in *mdx* mice is primarily dependent on the restored electrophysiological function of the sarcolemma and other sites downstream.[25] Baumann et al. (2020) demonstrated that the difference in recovery between *mdx* and wild-type mice reflects the difference in injury mechanisms. There is much more to uncover in the mechanisms of strength loss and recovery in *mdx* mice and significant work to evaluate the best way to translate these findings into studies to address exercise risks and benefits in males with DMD.

Exercise research in males with DMD

The safety of exercise and activity for individuals with neuromuscular disease, including males with DMD, has been debated for years.[12,19,100] Extrapolating data from animal studies to humans needs to be done with caution because of biomechanical differences and differences in phenotype expression between human participants and animal models of muscular dystrophy.[8,101] Based on the currently available evidence and clinical experience, international guidelines recommend rehabilitation for boys with DMD to include gentle stretching and active exercises (such as swimming and range of motion) and avoidance of strenuous (resisted or eccentric) exercises.[20,21,42,134] Others emphasize avoidance of any exercise and overexertion due to fear of further exacerbating the disease.[20,21,135] It

has been suggested that exercise with any muscle pain or fatigue production would indicate overexertion and contraction-induced injury and should be avoided.[135] Many factors need to be considered before denouncing or supporting physical activity for males with DMD. Limited research has been done on the type, frequency, and intensity of optimal exercise and physical activity in DMD.[6,8,12,20,21,42,100] There have been comprehensive reviews on exercise for DMD; however, current evidence is limited to a handful of human studies on exercise risks or benefits.[11,12,18,19,128] Little is known about the mechanisms that cause strength loss by dystrophin-deficient muscle in males with DMD during and immediately after repetitive muscle contractions[1,24,58,130] or about the rate and process of recovery.[25]

Early literature on exercise in neuromuscular disease divided patients into groups with multiple muscular dystrophy types and reported mixed results from resistive exercises.[13] One of the first studies that aimed to evaluate the impact of exercise in DMD was a case-controlled study of 4 boys age 4-11.[14] In this study, quadriceps muscles of one leg of each participant underwent a submaximal isokinetic exercise program 4-5 days/week for 6 months while the other leg did not. At the end of the 6 months and at 12, 18 and 24-months post-exercise program, the leg that exercised was able to produce greater maximal torque than the leg that did not exercise. These results suggest that submaximal exercise has no negative effect and a potentially positive effect on muscle strength.[14] A limitation of this work was the small sample size and the need for antigravity quadriceps muscle strength to participate.

Two other studies, one for the lower extremity[15] and one for the upper extremity[136], each compared two different exercise treatment types. The lower extremity study found no differences in strength between at-home manual resistance exercise and at-home active ROM exercise.[15] More significant gains were made in muscle endurance, upper extremity functional performance, and ambulation from exercise with an arm ergometer compared to a home ROM exercise program in the upper extremity study. There was no measurable change in muscle strength with either exercise program or no deleterious effects.

A hallmark study, *No Use is Disuse*, was the first randomized controlled trial in boys with DMD to examine whether exercise in the form of assisted bicycle training was feasible and safe.[16] The results demonstrated that assisted bicycle exercise training preserved motor function and muscle endurance in boys with DMD. No serious adverse events were recorded. There were no measured changes in muscle strength or ROM in either group. The No Use is Disuse study filled in knowledge about the possible effects of exercise in boys with DMD; however, it did not address moderate exercise such as resisted bicycle training. I am aware of a current clinical trial investigating the safety and efficacy of isometric exercise and dosing options in ambulatory boys with DMD, publication pending (NCT02421523). Much is still unknown about exercise prescription, fatigue or strength loss mechanisms, and exercise as an adjuvant to current therapeutics.

The literature makes repeated references to the potential for deleterious effects of exercise. References for exercise avoidance was based on studies in the literature that used eccentric muscle contractions to produce muscle injury in the

mdx mouse. [6,8,12,18,20,21,42,100,134,136] One human case study reports episodes of myoglobinuria related to physical exercise in two boys with DMD[135]; however, another reported no changes in MRI of the lower extremity when stepping exercises were performed four days before imaging. None of the human literature has reported severe adverse events thus far related to submaximal exercise training.

The lack of translational research investigating strength loss and the mechanisms contributing to the strength loss immediately after various exercise types in individuals with Duchenne muscular dystrophy supports physical inactivity. Little is known about the recovery of strength loss, which also adds to concerns around exercise prescription. As rapid advances are made in gene and cell therapies, and muscle pathology improves in males diagnosed with DMD, understanding of the physiology of transient strength loss due to muscle contractions will be needed to optimize the impact of these therapies with adjuvant exercise prescription.[11]

2.5 Conclusions and Aims of Thesis Work

Physical activity and exercise are essential for a healthy lifestyle. Boys with DMD are instructed to limit exercise because of a fear of further muscle damage and weakness. Limiting activity and exercise contribute to deconditioning and other sequelae due to physical inactivity. However, we do not know if exercise will benefit the boys or cause harm. To decide, “is exercise good medicine for boys with DMD, and if so, what type?” we first need to understand more about the response of dystrophin-deficient skeletal muscle to controlled, quantifiable, repeated muscle contractions. Three questions raised were: Is strength loss the same in males with DMD as in aged-matched control? Does the loss of strength occur because of loss of muscle excitability? Does dystrophic muscle recover after repeated isometric submaximal contractions? This work aimed to address these questions.

Aim 1: Before assessing mechanisms of exercise-induced strength loss, a standardized, repeatable exercise and measurement protocol needed to be designed. My first objective was to establish method feasibility, safety, reliability, discriminative validity for a combined protocol of maximal voluntary contractions and evoked muscle contractions from peripheral magnetic stimulation using EMG and a customized upper extremity strength measurement apparatus to measure torque and muscle fiber excitability of the wrist extensor muscles.

Hypothesis 1: Young men with DMD will be able to participate and perform a protocol of voluntary and evoked muscle strength and excitability measurements without adverse events or loss of strength

Hypothesis 2: The measurement protocol will be reliable within visit and between visits differentiating between controls and males with DMD

Aim 2: Evaluate the effect of repeated contractions on muscle excitability and strength loss in males with DMD

Hypothesis: Males with DMD will not differ from age-matched controls in exercise duration or strength loss.

Hypothesis: Males with DMD will have a greater loss of muscle excitability corresponding to a loss of torque from repeated muscle contractions than age matched controls during a bout of exercise

Aim 3: The final aim was to explore and compare short-term recovery of strength and muscle excitability after contraction-induced torque loss for males with DMD and aged-matched controls.

Hypothesis: Males with DMD will recover from an intermittent submaximal isometric exercise protocol in a shorter time than controls, as demonstrated by transient strength loss.

This thesis work begins to translate *mdx* mouse research on dystrophic muscle response to contraction-induced strength loss in males with DMD. It contributes to a greater understanding of how dystrophic muscle in males with DMD responds to and recovers from exercise. As therapeutics are created, novel interventions of exercise and therapy will need to be initiated to optimize the interventions. Methods to measure and monitor the response to therapeutics and

exercise are also needed for greater inclusion in clinical trials by males with DMD of all ages.

Chapter 3: Feasibility, safety, reliability, and validity of a voluntary and magnetically evoked muscle contraction protocol in males with Duchenne muscular dystrophy

The contents of this chapter have been accepted pending revisions to *Muscle & Nerve* (October 2020).

Joyce P. Trost, Mo Chen, Molly M. Stark, James S. Hodges, Sara Richter, Angus Lindsay, Gordon L. Warren, Dawn A. Lowe, Teresa J. Kimberley

Overview

Clinical trials addressing Duchenne Muscular Dystrophy (DMD) treatments require reliable and valid measurement protocols of muscle contractile function across all disease levels. This work aimed to evaluate a novel protocol, combining voluntary and evoked contractions to measure strength and excitability of wrist extensor muscles for safety, feasibility, reliability and discriminant validity between males with DMD and controls. Wrist extensor muscle strength and excitability were assessed in males with DMD (N=10; mean 15.4 [SD 5.9] years) (Brooke 1-6) and age-matched healthy male controls (N=15; mean 15.5 [SD 5.0] years). Torque and EMG measurements were analyzed under maximum voluntary and stimulated conditions at two visits. A protocol of multiple maximal voluntary contractions (MVC) and evoked twitch contractions was feasible and safe, with 96% of the participants completing the protocol and >93% strength maintained both for DMD and control males ($P \geq 0.074$). Reliability was excellent for voluntary and evoked torque measurements and EMG (ICC>0.90 and >0.85 within and between visits, respectively). Torque, EMG and timing of twitch onset measurements discriminated between DMD and controls ($P < 0.001$). Twitch contraction time did not differ significantly between groups ($P = 0.10$). This study evaluated a protocol that can be used to measure skeletal muscle function in clinical trials in males with DMD across a range of ages and disease levels. A protocol of multiple MVCs and evoked contractions is a safe, feasible, reliable and valid method to measure changes in strength and excitability of wrist extensors in DMD.

Introduction

Duchenne muscular dystrophy (DMD) is an x-linked recessive neuromuscular disorder affecting 1 in 3600-6000 male births.[7] DMD is caused by mutations in the *DMD* gene encoding dystrophin.[137] The resulting deficiency of dystrophin in skeletal muscle is linked to, among other things, decreased muscle torque generating capacity and reduced muscle excitability.[20,138-141] These decrements are progressive with overall muscle weakness beginning as early as age two, advancing to teenage boys typically requiring a wheelchair for community mobility. During their lifespan, males with DMD undergo many clinical treatments that may include extensive use of corticosteroids and other pharmaceuticals, physical therapy, and orthopaedic surgery to attempt to slow their functional decline, improve health, and preserve quality of life.[20,142]

Clinical trials are being designed to test interventions to increase dystrophin production, reduce muscle pathology, improve muscle function, and evaluate the benefits of exercise.[12,20] Exciting developments in therapeutics create a pressing need for broad participant inclusion across the DMD population, including those with limited mobility.[11,27,97,98,101] Thus, methods are needed for measuring muscle performance in patients with DMD at all levels of disease status.[99] Typical approaches to measure skeletal muscle function, and severity of disease are manual muscle testing[105], hand-held dynamometry[106], timed activities,[108,109] performance-based assessments[113] and patient-reported scales.[118-120] These outcome measures have value and shortcomings in assessing treatment efficacy. They require volitional effort, maybe insensitive to small changes in strength or

performance, cannot be used across all mobility levels, and do not provide insight into the physiology underlying muscle strength loss.[98,107,124,143,144] Adding evoked contractions to an assessment protocol of muscle function may address these shortcomings and open up clinical trial participation to individuals of all disease levels. Evoked contractions add the ability to measure twitch torque, electromechanical delay (i.e., the time lag between stimulation and onset of twitch torque), twitch contraction time (i.e., the time from twitch torque onset to peak twitch torque)[144,145], and M-wave amplitude[145], which cannot be measured with voluntary contractions.

Skeletal muscle of *mdx* mice (an animal model for DMD) is typically tested with electrically-evoked contractions and can include simultaneous electromyography (EMG) to evaluate mechanisms of contraction-induced torque loss of muscle function in response to treatments.[1,25,58,146] Electrical stimulation has been used for research in healthy adults to assess muscle function. However, it has not been adopted for routine clinical assessment and may not be appropriate for young participants due to discomfort, especially in protocols that require repeated stimulations.[27] In contrast, peripheral magnetic nerve stimulation (PMNS) has been used in studies of healthy adults and adults with various conditions because it can evoke complete nerve trunk stimulation while possibly inducing less painful stimuli in the skin.[27,29,54,75,88,90] Using PMNS to elicit muscle contraction has not yet been examined in children or young adults with neuromuscular disease. This study's purpose was to determine if young males with DMD could complete a combined protocol of repetitive maximal voluntary and PMNS-evoked measurements of wrist

extensor muscle function safely. The study also evaluated the reliability and validity of the protocol to measure muscle strength, excitability and timing of torque production. This type of protocol may provide an alternative or supplemental approach for assessing skeletal muscle contractile function in clinical trials evaluating new treatments for DMD.

Methods

Study population

Healthy control males and males with DMD between 8 and 25 years old were recruited at the University of Minnesota and Gillette Children's Specialty Healthcare using e-mail and flyers. Control participants could not have a neuromuscular disorder or any upper extremity mobility limitations. DMD participants had to have a DNA-established diagnosis of DMD with no other neuromuscular diagnosis. Participants could not have any implanted medical devices and had to obtain a neutral wrist extension position and perform a 3-sec contraction of the wrist extensor muscles. All participants were asked not to consume caffeine on the day of the assessment. The study was approved by the Institutional Review Board of the University of Minnesota. Before participation, written informed consent was obtained from parents for those under 18 years of age with assent obtained from the participant. Males 18 years and over provided their consent.

Study Design

The study used a repeated measures design with two groups and two visits. For each of the two visits, familiarization occurred and involved introducing equipment, determining optimal magnetic coil placement, and conducting maximal voluntary contractions (MVCs) of the wrist extensors. The first visit (Visit One) included baseline (*Baseline*$_1$) and re-test (*Re-Test*) measurements. The second visit (Visit Two) also included baseline (*Baseline*$_2$) measurements. During each of *Baseline*$_1$, *Re-Test* and *Baseline*$_2$, three MVCs were performed 1 min apart,

followed by stimulation to evoke a twitch. Baseline and re-test values were the averages of three measurements within that phase. Ten minutes of rest separated *Baseline$_1$* from *Re-Test* during Visit One.

During Visit One, height, weight, caffeine consumption, dominant arm, and medications were recorded. Additionally, for participants with DMD, genetic test results were extracted from medical records and ambulation status was recorded. Each participant with DMD was classified based on his arm function using the Brooke Upper Extremity Functional Rating Scale (Brooke) and overall disease severity using the Egen Klassification Version 2 (EK2) scale. Brooke scores range from 0 (full function) to 6 (no use of hands).[38,116] EK2 scale scores range from 0 to 51, with higher scores associated with lower function.[118,119,147]

Familiarization and set-up

Participants touched and interacted with the EMG electrodes, force transducer, and the magnetic stimulation coil for familiarization. Response of the investigator's arm muscles to the PMNS was observed, and participants experienced the magnetic stimulator at 65-100% intensity on the arm that was not being assessed. Ear protection was offered to participants to dampen the snapping sound of the magnetic stimulator.

Participants were seated in an adjustable chair or their wheelchair. Their non-dominant arm was positioned in a custom-built device with the shoulder abducted 45 degrees, the elbow flexed to 90 degrees and the forearm pronated, so the wrist was in a neutral position (Figure 3.1). The hand was positioned directly under a contoured, padded 7 cm block that housed a force transducer with the edge

of the block aligned with the wrist joint axis of rotation. A Velcro® stabilizing strap was placed proximal to the head of the ulna to secure the forearm to the device (Figure 3.1).

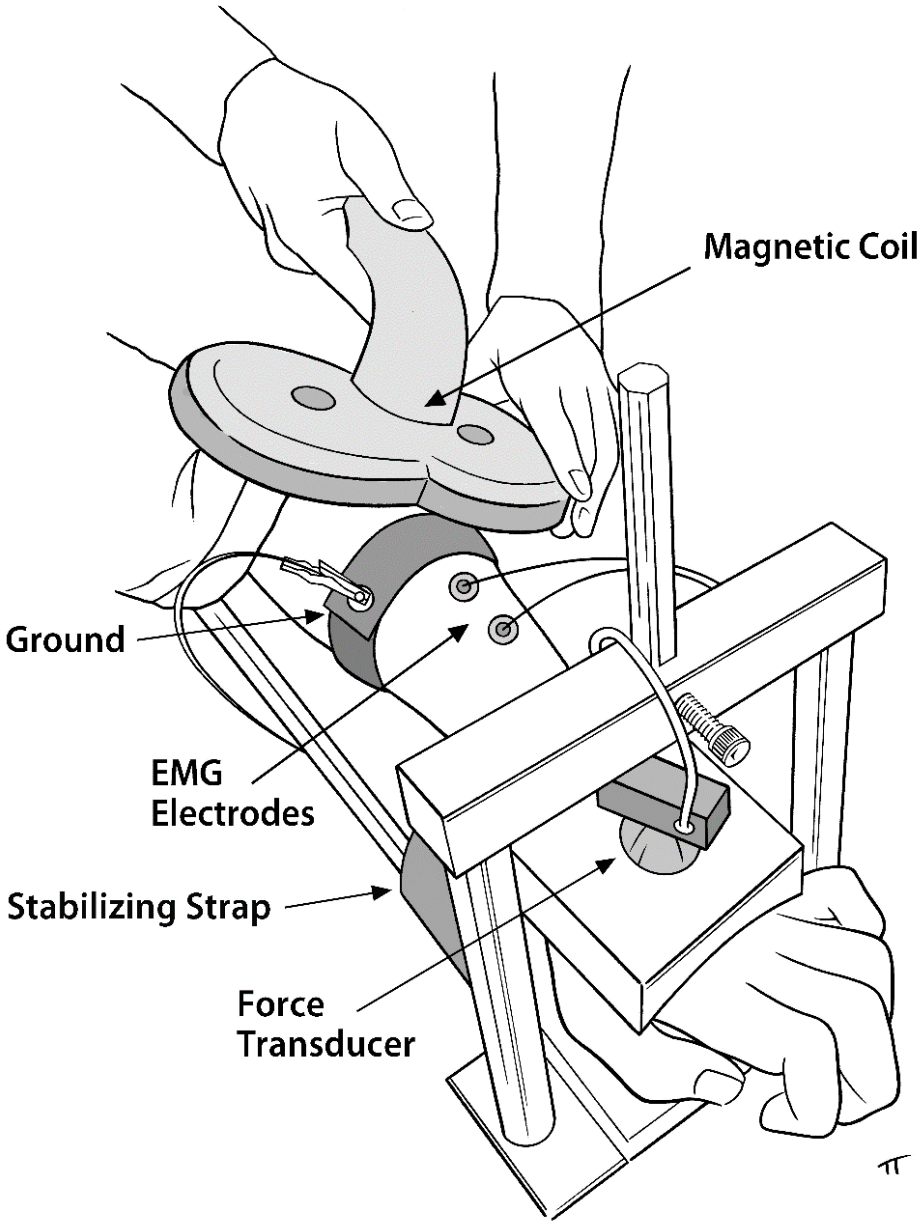

Figure 3.1 Upper extremity measurement device showing peripheral magnetic stimulation coil, force transducer, placement of EMG ground and electrodes and Velco® stabilizing strap.

The magnetic coil was placed over the dorsal aspect of the forearm just below the elbow at the region of the radial nerve (Figure 3.1). Five to ten evoked contractions were completed with incremental adjustments made between contractions to find the position that produced wrist extensors maximal twitch torque. Three to five practice MVCs one minute apart were also performed to estimate maximal torque used for setting the computer visual feedback for *Baseline* and *Re-Test* measurements.

EMG recording

The forearm skin was cleaned using alcohol pads, and arm length was measured from the olecranon to the distal end of the styloid process. A ground electrode strap (TD-431, Discount Disposables; St. Albans, VT) was placed 1/3 the distance from the olecranon to the styloid (Figure 3.1). A pair of stainless steel disc surface EMG electrodes (302139-200, Cadwell Inc.; Kennewick, WA) with an inter-electrode distance of 2 cm was taped distal to the ground electrode over the muscle belly of the extensor digitorum longus muscle; the center of the electrode pair was 2.5 cm distal to the ground electrode (Figure 3.1). EMG signals were amplified by an amplifier with a gain of x300 (Motion Lab Systems, Inc.; Baton Rouge, LA) and passed through a bandpass filter (10Hz-2000Hz). EMG signal quality was assessed by checking the resting baseline noise level, which was deemed acceptable if lower than ±10 μV. If an acceptable noise level was not achieved, the forearm was shaved, and the procedure was repeated. Electrodes were further secured with self-adherent Coban™ wrap (3M, St. Paul, MN) to minimize motion artifact.

Force recording

Force was measured with a transducer (MLP-50, Transducer Techniques LLC; Temecula, CA) and amplified using a bridge amplifier (Model 544, Therapeutics Unlimited Inc.; Iowa City, IA). Both EMG and force were recorded using an NI 9234 24-bit A/D converter (National Instruments Corporation; Austin, TX) at a sampling rate of 6.4 kHz, with data acquisition via a program developed in the LabVIEW software package environment (National Instruments Corporation). All wrist extensor strength measurements were completed under isometric conditions. Participants were instructed to increase effort from rest to maximum voluntary effort over 2 s, with the maximal effort held for 3 s.[148] Strong verbal encouragement and visual computer feedback including a goal of 110% of initial MVC was provided for all maximal contractions. Participants were coached throughout the protocol not to use shoulder motion or other compensatory muscle activity of the hand to produce MVC.

Magnetic stimulation

PMNS was applied using a D70^2 figure-of-eight magnetic coil (Figure 3.1) connected to Magstim Bistim and 200^2 magnetic stimulator set (Magstim Co. Ltd; Whitland, UK). Paired pulse stimulations were delivered at 100% stimulator output, with 10 ms inter-stimulus interval.

