DUCHENNE MUSCULAR DYSTROPHY – INSIGHT AND TREATMENT

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Duchenne Muscular Dystrophy (DMD) is a genetic disorder characterized by progressive degeneration of muscle fibers and dystrophic changes on muscle biopsy. DMD accounts for approximately 50% of all dystrophinopathies, with around 21,000 male babies born with the disease each year. It is also the most lethal X-linked recessive disorder as phenotypic traits are not immediately present at birth. Patients usually do not live past their 20’s without medical intervention to treat associated respiratory and cardiac dysfunctions. For these reasons DMD remains one of the greatest threats, amongst a range of pediatric pathologies, to the normalcy of child development and parental care. Although treatment options have shown to mitigate the progression of DMD, most are controversial and costly - the estimated annual treatment cost of DMD per patient is $50,953. In light of this, disease awareness and public health education are critical components for acquiring funds needed for research towards a cure. My hope is that through this integrated overview of DMD, the medical layman will better understand the depths of this lethal disease, and how it can be detrimental to both the affected child and his caretaker.
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Foreword

My experience observing Pediatric Physical Therapist at the Children’s Center for Rehab in Tucson, Arizona, has revealed to me the many physical ailments of children (neuromuscular, genetic, cardiac). In my observations, I witnessed how a single genetic mutation expressed as a phenotype can adversely affect the livelihood of both child and caretaker, and the various obstacles posed to pediatric physical therapists that are otherwise almost non-existent in an orthopedic outpatient clinic. I have come to realize that success in pediatrics does not usually amount to full recovery, but the betterment of daily functioning in the development of both child and adult. I dedicate my honors thesis to the steadfast and patient therapists who selflessly strive to better the lives of children. To date, a cure for Duchenne Muscular Dystrophy has yet to be found. But because of the continuous input by researchers, therapists, doctors, and the general public alike, there remains an optimistic prognosis for neuromuscular diseases in the future.
Chapter 1
The Muscular System

Constituting 40-50% of the average adult human weight, the muscular system is made up of 3 types of tissue: skeletal, cardiac, and smooth muscle. The primary function of skeletal muscle is to generate force and movement in response to physiological stimuli, which is essential for movement and rotation of bones around a joint - resulting in characteristic mobility.

From a young age, patients with DMD present delayed achievement of motor performance - reflecting early skeletal muscle weakness then relatively quick progression to a non-ambulatory state with wheelchair assistance. Most clinical mortalities of DMD can be attributed to both skeletal and cardiac muscle abnormalities, thus we will concentrate most of our efforts on the anatomy and physiology of these tissues.

Skeletal Muscle Anatomy

Skeletal muscles are so named because most are attached to and move bones. There are both voluntary and involuntary skeletal muscles. Voluntary skeletal muscles, such as the bicep or deltoids, are consciously controlled by the central nervous system. Involuntary skeletal muscles, such as the diaphragm do not require conscious control for breathing, but both types are affected by DMD.

Figure 1: Skeletal Muscle Components
A cartoon-schematic illustrating the individual smaller units of a muscle.

Though all skeletal muscles are highly structured and exist as individual organs, they share similar connective tissue components and microscopic anatomy with one another. Figure 1 illustrates a single skeletal muscle as a collection of muscle fiber bundles that are held together...
and protected by 3 layers of connective tissue: epimysium (surrounds the entire muscle), perimysium (surrounds a group of muscle fibers), and fascicle (surrounds a single muscle fiber or muscle cell)\textsuperscript{5}.

The most immediate observable feature of a muscle fiber is its size. The diameter of a single muscle fiber can range from 10-120µm\textsuperscript{13}. Its typical length is about 10cm, but may also extend the entire length of the skeletal muscle and is multinucleated to fulfill the tasks of the cell\textsuperscript{5}. Deep to the fascia, each muscle fiber is encompassed by a plasma membrane, called the sarcolemma, which contains a mass of invaginations that tunnel into the sarcoplasm (cytoplasm) like tubes\textsuperscript{13}. These tubes are termed Transverse (T)–tubules and are crucial for the conduction of action potentials to penetrate deep into the interior of the cell\textsuperscript{5}.

