Protocol for:

Impact and interplay of corticosteroid regimen and exercise training on DMD muscle function

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Impact and Interplay of Corticosteroid Regimen and Exercise Training on DMD Muscle Function

2. Investigators:

Principal Investigator:

- Tanja Taivassalo, PhD (Physiology & Functional Genomics)

Co-investigators and study staff:

- Warren Dixon, PhD (Mechanical & Aerospace Engineering)
- Kimberly Stubbs, PhD student (under Warren Dixon)
- Donovan Lott, PhD (Physical Therapy)
- Sean Forbes, PhD (Physical Therapy)
- John Sladky, MD (Pediatric Neurology)
- Lee Sweeney, PhD (Pharmacology & Therapeutics)
- Krista Vandenborne, PhD, PT (Physical Therapy)
- David Fedel, PhD, ABPP (Child Psychology)
- Catherine Powers (Physical Therapy)
- Samuel Riehl (Physical Therapy)

3. Abstract:

In the search for effective treatments for patients with Duchenne Muscular Dystrophy (DMD), the question of optimal glucocorticoid steroid (GC) administration regimen persists. Compelling preclinical data suggests that transient low dose GC administration provides the same efficacy with fewer side-effects as daily use, and may have ergogenic impacts on muscle similar to exercise. No study has yet investigated the potential of such low-dose twice weekly GC use as an optimal therapeutic strategy in boys with DMD. Furthermore, the role of exercise is increasingly being considered for boys with DMD. Recent preclinical data [1] and our own data in boys with DMD[2] have demonstrated that isometric strengthening exercise at a low to moderate intensity is not only safe but improves muscle strength and function whereas others [3,4] have demonstrated that dynamic cycling exercise is safe and therapeutic for boys with DMD. The purpose of this work is to determine the potential of twice weekly (2x/week) administration of low-dose GC and whether exercise training can synergize to delay disease progression, reverse secondary effects of disuse and induce benefit on muscle strength and physical function in boys with DMD. This proposal is innovative in its use of non-invasive magnetic resonance imaging and spectroscopy to assess the impact of GC and exercise on lower extremity muscle remodeling and disease progression in DMD. The exercise program combines dynamic cycling and isometric leg strengthening performed at the patient's and supervised remotely by the study team.

Up to 32 steroid-naïve boys (ages 4.0-8.0 years old) will be recruited and randomized to undergo the 2x/week GC regimen for 6 months followed by a continuation of the regimen for another 6 months with or without exercise training. To compare to the standard daily GC dosing regimen, 1-year data from age-matched DMD patients (part of the ImagingDMD natural history study) will be used. To compare the interplay of GC regimen and exercise, up to 16 age-matched boys with DMD (ages 5.0 to 9.0 years old) on daily GC will be recruited to undergo 6 months of exercise. Physical function, blood and urine samples and magnetic resonance imaging (MRI) and spectroscopy (MRS) will be used to monitor the intervention impact on disease pathophysiology and muscle remodeling. This project has promise to impact clinical practice, DMD patient care and quality of life, by providing information on a GC regimen at a dose/paradigm different from what is most commonly prescribed, and by providing information on exercise prescription and its impact and interplay with GC on physical function and disease progression in boys with DMD. Current clinical advice regarding exercise is non-specific. Our findings will not only advance the use of exercise in the field, but form a foundation for future assessment of adjuvant therapies as clinical trials advance. The researchers have extensive experience working with DMD patients, exercise and muscle physiology and MRI/MRS.