Safety

Adverse events were collected, and percent strength loss was calculated from *Baseline$_1$* and *Re-test* torque measurements to assess protocol safety. Participants were also asked how tired or sore their wrist and arm muscles were

after Visit One to assess the impact of performing a protocol of repeated MVCs and evoked twitches. A 10-point ordinal scale was used to quantify self-reported “how tired are your arm muscles?” at the end of Visit One (0 = not at all, 10 = extreme tiredness). Delayed impact of the assessment protocol on arm muscle soreness was collected by self-report the day after the visit via phone interview asking the participant to rate “how sore are your arm muscles?” (0 = not at all, 10 = extreme soreness).

Data Processing

Outcome measures describing muscle function, including contractile characteristics of voluntary muscle torque and excitability and evoked torque and excitability, were processed using Matlab (v2016b, Mathworks, Natick MA). Torque estimates were calculated from force output using the equation $\tau = F \times k$, where k is a constant, the distance from the estimated wrist joint axis to the midpoint of the force transducer. Maximal torque during MVC was calculated as the greatest average torque during a 250 ms moving average in a trial (Figure 3.2 A).

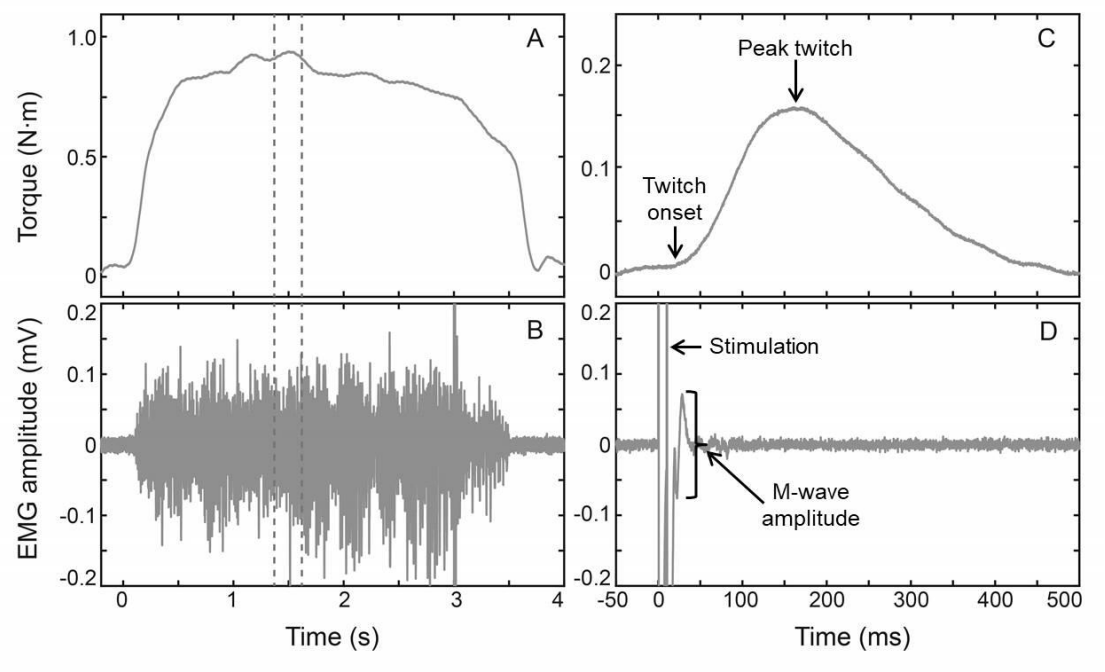

Figure 3.2 (A) Representative tracings of torque from the MVC (A) and (B) simultaneous EMG (B) and (C) evoked twitch torque (C) and (D) simultaneous M-wave (D) from one participant with DMD. Dashed vertical lines in A&B represent the 250ms time interval for MVC and RMS data extraction. Time to twitch onset is calculated as the time between Stimulation and Twitch onset; Twitch contraction time is calculated as the time between Twitch onset and Peak twitch (C). M-wave amplitude peaks are identified (D).

The amplitude of the EMG signal during the chosen 250 ms window of MVC was calculated using the root-mean-square (RMS) (Figure 3.2 B). Twitch torque was measured as the peak torque occurring within 200 ms after stimulation (Figure 3.2 C). Time to twitch onset was measured as the time from stimulation to twitch onset, and twitch contraction time was time from twitch onset to peak twitch torque (Figure 3.2 C). EMG M-wave amplitude peaks (M-wave) were manually identified between 13 and 30 ms after stimulation and the peak-to-peak amplitude recorded (Figure 3.2 D).

Intraclass correlation coefficients (ICC) with 95% confidence intervals were calculated for each variable within Visit One and between Visit One and Visit Two from an averaged measurement, absolute agreement, two-way random effects model to assess test-retest reliability.[149,150] Reliability of the protocol measurements was classified as excellent (ICC > 0.90), good (0.75<ICC≤0.90) or moderate (0.50<ICC≤0.75). [151] Bland-Altman plots were used to show agreement between the two measurements of strength and also between the two measurements of excitability through plotting the average of two measures against the difference of two measures. [152-154] To display plots for both groups on the same scale, measures were standardized to z-scores $Z = (value - mean)/SD)$.

The standard error of measurement (SEM), expressed in the original units of measurement to improve interpretability, was used to describe the observed error between trials in the repeated measurements ($SEM = SD \times \sqrt{1 - ICC}$). Spearman's rank correlation coefficients were used to investigate the relationships of the protocol measurements with age, Brooke, and EK2 scale. A priori interpretations for correlations were: < 0.30 poor, 0.30 - 0.59 fair, 0.60 - 0.79 strong, and 0.80 - 0.99 very strong.[155] A two-sample t-test was used to assess the ability of protocol measurements to differentiate DMD participants from control. Analyses were done using SAS version 9.4 (SAS Institute Inc.; Cary, NC) with ICCs computed in IBM SPSS Statistics for Windows, version 22 (IBM Corp.; Armonk, NY). P-values less than 0.05 were considered statistically significant.

Results

Participant Characteristics. Fourteen young males with DMD were identified who met the study inclusion criteria, and ten were enrolled. Four chose not to participate due to scheduling and travel constraints. Fifteen age-matched control participants were also recruited and enrolled. Table 3.1 shows participant characteristics. Participants with DMD were heterogeneous for disease severity, ambulatory status and corticosteroid usage (Table 3.2). They and had a median Brooke score of 2 (range 1 to 6) and median EK2 score of 8.5 (range 0 to 32) (Table 3.2).

Table 3.1 Participant demographics

Demographics	Controls (n=14)	DMD (n=10)	P-value
Age (yr)	15.9 (5.0)	15.4 (5.9)	0.950
Height (cm)	168.4 (23.8)	143.9 (14.1)	0.005
Weight (kg)	60.8 (22.4)	45.2 (14.9)	0.075

Values are means (SD); DMD, Duchenne muscular dystrophy; P-value calculated using a two-sample t-test.

Table 3.2 Detailed Duchenne participant demographics

Participant	Age	Genetic mutation	Ambulatory Status	FVC	FEV1/FVC ratio	EK2	Brooke	Steroids
1	11.3	Exon 50 deletion	Y	2	98	2	1	Deflazacort
2	10.7	Exon 55 deletion	Y	1.51	67	5	1	Prednisone
3	11.9	Exon 55 deletion	N	1.83	68	7	2	None
4	13.5	duplication of A within exon 40	N	1.38	79	10	2	Deflazacort
5	18.4	Exon 46-60 deletion	N	2.59	62	13	3	Deflazacort
6	8.9	Exon 18-25 deletion	Y	2.05	115	0	1	Deflazacort
7	22.4	Exon 3-9 deletion	N	0.97	21	32	6	None
8	10.1	Exon 43 deletion	Y	2.05	111	0	1	Deflazacort
9	25.5	premature stop at exon 5	N	1.94	48	16	4	Deflazacort
10	21.1	Exon 44 deletion	N	2.4	105	17	5	None

Age, age at first visit; FVC, forced vital capacity; FEV1/FVC, forced expiratory volume in one second divided by forced vital capacity; EK2, Egen Klassification scale version 2, Brooke, Brooke Upper extremity scale.

Safety and Feasibility

All 10 participants with DMD completed both visits. One adverse event (fainting) occurred in the control group during familiarization in Visit One. This participant withdrew from the study, which resulted in fourteen controls completing both visits. All remaining participants followed directions to complete repeated MVCs and tolerate 100% stimulator power output for evoked contractions. Percent MVC torque decrement (mean (SD)) from *Baseline*$_1$ to *Re-test* in Visit One was not significant for either group; DMD 1 (18) % (P=0.77) and control 6 (11) % (p=0.074). Evoked twitch torque also did not decrease from *Baseline*$_1$ to *Re-test* with decrements of 5 (50) % for DMD (P=0.084) and 7 (14) % for controls (P=0.15). Self-reported arm muscle tiredness was minimal with the median 0 (range 0-1) for the DMD group and median 1 (range 0-2) for the control group 1 (range 0-2), with DMD significantly less than controls (P=0.028). There was no self-reported arm muscle soreness the next day for the DMD group and minimal soreness in the control group (median 0 with range 0-3) and no difference between groups (P=0.24). Our protocol of combined voluntary and evoked muscle function measurements was deemed safe and feasible in these populations.

Reliability

Measurements were stable within and across visits. Measurement means did not change systematically between *Baseline*$_1$ and *Re-Test* within Visit One (Table 3.3) or across visits between *Baseline*$_1$ and *Baseline*$_2$ (Table 3.4). These results indicate that the protocol did not impact muscle torque and EMG measurement within a visit (e.g., due to muscle fatigue). The protocol was reliable

between visits (mean of 32 days between visits for DMD). ICC was >0.91 for all measures of torque and EMG, demonstrating excellent reliability for Visit One within each group (Table 3.3). Across visits, ICC was >0.85, indicating good to excellent reliability within each group (Table 3.4). SEM values are smaller for the DMD group than controls for both within and across visits as expected because of the smaller measurements in the DMD group (Tables 3.3 and 3.4).

Table 3.3 Descriptive and reliability values within Visit One

Assessments	$Baseline_1$	Re-Test	Mean Diff (SD)	P-value	ICC (95% CI)	SEM
Control n=14						
MVC (N·m)	3.50 (1.69)	3.23 (1.48)	-0.27 (1.05)	0.074	0.99 (0.97-0.99)	0.49
RMS (mV)	0.39 (0.12)	0.41 (0.13)	0.02 (0.06)	0.235	0.96 (0.91-0.99)	0.03
Evoked twitch (N·m)	0.78 (0.48)	0.70 (0.39)	-0.07 (0.14)	0.084	0.99 (0.98-0.99)	0.10
M-wave (mV)	3.23 (1.66)	3.10 (1.76)	-0.27 (0.52)	0.128	0.98 (0.95-0.99)	0.21
Time to twitch onset (ms)	22.9 (1.5)	22.9 (1.8)	0.1 (1.0)	0.838	0.91 (0.80-0.97)	0.6
Twitch contraction time (ms)	110.5 (5.0)	109.6 (4.4)	-0.9 (3.6)	0.363	0.94 (0.86-0.98)	1.2
DMD n=10						
MVC (N·m)	0.69 (0.32)	0.68 (0.29)	-0.01 (0.09)	0.772	0.96 (0.91-0.99)	0.16
RMS (mV)	0.07 (0.04)	0.07 (0.03)	-0.002 (0.007)	0.385	0.98 (0.95-0.99)	0.005
Evoked twitch (N·m)	0.12 (0.12)	0.08 (0.06)	-0.04 (0.08)	0.145	0.94 (0.84-0.98)	0.06
M-wave (mV)	0.62 (0.30)	0.48 (0.34)	-0.06 (0.17)	0.402	0.98 (0.94-0.99)	0.05
Time to twitch onset (ms)	37.7 (10.7)	38.1 (8.2)	0.4 (7.7)	0.867	0.91 (0.76-0.98)	3.3
Twitch contraction time (ms)	123.5 (43.7)	112.5 (25.5)	-11.0 (23.2)	0.168	0.97 (0.92-0.99)	6.5

Values are means (SD); RMS is EMG from MVC; M-wave is EMG from evoked twitch; $Baseline_1$ = Mean Visit One (trials 1-3); Re-Test = Mean Visit One (trials 4-6); Mean Diff, Changes in the mean for each participant are aggregated and expressed here as mean (SD); ICC, Intraclass Correlation Coefficient; CI, Confidence Interval; SEM, Standard error of measure; P-value calculated using a paired t-test.

Table 3.4 Descriptive and reliability values between Visit One and Visit Two

Assessments	$Baseline_1$	Re-Test	Mean Diff (SD)	P-value	ICC (95% CI)	SEM
Control n=14						
MVC (N·m)	3.50 (1.69)	3.62 (1.36)	0.05 (1.04)	0.877	0.97 (0.94-0.99)	0.64
RMS (mV)	0.39 (0.12)	0.46 (0.12)	0.07 (0.13)	0.084	0.88 (0.75-0.96)	0.04
Evoked twitch (N·m)	0.78 (0.48)	0.78 (0.45)	0.05 (0.23)	0.468	0.99 (0.97-0.99)	0.14
M-wave (mV)	3.23 (1.66)	2.75 (1.79)	-0.21 (1.62)	0.688	0.97 (0.90-0.99)	0.31
Time to twitch onset (ms)	22.9 (1.5)	22.5 (1.0)	-0.3 (1.0)	0.260	0.85 (0.68-0.95)	0.6
Twitch contraction time (ms)	110.5 (5.0)	110.9 (5.4)	0.5 (4.8)	0.699	0.93 (0.84-0.97)	1.5
DMD n=10						
MVC (N·m)	0.69 (0.32)	0.79 (0.25)	0.11 (0.32)	0.331	0.87 (0.70-0.97)	0.29
RMS (mV)	0.07 (0.04)	0.08 (0.06)	0.0062 (0.025)	0.461	0.97 (0.92-0.99)	0.008
Evoked twitch (N·m)	0.12 (0.12)	0.08 (0.06)	-0.04 (0.07)	0.105	0.94 (0.86-0.99)	0.06
M-wave (mV)	0.62 (0.30)	0.57 (0.44)	0.028 (0.43)	0.861	0.90 (0.72-0.98)	0.13
Time to twitch onset (ms)	37.7 (10.7)	42.6 (13.7)	4.9 (14.6)	0.319	0.85 (0.60-0.96)	5.4
Twitch contraction time (ms)	123.5 (43.7)	108.8 (27.3)	14.65 (30.7)	0.166	0.95 (0.87-0.97)	8.7

Values are means (SD); RMS is EMG from MVC; M-wave is EMG from evoked twitch; $Baseline_1$ = Mean Visit One (trials 1-3); Re-Test = Mean Visit One (trials 4-6); Mean Diff, Changes in the mean for each participant are aggregated and expressed here as mean (SD); ICC, Intraclass Correlation Coefficient; CI, Confidence Interval; SEM, Standard error of measure; P-value calculated using a paired t-test.

Validity

Measures of strength and excitability from the voluntary and evoked contractions were 80-85% lower in the DMD than the control group during *Baseline*$_1$ (P<0.001) (Table 3.5). These findings support what is referred to as discriminant validity. Time of twitch onset was delayed by 65% in the DMD group compared to the control group (P<0.001), whereas the twitch contraction time did not differ significantly between groups (P=0.10; Table 3.5).

Table 3.5 Discriminant Validity

	Control	DMD	P-value
MVC (N·m)	3.50 (1.69)	0.69 (0.32)	<0.001
RMS (mV)	0.39 (0.12)	0.07 (0.04)	<0.001
Evoked twitch (N·m)	0.78 (0.48)	0.12 (0.12)	<0.001
M-wave (mV)	3.23 (1.66)	0.62 (0.30)	<0.001
Time to twitch onset (ms)	22.9 (1.5)	37.7 (10.7)	<0.001
Twitch contraction time (ms)	110.5 (5.0)	123.5 (43.7)	0.103

Values are means (SD) from Baseline$_1$; P-value is a two-sample t-test

Torque from MVC and evoked twitch were significantly associated with each other and age in the control group (Table 3.6). Evoked twitch torque and time of twitch onset were significantly associated with activity scales and age in the DMD group. Correlations were poor between MVC and Brooke, EK2 and age in the DMD group. Torque from MVC and evoked twitch were poorly correlated in the DMD group (Table 3.6).

Table 3.6 Spearman's correlation between evoked and voluntary measures of torque and EMG within groups

DMD (Top→) Control (Bottom↓)	Brooke*	EK2*	Age*	MVC	RMS	Evoked Twitch	M-wave	Time to twitch onset	Twitch contraction time
Brooke*		0.97	0.93	-0.33	-0.56	-0.78	-0.15	0.60	-0.09
		(<0.001)	(<0.001)	(0.348)	(0.094)	(0.013)	(0.719)	(0.082)	(0.824)
EK2*			0.95	-0.274	-0.657	-0.83	-0.31	0.71	-0.16
			(<0.001)	(0.444)	0.039	(0.006)	(0.453)	(0.032)	(0.683)
Age*				-0.12	-0.588	-0.85	-0.24	0.73	-0.38
				(0.751)	(0.074)	(0.004)	(0.570)	(0.025)	(0.309)
MVC			0.85		0.44	0.13	-0.09	-0.04	-0.28
			(<0.001)		(<0.001)	(0.240)	(0.474)	(0.706)	(0.002)
RMS			0.37	0.42		0.56	0.38	-0.41	-0.20
			(0.197)	(<0.001)		(<0.001)	(0.001)	(<0.001)	(0.030)
Evoked twitch			0.87	0.90	0.32		0.50	-0.72	0.32
			(<0.001)	(<0.001)	(0.001)		(<0.001)	(<0.001)	(0.001)
M-wave			0.27	0.34	0.57	0.30		-0.28	0.21
			(0.446)	(0.001)	(<0.001)	(0.004)		(0.017)	(0.043)
Time to twitch onset			-0.55	-0.51	-0.40	-0.48	-0.14		-0.34
			(0.050)	(<0.001)	(<0.001)	(<0.001)	(0.185)		(<0.001)
Twitch contraction time			0.484 (0.094)	0.45 (<0.001)	0.32 (<0.001)	0.50 (<0.001)	0.23 (0.012)	-0.35 (<0.001)	

*Visit One Trial 1 used for correlation analyses of age, EK2 and Brooke. All others used *Baseline*$_1$, *Re-Test* and Baseline$_2$ values for correlation analysis; No Spearman calculated for controls involving Brooke and EK2 data (grey boxes); Spearman correlation p-value

Graphical presentation of MVC and evoked twitch torques using Bland-Altman plots with 95% confidence intervals show a tighter limit of agreement in the control compared to the DMD group (Figure 3.3 A and B). Plots of RMS and M-wave show good agreement for both DMD and control groups (Figure 3.3 C and D).

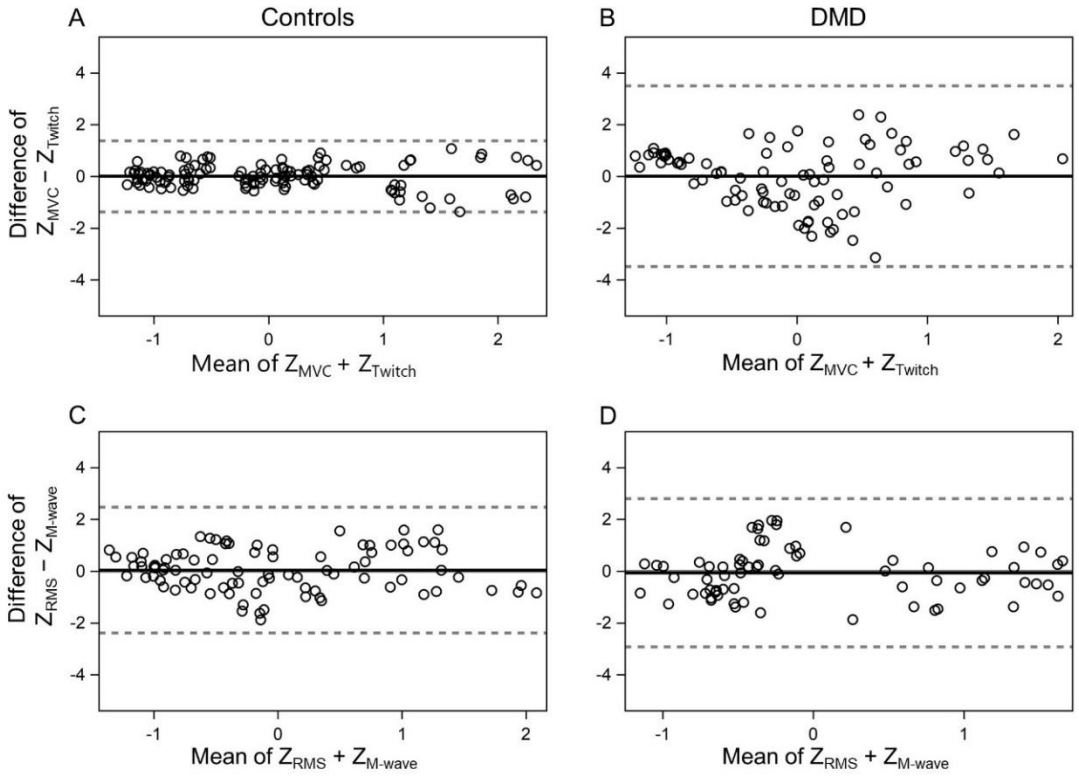

Figure 3.3 Bland–Altman plots of Z-scores of differences vs. mean with 95% limits of agreement between MVC and evoked twitch torques for (A) control and (B) DMD and RMS and M-wave for (C) control and (D) DMD. Data included 3 trials each from Visit One Baseline, Visit One Re-Test and Visit Two Baseline.from Visit One Baseline, Visit One Re-Test and Visit Two Baseline.