\textbf{Figure 2: Microscopic Anatomy of A Muscle Fiber}\textsuperscript{14}  
\textit{A cartoon-schematic representing various organelles present on a section of single muscle fiber}

Like a Russian doll, Figure 2 shows that each muscle fiber is further organized into elastic bundles of protein called myofibrils that extend the entire length of the muscle fiber. Myofibrils are surrounded by a series of membranous sacs called the sarcoplasmic reticulum (SR), which store calcium ions essential for muscle contraction\textsuperscript{5}. Each myofibril is further composed of multiple subunits called sarcomeres that are joined end to end by structural proteins called Z-discs (shown on figure 2 as z-line)\textsuperscript{13}. The sarcomere is the simplest functional unit of a muscle fiber, and accounts for the contractile property of both skeletal and cardiac muscle\textsuperscript{5, 13}. 
Under the light microscope, skeletal and cardiac muscles appear striated. This is due to the overlapping contractile fibers of the sarcomere, known as thin and thick filaments, which are arranged in parallel\textsuperscript{10, 13}. Figure 3 illustrates the arrangement of a single sarcomere, with the thin filaments attached at a single end to the Z-line and thick filaments held in the middle by myomesin (proteins that form the M-line)\textsuperscript{5}. These filaments consist of essential proteins that allow the contractibility of muscle.

Thin filaments are made of long double-helical chains of actin molecules along with regulatory proteins such as tropomyosin and troponin\textsuperscript{13}. On each actin molecule is a myosin-binding site that is covered by tropomyosin during relaxation; upon calcium binding, the regulatory protein troponin moves tropomyosin out of the way for muscle contraction\textsuperscript{5}. Thick filaments are made of hundreds of myosin molecules\textsuperscript{13}. Each molecule consists of a tail and two myosin heads that bind to myosin-binding sites on thin filament actin molecules\textsuperscript{5}. Contraction of muscle utilizes these contractile proteins in a process called the Sliding Filament Mechanism.
Dystrophin

Figure 4: Cartoon Illustration of Dystrophin-Glycoprotein Complex

The functional importance of dystrophin has been studied for many years by correlating mutations on chromosome Xp21 with clinical and biochemical representation in DMD patients. Shown in Figure 4, Purification of the dystrophin-glycoprotein complex (DGC) reveals that dystrophin plays a vital structural role in linking cytoskeletal microfibers to sarcolemmal glycoproteins: dystroglycan, sarcoglycan, syntrophins, which bind to extracellular matrix elements such as laminin. As an intracellular linker-protein, dystrophin is essential to the structural integrity of the muscle fiber as well as the successful transmission of tension from muscle fibers to bone. Patients with DMD are born with a genetic mutation on the dystrophin gene – carried on the X Chromosome - that renders them dystrophin deficient.

Figure 5: Frameshift Mutation of Dystrophin mRNA

A cartoon representation of mRNA splicing and mutation

The dystrophin gene is the largest known gene in the human body and spans at least 2.4Mb on the X-chromosome. Not surprisingly, more than 130 different point mutations have been detected on this gene alone. DMD is an X-linked recessive disorder. It occurs mostly in...
males (XY) because they only require one faulty copy of the dystrophin gene. Females (XX) however, can be carriers without realizing their deficiency, until later in life, due to a healthy “back up copy” of the dystrophin gene\textsuperscript{34, 59}. Sixty-four percent of DMD cases result from a gross deletion on the dystrophin gene, and other mutations include: small deletions, duplication of DNA within the gene itself, insertion, and missense mutation\textsuperscript{20}. Figure 5 illustrates a common frameshift and a premature stop codon mutation on mRNA upon splicing\textsuperscript{22}. In effect, due to these mutations, abnormal-mRNAs that are transcribed do not usually survive to become translated into functional dystrophin proteins\textsuperscript{20, 30}. Without dystrophin present to mediate the connection between the sarcolemma and extracellular cytoskeleton, muscle fibers degrade and scar, which eventually result in loss of function.