4. Background:

Current standard of care in DMD: DMD is a devastating, genetically linked neuromuscular disease characterized by rapidly progressive muscle atrophy and weakness, resulting in loss of ambulation early in the second decade of life, with death from respiratory muscle or cardiac failure occurring in the subsequent decade [5]. DMD is currently incurable and associated with poor quality of life, and GC are the only pharmacological intervention shown to delay disease progression[6]. DMD is caused by a deficiency of the structural sarcolemma-stabilizing protein dystrophin, which when absent leads to mechanical weakening, increased membrane damage resulting in cycles of myofiber degeneration, regeneration and eventual replacement of muscle with fat and fibrotic tissue [7,8]. These circumstances provoke an abnormal persistence of inflammatory macrophages within the muscle, contributing to the dystrophic pathology. GC are believed to act through immune system modulation and reduction of fibrosis within dystrophic muscle [9,10]. Treatment with prednisone or deflazacort is recommended to start between the ages of 4-6 years, resulting in an apparent improvement in muscle strength and ambulation in most boys for 6-12 months, followed by stabilization for about 2 years and long-term slower deterioration than untreated patients [10]. Despite these benefits, long-term daily treatment is associated with a high incidence of clinically adverse effects, which include excess weight gain beginning early in disease progression, cushingoid features, behavioral disturbances, short stature, osteopenia and risk of fractures, insulin resistance/diabetes, and others [11] with the first four features being the most common side effects [12] and occurring in 60 to 100% of DMD boys. In a recent retrospective analysis of GC use in US males with DMD as part of the Duchenne Registry, 25% of patients reported never using GC with the primary reason being concerns about side-effects [13]. Another 12% of patients reported discontinuing GC treatment with the primary reason being problems with side-effects. Concerns about the side effects have led to the use of many alternative regimens. Up to 29 different regimens have been identified and are in regular use in clinics around the world [14]. However data on the efficacy of potential alternative regimens vs. placebo, or vs. daily GC, are limited [15]. There is a clear uncertainty concerning the best treatment regimen (maximizing benefit and minimizing side effects). Patients and families have documented a high level of frustration with the status quo, asking explicitly for more information to be generated to guide best practice [10,14].

Previous work on weekend GC dosing: Escolar and colleagues [16] compared a regimen of high-dose GC pulses on weekends (5 mg/kg/day on Saturday and Sunday) vs daily (0.75 mg/kg/day) prednisone in DMD boys aged 4–10 years, reporting that weekend was equivalent to daily dosing over 12 months based on similar increases in leg and arm muscle strength. Increases in body mass index were still detected on the weekend regimen, but linear growth was greater and lumbar mineral density was improved compared to the daily dosing group. The authors concluded that the weekend prednisone strategy provides a safe and effective alternate to daily dosing. However, the total weekly dose for weekend therapy (10 mg/kg) was almost two times higher than the total daily dosage each week (5.25 mg/kg). There was no justification for this high weekend dose other than it was previously used in children with epilepsy with minimal side effects [17]. In their lead-up pilot study, 30% of DMD boys or their parents reported irritability or sleep disturbance during 1– 2 days following prednisone administration, and two children discontinued medication because of the severe

side effect time locked to 2 days following the high dose given. These findings lead to consideration of a lower dose strategy: one that uses the standard daily dose taken twice a week as a more optimal alternate GC strategy for patients with DMD.