Discussion

This study demonstrates a novel, safe, feasible, reliable and valid protocol for evaluating muscle function in males with DMD across a range of ages and disease levels. We report on the potential impact of a combined protocol of MVCs with evoked twitch contractions to measure muscle torque, excitability, time of twitch onset, and twitch contraction time in males with DMD. Our results suggest that measurements of voluntary and evoked contractions of the wrist extensor muscles are reliable across all disease status levels in males with DMD. A combined voluntary and evoked muscle contraction protocol has the potential to expand clinical trial participation for males with DMD across the life span even as the disease progresses and activity levels change.

Our protocol of a combination of maximal voluntary and evoked contractions is feasible to evaluate muscle strength and excitability, as evidenced by 96% of participants completing the protocol. We believe one aspect that contributed to success of the protocol completion was familiarization. All participants interacted with the equipment, practiced performing and holding an MVC and experienced the PMNS before starting the protocol. Younger participants often requested to see how the magnet would evoke contractions and move the wrist of their caregiver or the investigator before experiencing it themselves. The single control participant who could not complete the protocol withdrew due to fainting during familiarization to the magnetic stimulator. We later learned that this participant had a family history of vasosyncopy during medical procedures.

Nominal torque decrements demonstrate evidence of the safety of repeated MVCs and evoked contractions in young males with DMD and age-matched healthy controls during the protocol within a visit and minimal or no reports of tired or sore wrist extensor muscles following the protocol. Males with DMD self-reported less tired arm muscles than controls after completing the protocol and had no complaints of sore muscles the day after the protocol. This may be because the torque produced during the multiple MVCs by the males with DMD was near their typical torque production required for ADLs. Consistency of strength and excitability measurements from *Baseline*$_1$ to *Re-Test* demonstrates protocol safety. The protocol did not cause a systematic decrement in torque or EMG due to the vulnerability of the dystrophin-deficient muscle (Table 3.3). *Baseline*$_1$ and *Baseline*$_2$ measurement means did not significantly decrease between visits indicating that disease progression within one month will not affect this protocol's ability to measure change across visits (Table 3.4).

Correlations of MVC and evoked twitch torque with age were very strong in controls, which is similar to results in quadriceps muscle of healthy adults.[29] These results indicate that voluntary and evoked torque measures in the control group are comparable to the extent that one might replace the other with sufficient accuracy to measure strength. However, for the DMD group, the interpretation is not as straightforward. As expected, evoked torque in the DMD group showed a strong inverse correlation with age and disease (Brooke and EK2). An unanticipated result was the lack of correlations in the DMD group between MVC and age, Brooke or EK2.

Further, Bland-Altman plots graphically displayed a wider interval for DMD males between MVC and evoked twitch torque compared to controls (Figure 3.3). One possible explanation for the lack of MVC correlates that despite verbal cueing, the DMD participants had a more challenging time than controls in eliminating compensatory motions such as shoulder elevation, wrist eversion, and thumb tucking maximal effort. These results suggest that evoked contractions may be superior to MVC for strength measurement in males with DMD because they have difficulty isolating motions while producing a maximal contraction.

Similar protocols have assessed quantitative maximal voluntary muscle torque reliability in healthy children and adults and patients with limb-girdle muscular dystrophy[107], surface EMG of the biceps in DMD, and quadriceps strength and fatigue with evoked contractions in adults.[27,29] However, reliability of a comprehensive protocol including both MVCs and evoked twitches for upper extremity muscle strength and excitability had not been assessed in healthy children or young males with DMD. As measured by ICC in the present study, reliability was excellent for all voluntary and evoked measures within visits for both groups of participants. Reliability across visits for measurements of strength, excitability and timing of twitch was good to excellent.

Importantly, voluntary and evoked strength and excitability measures differentiated males with DMD from controls ($P<0.001$), consistent with previous literature that measured MVC and EMG of multiple UE muscle groups in both DMD and controls.[140] As expected, measures of strength and excitability from the voluntary and evoked contractions were 80-85% lower in the DMD group than in

controls (Table 3.5). Time of twitch onset was 65% longer for DMD participants than controls, whereas twitch contraction time did not differ (Table 3.5). The delayed time of twitch onset is consistent with previous work that measured delay between electrical stimulation and twitch onset of the biceps brachii muscle in males with DMD.[144] Lacourpaille et al. provided evidence that the electromechanical delay was due to prolonged force transmission, which they speculated was due to structural abnormalities of the muscle due to the absence of dystrophin.[144] An alternative or additional explanation is that muscle lacking dystrophin has a compromised sarcolemma and thus slowed propagation of action potentials or impaired excitation-contraction coupling, which have been demonstrated in *mdx* mouse muscle.[25,156] Thus, measurements of twitch onset and twitch contraction time from evoked stimulations has the potential to provide insight into mechanisms underlying muscle weakness and strength loss in patients with DMD.

Muscle excitability, as measured by RMS during the MVC, was in good agreement with M-wave from the evoked twitch (Figure 3.3). RMS had strong negative correlations with Brooke, EK2 and age in the DMD group. However, evoked M-wave in the DMD group was not correlated with Brooke, EK2 or age despite the strong correlation of evoked twitch torque with activity level and age. These results suggest that PMNS targeted to the extensor digitorum longus may have activated motor units in additional wrist extensor muscles. Simultaneously, EMG was collected more specifically from the extensor digitorum longus muscle, indicating a potential limitation of our approach. In the future, this limitation could be mitigated by using PMNS on a larger muscle such as the biceps femoris or

quadriceps or using electrical stimulation, if tolerated, to better isolate the stimulation to a single muscle or muscle group.

Evaluating new therapies for the DMD population requires a measurement protocol that will not induce systematic muscle strength changes, is not limited by motivation or disease level, and is safe and reliable. Our protocol, which combines measurements from maximal voluntary and evoked contractions, was found to fit these criteria and showed discriminant validity for males with DMD and controls.

Conclusion and Next Steps

I designed a novel protocol to address the first objective of this dissertation. Voluntary and evoked contractions to measure strength and excitability of wrist extensor muscles of males with DMD and controls were combined into one protocol. In Chapter 3, I report on the evaluation of the safety, feasibility, and reliability and discriminant validity of the measurement protocol, which was essential to further explore strength loss and muscle fiber inexcitability after submaximal isometric contractions and during recovery in males with DMD. There are no current outcome measures for DMD which quantitatively measure voluntary and evoked strength along with muscle excitability over disease progression or in response to an intervention. Torque and EMG measurements under maximum voluntary and stimulated conditions were feasible and safe, with 96% of the participants completing the assessment protocol with >93% strength maintained both for DMD and controls within a visit.

Establishment of measurement protocol reliability and discriminate validity demonstrated a protocol that is useful for measuring skeletal muscle function in clinical trials in males with DMD across a range of ages and disease levels. Once established, the second part of the study aimed to address the concern of injury to dystrophic deficient skeletal muscle during and after repeated contractions (exercise) that result in acute strength loss. Knowing that hypersensitivity to strength loss from eccentric exercise, due to fibers becoming unexcitable, was a feature of dystrophin-deficient skeletal muscle in the *mdx* mouse model, I wanted to explore how this translated to humans lacking dystrophin. Chapter 4 addresses concern

about exercise by individuals with DMD through a quanitative method of voluntary and evoked measurements. I address our hypotheses that there would be no difference between males with DMD and controls in percent strength loss during exercise and that males with DMD would have more significant impairment in muscle excitability corresponding with a loss of strength than age-matched controls. To accomplish this, males with DMD and a group of age-matched controls performed a sub-maximal voluntary isometric wrist extensor exercise protocol with voluntary and evoked force and EMG measured before, during, and after exercise. Lastly, the recovery of both strength and muscle excitability was explored and compared between groups.

Chapter 4: Inexcitability of the muscle sarcolemma, strength loss and recovery in males with DMD after isometric exercise

The contents of this chapter are under final preparation for submission to *Neuromuscular Disorders*

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Overview

Repeated contractions by skeletal muscle can result in strength loss, which can be concerning in the context of a neuromuscular disease. A feature of dystrophin-deficient skeletal muscle in the *mdx* mouse model is a hypersensitivity to strength loss from eccentric exercise due to fibers becoming unexcitable. It is unknown how this feature translates to humans lacking dystrophin. We evaluated the strength loss and muscle inexcitability of wrist extensor muscles of males with DMD to intermittent submaximal isometric exercise and the recovery from this protocol and made three main findings.

Males with DMD and a group of controls performed a sub-maximal voluntary isometric wrist extensor exercise protocol until 55% of maximal voluntary contraction (MVC) could no longer be sustained. Voluntary and evoked torque and EMG were assessed before, during, and after exercise.

Exercise duration and strength loss measured by MVC and evoked twitch torque did not differ between males with DMD and controls. There was a significant interaction (time*group; $p<0.001$) for MVC torque during exercise, suggesting that the two groups differed in how they reached muscle fatigue. There was no difference in the group main effect ($P=0.133$) with MVC torque decrement, 55% in the DMD group and 45% in the control group at exercise cessation. Evoked twitch torque decreased by 34% for DMD and 36% for control with no group*time interaction observed ($p=0.834$). Second, in contrast to *mdx* and wild type mice undergoing eccentric contractions, muscle inexcitability during and after exercise did not differ between DMD and control groups for RMS EMG decrement ($P=0.986$) or

M-wave decrement (P=0.911). This finding does not support our hypothesis that the DMD group would have a more considerable decline in muscle excitability with fatigue during exercise. Lastly, strength loss was transient, indicative of muscle fatigue, and the recovery of strength was more rapid in males with DMD than in controls. The DMD group recovered evoked torque by 5 minutes (P=0.266) and MVC baseline strength by 10 minutes post-exercise (P=0.530). In contrast, controls were still different from MVC and evoked torque baseline at 15 minutes after exercise (P<0.002).

This study indicates that the muscle excitability decrement during strength loss from the submaximal intermittent isometric exercise does not differ between males with DMD and controls. Strength loss is transient and recovers faster in males with DMD than in controls, suggesting different peripheral fatigue mechanisms in the two groups.

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked, devastating, and ultimately fatal disease affecting 1 in 3600-6000 male births. [7] The disease results from a mutation in the *DMD* gene that encodes a structural protein at the sarcolemma in skeletal muscle that is missing or non-functional.[137] The deficiency of dystrophin in skeletal muscle results in, among other things, progressive muscle weakness, fibrosis, inflammation, fatigue, and necrosis.[20,138-141] Treatments are being developed and tested for males with DMD to increase dystrophin expression[52,157] and produce full-length functional dystrophin protein[158], thereby slowing the decline produced by the disease. As treatments continue to be developed, there is an opportunity for a more active lifestyle for males with DMD, and exercise as an adjuvant to those treatments will be necessary.[11,12] Physical inactivity, deconditioning, and fear of exercise have been common concerns for individuals with DMD. Healthcare providers do not have useful measures or evidence for prescribing and evaluating exercise programs, perpetuating the fear.[9] How frequent, at what intensity and duration, as well as what types of exercise will contribute to improvement in health and function and not exacerbate the progression of the disease or cause muscle damage remain valid and unanswered questions for individuals with DMD. [6]

Few studies have examined the response of males with DMD to exercise[16,19,24,128]; however, animal studies specifically with the *mdx* mouse (the mouse model of DMD) have extensively explored dystrophic muscle responses to exercise.[12] Eccentric contractions are a common approach to study dystrophic

muscle response to treatments in *mdx* mice.[23] Contraction-induced strength loss may be due to alterations in neuromuscular transmission, excitation-contraction coupling, availability of K^+ and Ca^{2+}, blood flow, and the contractile apparatus's impaired performance.[72] Eccentric contraction-induced strength loss in normal skeletal muscle primarily results from impaired Ca^{2+} release from the sarcoplasmic reticulum.[159,160] However, in dystrophic muscle, inexcitability has been indicated as a significant contributor to strength loss after eccentric contractions.[1,25,58,161] Mechanical disruption of action potential propagation along the sarcolemma due to lack of dystrophin stability is plausible as a contributor to strength loss after high intensity, high volume, eccentric contractions in dystrophic muscle.[1,25] Others have suggested that a primary mechanism of strength loss induced through eccentric contractions in *mdx* muscle is a discontinuous motor end-plate of the neuromuscular junction.[146,161] M-wave EMG decrement in *mdx* mice after eccentric contractions was 50-65% with minimal or no decrement measured in wild type mice.[1,25] Interestingly, fiber excitability was restored within 24 hours in *mdx* mice[1,25], and strength was restored faster in *mdx* mice than in wild-type mice during recovery.[59,162]

The primary objective of this study was to translate the preclinical findings from *mdx* mouse research to determine if repeated muscle contractions causing strength loss in males with DMD were primarily due to loss of muscle excitability. Furthermore, we wanted to test whether strength loss, muscle excitability and exercise duration differed in age-matched control participants. A secondary objective was to explore transient strength loss during recovery, i.e., peripheral fatigue through measurement of muscle strength and excitability for males with

DMD. To address these objectives, we first established a protocol to elicit strength loss of the wrist extensors and methods to measure voluntary and evoked torque and EMG during and after an exercise protocol. We chose a submaximal intermittent isometric exercise protocol (subsequently referred to as 'exercise' or 'exercise protocol') with intermittent measurements of voluntary and evoked torque and EMG during the exercise protocol. The protocol allowed simultaneous tracking of decrements in muscle strength and excitability.[29,163] The protocol was designed with submaximal contractions to simulate activities of daily living (ADLs) and exercise activities that may be prescribed for males with DMD. Isometric muscle contractions were used in this initial study instead of concentric or eccentric contractions, for safety.

Methods

Study population

The Institutional Review Board of the University of Minnesota approved the study. Healthy control males and males with DMD were recruited at the University of Minnesota (Minneapolis, MN) and Gillette Children's Specialty Healthcare (St. Paul, Minnesota). Inclusion criteria were males between eight and twenty-five years of age, no implanted medical devices and able to perform a 3-sec contraction of the wrist extensor muscles. Control participants could have no neuromuscular disorder and no upper extremity mobility limitations. DMD participants had to have a DNA-established diagnosis of DMD and be able to obtain a neutral wrist extension. The protocol and equipment were explained to all participants as part of the consent process (Appendix B). For those under 18 years of age, written informed consent was obtained from a parent with assent obtained from the participant (Appendix A). Males 18 years and over provided their consent (Appendix A). All participants were asked not to consume caffeine on the day of the study.

Study Design

Safety and feasibility of the assessment protocol were previously established. (Chapter 3) The study used a repeated measures design with two groups. For participants with DMD, ambulation status, Brooke Upper Extremity Functional Rating Scale (Brooke) [38,116] and the Egen Klassification Version 2 (EK2) scale [118,119,147] were administered. Before data collection, set up and familiarization occurred as described in (Chapter 3). Optimal magnetic coil placement for stimulation of the extensor digitorum longus was determined and marked for the

visit. I assessed supramaximality of the stimulations as well as instructed participants during practice of holding maximal voluntary contractions (MVCs) for 3 sec. The study protocol included baseline, exercise and recovery measurements of voluntary and evoked contractions (Figure 4.1).

Physiological outcome measures of the wrist extensors collected at each time point of interest were: tetanic torque during maximal voluntary contractions (MVC), simultaneous EMG root mean squared (RMS), evoked torque (Twitch), simultaneous EMG M-wave amplitude (M-wave). Torque from three initial MVCs was measured before beginning the baseline measurements. The largest initial MVC torque was used to set targets for visual feedback during the maximal voluntary contractions (110% of MVC) and submaximal contractions (55-65% of MVC). Each submaximal contraction was maintained for 6 sec, followed by 4 sec of rest during the exercise protocol (Figure 4.1). The exercise protocol continued until the participant could not produce 55% of MVC torque during two subsequent MVC measurements. At this point, the exercise was stopped, and recovery began (Figure 4.1). Adverse events were recorded. Self-reported fatigue was collected from participants 15 min after the exercise protocol ended, at the end of the recovery time, by asking them to rate how tired their arm muscle was on a scale from 0-10 using a fatigue visual analog scale.[164] Arm muscle soreness was collected by self-report on the day after the visit via phone interview, asking the participant to rate "how sore are your arm muscles?" (0 = not at all, 10 = extreme soreness", to quantify delayed impact of the exercise protocol.

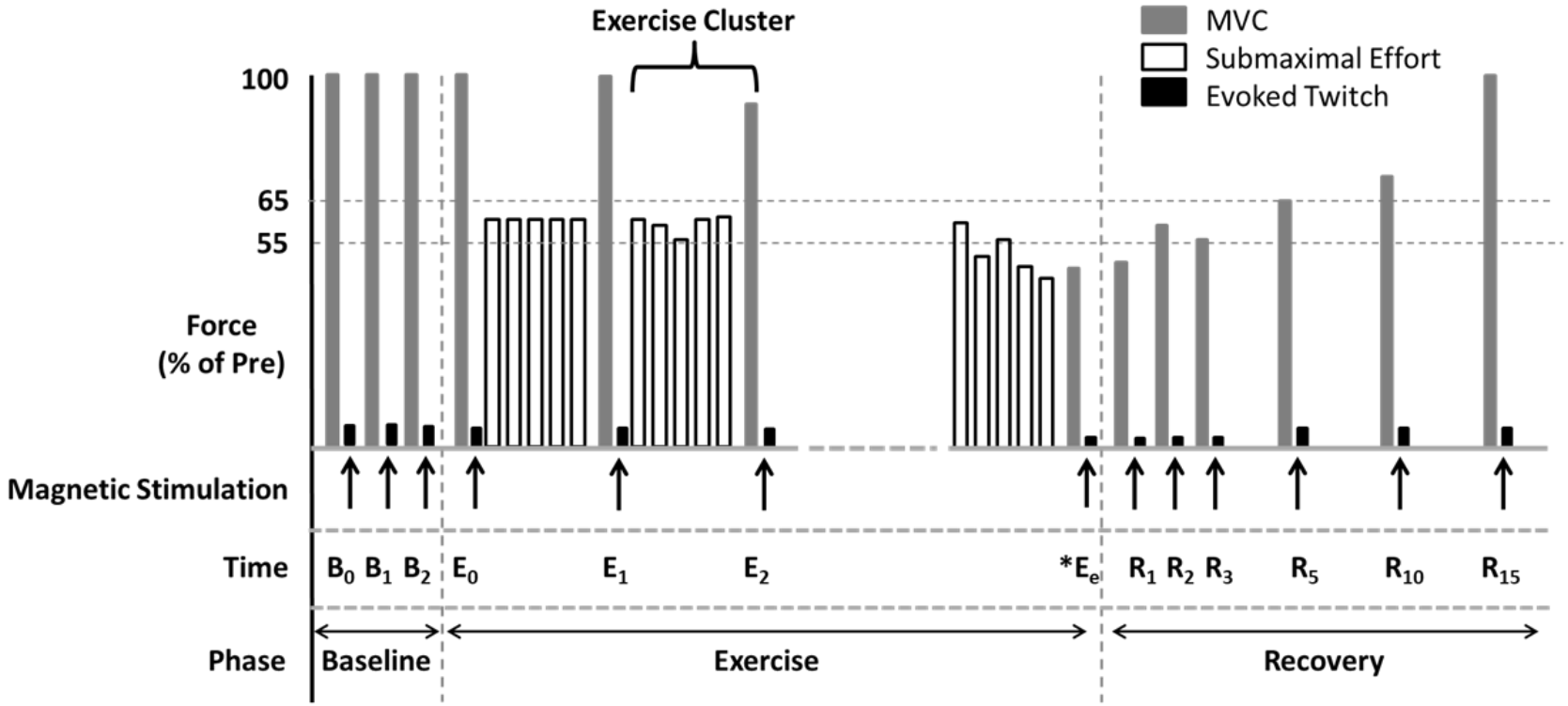


Figure 4.1 Overview of the experimental protocol. Subscripts denote minutes; B_n, baseline measurements; E_n, exercise measurements; R_n, recovery measurements after exercise cessation. Exercise cluster, five submaximal contractions followed by an MVC and evoked twitch. Time and force are not to scale in the schematic

Procedures and Measures

EMG and force data were collected as previously described, which will be briefly summarized here (Chapter 3). Surface EMG was collected with stainless steel disc surface EMG electrodes (302139-200, Cadwell Inc.; Kennewick, WA, USA) with an inter-electrode distance of 2 cm positioned over the muscle belly of the extensor digitorum longus muscle. EMG signals were amplified with a gain of x300 (Motion Lab Systems, Inc.; Baton Rouge, LA) and passed through a bandpass filter (10Hz-2000Hz). Torque was measured with a transducer (MLP-50, Transducer Techniques LLC; Temecula, CA) and amplified using a bridge amplifier (Model 544, Therapeutics Unlimited Inc.; Iowa City, IA). Both EMG and force were recorded using an NI 9234 24-bit A/D converter (National Instruments Corporation; Austin, TX) at a sampling rate of 6.4 kHz, with data acquisition via a LabVIEW software package environment (National Instruments Corporation). Evoked contractions were produced by a D70^2 figure-of-eight magnetic coil connected to Magstim Bistim and 200^2 magnetic stimulator set (Magstim Co. Ltd; Whitland, UK). Paired pulse stimulations were delivered at 100% stimulator output, with 10 ms inter-stimulus intervals.