In addition to its mechanical function, the DCG is also a key mediator in cellular signaling. In a healthy human, the DCG binds nNOS (neuronal Nitric Oxide Synthase), an essential enzyme known for signaling downstream proteins to decrease vasoconstriction as well as binding caveolin-3, which acts as a membrane scaffold to organize membrane proteins and structure. In the absence of dystrophin, the DCG fails to form and nNOS is no longer localized on the sarcolemma, and thus loses its function. With unregulated vasoconstriction and loss of caveolin-3, myofiber apoptosis increases in both skeletal and cardiac muscle\textsuperscript{43}. The loss of intracellular signaling and sarcolemma structure of muscle can be observed on a patient’s phenotype. Patients with DMD will initially experience pseudohypertrophy of their gastrocnemius (due to scaring and adipose deposits) and then progressive and severe weakness in both cardiac and skeletal muscle\textsuperscript{4}.

Aside from striated muscles, no other affected tissues manifest clear phenotypic consequences of dystrophin gene mutations. Mental retardation in DMD patients remains one of the least understood features of the disease, because there is no clear correlation between specific types or locations of mutations.

The Physiology of Movement

The main goal of muscle function is force production, and the smallest unit of voluntary force production is the motor unit\textsuperscript{13}. The movement plan first begins as an electrical signal formulated in the prefrontal cortex and the primary motor cortex. From large pyramidal neurons, the movement-plan travels toward the spinal cord and exit via motor neurons of the ventral horn.
to branch and synapse at various muscle fibers, forming the Neuromuscular Junction (NMJ) shown in figure 613.

Figure 6: The Neuromuscular Junction

As the electrical signal approaches the axon button, voltage gated calcium channels open to allow the influx of calcium. The influx of calcium causes synaptic vesicles filled with the neurotransmitter acetylcholine (Ach) to fuse with the presynaptic membrane. Once released, Ach travels down its concentration gradient (across the synaptic cleft) to bind with nicotinic Ach-receptors, opening non-selective cation gated channels, which results in net cation influx into the muscle fiber13, 21. As positive charge flows into the muscle cell, a local potential known as the end plate potential (EPP) is initiated13. This activates voltage gated sodium channels in close proximity, allowing influx of sodium ions into the sarcolemma. The resulting action potential propagates along the sarcolemma down the T-tubules then, at the triad, activates voltage gated calcium channels at the terminal cisternae (figure 2-enlarged portion of the SR) to release calcium into the sarcoplasm.

The calcium released into the sarcoplasm is quickly bound by troponin-c on sarcomere thin filaments, causing a conformational change in the troponin-tropomyosin complex and exposes myosin-binding sites on G-actin13. By a process known as the “Sliding Filament Mechanism”, myosin establishes a firm bond with actin on its binding site, forming a cross-bridge13. Bending at its neck, myosin moves the thin filaments towards the M-line in a rowing fashion, shortening the sarcomere13. ATP then binds at the myosin head, allowing dissociation of the actin-myosin bond. The process continues until calcium is resequusted into the SR by a
transport protein called SERCA. On the macroscopic scale, the Sliding Filament Theory translates into muscle contraction – utilizing intracellular proteins such as dystrophin to translate contractile tension to tendons, as well as to maintain cell structural integrity under continuously stressful conditions.

The pathological muscle wasting in DMD was first observed at the microscopic level in 1975, when Mokri and Engle found large lesions on the sarcolemma of DMD biopsies\textsuperscript{33}. It seems that in the absence of dystrophin, the sarcolemma becomes structurally compromised as the DGC is no longer expressed, allowing the unregulated passage of cytosolic and extracellular proteins and ions through the muscle fiber due to the “leakiness” of the plasma membrane\textsuperscript{20, 33}. This hypothesis is strongly supported by the observation of abnormally high levels of sarcoplasmic enzymes in serum\textsuperscript{20}. Additionally, membrane lesions have been hypothesized to allow pathological influx of calcium ions into the muscle fiber, which activates intracellular proteases and the accumulation of harmful reactive oxygen species\textsuperscript{20, 39}. The abnormal permeability of the sarcolemma is worsened by the mechanical stress placed on the muscle fiber during contraction\textsuperscript{33}. Eventually, constant deviation from homeostasis leads to the grouped necrosis then fibrosis of muscle fibers\textsuperscript{24}. This is witnessed early in childhood as DMD patients first display a thickened gastrocnemius (due to accumulation of scar tissue) followed by progressive muscle weakness in their delayed achievement of motor milestones.