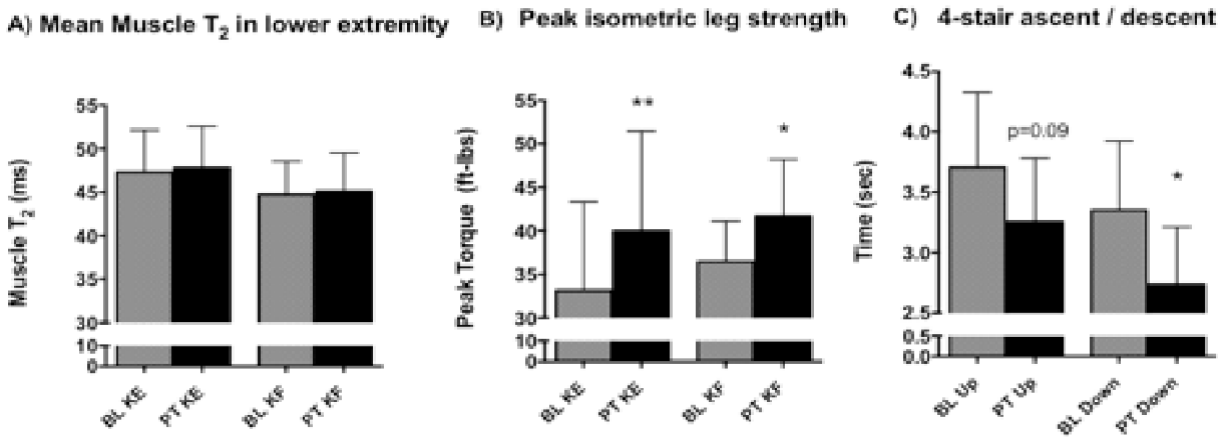
Data from animal studies support this lower dose 2x/week paradigm. The impact of a single weekly administration of a daily dose was recently compared to daily administration over 4 weeks in the mdx mouse model [18]. Both dosing regimens improved sarcolemmal injury and fibrosis comparably, however weekly dosing also improved muscle performance (time to exhaustion on treadmill and grip strength), resistance to fatigue, myofiber cross sectional area (CSA) and increased expression of genes associated with muscle remodeling whereas daily dosed mice ran less than controls, lost body mass and demonstrated induction of muscle atrophy markers. Given the broad range of health outcomes that were differently impacted and improved by weekly compared to daily dosing, 2x/week administration of the GC daily dose should be considered for DMD patients.

Postulated mechanism of action of weekly GC dosing based on preclinical models: GC are known to have both beneficial and detrimental effects on skeletal muscle, with the frequency and dose playing an important role in therapeutic outcome. Chronic GC exposure induces muscle atrophy pathways[19,20], whereas transient exposure has been documented to enhance muscle performance and produce ergogenic effects in both animals and humans (i.e. doping by athletes) [21-24]. The mechanism underlying the therapeutic effect is thought to relate to their anti-inflammatory effects, however other mechanisms may be involved. Recently, a distinct GC-induced gene regulatory pathway (KLF15 axis) in skeletal muscle was identified and reported to mediate the ergogenic effects of GC by regulating muscle substrate utilization (lipid metabolism), with no impact on muscle atrophy [20]. Results show that in dystrophic muscle (both patient and mice), the expression of this key metabolic transcription factor (KLF15) was low relative to control, and transient GC administration in mdx mice increased KLF15 expression, and this was associated with improved exercise performance as measured by treadmill distance/time to exhaustion and grip strength. Interestingly, in the above-mentioned study on weekly GC dosing in mdx mice [18], genes associated with this KLF15 pathway were upregulated after weekly administration of a daily dose of GC over 4 weeks, but downregulated after daily dosing over that time period. The authors suggest that weekly GC dosing better harnesses the dual beneficial steroid effect on muscle by improving sarcolemmal repair as well as inducing ergogenic remodeling, and that when chronic treatment is required, weekly low dose administration may be associated with the benefit without driving atrophic pathways [18].

Exercise training as a therapeutic strategy in DMD: Although exercise is well-known to be a potent inducer of signaling pathways that lead to remodeling and physiological adaptations improving muscle strength and cardiorespiratory fitness in healthy individuals, very little is known about its impact in DMD. The few studies that have assessed the impact of either aerobic or strength exercise are summarized in [25]. Overall, the DMD community has been cautious in its approach to exercise training, [26,27]. Longstanding clinical concerns have appropriately been based on the fact that high intensity and eccentric muscle actions are damaging to dystrophic muscle [28], however, similar to GC administration, the frequency and dose (intensity) of exercise play an important role in functional and therapeutic outcome. This is becoming more recognized in clinical application of exercise to DMD, with recent studies

indicating that submaximal exercise may be beneficial [3,4]. To this point, the "No Use is Disuse" study was the first randomized clinical trial (RCT), comparing the effects of assisted cycle training to usual care in late ambulatory or recently wheelchair-dependent DMD boys [4]. In addition to demonstrating safety and feasibility over 24 weeks of exercise, it highlighted the potential of exercise to delay functional deterioration of motor abilities (as the usual care group demonstrated a 6% decline in that time period). However, improvements in muscle function were not detected, likely relating to the low intensity of the assisted cycling exercise, which was aimed at reducing the amount of secondary disuse.