Data Processing

Muscle force and EMG measures were processed using Matlab (v2016b, Mathworks, Natick MA). During MVC, the maximum force was calculated as the greatest average force during a 250 ms moving average in a trial. The amplitude of the EMG signal during this same time was calculated using the root-mean-square (RMS) method. Evoked twitch force was measured as the peak force occurring

within 200 ms after stimulation. Torque was calculated from force output ($\tau = F \times k$) where k is a constant, the distance between the midpoint of the force transducer and the estimated wrist joint axis. Peak EMG m-wave amplitude (M-wave) was manually identified between 13 and 30 ms after stimulation.

Data Analyses

Values are reported as mean (SD). Differences between groups in demographics and exercise duration were assessed using a 2-sample t-test. Because total exercise time varied between participants and between groups, minutes of exercise were converted to percent of exercise (minute of exercise/total number of minutes of exercise) and grouped into deciles of exercise for comparison. Repeated measures regression models (mixed-effects model) were used to assess trends over time within each group and between groups. In the models assessing trends over time within group, measurements were used as the dependent variable with time as the independent variable. In the combined models assessing differences between groups over time, percent of baseline measure was used as the dependent variable with group, time, and group by time interaction as independent variables. Measures of exercise and of recovery were analyzed separately. Two-sided testing was used throughout. An α level of 0.05 was used for all analyses to report significance. Analyses were conducted in SAS v9.4 (SAS Institute, Cary, NC).

Results

Ten males with DMD and ten controls completed the protocol for all baseline measurements, exercise and recovery. Demographics are summarized for DMD and controls (Table 4.1). Detailed DMD demographics, steroid usage, and genetic deletions are also summarized (Table 4.2). Baseline strength and EMG actual values for torque and EMG differed between groups (P<.001; Table 4.3).

Table 4.1 Participant demographics – Exercise protocol

Demographics	Controls (n=10)	DMD (n=10)	P-value
Age (yr)	16.6 (5.0)	15.7 (5.9)	0.704
Height (cm)	170.0 (23.7)	143.9 (14.1)	0.008
Weight (kg)	61.2 (23.1)	45.2 (14.9)	0.083

Values are means (SD); DMD, Duchenne muscular dystrophy; VAS, visual analog scale; P-value calculated using a two-sample t-test.

Table 4.2 DMD Participant Detailed Demographics

Participant	Age	Genetic mutation	Ambulatory Status	FVC	FEV1/FVC ratio	EK2	Brooke	Steroids
1	11.3	Exon 50 deletion	Y	2	98	2	1	Deflazacort
2	10.7	Exon 55 deletion	Y	1.51	67	5	1	Prednisone
3	11.9	Exon 55 deletion	N	1.83	68	7	2	None
4	13.5	duplication of A within exon 40	N	1.38	79	10	2	Deflazacort
5	18.4	Exon 46-60 deletion	N	2.59	62	13	3	Deflazacort
6	8.9	Exon 18-25 deletion	Y	2.05	115	0	1	Deflazacort
7	22.4	Exon 3-9 deletion	N	0.97	21	32	6	None
8	10.1	Exon 43 deletion	Y	2.05	111	0	1	Deflazacort
9	25.5	premature stop at exon 5	N	1.94	48	16	4	Deflazacort
10	21.1	Exon 44 deletion	N	2.4	105	17	5	None

Age, age at first visit; FVC, forced vital capacity; FEV1/FVC, forced expiratory volume in one second divided by forced vital capacity; EK2, Egen Klassification scale version 2, Brooke, Brooke Upper extremity scale.

Table 4.3 Absolute values of torque and EMG measures at baseline, the end of exercise, and during recovery

Measurements	Baseline	Exercise End	R_1	R_2	R_3	R_5	R_{10}	R_{15}
Control n=10								
MVC torque (N·m)	3.67 (1.48)	1.95 (0.69)	2.20 (0.65)	2.39 (0.74)	2.21 (0.68)	2.43 (0.86)	2.77 (0.83)	3.00 (1.07)
RMS EMG (mV)	0.43 (0.11)	0.28 (0.08)	0.27 (0.07)	0.27 (0.10)	0.27 (0.10)	0.31 (0.11)	0.34 (0.10)	0.35 (0.10)
Evoked twitch torque (N·m)	0.83 (0.45)	0.55 (0.28)	0.45 (0.26)	0.48 (0.29)	0.46 (0.28)	0.44 (0.26)	0.40 (0.20)	0.40 (0.23)
M-wave EMG (mV)	2.83 (1.80)	2.12 (1.69)	2.22 (1.62)	2.29 (1.75)	2.31 (1.78)	2.21 (1.45)	2.50 (1.83)	2.39 (1.72)
DMD n=10								
MVC torque (N·m)	0.82 (0.25)	0.33 (0.12)	0.53 (0.22)	0.56 (0.27)	0.58 (0.26)	0.64 (0.37)	0.76 (0.27)	0.74 (0.30)
RMS EMG (mV)	0.08 (0.05)	0.05 (0.04)	0.05 (0.04)	0.05 (0.03)	0.05 (0.04)	0.05 (0.03)	0.06 (0.04)	0.05 (0.03)
Evoked twitch torque (N·m)	0.08 (0.06)	0.05 (0.05)	0.06 (0.05)	0.06 (0.05)	0.05 (0.04)	0.07 (0.06)	0.09 (0.09)	0.08 (0.08)
M-wave EMG (mV)	0.57 (0.43)	0.44 (0.45)	0.43 (0.47)	0.39 (0.44)	0.42 (0.41)	0.40 (0.36)	0.38 (0.32)	0.35 (0.28)

Values are means (SD); Baseline = Mean of 3 baseline trials and first exercise trial; Exercise End = final exercise trial; R = recovery measurements after exercise cessation, subscripts denote minutes

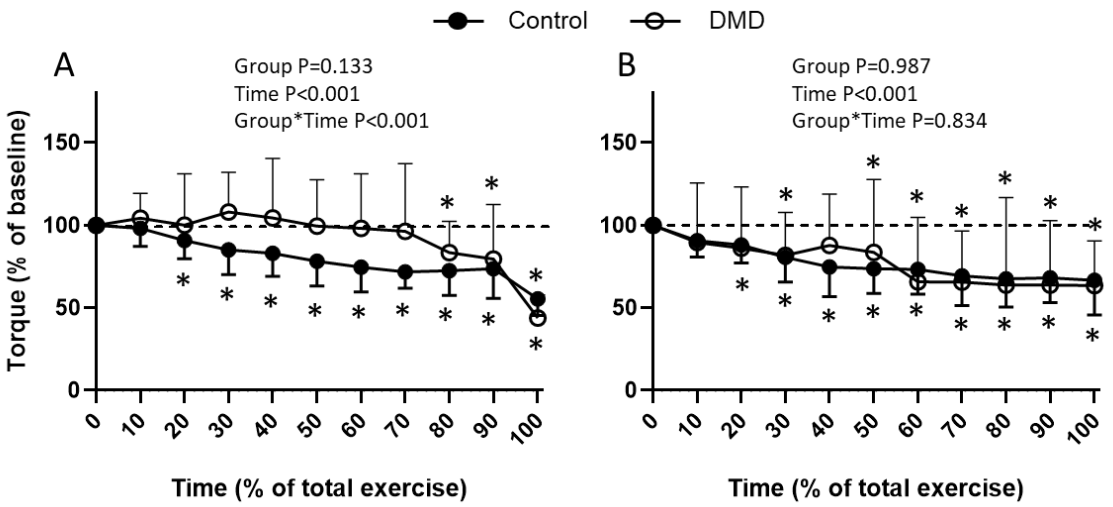


Figure 4.2 Strength loss of wrist extensors during repetitive isometric contractions in controls and males with DMD. (A) Torque from maximal voluntary contractions for each decile of exercise; (B) torque from evoked twitches for each decile of exercise. Dashed horizontal lines in A&B represent baseline. P-values associated with the interaction and main effect of the mixed model are indicated. * signifies a significant difference (P<0.05) within group from baseline.

We successfully obtained strength loss through our exercise protocol (by design) as measured by decrements in maximal voluntary contraction torque (Figure 4.2 A) and evoked twitch contraction torque (Figure 4.2 B). The significant group*time interaction for MVC torque indicates that the DMD and control groups differed in how they exhibited strength loss over time during exercise (Figure 4.2 A). MVC torque decrement was 55% in the DMD group and 45% in the control group at exercise protocol cessation. There was no group*time interaction for evoked twitch torque with the control group decrement 34% and the DMD group decrement 36% at the time of exercise protocol cessation (Figure 4.2 B). Exercise duration (the time to reach MVC decrement of 45% of baseline) in the control group was 20.9 (SD 8.9)

min (range 10-39) and in the DMD group 13.4 (SD 7.5) min (range 5-27) and did not quite differ significantly between groups (P = 0.051, Figure 4.3).

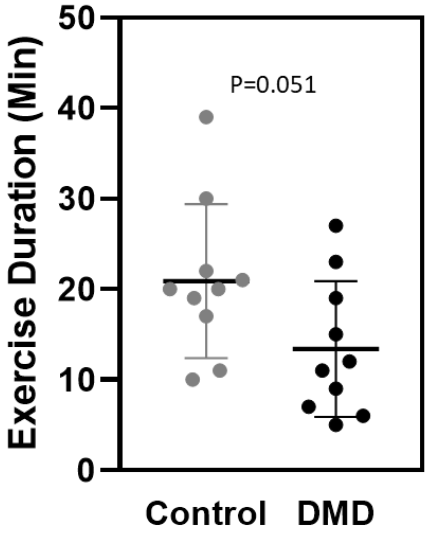


Figure 4.3 Exercise duration of wrist extensors during repetitive isometric contractions in controls and males with DMD was the time to reach a MVC decrement of 45% of initial MVC for two successive attempts. DMD, Duchenne muscular dystrophy.

EMG for both MVC and evoked contractions was analyzed to determine how muscle excitability was affected during exercise strength loss. During the exercise protocol, the RMS EMG decrement did not differ for the DMD group compared to the controls (Figure 4.4 A). The RMS EMG decrement as a percent of baseline was 30% in the control group and 26% in the DMD group at the end of the exercise, with no difference between groups (Figure 4.4 A). M-wave collected during evoked contractions showed a significant group-by-time interaction with the two groups responding differently in muscle excitability as torque was lost throughout the exercise (Figure 4.4 B). The control group M-wave declined early in the exercise

protocol and then plateaued, whereas the DMD group M-wave declined toward the end of exercise. The decrease in M-wave from baseline to the end of the exercise was 26% in the control group and 32% in the DMD group, with no difference between groups (Figure 4.4 B).

Regression analysis found that RMS EMG is a strong predictor of MVC contraction-induced torque in the control group, explaining 92% of the variance. In contrast, it only explained 43% of the variance in the DMD group (Figure 4.5 A). One outliner in the DMD group contributed to this finding. For the evoked measurements, M-wave variance explained 74% of the torque variance for the DMD group and 59% for the control group (Figure 4.5 B). Torque decreased proportionally with EMG for both voluntary and evoked measurements (Figure 4.5). For every 1 percent of baseline decrement in RMS, the mean MVC decreased by 1.38 (standard error [SE] 0.528) in the DMD group, which did not differ from the slope in the control group (1.25, [SE] 0.29) (Figure 4.5A; P=0.819). Findings were similar for evoked measurements where there was also a proportional decrease in torque with a decrement in M-wave (Figure 4.5B). The regression slope was ~1 for both the DMD group (0.97, [SE] 0.19) and the control group (1.03, [SE] 0.29; P=0.883), implying a given reduction in M-wave caused a similar decrease in evoked torque.

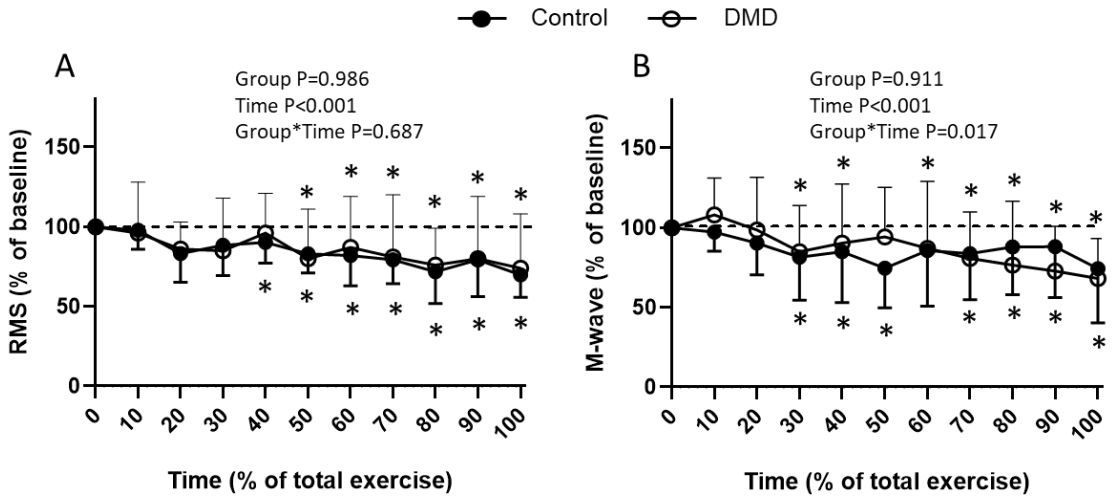


Figure 4.4 (A) EMG RMS, electromyography root mean squared during maximal voluntary contraction for each decile of exercise expressed as a percent of baseline; (B) EMG M-wave during evoked twitch contractions for each decile of exercise expressed as a percent of baseline; Dashed horizontal lines in A&B represent baseline. * signifies a significant difference (P<0.05) within group from baseline.

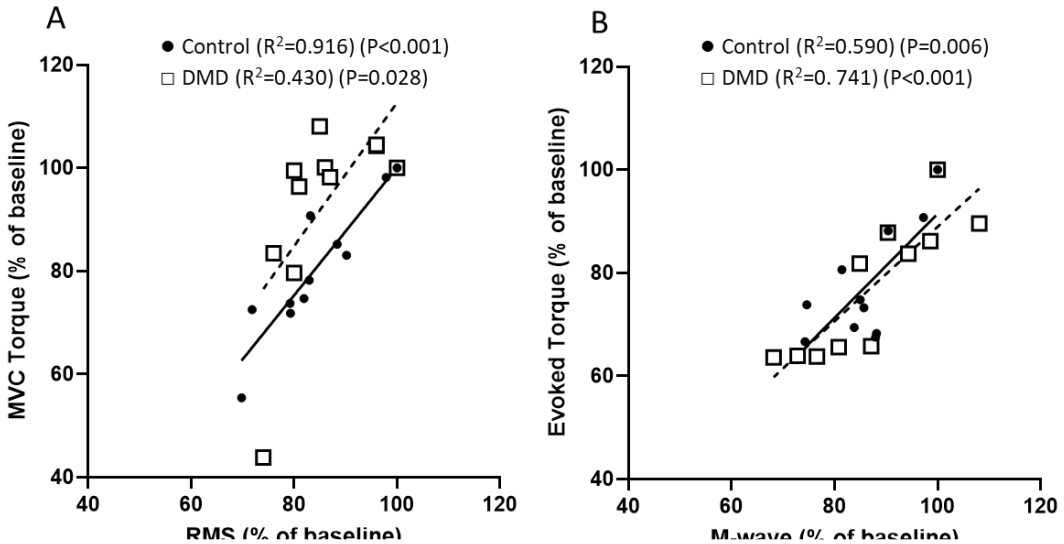


Figure 4.5 Analysis of (A) voluntary strength vs. simultaneous RMS EMG; (B) evoked strength vs. simultaneous M-wave EMG during exercise for controls and DMD; DMD Duchenne muscular dystrophy

We continued to measure MVC torque and simultaneous RMS and evoked twitch torque and simultaneous M-wave while the participants recovered from the exercise. Recovery of voluntary muscle torque did not differ between the DMD and age-matched controls over time (Figure 4.6 A). DMD and control MVC torque returned to baseline by 15 min after exercise. There is a group-by-time interaction during evoked twitch torque recovery (Figure 4.6 B): the DMD group recovered twitch torque by 10 minutes post-exercise, whereas the control group did not recover by 15 minutes, suggesting different muscle recovery mechanisms in the two groups (Figure 4.6 B).

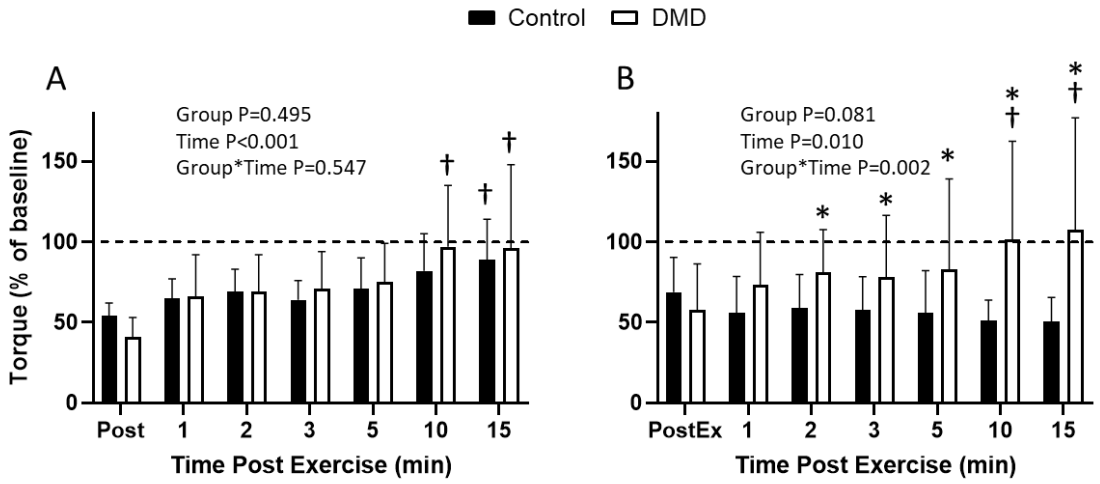


Figure 4.6 (A) Maximal voluntary contraction torque for post exercise and 1, 2, 3, 5, 10 and 15 minutes post exercise; (B) evoked twitch torque for post exercise and 1, 2, 3, 5, 10 and 15 minutes post exercise; Dashed horizontal lines in A&B represent baseline; † not significantly different within group from baseline (P>0.05); * DMD is significantly different from control (P<0.05).

There was no group-by-trial interaction for muscle excitability recovery as measured by RMS EMG (Figure 4.7 A) and M-wave EMG (Figure 4.7 B). DMD and control RMS and M-wave still differed from baseline by 15 min after exercise.

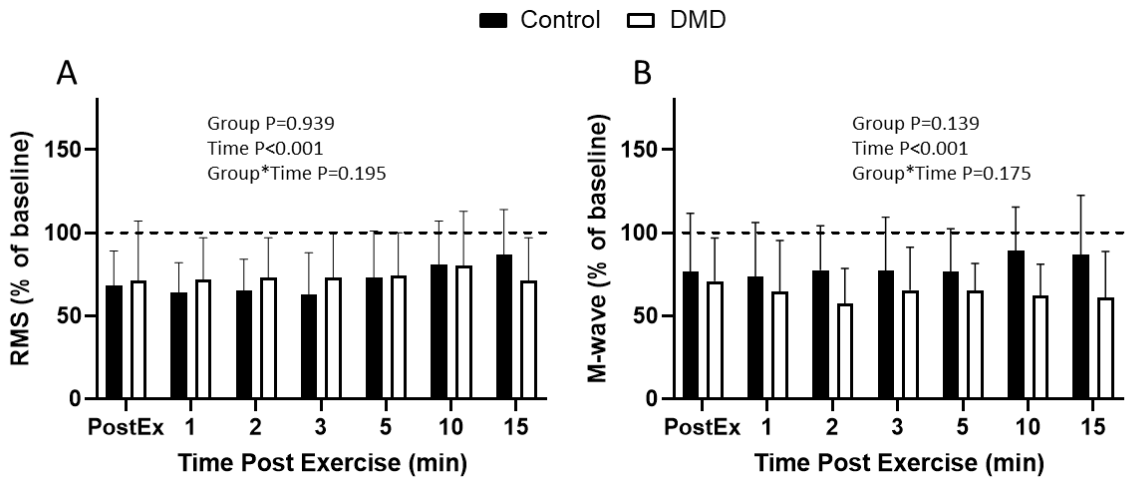


Figure 4.7 (A) Maximal voluntary contraction RMS EMG for post exercise and 1, 2, 3, 5, 10 and 15 minutes post exercise; (B) evoked twitch M-wave EMG for post exercise and 1, 2, 3, 5, 10 and 15 minutes post exercise; Dashed horizontal lines in A&B represent baseline; no trials for either group were not significantly different from baseline (P>0.05).