The Motor Unit

A motor unit consists of a single motor neuron and all the muscle fibers it innervates\textsuperscript{13}. Motor units comprised of large numbers of muscle fiber are often utilized for forceful movement, while motor units comprised of smaller numbers of muscle fiber tend to be used for subtle and precise movements\textsuperscript{16}. Motor units can vary by magnitude and velocity of force production, most of which has to do with the type and number of muscle fibers that make up the motor unit\textsuperscript{16}.

All fibers in a motor unit are typically homogenous in composition, meaning that fiber type usually denotes the type of motor unit. Muscle fibers are categorized into 2 types, slow and fast twitch, or type 1 and 2 respectively according to myosin types\textsuperscript{13, 16}. The latter, however, can be further subcategorized into Type 2A and 2X in humans\textsuperscript{16}. Figure 7 shows the various components of muscle fiber types that make them unique.
Notice that “twitch (contraction) time” for types 1 and 2 differ dramatically. This is primarily due to the different myosin ATPase isoforms present on each type. Type 2 fibers have myosin ATPase isoforms that catalyze ATP hydrolysis much faster than the ones on type 1 fibers. Hence, type 2 fibers have more cross bridge formations and detachments per unit time, and thus faster speed of contraction across fast (FOG/FG) motor units.

The magnitude of force production is subject to the size of individual fibers, the number of muscle fibers, and the innervation ratio in that motor unit. Compared to smaller fibers, large muscle fibers contain more sarcomeres per unit volume - meaning more cross bridges that can be formed. Incidentally, as seen in Figure 7, fiber types also account for the individual diameter of each muscle fiber. With increasing number of larger diameter fibers, the motor unit also increases in magnitude of force production. Essentially, the total cross sectional area of a motor unit determines force production potential.

Overall, it is important to note that while our muscles may have the same basic components, not all function to do the same tasks. Type 1 muscle fibers are primarily used for endurance exercises such as walking and maintaining posture, while type 2 muscles are used for short and explosive movements such as running and jumping. Patients with DMD usually show early signs of muscular weakness in proximal muscles, which vary in fiber type composition. Observations of DMD patients also reveal difficulty in recruiting both type 1 (slow) fibers for activities such as walking (gait) and standing, and type 2 (fast) fibers for activities such as running. This is followed by substantial and insidious fatigue in these fibers, often leading to...
wheelchair assistance by the age of 13\textsuperscript{18}. However, one of the primary causes of death in DMD patients is not due to degradation of ambulatory muscles, but ventilatory insufficiency due to diaphragm pseudohypertrophy\textsuperscript{28}. Additionally, intercostal muscles begin to fail during the teenage years resulting in decreased capacity for forced inhalation. Eventually, further stiffening of the diaphragm due to scaring will result in: decreased lung capacity, decreased cough efficacy, and REM sleep hyponemia\textsuperscript{19}. Consequently, in the past, 70\% of DMD mortalities were due to respiratory complications\textsuperscript{19}. As understanding of this disease has progressed significantly over the years, DMD patient prognosis has improved as ventilatory aids (such as nocturnal ventilation), cough assist devices (to remove bronchial secretions), and spinal surgery (to correct air-passage), amongst an array of treatment options are now offered as part of their treatment plan\textsuperscript{36, 37}. With the help of these treatments, life expectancy for DMD patients has been improved from 22 years to 30 years on average, significantly decreasing the mortality of DMD patients due to ventilatory failure\textsuperscript{37}. Unfortunately, cardiomyopathies have become the leading cause of death in DMD patients\textsuperscript{36, 38}.

Cardiac Anatomy and Function

The heart is the primary organ responsible for pumping blood throughout the body. It is divided into 4 chambers with left and right sides housing the atria at the base, and the ventricles at the apex. While the heart is a single organ, it actually functions as 2 separate pumps\textsuperscript{25}. The right segment of the heart receives deoxygenated blood from the body and pumps it through the pulmonary system for gas exchange. Oxygenated blood then feeds into the left segment, which is pumped throughout the systemic circulation. Due to the longer distance and higher pressure of the systemic circulation, the left ventricle is much more muscular than the right ventricle\textsuperscript{25, 26}.