Early exploratory studies assessing strength training in DMD revealed improvements in muscle strength with no ill-effects and advocated for starting exercise early in the course of disease when there is a maximum amount of functional muscle [29-31]. Despite these early reports, there have been no published reports on strength training in DMD until a recent publication by our laboratory[2]. We assessed a 12-week leg strength training study in seven boys with DMD with the aim of determining safety and feasibility of a home-based remotely supervised trial (Principal Investigator D. Lott, NIH grant R21 AR064949-01A1). Isometric (where the muscle generates force without changing length) exercise was chosen as the modality because of the advantage that it does not expose the muscle to potential eccentric actions (which are known to cause damage to dystrophic muscle). Patients underwent three exercise sessions of their knee extensors and knee flexors per week, ~1.5 hours per session at an intensity of 50% of maximal voluntary contraction. Overall compliance to training was high (85%) and notably, we detected no evidence of muscle damage (as measured by MRI



transverse relaxation time, T_2, a construct of muscle damage, inflammation, and edema) and no increase in serum creatine kinase (a blood marker of muscle damage) after 12 weeks of training. Interestingly, improvements in muscle strength and function were detected in the boys with DMD after training. Increases in mean peak torque of KE (23%, $p< 0.05$) and KF (17%, $p=0.09$) were detected and the time to ascend or descend 4-stairs decreased.

Figure 1. Baseline (BL) measures compared to post-training (PT) of the knee extensors (KE) and flexors (KF) after 12 weeks of moderate intensity isometric exercise training in DMD boys. No increase in MRI T_2 was noted (A), but significant improvements occurred for both strength (B) and functional ability (C). Differences noted between BL and PT (*$p<0.05$, **$p< 0.01$).

Collectively, these findings demonstrate safety, feasibility and benefit of leg isometric

resistance training in DMD but also support the safety of dynamic, aerobic exercise training, as aerobic exercise by definition elicits less mechanical load compared to resistance exercise.

Potential adaptive impact of exercise on dystrophic muscle: The lack of dystrophin leads to numerous cell and vascular abnormalities including mechanical weakening of the sarcolemma, abnormal calcium homeostasis, mitochondrial dysfunction, increased oxidative stress, apoptosis and functional ischemia [32]. Based on preclinical studies, exercise training can benefit dystrophic muscle by 1) preventing functional decline caused by secondary muscle disuse and 2) modulating cellular and molecular signaling cascades that promote muscle adaptation and improve the underlying pathophysiology [33]. Aerobic and strength exercises typically induce divergent molecular mechanisms and signaling pathways in muscle, resulting in oxidative versus hypertrophy phenotypes respectively [34]. Aerobic muscle have more mitochondria, capillaries, anti-oxidant enzymes and express higher levels of utrophin along the sarcolemma, and are more resilient to injury and fatigue as compared to fast, glycolytic muscle. Targeting mitochondrial biogenesis and promotion of the oxidative phenotype is therefore an emerging and promising physiologically-relevant avenue of therapeutic research for DMD, with various pharmacological agents in the research pipeline [26,33,35]. Indeed, exercise in the form of voluntary wheel running can increase muscle utrophin levels, mitochondrial function, Type 1 fibers proportions, antioxidants enzymes and fatigue resistance in *mdx* mice [36]; whether aerobic exercise induces these adaptations in DMD is currently unknown. Strength training increases synthesis of contractile proteins, resulting in fiber hypertrophy and increases in specific and absolute maximal force production. In the animal literature, the large majority of studies assessing exercise use voluntary wheel running as opposed to true strengthening exercise, and report improvements in both aerobic pathways (increase in mitochondrial biogenesis,[37] and in hypertrophy related pathways (increase contractile proteins, [36]; specific and absolute force [38,39]). Although our recent study of isometric strength training resulted in increased leg muscle strength (see above), it was not designed to address underlying mechanisms. The detected improvements are likely a combination of improved neural recruitment and muscle hypertrophy. This current proposal will measure potential increases in muscle cross sectional area as a marker of hypertrophy.