Self-reported fatigue after 15 minutes of recovery did not differ between groups (Figure 4.8 A). Muscle soreness, as reported 24 hours after exercise, was significantly greater in the control group than in the DMD group (Figure 4.8 B).

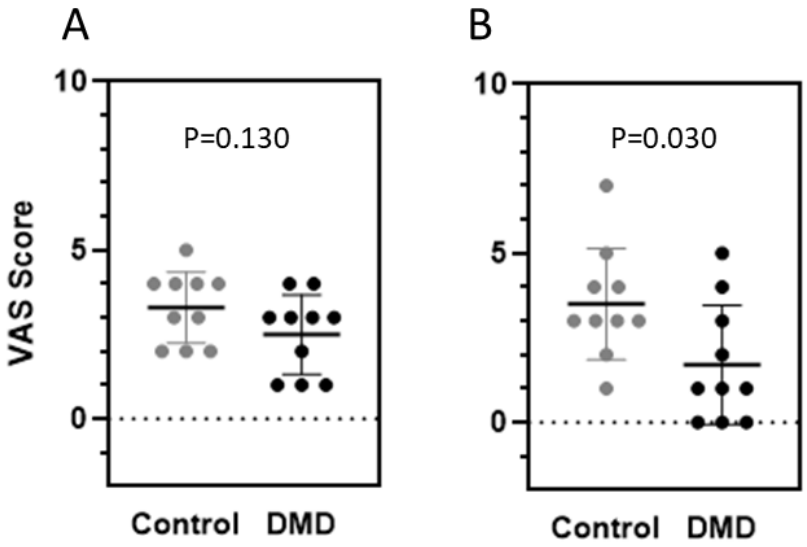


Figure 4.8 (A) Experienced fatigue for wrist extensor muscles 15 minutes after exercise as rated from 0-10 on the visual analog scale (VAS), 0 = not at all, 10 = extreme muscle fatigue; (B) Muscle soreness of wrist extensor muscles 24 hours after exercise as rated from 0-10 on the visual analog scale (VAS), 0 = not at all, 10 = extreme muscle soreness; DMD, Duchenne muscular dystrophy.

Discussion

This study, which evaluated the response of wrist extensor muscles to intermittent submaximal isometric exercise and the recovery from this protocol, produced three main results. First, exercise duration and the loss of strength measured by MVC and evoked twitch torque did not differ between males with DMD and controls. However, males with DMD and controls exhibited different patterns of voluntary strength loss over the time of exercise. Second, in contrast to *mdx* and wild type mice undergoing eccentric contractions, muscle inexcitability during and after exercise did not differ between DMD and control groups. Lastly, strength loss was transient, indicative of muscle fatigue, and recovery of strength was more rapid in males with DMD than in controls.

I designed the study to accommodate the differences in strength between males with DMD and controls by setting the submaximal exercise protocol target to 55-65% of each participant's MVC. It was not surprising then that the males with DMD had exercise duration that was not quite significantly shorter than the controls (~35% shorter). Exercise was stopped when participants could not produce an MVC 55% of their initial MVC for two consecutive attempts. Males with DMD behaved differently than controls for MVC strength loss during exercise. In the DMD group, MVC torque was maintained until 80% of exercise, after which there was a rapid decline just before exercise cessation. The decrement pattern in the DMD group is remarkably different from the control group, where MVC torque differed from baseline by 20% of exercise with a gradual decline thereafter (Figure 4.2 A). Differences between groups in ability to produce MVC torque suggest the use of

compensatory strategies and muscle substitution patterns by the males with DMD.[98,165] MVC measurements of certain muscle groups prove to be extremely difficult because of an overwhelming urge by most participants to increase activation of synergists to generate the target force as fatigue progresses.[165] Males with DMD may have learned 'load sharing' where during fatigue, they use synergistic muscles to be efficient, decrease total energy cost, and maintain workload.[165] Evoked strength loss became different from baseline at 30% of exercise duration for both groups suggesting similar peripheral fatigue in both groups (Figure 4.2 B).

Secondly, we measured muscle inexcitability during and after exercise. We found that in contrast to *mdx* mice undergoing contraction-induced force loss,[1,25,58] loss of muscle excitability after exercise did not differ between the DMD and control groups. In our human study, an exercise protocol of repeated isometric contractions was designed to produce a loss of voluntary strength of only ~60%. In contrast, *mdx* mice are subjected to a maximal eccentric contraction protocol that elicits up to 80% loss of strength. Others have found that little muscle excitability decrement occurs when the *mdx* mice undergo an isometric or concentric protocol.[1,58] Translating findings from various exercise contraction-induced strength loss protocols in *mdx* mice to males with DMD should be done carefully. Fatigue or muscle injury mechanisms are highly dependent on the type, frequency, duration, and intensity of the muscle contraction during an exercise bout.[166,167]

In our study, the relationship between M-wave decrement and torque decrement (slope) during exercise was ~1 for evoked contractions for both DMD and controls and slightly greater than 1 for voluntary contractions but again not

different from controls. This finding is similar to the proportional decrement in force in *mdx* mice when a pharmacological reduction in compound motor action potential was created to mimic the reduction seen during contractions.[58] These results suggest that muscle excitability contribution to torque in males with DMD during a isometric submaximal exercise protocol is similar to the findings in *mdx* mice when muscle contraction type is considered.[25]

This study's third important finding was that strength loss is transient in males with DMD after an intermittent submaximal isometric exercise protocol. Recovery of strength as measured by both MVC and evoked contractions occurred by 10 minutes after exercise cessation. This time to recovery indicates that peripheral muscle fatigue and not muscle injury occurred in response to the submaximal exercise protocol. This finding is consistent with strength recovery measured in *mdx* mice after low-intensity training.[130] However, *mdx* mice after eccentric contractions take days to recover. Recovery of evoked contractions was markedly different in the DMD group (Figure 4.6 B) compared to the control group, suggesting a difference between groups in the primary mechanism of peripheral fatigue.[168] The fact that evoked twitch torque did not recover within 15 min for the control group has been previously described in the literature. It is characteristic of excitation-contraction uncoupling, a disruption between the electrical events occurring at the sarcolemma and the Ca^{2+} release.[25,166] The significantly shorter strength recovery time of evoked contractions in males with DMD suggests that excitation-contraction uncoupling is likely not the primary mechanism for fatigue during this protocol. Rapid recovery in the DMD group implies action potential

conduction failure as a possible contributing mechanism, similar to *mdx* mice who rapidly recover from isometric or concentric contractions.[1,58]

Transient strength loss (Figure 4.6) and muscle excitability that did not differ from controls in recovery (Figure 4.7) are key findings to support prescription of submaximal intermittent isometric contractions for males with DMD. To ensure strength recovery, exercise should be closely monitored and stopped when fatigue surpasses ~60% of baseline MVC. Evoked contractions are useful in monitoring fatigue during exercise as we observed males with DMD using synergistic muscles to compensate for primary muscle fatigue. Self-reported fatigue and muscle soreness scores that were not different or less than controls, respectively, confirm that this exercise protocol was not detrimental. Two previous studies, one for the lower extremity[15] and one for the upper extremity[136], demonstrated that active range of motion or ergometry exercise programs produced no measurable change in muscle strength and had no deleterious effects on boys with DMD. A hallmark study, No Use is Disuse, found that assisted bicycle training in boys with DMD was feasible and safe.[16] Our results are consistent with previous studies of exercise in DMD, finding no detrimental effects when a submaximal exercise protocol is completed.

This work has made progress in understanding contraction-induced strength loss, muscle excitability, and recovery from a submaximal exercise protocol in males with DMD. If prescribed, carried out, and monitored appropriately, exercise can improve general health, well-being, and quality of life for males with DMD. With new therapies being developed to treat dystrophin gene expression, concurrent adjuvant

therapies such as exercise will impact outcomes and quality of life if they use the right type, intensity and frequency of exercise.[12] This translational research study begins to address the fear of exercise that has contributed to a sedentary lifestyle and consequential co-morbidities in many males with DMD.[8]

Chapter 5: Conclusion

The studies in this thesis contribute to understanding dystrophic deficient muscle response to exercise in males with DMD. Chapter 3 reports on a novel protocol, combining voluntary and evoked contractions to measure the strength and excitability of wrist extensor muscles for safety, feasibility, reliability, and discriminant validity. Wrist extensor muscle strength and excitability were assessed in males with DMD and age-matched healthy controls. Torque and EMG measurements were analyzed under maximum voluntary and stimulated conditions. The assessment protocol of multiple maximal voluntary contractions (MVC) and evoked twitch contractions was feasible and safe, with 96% of the participants completing the protocol with 93% of strength maintained for both DMD and control groups. Reliability was excellent, with strength and excitability measurements discriminating between DMD and controls. In conclusion, we found that a protocol of multiple MVCs and evoked contractions are a safe, feasible, reliable and valid method to measure changes in strength and excitability of wrist extensors in DMD.

In Chapter 4, I described the use of repeated intermittent submaximal isometric contractions (exercise) to induce strength loss in and evaluate muscle inexcitability of wrist extensor muscles of males with DMD. We also explored recovery of strength and excitability after this protocol. There were three main findings. Exercise duration and the loss of strength measured by MVC and evoked twitch torque did not differ between males with DMD and controls. The two groups differed in how they reached muscle fatigue during voluntary contractions but did not differ when peripheral fatigue was measured through evoked contractions. Our

hypothesis that males with DMD would have a more considerable decline in muscle excitability with strength loss during exercise was not supported. The difference of isometric (males with DMD) vs. eccentric (*mdx* mice) contractions and differences in intensity and severity compared to the *mdx* mice would have influenced this result. Lastly, strength loss was transient, indicative of muscle fatigue and not muscle injury. Recovery of strength was more rapid in males with DMD than in controls suggesting different peripheral fatigue mechanisms in the two groups.

Limitations

This work has several limitations that should be considered in subsequent research in this area. The first is that this study had a small sample size (DMD n=10; control n=14). Due to the limited sample size, I may not have had adequate power to detect some differences between groups. Heterogeneity of disease progression, phenotypic variability, and current medications (i.e., steroids, cardiac medications, and investigative therapeutics) add to data interpretation complexity and are a challenge faced by most DMD research. The heterogeneity does bolster the feasibility, safety, reliability and validity aspect of the work. However, when drawing conclusions regarding strength loss, muscle excitability and recovery, some differences may not have been detected due to heterogeneity and variation within the DMD group. Future work could mitigate this by focusing on either ambulatory or non-ambulatory subgroups and tightening inclusion criteria by only including males with DMD on specific therapeutics.

Another limitation was the use of wrist extensor muscles, specifically the extensor digitorum longus, for evoked contractions. The non-dominant arm was

selected to minimize any impairment of ADLs if, by chance, strength loss did not recover immediately. The small anatomical size of the extensor digitorum, especially of the males with DMD, proved technically challenging in conducting this protocol. The placement and angle of the magnetic stimulator needed to be consistent from trial to trial, which required expertise by the investigator without real-time feedback. Motion capture technology used to guide stimulator navigation may have allowed greater peripheral magnetic stimulation precision. However, use of motion capture feedback would have slowed down the measurements substantially, which was deemed not practical because it was critical to measure contraction-induced strength loss in a time-sensitive manner. The wrist also has many degrees of freedom and multiple muscle groups contributing to wrist extension during voluntary contractions. Synergistic muscles may have contributed to the maintenance of torque during voluntary contractions as fatigue ensued. An alternative muscle for future studies would be the biceps brachii muscle because of its size, access for stimulation, and smaller opportunity for compensations and synergistic muscles to increase torque production when performing elbow flexion.

Measurement of voluntary and evoked contractions together gives insight into both central and peripheral components of fatigue after exercise. The evoked contractions inform about components of peripheral fatigue that occur from the neuromuscular junction through the actin-myosin cross-bridges. Voluntary contractions are a combination of central and peripheral components of fatigue. If we had incorporated data from superimposed stimulations on the MVCs through the interpolated twitch technique analysis that would have further informed how central

components from the brain to the neuromuscular junction contribute to strength loss in males with DMD.

Lastly, the study design allowed the visit 2 data to be collected within three months of the initial visit. This large window for the second visit was set to increase enrollment. Some DMD participants traveled several hours for the research visits and therefore needed to complete the research visit when they returned for their clinic visit. The potential exists that during this period, the males with DMD may have lost strength due to disease progression that was not reflected in a change in Brooke or EK2 scores, which may have affected the reliability and validity measures.

Future Directions

Further research with a larger sample size is needed to understand the full utility of this type of protocol across the spectrum of patients with DMD. By increasing sample size, sub-analyses could be done for ambulatory and non-ambulatory males, giving further insight into mechanisms that may contribute to strength loss at different stages of the disease process. Several other study design modifications could also contribute to further understanding of the mechanisms of strength loss in males with DMD. Future work should consider systematically evaluating iterations of exercise with various durations, patterns (sustained or intermittent contractions) and types of contractions (isometric or concentric).

Ultimately, other muscle groups such as the biceps brachii and a weight-bearing muscle such as the quadriceps should be incorporated to assess whether changes in strength and excitability after exercise are different in muscles with

different fiber types. Future work could further explore mechanisms for strength loss and contributing aspects of central and peripheral fatigue by also incorporating outcome measures such as the Performance of the Upper Limb (PUL), creatine kinase measurement, muscle biopsy, muscle MRI, and the interpolated twitch technique. Adding these assessments will add time, expense and complexity to the study design and should be weighed for their contribution.

Many new therapies are being developed for males with DMD to increase dystrophin expression by skipping over the mutation, creating a milder Becker-like phenotype. Using a combined protocol of voluntary and evoked contractions to measure muscle strength and excitability longitudinally in clinical trials has the potential to increase recruitment of males with DMD at all disease levels and to be sensitive enough to capture small changes in muscle function. Collection and analysis of this type of data can complement current outcome measures such as the PUL and 6-minute walk test and inform about changes in phenotypic expression and improved muscle function.

As new therapeutics decrease the severity of the phenotype, more exercise will need to be built into the lives of males with DMD to optimize the strength available with a milder phenotype. Continued research is needed on contraction-induced strength loss and muscle excitability and recovery after exercise in males with DMD to determine an exercise prescription that is safe yet effective. Research should inform individualized exercise prescription where physical activity is monitored to optimize the outcomes of strength and function while minimizing injury or deconditioning risks. There are unlimited opportunities to continue to explore the

effects of exercise, including risks, benefits and mechanisms of strength loss in DMD.

Bibliography

1. Call JA, Warren GL, Verma M, Lowe DA. Acute failure of action potential conduction in mdx muscle reveals new mechanism of contraction-induced force loss. *J Physiol.* 2013;591(15):3765-3776.
2. Lou JS. Techniques in assessing fatigue in neuromuscular diseases. *Phys Med Rehabil Clin N Am.* 2012;23(1):11-22, ix.
3. Edwards RHT. Biochemical bases of fatigue in exercise performance: catastrophe theory of muscular fatigue. In: Knuttgen JA, Vogel JA, Poortmans J, eds. *Biochemistry of Exercise.*1983:3-28.
4. Williams C, Ratel S. *Human Muscle Fatigue.* USA: Routledge; 2009.
5. Rybakova IN, Ervasti JM. Dystrophin-glycoprotein complex is monomeric and stabilizes actin filaments in vitro through a lateral association. *J Biol Chem.* 1997;272(45):28771-28778.
6. Markert CD, Ambrosio F, Call JA, Grange RW. Exercise and Duchenne muscular dystrophy: toward evidence-based exercise prescription. *Muscle Nerve.* 2011;43(4):464-478.
7. Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol.* 2010;9(1):77-93.
8. Markert CD, Case LE, Carter GT, Furlong PA, Grange RW. Exercise and Duchenne muscular dystrophy: where we have been and where we need to go. *Muscle Nerve.* 2012;45(5):746-751.
9. Jansen M, de Groot IJM, van Alfen N, Geurts ACH. Physical training in boys with Duchenne Muscular Dystrophy: the protocol of the No Use is Disuse study. *Bmc Pediatrics.* 2010;10:55-2431-2410-2455.
10. Bianchi ML, Biggar D, Bushby K, Rogol AD, Rutter MM, Tseng B. Endocrine aspects of Duchenne muscular dystrophy. *Neuromuscul Disord.* 2011;21(4):298-303.
11. Kostek M. Precision medicine and exercise therapy in Duchenne muscular dystrophy. *Sports (Basel, Switzerland).* 2019;7(3).

12. Kostek MC, Gordon B. Exercise Is an Adjuvant to Contemporary Dystrophy Treatments. *Exerc Sport Sci Rev.* 2018;46(1):34-41.
13. Vignos PJ, Jr., Watkins MP. The effect of exercise in muscular dystrophy. *JAMA.* 1966;197(11):843-848.
14. de Lateur BJ, Giaconi RM. Effect on maximal strength of submaximal exercise in Duchenne muscular dystrophy. *Am J Phys Med.* 1979;58(1):26-36.
15. Scott OM, Hyde SA, Goddard C, Jones R, Dubowitz V. Effect of exercise in Duchenne muscular dystrophy. *Physiotherapy.* 1981;67(6):174-176.
16. Jansen M, van Alfen N, Geurts ACH, de Groot IJM. Assisted Bicycle Training Delays Functional Deterioration in Boys With Duchenne Muscular Dystrophy: The Randomized Controlled Trial "No Use Is Disuse". *Neurorehabilitation and Neural Repair.* 2013;27(9):816-827.
17. Grange RW, Call J.A. Recommendations to Define Exercise Prescriptionfor Duchenne Muscular Dystrophy. *Sport Sci Rev.* 2007;35:12-17.
18. Spaulding HR, Selsby JT. Is Exercise the Right Medicine for Dystrophic Muscle? *Medicine and science in sports and exercise.* 2018.
19. Gianola S, Pecoraro V, Lambiase S, Gatti R, Banfi G, Moja L. Efficacy of muscle exercise in patients with muscular dystrophy: a systematic review showing a missed opportunity to improve outcomes. *PLoS One.* 2013;8(6):e65414.
20. Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol.* 2018;17(3):251-267.
21. Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol.* 2010;9(2):177-189.
22. Ng SY, Manta A, Ljubicic V. Exercise biology of neuromuscular disorders. *Appl Physiol Nutr Metab.* 2018;43(11):1194-1206.

23. Lindsay A, Baumann CW, Rebbeck RT, et al. Mechanical factors tune the sensitivity of mdx muscle to eccentric strength loss and its protection by antioxidant and calcium modulators. *Skeletal muscle.* 2020;10(1):3.
24. Lindsay A, Larson AA, Verma M, Ervasti JM, Lowe DA. Isometric resistance training increases strength and alters histopathology of dystrophin-deficient mouse skeletal muscle. *Journal of applied physiology (Bethesda, Md : 1985).* 2019;126(2):363-375.
25. Baumann CW, Warren GL, Lowe DA. Plasmalemma function is rapidly restored in mdx muscle after eccentric contractions. *Medicine and science in sports and exercise.* 2020;52(2):354-361.
26. Kogelman B, Putker K, Hulsker M, et al. Voluntary exercise improves muscle function and does not exacerbate muscle and heart pathology in aged Duchenne muscular dystrophy mice. *Journal of Molecular and Cellular Cardiology.* 2018;125:29-38.
27. Man WD, Moxham J, Polkey MI. Magnetic stimulation for the measurement of respiratory and skeletal muscle function. *Eur Respir J.* 2004;24(5):846-860.
28. Man WD, Soliman MG, Nikoletou D, et al. Non-volitional assessment of skeletal muscle strength in patients with chronic obstructive pulmonary disease. *Thorax.* 2003;58(8):665-669.
29. Polkey MI, Kyroussis D, Hamnegard CH, Mills GH, Green M, Moxham J. Quadriceps strength and fatigue assessed by magnetic stimulation of the femoral nerve in man. *Muscle Nerve.* 1996;19(5):549-555.
30. Swallow EB, Gosker HR, Ward KA, et al. A novel technique for nonvolitional assessment of quadriceps muscle endurance in humans. *Journal of applied physiology.* 2007;103(3):739-746.
31. Dalkilic I, Kunkel LM. Muscular dystrophies: genes to pathogenesis. *Curr Opin Genet Dev.* 2003;13(3):231-238.
32. Fairclough RJ, Bareja A, Davies KE. Progress in therapy for Duchenne muscular dystrophy. *Exp Physiol.* 2011;96(11):1101-1113.