Overall, 99\% of cardiac muscle cells are cardiomyocytes while 1\% are autorhythmic cells\textsuperscript{27}. While cardiomyocytes are small and contain only a single nucleus, their cellular components are arranged much like skeletal muscles – each cardiac myofiber is an organization of myofibril bundles composed of sarcomeres. However, unlike skeletal muscle, cardiomyocytes are tightly connected to one another via intercalated discs, which contain both desmosomes and gap junctions. Essentially, cardiomyocytes are arranged so that an electrical signal forces the left and right atria then ventricles to contract as a functional syncytia.
Autorhythmic cells are a collection of cardiac cells that are capable of spontaneously producing action potentials. As part of the conduction system, these cells are the primary reason why the heart can continue beating on its own without nervous input\textsuperscript{31}. The conduction system is composed of the: sinoatrial (SA) node, atrial-ventricular (AV) node, Bundle of His, left and right bundle branches, and Purkinje fibers – listed in their respective order of activation. The SA node is the primary pacemaker of the heart and forces the conduction system to follow its rate of firing (around 70 per minute) in a mechanism known as suppression overdrive\textsuperscript{27}. As the SA node fires, an electrical signal is transduced along the inter-atrial pathway, inducing left and right atrial contraction (kick). As a benefit to atrial emptying, the AV node takes a longer time to conduct the action potential for ventricular contraction. Upon conduction, the AV node propagates the action potential quickly to the Bundle of His and eventually to the Purkinje fibers – initiating ventricular myocyte contraction. It is important to note that the AV node is the only point of electrical contact between atria and ventricles, and all of these electrical activities occur in a single cardiac cycle (heart beat)\textsuperscript{27}.

Though mostly asymptomatic in early childhood, cardiomyopathies progress quickly over time. Over 80% of DMD patients over 18 years old have some form of cardiac dysfunction, and almost all who survive into the 3\textsuperscript{rd} decade of life display cardiomyopathy\textsuperscript{34,39}. Interestingly, the severity of dysfunction does not correlate with the severity of skeletal muscle weakness, and female carriers are more likely to show signs of cardiomyopathy rather than skeletal muscle deterioration\textsuperscript{34}. Most DMD patients with heart abnormalities first display sinus tachycardia (abnormally fast heart rate arising from SA nodal dysfunction) on the ECG, followed by progression to heart failure\textsuperscript{34,35}. Studies have shown that most DMD patients with tachycardia are more likely to progress to cardiomyopathy, hypertrophic ventricles, then end-stage dilation (heart failure) within 5 years\textsuperscript{35}.

In DMD patients, the lack of dystrophin in cardiac myocytes elicits similar pathobiochemical responses as seen in skeletal muscles\textsuperscript{40}. This strongly supports the current notion that the lack of concomitant DGC expression is a direct cause for the wasting and stiffening of cardiac muscle cells with constant contractions – most notably diagnosed first as tachycardia then dilated cardiomyopathy\textsuperscript{40}. Following poor cardiac function, the renin-angiotensin system is activated to conserve blood pressure and volume in the systemic
circulation. Amongst its array of actions, angiotensin II is potent stimulator of TGF-β, which stimulates degradation and fibrosis of cardiac muscle – contributing to heart failure.

Additionally, recent studies reveal that a deletion of the dystrophin gene is a harbinger for enhanced enterovirus-induced cardiomyopathy\textsuperscript{41}. Enterovirus introduction to highly permeable cardiac cell membranes initiates an inflammatory response that precipitates heart failure. In a study conducted by Mavrogeni et. al, all 6 DMD patients who contracted myocardial inflammation progressed to significant left ventricular dysfunction (decreased ejection fraction) within a year\textsuperscript{41}. This, along with other dmx mice studies, provides evidence for dystrophin’s probable role in the signaling pathway of inflammation\textsuperscript{43}. The prognosis for DMD patients with myocardial inflammation or signs of tachycardia is grave – most will progress to heart failure if there is no early intervention.