A key obstacle to studying the impact of exercise in DMD boys has been the difficulty to noninvasively monitor how dystrophic muscle responds in a repeatable and valid manner [40]. To overcome this, **a key strength of this study is our use of quantitative magnetic resonance imaging (MRI) and spectroscopy (MRS),** which are emerging as powerful, non-invasive outcome measures for assessing muscle pathophysiology and disease progression in DMD, as primary endpoints to monitor the muscle response to exercise in DMD. Through ImagingDMD, a multicenter natural history study of MR biomarkers in boys with DMD led by Dr. Vandenborne, they have shown that lower extremity measures are reproducible and standardized [41]; discriminate patients from controls[41], responsive to disease progression [42,43]; responsive to GC intervention [44] and correlate with clinically meaningful outcomes suggesting that quantitative MR biomarkers may be appropriate surrogates of ambulatory function and have the potential to help demonstrate efficacy/inefficacy of therapeutic GC in clinical trials [45].

Rationale for combined exercise and low dose 2x/week-GC: Considering that >85% of

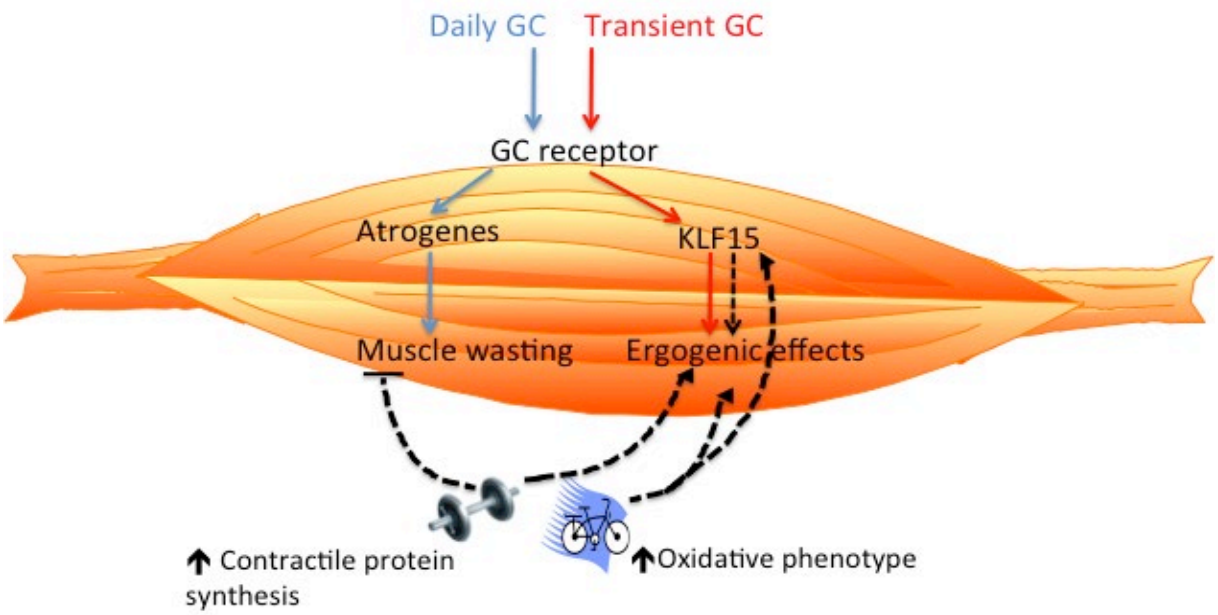


Figure 2: Impact and interplay of GC and exercise in skeletal muscle. Transient (low dose WE) GC (red) bind to the GC receptor to trigger the KLF15 axis resulting in ergogenic effects whereas chronic GC (blue) triggers atrophy signaling. Exercise optimizes the adaptive response; aerobic (cycling) induces both the KLF15 axis and promotes an oxidative muscle phenotype with increased ability to metabolize fat. Strength exercise stimulates hypertrophy signaling and inhibits atrophy. We propose the combination of low-dose 2x/week-GC and exercise will be synergistic, and protect the muscle membrane, attenuate muscle injury and preserve muscle function in patients with DMD.

DMD patients are on GC[16], understanding the interplay between GC dosing strategy and exercise is necessary for patient management and therapeutic application. Although this is true for both daily as well as alternate GC regimens, chronic GC have been shown to decrease muscle protein synthesis in human muscle [46]. Given the compelling pre-clinical data on the ergogenic effects of weekly/transient GC administration along with findings that one bout of aerobic exercise markedly increases KLF15 expression in healthy human locomotor muscle [47] and that KLF15 levels are low in DMD patient muscle [20], we propose that the combination of 2x/week-GC and exercise may represent an optimal therapeutic strategy DMD (see **Figure 1**). Not only do both strategies converge on the