33. Monaco AP, Bertelson CJ, Liechti-Gallati S, Moser H, Kunkel LM. An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. *Genomics.* 1988;2(1):90-95.
34. Angelini C, Pegoraro E, Turella E, Intino MT, Pini A, Costa C. Deflazacort in Duchenne dystrophy: study of long-term effect. *Muscle Nerve.* 1994;17(4):386-391.
35. Deconinck N, Dan B. Pathophysiology of duchenne muscular dystrophy: current hypotheses. *Pediatr Neurol.* 2007;36(1):1-7.
36. Goldstein JA, McNally EM. Mechanisms of muscle weakness in muscular dystrophy. *J Gen Physiol.* 2010;136(1):29-34.
37. Emery AEH. The muscular dystrophies. *The Lancet.* 2002;359(9307):687-695.
38. Brooke MH, Fenichel GM, Griggs RC, et al. Duchenne muscular dystrophy: patterns of clinical progression and effects of supportive therapy. *Neurology.* 1989;39(4):475-481.
39. McDonald CM, Abresch RT, Carter GT, et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. *Am J Phys Med Rehabil.* 1995;74(5 Suppl):S70-92.
40. Mendell JR, Province MA, Moxley RT, 3rd, et al. Clinical investigation of Duchenne muscular dystrophy. A methodology for therapeutic trials based on natural history controls. *Arch Neurol.* 1987;44(8):808-811.
41. Kinali M, Arechavala-Gomeza V, Cirak S, et al. Muscle histology vs MRI in Duchenne muscular dystrophy. *Neurology.* 2011;76(4):346-353.
42. Kinnett K, Rodger S, Vroom E, Furlong P, Aartsma-Rus A, Bushby K. Imperatives for DUCHENNE MD: a Simplified Guide to Comprehensive Care for Duchenne Muscular Dystrophy. *PLoS Curr.* 2015;7:10.1371/currents.md.87770501e87770586f87770536f87770501c87770571e87770500a87775882ed87770509ba87770501.
43. Angelini C, Tasca E. Fatigue in muscular dystrophies. *Neuromuscul Disord.* 2012;22 Suppl 3(0):S214-220.
44. El-Aloul B, Speechley KN, Wei Y, Wilk P, Campbell C. Fatigue in young people with Duchenne muscular dystrophy. *Dev Med Child Neurol.* 2019.

45. Schillings ML, Kalkman JS, Janssen HM, van Engelen BG, Bleijenberg G, Zwarts MJ. Experienced and physiological fatigue in neuromuscular disorders. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology.* 2007;118(2):292-300.
46. McDonald CM, Henricson EK, Abresch RT, et al. Long-term effects of glucocorticoids on function, quality of life, and survival in patients with Duchenne muscular dystrophy: a prospective cohort study. *The Lancet.* 2018;391(10119):451-461.
47. Schreiber A, Brochard S, Rippert P, et al. Corticosteroids in Duchenne muscular dystrophy: impact on the motor function measure sensitivity to change and implications for clinical trials. *Dev Med Child Neurol.* 2018;60(2):185-191.
48. Connolly AM, Schierbecker J, Renna R, Florence J. High dose weekly oral prednisone improves strength in boys with Duchenne muscular dystrophy. *Neuromuscul Disord.* 2002;12(10):917-925.
49. de Valle KL, Davidson ZE, Kennedy RA, Ryan MM, Carroll KM. Physical activity and the use of standard and complementary therapies in Duchenne and Becker muscular dystrophies. *Journal of pediatric rehabilitation medicine.* 2016;9(1):55-63.
50. Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. *Lancet Neurol.* 2018;17(4):347-361.
51. Petrof BJ, Shrager JB, Stedman HH, Kelly AM, Sweeney HL. Dystrophin protects the sarcolemma from stresses developed during muscle contraction. *Proc Natl Acad Sci U S A.* 1993;90(8):3710-3714.
52. Mendell JR, Goemans N, Lowes LP, et al. Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. *Ann Neurol.* 2016;79(2):257-271.
53. Atamturk H, Atamturk A. Therapeutic effects of aquatic exercises on a boy with Duchenne muscular dystrophy. *J Exerc Rehabil.* 2018;14(5):877-882.
54. Bachasson D, Temesi J, Bankole C, et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: benefits and limits of

femoral nerve magnetic stimulation. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology.* 2014;125(2):396-405.

55. McGreevy JW, Hakim CH, McIntosh MA, Duan D. Animal models of Duchenne muscular dystrophy: from basic mechanisms to gene therapy. *Dis Model Mech.* 2015;8(3):195-213.
56. Bulfield G, Siller WG, Wight PA, Moore KJ. X chromosome-linked muscular dystrophy (mdx) in the mouse. *Proc Natl Acad Sci U S A.* 1984;81(4):1189-1192.
57. Partridge TA. The mdx mouse model as a surrogate for Duchenne muscular dystrophy. *The FEBS journal.* 2013;280(17):4177-4186.
58. Roy P, Rau F, Ochala J, et al. Dystrophin restoration therapy improves both the reduced excitability and the force drop induced by lengthening contractions in dystrophic mdx skeletal muscle. *Skeletal muscle.* 2016;6:23.
59. Call JA, Eckhoff MD, Baltgalvis KA, Warren GL, Lowe DA. Adaptive strength gains in dystrophic muscle exposed to repeated bouts of eccentric contraction. *Journal of applied physiology (Bethesda, Md : 1985).* 2011;111(6):1768-1777.
60. Frontera WR, Ochala J. Skeletal muscle: a brief review of structure and function. *Calcif Tissue Int.* 2015;96(3):183-195.
61. Lovering RM, De Deyne PG. Contractile function, sarcolemma integrity, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *Am J Physiol Cell Physiol.* 2004;286(2):C230-238.
62. Cohn RD, Campbell KP. Molecular basis of muscular dystrophies. *Muscle Nerve.* 2000;23(10):1456-1471.
63. Gao QQ, McNally EM. The Dystrophin Complex: Structure, Function, and Implications for Therapy. *Compr Physiol.* 2015;5(3):1223-1239.
64. Shishmarev D. Excitation-contraction coupling in skeletal muscle: recent progress and unanswered questions. *Biophys Rev.* 2020;12(1):143-153.
65. Heydemann A. Skeletal Muscle Metabolism in Duchenne and Becker Muscular Dystrophy—Implications for Therapies. *Nutrients.* 2018;10(6):796.

66. Houang EM, Sham YY, Bates FS, Metzger JM. Muscle membrane integrity in Duchenne muscular dystrophy: recent advances in copolymer-based muscle membrane stabilizers. *Skeletal muscle.* 2018;8(1):31.
67. Bigland-Ritchie BR, Dawson NJ, Johansson RS, Lippold OC. Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. *J Physiol.* 1986;379:451-459.
68. Enoka RM. Muscle fatigue--from motor units to clinical symptoms. *J Biomech.* 2012;45(3):427-433.
69. Vollestad NK. Measurement of human muscle fatigue. *J Neurosci Methods.* 1997;74(2):219-227.
70. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev.* 2001;81(4):1725-1789.
71. Enoka RM, Stuart DG. Neurobiology of muscle fatigue. *Journal of applied physiology (Bethesda, Md : 1985).* 1992;72(5):1631-1648.
72. Bigland-Ritchie B. EMG/force relations and fatigue of human voluntary contractions. *Exerc Sport Sci Rev.* 1981;9:75-117.
73. Merton PA. Voluntary strength and fatigue. *J Physiol.* 1954;123(3):553-564.
74. Enoka RM, Duchateau J. Muscle fatigue: what, why and how it influences muscle function. *J Physiol.* 2008;586(1):11-23.
75. Bachasson D, Millet GY, Decorte N, Wuyam B, Levy P, Verges S. Quadriceps function assessment using an incremental test and magnetic neurostimulation: a reliability study. *J Electromyogr Kinesiol.* 2013;23(3):649-658.
76. Kremenic IJ, Ben-Avi SS, Leonhardt D, McHugh MP. Transcutaneous magnetic stimulation of the quadriceps via the femoral nerve. *Muscle Nerve.* 2004;30(3):379-381.
77. Kremenic IJ, Glace BW, Ben-Avi SS, Nicholas SJ, McHugh MP. Central fatigue after cycling evaluated using peripheral magnetic stimulation. *Medicine and science in sports and exercise.* 2009;41(7):1461-1466.
78. Lampropoulou SI, Nowicky AV, Marston L. Magnetic versus electrical stimulation in the interpolation twitch technique of elbow flexors. *J Sports Sci Med.* 2012;11(4):709-718.

79. O'Brien TD, Reeves ND, Baltzopoulos V, Jones DA, Maganaris CN. Assessment of voluntary muscle activation using magnetic stimulation. *Eur J Appl Physiol.* 2008;104(1):49-55.
80. Park ND, Maresca RD, McKibans KI, Morgan DR, Allen TS, Warren GL. Caffeine's Beneficial Effect on Maximal Voluntary Strength and Activation in Uninjured but Not Injured Muscle. *International Journal of Sport Nutrition and Exercise Metabolism.* 2008;18:639-652.
81. Herbert RD, Gandevia SC. Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation. *Journal of neurophysiology.* 1999;82(5):2271-2283.
82. Cooper MA, Herda TJ, Walter-Herda AA, Costa PB, Ryan ED, Cramer JT. The reliability of the interpolated twitch technique during submaximal and maximal isometric muscle actions. *Journal of strength and conditioning research.* 2013;27(10):2909-2913.
83. Taylor JL. Last Word on Point:Counterpoint: The interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *Journal of Applied Physiology.* 2009;107(1):367-367.
84. Taylor JL. Point: the interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *Journal of applied physiology (Bethesda, Md : 1985).* 2009;107(1):354-355.
85. de Haan A, Gerrits KH, de Ruiter CJ. Counterpoint: the interpolated twitch does not provide a valid measure of the voluntary activation of muscle. *Journal of applied physiology (Bethesda, Md : 1985).* 2009;107(1):355-357; discussion 357-358.
86. Huang YM, Hsu MJ, Lin CH, Wei SH, Chang YJ. The non-linear relationship between muscle voluntary activation level and voluntary force measured by the interpolated twitch technique. *Sensors (Basel).* 2010;10(1):796-807.
87. Millet GY, Bachasson D, Temesi J, et al. Potential interests and limits of magnetic and electrical stimulation techniques to assess neuromuscular fatigue. *Neuromuscul Disord.* 2012;22 Suppl 3(Suppl 3):S181-186.

88. Han TR, Shin HI, Kim IS. Magnetic stimulation of the quadriceps femoris muscle: comparison of pain with electrical stimulation. *Am J Phys Med Rehabil.* 2006;85(7):593-599.
89. Tomazin K, Verges S, Decorte N, Oulerich A, Millet GY. Effects of coil characteristics for femoral nerve magnetic stimulation. *Muscle Nerve.* 2010;41(3):406-409.
90. Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. *Journal of applied physiology (Bethesda, Md : 1985).* 2009;106(2):701-710.
91. Froyd C, Beltrami FG, Millet GY, Noakes TD. No Critical Peripheral Fatigue Threshold during Intermittent Isometric Time to Task Failure Test with the Knee Extensors. *Front Physiol.* 2016;7:627.
92. Froyd C, Beltrami FG, Noakes TD. Neuromuscular Fatigue at Task Failure and During Immediate Recovery after Isometric Knee Extension Trials. *Sports (Basel, Switzerland).* 2018;6(4).
93. Kufel TJ, Pineda LA, Mador MJ. Comparison of potentiated and unpotentiated twitches as an index of muscle fatigue. *Muscle Nerve.* 2002;25(3):438-444.
94. Rossini PM, Burke D, Chen R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology.* 2015;126(6):1071-1107.
95. Machetanz J, Bischoff C, Pichlmeier R, et al. Magnetically induced muscle contraction is caused by motor nerve stimulation and not by direct muscle activation. *Muscle Nerve.* 1994;17(10):1170-1175.
96. Matsumoto H, Hanajima R, Terao Y, Ugawa Y. Magnetic-motor-root stimulation: review. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology.* 2013;124(6):1055-1067.

97. Domingos J, Muntoni F. Outcome measures in Duchenne muscular dystrophy: sensitivity to change, clinical meaningfulness, and implications for clinical trials. *Dev Med Child Neurol.* 2018;60(2):117.
98. Servais L, Deconinck N, Moraux A, et al. Innovative methods to assess upper limb strength and function in non-ambulant Duchenne patients. *Neuromuscul Disord.* 2013;23(2):139-148.
99. Bendixen RM, Morgenroth LP, Clinard KL. Engaging participants in rare disease research: a qualitative study of Duchenne muscular dystrophy. *Clinical therapeutics.* 2016;38(6):1474-1484 e1472.
100. Stefanetti RJ, Blain A, Jimenez-Moreno C, et al. Measuring the effects of exercise in neuromuscular disorders: a systematic review and meta-analyses. *Wellcome Open Res.* 2020;5:84.
101. Lynn S, Aartsma-Rus A, Bushby K, et al. Measuring clinical effectiveness of medicinal products for the treatment of Duchenne muscular dystrophy. *Neuromuscul Disord.* 2015;25(1):96-105.
102. Organization WH. Towards a Common Language for Functioning, Disability and Health ICF. 2002.
103. Ricotti V, Selby V, Ridout D, et al. Respiratory and upper limb function as outcome measures in ambulant and non-ambulant subjects with Duchenne muscular dystrophy: A prospective multicentre study. *Neuromuscular Disorders.* 2019;29(4):261-268.
104. Bushby K, Connor E. Clinical outcome measures for trials in Duchenne muscular dystrophy: report from International Working Group meetings. *Clin Investig (Lond).* 2011;1(9):1217-1235.
105. Florence JM, Pandya S, King WM, et al. Intrarater reliability of manual muscle test (Medical Research Council scale) grades in Duchenne's muscular dystrophy. *Phys Ther.* 1992;72(2):115-122; discussion 122-116.
106. Hebert LJ, Maltais DB, Lepage C, Saulnier J, Crete M, Perron M. Isometric muscle strength in youth assessed by hand-held dynamometry: a feasibility, reliability, and validity study. *Pediatr Phys Ther.* 2011;23(3):289-299.

107. Decostre V, Canal A, Ollivier G, et al. Wrist flexion and extension torques measured by highly sensitive dynamometer in healthy subjects from 5 to 80 years. *BMC Musculoskelet Disord.* 2015;16:4.
108. McDonald CM, Henricson EK, Han JJ, et al. The 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy. *Muscle & Nerve.* 2010;41(4):500-510.
109. McDonald CM, Henricson EK, Abresch RT, et al. THE 6-minute walk test and other endpoints in Duchenne muscular dystrophy: Longitudinal natural history observations over 48 weeks from a multicenter study. *Muscle & Nerve.* 2013;48(3):343-356.
110. Mayhew A, Cano S, Scott E, et al. Moving towards meaningful measurement: Rasch analysis of the North Star Ambulatory Assessment in Duchenne muscular dystrophy. *Dev Med Child Neurol.* 2011;53(6):535-542.
111. Mazzone E, Martinelli D, Berardinelli A, et al. North Star Ambulatory Assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. *Neuromuscular Disorders.* 2010;20(11):712-716.
112. Berard C, Payan C, Hodgkinson L, Fermanian J, Grp MCS. A motor function measure scale for neuromuscular diseases. Construction and validation study. *Neuromuscular Disorders.* 2005;15(7):463-470.
113. Mayhew A, Mazzone ES, Eagle M, et al. Development of the Performance of the Upper Limb module for Duchenne muscular dystrophy. *Dev Med Child Neurol.* 2013;55(11):1038-1045.
114. Mayhew AG, Coratti G, Mazzone ES, et al. Performance of Upper Limb module for Duchenne muscular dystrophy. *Dev Med Child Neurol.* 2020;62(5):633-639.
115. Duong T. Performance of the Upper Limb. 2016.
116. Brooke MH. Clinical trial in Duchenne dystrophy. I. The Design of the Protocol. *Muscle & Nerve.* 1981;4:186-197.
117. Connolly AM, Malkus EC, Mendell JR, et al. Outcome reliability in non-ambulatory boys/men with Duchenne muscular dystrophy. *Muscle Nerve.* 2015;51(4):522-532.

118. Steffensen B, Hyde S, Lyager S, Mattsson E. Validity of the EK scale: a functional assessment of non-ambulatory individuals with Duchenne muscular dystrophy or spinal muscular atrophy. *Physiother Res Int.* 2001;6(3):119-134.
119. Mercuri E, Mayhew A, Muntoni F, et al. Towards harmonisation of outcome measures for DMD and SMA within TREAT-NMD; report of three expert workshops: TREAT-NMD/ENMC workshop on outcome measures, 12th--13th May 2007, Naarden, The Netherlands; TREAT-NMD workshop on outcome measures in experimental trials for DMD, 30th June--1st July 2007, Naarden, The Netherlands; conjoint Institute of Myology TREAT-NMD meeting on physical activity monitoring in neuromuscular disorders, 11th July 2007, Paris, France. *Neuromuscul Disord.* 2008;18(11):894-903.
120. Klingels K, Mayhew AG, Mazzone ES, et al. Development of a patient-reported outcome measure for upper limb function in Duchenne muscular dystrophy: DMD Upper Limb PROM. *Dev Med Child Neurol.* 2017;59(2):224-231.
121. Fujii T, Takeshita E, Iwata Y, et al. Cumulative jerk as an outcome measure in nonambulatory Duchenne muscular dystrophy. *Brain Dev.* 2019;41(9):796-802.
122. Verma S, Lin J, Travers C, McCracken C, Shah D. Quantitative electromyography in ambulatory boys with Duchenne muscular dystrophy. *Muscle & Nerve.* 2017;56(6):1168-1171.
123. Phillips MF, Quinlivan RM, Edwards RH, Calverley PM. Changes in spirometry over time as a prognostic marker in patients with Duchenne muscular dystrophy. *American Journal of Respiratory and Critical Care Medicine.* 2001;164:2191-2194.
124. Janssen M, Harlaar J, Koopman B, de Groot IJM. Unraveling upper extremity performance in Duchenne muscular dystrophy: A biophysical model. *Neuromuscul Disord.* 2019;29(5):368-375.
125. Senesac CR, Lott DJ, Forbes SC, et al. Longitudinal Evaluation of Muscle Composition Using Magnetic Resonance in 4 Boys With Duchenne Muscular Dystrophy: Case Series. *Physical Therapy.* 2015;95(7):978-988.

126. Edwards RH, Hill DK, Jones DA, Merton PA. Fatigue of long duration in human skeletal muscle after exercise. *J Physiol.* 1977;272(3):769-778.
127. Harris ML, Polkey MI, Bath PM, Moxham J. Quadriceps muscle weakness following acute hemiplegic stroke. *Clin Rehabil.* 2001;15(3):274-281.
128. Hyzewicz J, Ruegg UT, Takeda S. Comparison of Experimental Protocols of Physical Exercise for mdx Mice and Duchenne Muscular Dystrophy Patients. *J Neuromuscul Dis.* 2015;2(4):325-342.
129. Landisch RM, Kosir AM, Nelson SA, Baltgalvis KA, Lowe DA. Adaptive and nonadaptive responses to voluntary wheel running by mdx mice. *Muscle Nerve.* 2008;38(4):1290-1303.
130. Call JA, McKeehen JN, Novotny SA, Lowe DA. Progressive resistance voluntary wheel running in the mdx mouse. *Muscle Nerve.* 2010;42(6):871-880.
131. Baltgalvis KA, Call JA, Cochrane GD, Laker RC, Yan Z, Lowe DA. Exercise training improves plantar flexor muscle function in mdx mice. *Medicine and science in sports and exercise.* 2012;44(9):1671-1679.
132. Hayes A, Williams DA. Beneficial effects of voluntary wheel running on the properties of dystrophic mouse muscle. *Journal of applied physiology (Bethesda, Md : 1985).* 1996;80(2):670-679.
133. Zelikovich AS, Quattrocelli M, Salamone IM, Kuntz NL, McNally EM. Moderate exercise improves function and increases adiponectin in the mdx mouse model of muscular dystrophy. *Sci Rep.* 2019;9(1):5770.
134. Sejerson T, Bushby K, Excellence T-NENo. Standards of care for Duchenne muscular dystrophy: brief TREAT-NMD recommendations. *Adv Exp Med Biol.* 2009;652:13-21.
135. Garrood P, Eagle M, Jardine PE, Bushby K, Straub V. Myoglobinuria in boys with Duchenne muscular dystrophy on corticosteroid therapy. *Neuromuscul Disord.* 2008;18(1):71-73.
136. Alemdaroglu I, Karaduman A, Yilmaz OT, Topaloglu H. Different types of upper extremity exercise training in Duchenne muscular dystrophy: effects on functional performance, strength, endurance, and ambulation. *Muscle Nerve.* 2015;51(5):697-705.