Early detection and intervention of cardiomyopathy is key to DMD patient longevity. The current standard of care for DMD patients involves echocardiograms and ECG, both proven to be useful in detecting cardiopathologies. However, current studies reveal Cardiac Magnetic Resonance Imaging (CMR) as a more sensitive means to detect early cardiac involvement\textsuperscript{39}. CMR has shown to detect cardiac fibrosis even when an ECG appears normal\textsuperscript{39}. It is strongly advocated that CMR be in a DMD patient’s line of care due to its ability to help decide when cardioprotective treatment should be instituted\textsuperscript{39}. 
Chapter 2  
Treatment and Research

To date, no cure for DMD has been identified. Due to the progressive nature of the disease, the main goals for rehabilitation are to delay the decline in muscle performance and maximize function in the patient. However, the development of effective therapies for DMD has been a challenge throughout the years. While some ventilatory and cardiac treatments, already discussed, are relatively well established and have increased life expectancy of DMD patients, other therapeutic methods such as exercise and corticosteroid use have become hot topics for debate. In this chapter, the proposed efficacy of these highly controversial treatment methods will be closely examined through the lens of modern research. For a complete overview, future treatment methods such as gene therapy and stem cell treatment will also be discussed.

Exercise

Although the international guidelines recommend regular submaximal intensity exercise for DMD patients, there still remains a billow of doubt about its effectiveness. Concerned parents often ask: “Does exercise help or hurt my child?” On the surface, their concern seems justifiable as aggravating an already compromised muscular system contradicts the effects of treatment. Furthermore, past research using mdx (DMD) mice models purport the idea that exercise accelerates the progression of DMD. However, the main problem with these kinds of research is that the mdx mouse does not fully encapsulate the phenotype of a human DMD patient in terms of muscle regeneration and protein compensation. It is also unclear how the intensity and type of exercise induced on the mice model translates to similar detrimental effects for human DMD patients. Thus, such research must be considered cautiously.

Alternatively, small human studies have shown the positive effects of exercise on DMD patients. Alemdaroğlu et al. showed that submaximal upper extremity exercise (training with an arm ergometer) had positive effects on DMD subjects’ muscular endurance, performance of daily activities, arm function, and ambulation status, with almost no change in muscular strength. Because of their wheelchair confinement early in life, DMD patients often develop secondary functional deterioration. In an effort to confront the “No Use is Disuse” hypothesis, a randomized controlled trial was conducted by Groot et al. to determine the effects of dynamic
physical training on DMD patients\textsuperscript{49}. In his study, it was found that dynamic exercise, such as assisted bicycle training (with little to no resistance) delayed functional deterioration due to muscle disuse – contrary to the popular opinion that it accelerates disease progression\textsuperscript{49}. While these studies, and others alike, give evidence that some exercise can benefit DMD patients, there still remains an uncertainty and uneasiness when prescribing exercise for individual patients. From the current available evidence, establishing clearly defined protocols such as the: intensity, frequency, duration, and mode of exercise can be extremely difficult\textsuperscript{50}. In order for physical therapists to make an informed exercise prescription, there needs to be controlled studies that test various standard operating protocols for exercise training in DMD across all stages of progression\textsuperscript{44}.

\textbf{Corticosteroids}

Steroid therapy is considered the “gold standard” in treating DMD patients for both skeletal and cardiac muscle insult\textsuperscript{42, 54}. Thus far, 7 Class I studies have shown that acute administration of Prednisone and Deflazacort (not found in the USA) improved muscle strength and function in DMD. Patients who were given these corticosteroids showed increase in peak strength in 3 months and improvement in rising and walking times, along with later loss of ambulation than placebo groups. Many studies also give strong evidence that DMD patients treated with steroids prior to the onset of cardiac dysfunction show slower progression to heart disease - 93\% of steroid-treated DMD children maintain normal cardiac function compared to 53\% of untreated children\textsuperscript{42}. Though the mechanism of steroids and its benefits in DMD patients remain a mystery, many researchers offer promising explanations for their worth. One hypothesis is that muscle degeneration in DMD is delayed with steroid treatment due to its immunosuppressive actions on inflammatory agents that complement cell lysis. In DMD, TGF-\(\beta\) (a muscle regeneration inhibitor) and CD8\(^+\) cells (muscle specific cytotoxic T-cells) are strongly up regulated and contribute to the pathology of muscle degeneration. Corticosteroids down regulate genes involved in the production of TGF-\(\beta\) as well as reduce infiltration of muscle fibers and connective tissue proliferation in DMD\textsuperscript{54}. Another explanation is that corticosteroids reduce the rate of muscle necrosis by inhibiting proteolysis. Rifai et al. found that Prednisone seemed to increase muscle mass by mediating proteolysis rather than stimulation of protein synthesis\textsuperscript{55}. 
While early steroid therapy seems to preserve overall cardiac and skeletal muscular function, perhaps the most concerning aspect about their administration are the associated side effects. The Class I studies that evaluated daily Prednisone and Deflazacort treatment found that the most common side effects in DMD patients were weight gain, development of a Cushingoid facial appearance 6 to 18 months after treatment, and cataracts\textsuperscript{52, 53}. The American Academy of Neurology, backed by the Muscular Dystrophy Association (MDA), recommend a daily dose of 0.75mg/Kg of Prednisone and 0.9mg/Kg of Deflazacort for optimal results\textsuperscript{52, 53}. Dosage can be effectively lowered to 0.3mg/Kg if side effects such as excessive weight gain develop\textsuperscript{53}. Consequently, the decision to proceed with steroid treatment must include a discussion of both benefits and side effects between the caregiver and the attending physician. If parents decide to administer steroids, there are many methods to monitor their child’s disease progression as well as to mediate the side effects. Timed function tests, pulmonary function tests, and age at loss of ambulation are useful to assess benefits of steroid treatment\textsuperscript{52}. Much more research must be done to find an optimal therapy window, steroid, dosage, and therapy duration for DMD patients. As an alternative, ACE inhibitors (used to prevent the conversion of angiotensin I to angiotensin II) and β-blockers are widely used and recommended to prevent and treat cardiac fibrosis\textsuperscript{42}. Current recommendations continue to advocate the use of ACE inhibitors as a preventative therapy for DMD patients\textsuperscript{42}.

Gene Therapy

Gene therapy is an experimental technique that alters gene expression through: replacement of a mutated gene, “knock-out” of a mutated gene, and introduction of a new gene. Modified genes through gene therapy are supposed to produce their respective functioning proteins. In the case of DMD, the most commonly targeted gene is the dystrophin gene, which is unable to produce functioning dystrophin for the DGC due to mutations. However, the sheer size of the dystrophin gene has posed therapeutic challenges in DMD. Viral vectors are commonly used as a transport vesicle to deliver genetic materials into cells for gene transfer and replacement\textsuperscript{57}. However, in the case of DMD, viral vectors are unable to incorporate a gene of this enormous magnitude\textsuperscript{56}. Experiments with mdx mice models show that the dystrophin gene can be truncated with minimal impact in function\textsuperscript{51}. Further studies have also found that transgenic mdx mice that expressed the inserted truncated dystrophin cDNA did not develop
muscular dystrophy. Interestingly, muscles of these animal models displayed complete restoration of their DGC\textsuperscript{51}. The ability to rescue the DGC by means of gene replacement has spurred efforts to translate the same results to human subjects. MDA supported scientists recently ran a trial with synthesized dystrophin gene in DMD boys. Although the trial appeared to be safe, some boys experienced immune system rejection of the later synthesized dystrophin protein\textsuperscript{60}. Some of the main obstacles for this invasive method include: finding an efficient delivery method for gene insertion, generation of functional mini-gene cassetts, and prevention of inflammation\textsuperscript{60}.