137. Hoffman EP, Brown RH, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dytrophy locus. *Cell.* 1987;51(6):919-928.
138. Lobo-Prat J, Janssen M, Koopman B, Stienen AHA, de Groot IJM. Surface EMG signals in very late-stage of Duchenne muscular dystrophy: a case study. *J Neuroeng Rehabil.* 2017;14(1):86.
139. Lobo-Prat J, Nizamis K, Janssen M, et al. Comparison between sEMG and force as control interfaces to support planar arm movements in adults with Duchenne: a feasibility study. *J Neuroeng Rehabil.* 2017;14(1):73.
140. Janssen M, Harlaar J, Koopman B, de Groot IJM. Dynamic arm study: quantitative description of upper extremity function and activity of boys and men with duchenne muscular dystrophy. *J Neuroeng Rehabil.* 2017;14(1):45.
141. Mathur S, Lott DJ, Senesac C, et al. Age-related differences in lower-limb muscle cross-sectional area and torque production in boys with Duchenne muscular dystrophy. *Arch Phys Med Rehabil.* 2010;91(7):1051-1058.
142. Landfeldt E, Lindgren P, Bell CF, et al. Health-related quality of life in patients with Duchenne muscular dystrophy: a multinational, cross-sectional study. *Dev Med Child Neurol.* 2016;58(5):508-515.
143. Fujii T, Takeshita E, Iwata Y, et al. Cumulative jerk as an outcome measure in nonambulatory Duchenne muscular dystrophy. *Brain and Development.* 2019.
144. Lacourpaille L, Hug F, Guevel A, et al. New insights on contraction efficiency in patients with Duchenne muscular dystrophy. *J Appl Physiol.* 2014;117(6):658-662.
145. Desmedt JE, Emeryk B. Disorder of muscle contraction processes in sex-linked (Duchenne) muscular dystrophy, with correlative electromyographic study of myopathic involvement in small hand muscles. *Am J Med.* 1968;45(6):853-872.
146. Pratt SJ, Shah SB, Ward CW, Kerr JP, Stains JP, Lovering RM. Recovery of altered neuromuscular junction morphology and muscle function in mdx mice after injury. *Cell Mol Life Sci.* 2015;72(1):153-164.

147. Mayhew A, Eagle M, Steffenson B. 17th International Congress of The World Muscle Society: S.P.6 Exploratory Rasch analysis of the EK2 scale used in a population of Duchenne muscular dystrophy (DMD). 2012;22(9-10):877.
148. Lee M, Carroll TJ. The amplitude of Mmax in human wrist flexors varies during different muscle contractions despite constant posture. *J Neurosci Methods.* 2005;149(2):95-100.
149. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull.* 1979;86(2):420-428.
150. Rankin G, Stokes M. Reliability of assessment tools in rehabilitation: an illustration of appropriate statistical analyses. *Clinical Rehabilitation.* 1998;12(3):187-199.
151. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med.* 2016;15(2):155-163.
152. Dogan NO. Bland-Altman analysis: A paradigm to understand correlation and agreement. *Turk J Emerg Med.* 2018;18(4):139-141.
153. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1(8476):307-310.
154. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res.* 1999;8(2):135-160.
155. Chan YH. Biostatistics 104: correlational analysis. *Singapore Med J.* 2003;44(12):614-619.
156. Capote J, DiFranco M, Vergara JL. Excitation-contraction coupling alterations in mdx and utrophin/dystrophin double knockout mice: a comparative study. *Am J Physiol Cell Physiol.* 2010;298(5):C1077-1086.
157. Echigoya Y, Lim KRQ, Melo D, et al. Exons 45-55 Skipping using mutation-tailored cocktails of antisense morpholinos in the DMD gene. *Mol Ther.* 2019;27(11):2005-2017.
158. Landfeldt E, Lindberg C, Sejersen T. Improvements in health status and utility associated with ataluren for the treatment of nonsense mutation Duchenne muscular dystrophy. *Muscle Nerve.* 2020;61(3):363-368.

159. Warren GL, Ingalls CP, Lowe DA, Armstrong RB. Excitation-contraction uncoupling: major role in contraction-induced muscle injury. *Exerc Sport Sci Rev.* 2001;29(2):82-87.
160. Ingalls CP, Warren GL, Williams JH, Ward CW, Armstrong RB. E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *Journal of applied physiology (Bethesda, Md : 1985).* 1998;85(1):58-67.
161. Pratt SJP, Shah SB, Ward CW, Inacio MP, Stains JP, Lovering RM. Effects of in vivo injury on the neuromuscular junction in healthy and dystrophic muscles. *J Physiol.* 2013;591(2):559-570.
162. Brooks SV. Rapid recovery following contraction-induced injury to in situ skeletal muscles in mdx mice. *J Muscle Res Cell Motil.* 1998;19(2):179-187.
163. Hunter SK, Critchlow A, Shin IS, Enoka RM. Men are more fatigable than strength-matched women when performing intermittent submaximal contractions. *Journal of applied physiology (Bethesda, Md : 1985).* 2004;96(6):2125-2132.
164. Kim E, Lovera J, Schaben L, Melara J, Bourdette D, Whitham R. Novel method for measurement of fatigue in multiple sclerosis: Real-Time Digital Fatigue Score. *J Rehabil Res Dev.* 2010;47(5):477-484.
165. Mendes B, Firmino T, Oliveira R, et al. Effects of knee flexor submaximal isometric contraction until exhaustion on semitendinosus and biceps femoris long head shear modulus in healthy individuals. *Sci Rep.* 2020;10(1):16433.
166. Carroll TJ, Taylor JL, Gandevia SC. Recovery of central and peripheral neuromuscular fatigue after exercise. *Journal of applied physiology (Bethesda, Md : 1985).* 2017;122(5):1068-1076.
167. Enoka RM, Baudry S, Rudroff T, Farina D, Klass M, Duchateau J. Unraveling the neurophysiology of muscle fatigue. *J Electromyogr Kinesiol.* 2011;21(2):208-219.
168. Cairns SP, Knicker AJ, Thompson MW, Sjogaard G. Evaluation of models used to study neuromuscular fatigue. *Exercise and Sport Sciences Reviews.* 2005;33(1):9-16.

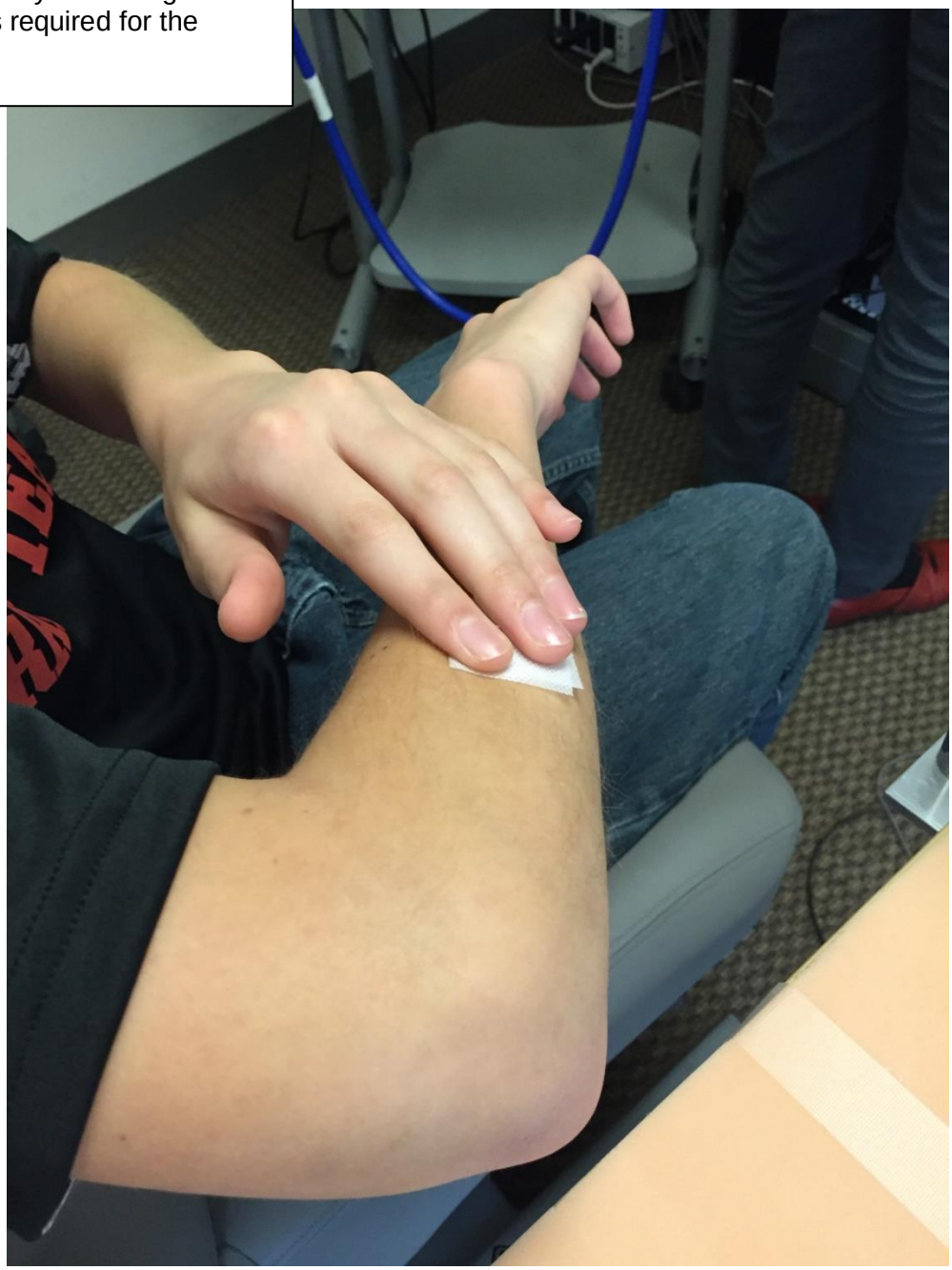
Appendix A – Participant pre-consent education handout

This is a series of pictures that will show you the equipment and process for doing the study that we are asking you to participate in. Please ask questions as we go along and look through the pictures.

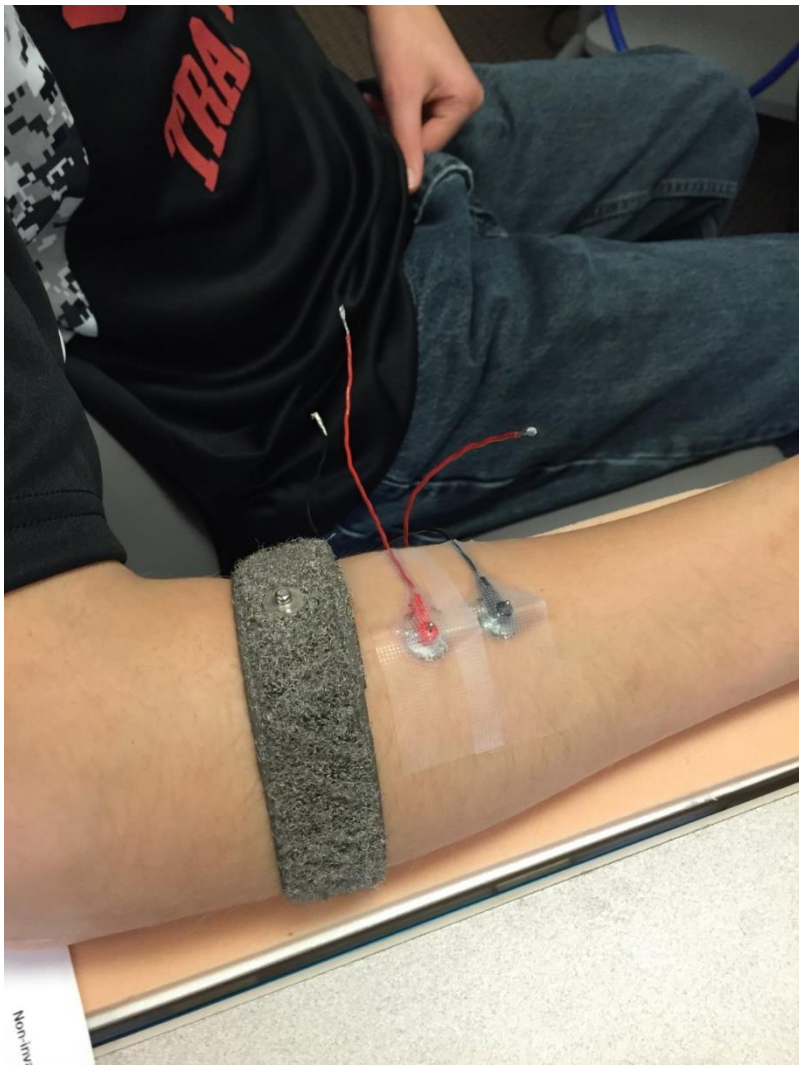
This is the equipment that will be used to study the muscles of your arm.

This is the machine that functions as a magnet to send impulses to your arm muscles to make them move.

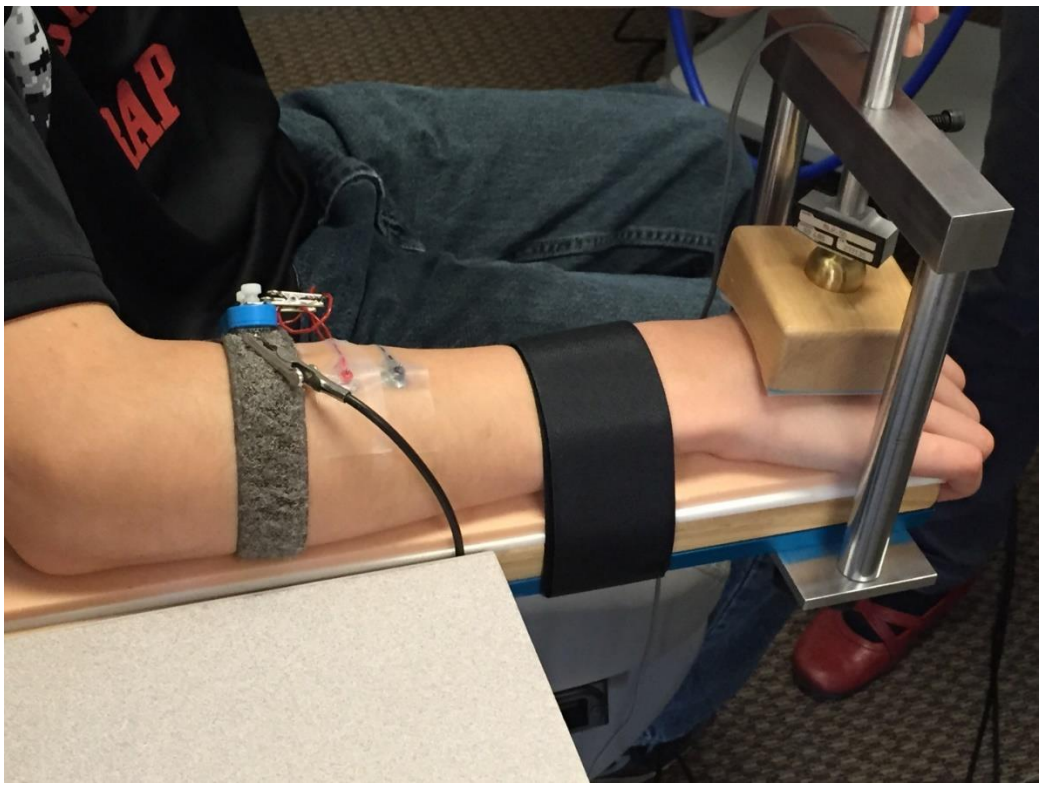
Now we will show you the process for getting ready for the study and doing the activities required for the study.



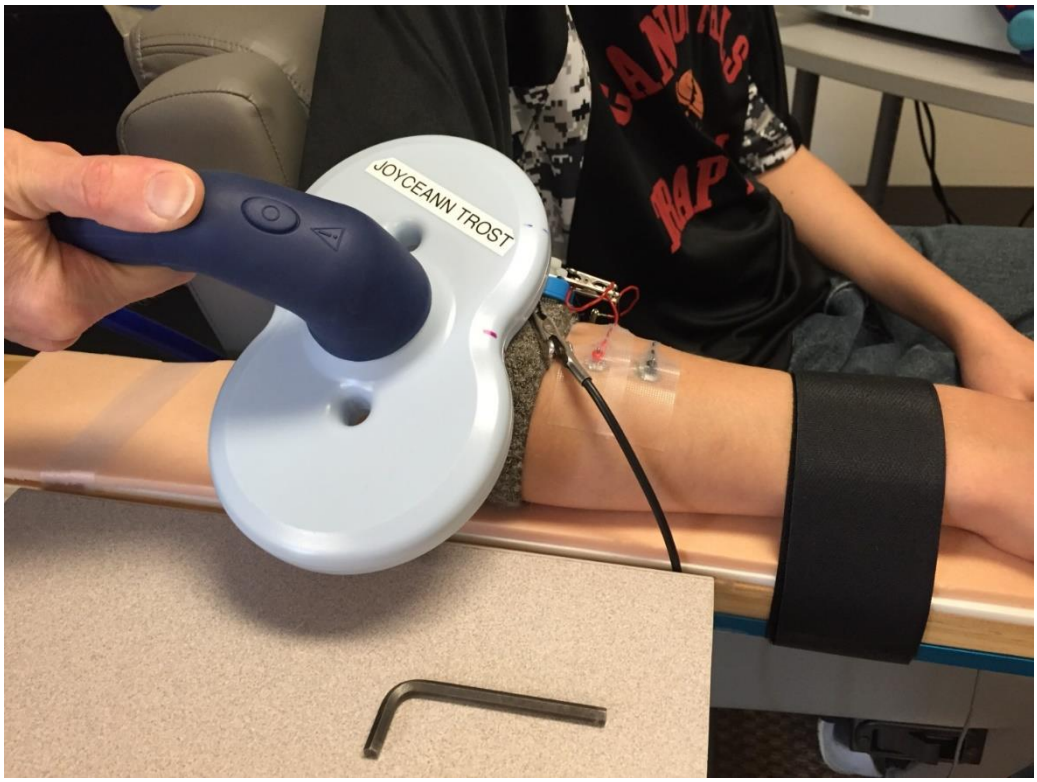
Before we put on the study equipment, we need to clean your arm with alcohol wipes.



Then we put on a strap and electrodes. The electrodes have red and black wires and a metal part that will be taped to your arm. The strap and electrodes allow us to measure the activity of your muscles.



This picture shows the complete set-up of your arm. There are straps to keep your arm in one position during the study. A block is placed on top of your hand to measure the force you can make when you move your wrist. The block is pressed down firmly on top of your hand to make sure the equipment is able to measure your wrist strength. Once you are all set up, wires go to the computers to record the reaction of your arm muscles.



The magnet, which is the grey figure eight item, is then held up next to your arm. It is activated to stimulate your arm muscles. When it is activated, you will hear a clicking sound and your wrist will move without you trying to move it.

Appendix B – Consents

Adult Consent Form

Inexcitability of the muscle plasmalemma and strength loss in Duchenne and Becker muscular dystrophy

You are invited to participate in a research study investigating the effect of fatigue on muscle strength and activity assessed using a non-invasive muscle stimulation device called a peripheral magnetic stimulator. You have been selected because you showed interest in the study when asked to participate during a recent clinic visit, or you have contacted us after receiving an introduction letter or a flyer. This study is being conducted by Joyce Trost, PT and Steven Smith, MD and Randall Richardson, MD of Gillette Children's Specialty Healthcare, and Dawn Lowe, PhD and Peter Karachunski, MD of the University of Minnesota - Paul and Sheila Wellstone Muscular Dystrophy Center. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

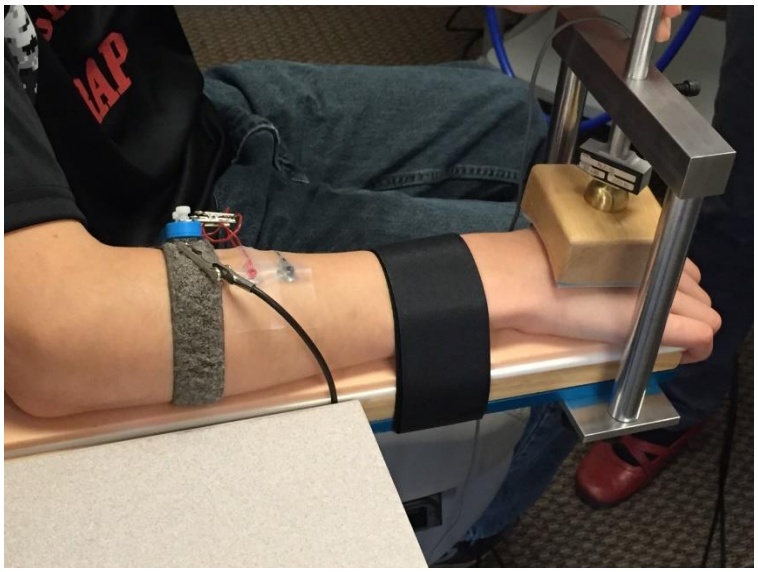
Study Purpose

We are testing young adult men and boys ages 8 to 25 years in this study who have Duchenne or Becker muscular dystrophy or who are healthy and acting as control participants. By applying a magnetic field to a nerve on your arm, electrical currents are produced within the nerve that can affect and measure muscle activity. By using peripheral magnetic stimulation, the muscle and nervous system can be studied to gain a greater understanding of what happens when muscles become fatigued in adult young men and boys with normally developing muscles compared to young men and boys with muscular dystrophy. This may assist physicians develop better ways to care for people diagnosed with muscular dystrophy.