Stem Cell Research

Stem Cells are also known as pluripotent cells. That is, they have the potential to become any specialized cell in the body given proper cell-cell communication and developmental factors. MDA scientists are currently isolating stem cells from muscle, blood vessels or bone marrow to regenerate muscles in dystrophin-deficient laboratory animals\textsuperscript{60}. A research conducted by Benchaouir et al. in 2007 showed that exon skipping of a DMD patient’s stem cell, and the eventual insertion into mdx mice, showed restoration of dystrophin synthesis and regeneration of dystrophic skeletal muscles. More recently, in 2010, French MDA researchers found a previously unknown muscle stem cell located between muscle fibers in mice\textsuperscript{60}. This remains an exciting prospect for stem cell research. Though DMD stem cell research has yet to translate to human trials, this form of therapy could very well be a cure for the disease.
Chapter 3
DMD Support

A diagnosis of DMD can be devastating to both patient and caretaker. As the DMD child develops, muscle weakness and accompanied fatigue will set in during the early stages of childhood. Progressive weakness in the trunk and limbs will lead to a non-ambulatory stage and eventually respiratory and cardiac complications. At various stages, caretaking for the patient can be difficult to bear. Parents sometimes quit their jobs to take care of their dystrophic child full-time, losing an average of $15,481 per year in income\(^5\)\(^8\). Non-medical expenditures including: special care, housing, and supplements, cost on average about $12,939 per year\(^5\)\(^8\). The loss of income combined with increased cost of living and day-to-day patient care can take a huge toll on caretakers. However, non-profit organizations such as the MDA have implemented programs that seek to support caretakers by improving the lifestyle of patients and their families.

The Muscular Dystrophy Association (MDA)

The MDA is the world’s leading nonprofit health organization that sponsors research seeking the causes of, and effective treatments for, neuromuscular diseases (MDA). Beyond their global research efforts, the MDA plays a vital role in community outreach and education throughout the United States. They’ve established support groups that are disease specific and topic focused for affected families. In these support groups, a facilitator oversees multiple families throughout their caretaking roles. Communal bonds are built as families fellowship and offer support to one another in a friendly environment. The MDA also has multiple specialized clinics stationed all over the United States. Tucson alone has 3 locations – one of which is found in Banner Health Medical Center at the University of Arizona. These clinics serve the underprivileged by offering free medical services to patients who are either not or under-insured. As an added bonus, the MDA also offers an all-expenses paid summer camp for dystrophic children - reminding both patient and caretaker that neuromuscular diseases should never be fought alone.
Closing Statements

“Education is the most powerful weapon with which to change the world” – Nelson Mandela.

My thesis research has taken me into the depths of DMD, a little-known muscular disease that affects close to 21,000 new born each year. Even more intriguing to me is the exhibition of tenacity and resilience in family members who have a son or brother diagnosed with DMD. To me, they are the epitome of human selflessness, compassion, and love. Contrary to popular belief, effective change doesn’t necessarily need to come from the surgical room or the laboratory. While the search for a cure is a priority, those of us watching from the sidelines must never forget that emotional, physical, and financial suffering occurs in real time for a lot of families affected by neuromuscular disorders. In reality, donations and grants are the main driving force for all the benefits provided by the MDA. Without the necessary public education and fundraising efforts, all of these important emotional and financial support elements would cease to exist. That is why I’ve decided to raise awareness and funds for the MDA by participating in this year’s MDA Tucson Muscle Walk. My hope is that through this thesis, and my outreach in the University of Arizona, students will become more knowledgeable about DMD and join the fight for progress.

As my undergraduate career comes to a close, I would like to honor the people who have made my honors experience a memorable one. I thank Dr. Keen for his light heartedness, time investment, and willingness to work with me on my thesis. I would have never aspired to complete such a passionate piece if it were not for his guidance and unwavering belief in me. I would also like to thank Dr. Rankin for her continuous support throughout my academic years. She has instilled in me the significance of community outreach and student involvement; without her encouragement and trust, I would not have become the leader and student I am today. Lastly, I thank my family and friends for their continual support and advise during my uninspired moments. I close this chapter with a full and grateful heart.
Appendix

Please join my efforts in fundraising by donating to the MDA. Attached is my personal MDA page for the October 24 2015 Tucson Muscle Walk.

http://www2.mda.org/site/TR/Walk/General?px=3450982&pg=personal&fr_id=19839


54. T.I.3 the Rationale for Immunosuppressive Treatment in DMD. Neuromuscular Disorders, 2008;18.9:830.