Study Procedures

If you agree to participate and you have Duchenne or Becker muscular dystrophy, you will be seen for three visits during the study. To see if you are eligible to participate in this study, you will be asked questions about your health history and will participate in a functional assessment at a screening visit. You will also be able to see and touch the equipment that will be used during the treatment visits at the screening visit or the first assessment visit. If you are a man with normally developing muscles, you will have your screening visit over the telephone. If you pass the screening visit, two assessment visits will occur about one month apart. One will measure magnetic current through your wrist muscles at baseline and after you do some wrist flexing, with opportunities for resting in between wrist flexes. The last visit will measure magnetic current while you do wrist flexes with little opportunity to rest and will measure fatigue in the wrist muscles.

For the testing assessment visits, you will be seated in a chair with your arm placed in an apparatus similar to the following:



You can pick which arm you would like to use for the study. Small surface electrodes will be placed on your arm with tape and connected to a machine to record the electrical response of your wrist muscles during the assessment.

Next, you will be asked to move your wrist up as far as it can go 3-5 times while your strength is measured. You will then be asked to do this while your arm muscle is stimulated with a magnetic field.

You will receive a series of stimulations to a nerve in your arm with a coil held over your elbow area and forearm. A very brief pulse of electric current will pass through the coil creating a magnetic field near the nerve in your arm, which can pass through the skin and activate the muscle. The magnetic field intensity and location will be adjusted until the best location is found which produces the highest twitch of the wrist muscles. Once this spot is found, the stimulation with the magnetic field will be done several times while your arm muscle is at rest and while you are contracting your wrist muscles. This procedure will be the same at both assessment visits.

At the first assessment visit, you will do this procedure and will be allowed to rest for up to 10 minutes before being assessed again.

At the second assessment visit, you will be asked to do the same procedure but with exercise of your wrist in between, so that we can see how your wrist muscles react when tired or fatigued.

Study personnel will ask you about any side effects experienced from the magnetic stimulation at your next visit, and also following the last assessment visit with a follow up phone call.

The device used in this study for magnetic stimulation is approved by the Food and Drug Administration for stimulation of muscles.

The total time of participation for each assessment visit will be approximately 2 hours. The screening visit will be approximately 1 hour for the functional assessment for men diagnosed with muscular dystrophy. For men with normally developing muscles, the screening assessment will last approximately 10 to 15 minutes.

Risks of Study Participation

Mild effects may include fatigue, muscle soreness, and stress. More serious excessive fatigue or muscle soreness and fainting from the stress of the assessment may occur but is not expected.

Compensation for Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to you or your insurance company. If you think that you have suffered a research related injury, please let us know right away.

Benefits of Study Participation

There are no direct benefits to you for participating in this study.

Study Costs/Compensation

You will receive $25 per visit for a total of $50 of compensation if both visits are completed. This compensation will be given at the second visit. In addition, you will receive $50 at each visit for travel costs.

Confidentiality

The records of this study will be kept private. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject. You will be assigned a Study ID number for confidentiality. Your record for the study may, however, be reviewed by the investigators and study personnel from Gillette Children's Specialty Healthcare and from the University of Minnesota involved with the study and departments at the University with appropriate regulatory oversight. To these extents, confidentiality is not absolute.

Protected Health Information (PHI)

Your PHI created or received for the purposes of this study is protected under the federal regulation known as HIPAA. Refer to the attached HIPAA authorization for details concerning the use of this information.

Voluntary Nature of the Study

Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect current or future relations with Gillette Children's Specialty Healthcare or with the University or the University of Minnesota Medical Center, Fairview. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Whom to Contact
A list of investigator names are below. You may ask any questions you have now, or if you have questions later, you are encouraged to contact them at:

Joyce Trost, PT	651-325-2339
Steven Smith, M.D.	651-578-5062
Peter Karachunski, M.D.	612-365-6777

You may also contact the research coordinator at 651-325-2314.

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the Fairview Research Helpline at telephone number 612-672-7692 or toll free at 866-508-6961. You may also contact this office in writing or in person at Fairview Research Administration, 2433 Energy Park Drive, St. Paul, MN 55108.

Or you may contact Patient Representative of the Quality Improvement Resources Department at Gillette Children's Specialty Healthcare, 200 East University Avenue, St. Paul MN 55101, Telephone 651-229-1706 or 1-800 719- 4040 (toll free) or e-mail qualityrep@gillettechildrens.com.

You may also send feedback by going to: **https://www.gillettechildrens.org/contact-us/** and completing the feedback form. You will be given a copy of this form to keep for your records.

Statement of Consent

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Name of participant (printed) _____

Signature of Participant _____Date_____

Signature of Person Obtaining Consent _____Date_____

Parent/Legal Guardian Consent Form

Inexcitability of the muscle plasmalemma and strength loss in Duchenne and Becker muscular dystrophy

Your child is invited to participate in a research study investigating the effect of fatigue on muscle strength and activity assessed using a non-invasive muscle stimulation device called a peripheral magnetic stimulator. Your child has been selected because you and your child showed interest in the study when asked to participate during a recent clinic visit, or you have contacted us after receiving an introduction letter or a flyer. This study is being conducted by Joyce Trost, PT and Steven Smith, MD and Randall Richardson, MD of Gillette Children's Specialty Healthcare, and Dawn Lowe, PhD and Peter Karachunski, MD of the University of Minnesota - Paul and Sheila Wellstone Muscular Dystrophy Center. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

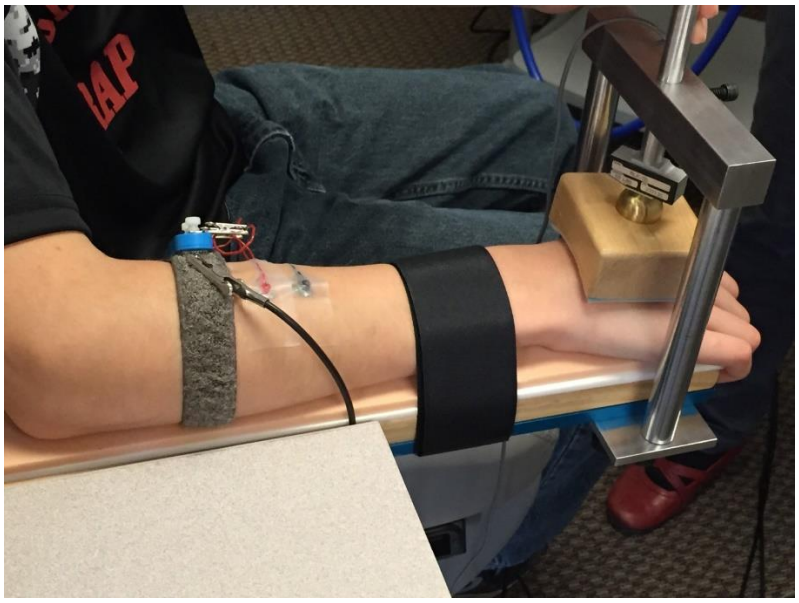
Study Purpose

We are testing young adult men and boys ages 8 to 25 years in this study who have Duchenne or Becker muscular dystrophy or who are healthy and acting as control participants. By applying a magnetic field to a nerve on your child's arm, electrical currents are produced within the nerve that can affect and measure muscle activity. By using peripheral magnetic stimulation, the muscle and nervous system can be studied to gain a greater understanding of what happens when muscles become fatigued in adult young men and boys with normally developing muscles compared to young men and boys with muscular dystrophy. This may assist physicians develop better ways to care for people diagnosed with muscular dystrophy.

Study Procedures

If you agree to allow your child to participate, and your child has Duchenne or Becker muscular dystrophy, your child will be seen for three visits during the study. To see if your child is eligible to participate in this study, you and your child will be asked questions about your child's health history and your child will participate in a functional assessment at a screening visit. Your child will also be able to see and touch the equipment that will be used during the treatment visits at the screening visit or the first assessment visit. If your child is a boy or man with normally developing muscles, you and your child will have a screening visit over the telephone. If your child passes the screening visit, two assessment visits will occur about one month apart. One will measure magnetic current through your child's wrist muscles at baseline and after your child does some wrist flexing, with opportunities for resting in between wrist flexes. The last visit will measure magnetic current while your child does wrist flexes with little opportunity to rest and will measure fatigue in the wrist muscles.

For the testing assessment visits, your child will be seated in a chair with his arm placed in an apparatus similar to the following:



Your child can pick which arm he would like to use for the study. Small surface electrodes will be placed on your child's arm with tape and connected to a machine to record the electrical response of your wrist muscles during the assessment.

Next, your child will be asked to move his wrist up as far as it can go 3-5 times while your child's strength is measured. Your child will then be asked to do this while his arm muscle is stimulated with a magnetic field.

Your child will receive a series of stimulations to a nerve in his arm with a coil held over his elbow area and forearm. A very brief pulse of electric current will pass through the coil creating a magnetic field near the nerve in his arm, which can pass through the skin and activate the muscle. The magnetic field intensity and location will be adjusted until the best location is found which produces the highest twitch of the wrist muscles. Once this spot is found, the stimulation with the magnetic field will be done several times while your child's arm muscle is at rest and while he contracts his wrist muscles. This procedure will be the same at both assessment visits.

At the first assessment visit, your child will do this procedure and will be allowed to rest for up to 10 minutes before being assessed again.

At the second assessment visit, your child will be asked to do the same procedure but with exercise of your child's wrist in between, so that we can see how your child's wrist muscles react when tired or fatigued.
Study personnel will ask you and your child about any side effects experienced from the magnetic stimulation at their next visit, and also following the last assessment visit with a follow up phone call.

The device used in this study for magnetic stimulation is approved by the Food and Drug Administration for stimulation of muscles.

The total time of participation for each assessment visit will be approximately 2 hours. The screening visit will be approximately 1 hour for the functional assessment for boys and men diagnosed with muscular dystrophy. For boys with normally developing muscles, the screening assessment will last approximately 10 to 15 minutes.

Risks of Study Participation

Mild effects may include fatigue, muscle soreness, and stress. More serious excessive fatigue or muscle soreness and fainting from the stress of the assessment may occur but is not expected.

Compensation for Injury

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to you or your insurance company. If you think that your child has suffered a research related injury, please let us know right away.

Benefits of Study Participation

There are no direct benefits to your child for participating in this study.

Study Costs/Compensation

You will receive $25 per visit for a total of $50 of compensation if both visits are completed. This compensation will be given at the second visit. In addition, $50 will be given at each visit for travel costs.

Confidentiality

The records of this study will be kept private. In any publications or presentations, we will not include any information that will make it possible to identify your child as a subject. Your child will be assigned a Study ID number for confidentiality. Your child's record for the study may, however, be reviewed by the investigators and study personnel from Gillette Children's Specialty Healthcare and from the University of Minnesota involved with the study and departments at the University with appropriate regulatory oversight. To these extents, confidentiality is not absolute.

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Your PHI created or received for the purposes of this study is protected under the federal regulation known as HIPAA. Refer to the attached HIPAA authorization for details concerning the use of this information.

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Whom to Contact
A list of investigator names are below. You may ask any questions you have now, or if you have questions later, you are encouraged to contact them at:

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Steven Smith, M.D.	651-578-5062
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Or you may contact Patient Representative of the Quality Improvement Resources Department at Gillette Children's Specialty Healthcare, 200 East University Avenue, St. Paul MN 55101, Telephone 651-229-1706 or 1-800 719- 4040 (toll free) or e-mail qualityrep@gillettechildrens.com.

You may also send feedback by going to: **https://www.gillettechildrens.org/contact-us/** and completing the feedback form.

You will be given a copy of this form to keep for your records.

Statement of Consent

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Name of child (printed) _____

Signature of Parent/Guardian _____Date_____

Signature of Person Obtaining Consent_____Date_____

Child Assent

8 to 17 years

Inexcitability of the muscle plasmalemma and strength loss in Duchenne muscular dystrophy

You are being invited to join a research study of how muscles get tired. You were selected as a possible participant because you or your parent saw an advertisement about this research, or you and your parent talked to your doctor when you were in clinic.

If you agree to be in this study, we will ask you to do a screening visit first to determine whether you are able to do the study. If you have Duchenne or Becker muscular dystrophy, we will ask you to come in for a screening visit to complete an assessment that measures how well your muscles and body function. If you do not have muscular dystrophy, we will conduct a short screening visit on the telephone with your parent.

There will be two more visits. We will ask you to wear cuffs and markers (like stickers) on your arm to measure your muscle strength and activity. We will ask you to move your wrist and arm several times. We will also put a magnet on your arm and stimulate it to make electricity that will make your muscles move without you trying. This may feel different but it will not hurt.

We will ask you to do the testing twice and it may take up to 2 hours each time. If you don't want to do the study, or you get tired in the middle of a visit and want to stop, that's okay. No one will get mad at you if you don't want to do it, or if you change your mind after you have started. If you don't want to do the study or you get tired or stressed in the middle of a visit and want to stop, that's okay. You will get $25 per assessment for a total of $50 if both visits are completed. There is no gift card for the screening visit. In addition, you or your parents will get $50 for travel costs at each visit.

You can ask any questions that you have about the study.

Signing below means that you have read this paper or had it read to you and that you are willing to be in this study. If you do not want to be in this study, then do not sign.

Signature of Participant_____ **Date**_____

Printed name of Participant _____

Signature of person obtaining assent _____ **Date**_____

Printed name of person obtaining assent _____

Appendix C – Egen Klassification Scale Version 2

Egen Klassifikation Scale Version 2 (EK2)[118] Steffensen 2008		
Name		**DOB**
Date of assessment		
Date of spinal surgery (please circle)		**Assessor**
NOTE: *Score the best you have done in the last two weeks especially if there is variation between good and bad days		
1	**Ability to use wheelchair** How do you get around indoors and outdoors?	**N/A**
	Able to use a manual wheelchair on flat ground, 10m < 1 minute	**0**
	Able to use a manual wheelchair on flat ground, 10m > 1 minute	**1**
	Unable to use manual wheelchair, requires power wheelchair	**2**
	Uses power wheelchair, but occasionally has difficulty steering	**3**

2	**Ability to transfer from wheelchair** How do you transfer from your wheelchair to a bed?	**N/A**
	Able to transfer from wheelchair without help	**0**
	Able to transfer independently from wheelchair, with use of aid	**1**
	Needs assistance to transfer with or without additional aids (hoist, easy glide)	**2**
	Needs to be lifted with support of head when transferring from wheelchair	**3**

3	**Ability to stand** Do you sometimes stand? How do you do this?	**N/A**
	Able to stand with knees supported, as when using braces	**0**
	Able to stand with knees and hips supported, as when using standing aids	**1**
	Able to stand with full body support	**2**
	Unable to be stood	**3**

4	**Ability to balance in the wheelchair** Can you bend forwards and to the sides and return to the upright position?	**N/A**
	Able to push himself upright from complete forward flexion by pushing up with hands	**0**
	Able to move the upper part of the body > 30 in all directions from the upright position, but cannot push himself upright as above	**1**
	Able to move the upper part of the body < 30 from one side to the other	**2**
	Unable to change position of the upper part of the body, cannot sit without total support of the trunk and head	**3**

5	**Ability to move the arms** Can you move your fingers, hands and arms against gravity?	**N/A**
	Able to raise the arms above the head with or without compensatory movements	**0**
	Unable to lift the arms above the head, but able to raise the forearms against gravity, ie. hand to mouth with / without elbow support	**1**
	Unable to lift the forearms against gravity, but able to use the hands against gravity when the forearm is supported	**2**
	Unable to move the hands against gravity but able to use the fingers	**3**

6	**Ability to use the hands and arms for eating** Can you describe how you eat?	**N/A**
	Able to eat and drink without elbow support	**0**
	Eats or drinks with support at elbow	**1**
	Eats and drinks with elbow support; with reinforcement of the opposite hand +or – aids	**2**
	Has to be fed	**3**

7	**Ability to turn in bed** How do you turn in bed during the night?	**N/A**
	Able to turn himself in bed with bedclothes	**0**
	Can turn in some directions in bed. Needs help to turn in bed. (Needs rail to pull on, someone else needs to position legs, covers)	**1**
	Unable to turn himself in bed. Has to be turned 0 - 3 times during the night	**2**
	Unable to turn himself in bed. Has to be turned > 4 times during the night	**3**

8	**Ability to cough** How do you cough when you have to?	**N/A**
	Able to cough effectively	**0**
	Has difficulty to cough but able to clear throat	**1**
	Always needs help with coughing. (Help could be: needs to adopt certain position, manual reinforcement, air-stacking)	**2**
	Unable to cough, Needs suction and/or hyperventilation techniques or IPPB in order to keep airways clear	**3**

9	**Ability to speak** Can you speak so that what you say can be understood if you sit at the back of a large room?	**N/A**
	Powerful speech. Able to sing and speak loudly	**0**
	Speaks normally, but cannot raise his voice	**1**
	Speaks with quiet voice and needs a breath after 3 to 5 words	**2**
	Speech is difficult to understand except to close relatives	**3**

10	**Physical well-being** This relates to respiratory insufficiency only (see manual) Use the categories as questions	**N/A**
	No complaints, feels good	**0**
	Easily tires. Has difficulty resting in a chair or in bed	**1**
	Has loss of weight, loss of appetite and associated poor sleep	**2**
	Experience additional symptoms to score 2: Palpitations and perspiring	**3**

11	**Daytime fatigue** Do you have to organise your day or take a rest to avoid getting too tired?	**N/A**
	Doesn't get tired during day	**0**
	Need to limit activity to avoid getting too tired	**1**
	Need to limit my activity **and** have a rest period to avoid getting too tired	**2**
	Get tired during day even if I rest and limit activity	**3**

12	**Head Control** How much head support do you need in your wheelchair?	**N/A**
	Does not need head support	**0**
	Needs head support when going up and down slope (15° standard ramp)	**1**
	Needs head support when driving wheelchair	**2**
	When sitting still in a wheelchair needs head support	**3**

13	**Ability to control Joystick** What kind of joystick do you use to control your chair?	**N/A**
	Uses a standard joystick without special adaptation	**0**
	Uses an adapted joystick or has adjusted wheelchair in order to use joystick	**1**
	Uses other techniques for steering than joystick such as blowing sucking systems or scanned driving	**2**
	Unable to operate wheelchair. Needs another person to operate it	**3**

14	**Food Textures** Do you have to modify your food in any way in order to eat it?	**N/A**
	Eats all textures of food	**0**

	Eats cut up or small pieces of food or avoids hard/chewy foods	**1**
	Eats minced/ pureed food	**2**
	Minimal oral intake	**3**

15	**Eating a meal** (with or without assistance) How long does it take to complete a whole meal?	**N/A**
	Able to consume a whole meal in the same time as others sharing the meal	**0**
	Able to consume a whole meal in the same time as others only with encouragement or needs some additional time (<10 min)	**1**
	Able to consume a whole meal but requires substantially more than 10 minutes extra compared to others eating the same meal or reduces portion size	**2**
	Unable to consume a whole meal even with additional time, assistance	**3**

16	**Swallowing** Do you ever have problems with swallowing?	**N/A**
	Never has problems when swallowing and never chokes on food/drink,	**0**
	May experience occasional (less than once a month) problems swallowing certain types of food or occasionally chokes	**1**
	Has regular trouble swallowing food/drink or chokes on food/drink (more than once a month)	**2**
	Has trouble swallowing saliva or secretions	**3**

17	Hand function Which of these activities can you do?	**N/A**
	Can unscrew the lid of a water of fizzy drink bottle and break the seal	**0**
	Can write two lines or use computer keyboard	**1**
	Can write signature or send text or use remote control	**2**
	Cannot use hands	**3**

	TOTAL SCORE / 51	

	Comments: reasons any items were not applicable (N/A)

Appendix D – Brooke Score

Brooke Score

Grade	Functional description Brooke scale for upper extremity
1	Starting with arms at the sides, the patient can abduct the arms in a full circle until they touch above the head
2	Can raise arms above head only by flexing the elbow (shortening the circumference of the movement) or using accessory muscles
3	Cannot raise hands above head, but can raise an 8-oz glass of water to the mouth
4	Can raise hands to the mouth, but cannot raise an 8-oz glass of water to the mouth
5	Cannot raise hands to the mouth, but can use hands to hold a pen or pick up pennies from the table
6	Cannot raise hands to the mouth and has no useful function of hands