KLF15-axis, but exercise may also recruit compensatory pathways increasing mitochondrial biogenesis and the oxidative phenotype, or promote hypertrophy and synthesis of contractile proteins and inhibit atrophy signaling within muscle thus enhancing the remodeling effect and leading to optimal benefit. Combinatorial treatment strategies are increasingly being considered for DMD, and there is evidence from preclinical studies that exercise training can augment the effect of pharmacological treatment strategies [36,48].

5. Specific Aims:

Aim 1: To determine the 12-month impact of a low dose (0.75 mg/kg x 2 days of prednisone) twice weekly regimen on steroid-associated changes in BMI, DMD muscle pathophysiology and physical function. These data will be compared to the retrospective data of boys with DMD who were on daily steroids and previously enrolled in IRB201700056. We hypothesize that compared to the standard daily regimen, DMD boys on 2x/week dosing will present with less change in BMI, similar slowing of fat fraction increase, and trend for improvement in physical function over the 1-year.

Aim 2: To determine whether a 6-month in-home, moderate intensity exercise training program modulates the GC effects on muscle pathophysiology and physical function in patients with DMD**.** The exercise intervention will involve a combination of aerobic and isometric leg strength training. We hypothesize that boys with DMD undergoing the exercise intervention will show increased physical function, strength and cross sectional area (CSA) of the trained muscles compared to boys who do not exercise, and that exercise will result in greater attenuation of disease progression and greater muscle remodeling in the 2x/week-GC exercise group compared to the daily-GC group.

6. Research Plan:

This exploratory study will be conducted over 3 years and is designed to compare the effects of a 12-month 2x/week-GC regimen (using a dose lower than previously used for weekend dosing) to the standard daily GC regimen on change in BMI, disease progression and physical function of boys with DMD, and investigate whether lower extremity exercise training for 6 months will modulate the GC effects to further improve physical function (see Overview of Study design, Figure 3).

Aim 1 will comprise of up to 32 steroid-naïve patients at baseline, who begin a 12-month treatment period with two days of prednisone per week (dose of 0.75 mg/kg per day). Half of the group will be randomized to continue 2x/week-GC for another 6 months (Aim 1), whereas the other half will begin a 6-month exercise-training program while continuing on the 2x/week-GC regimen (Aim 2). Patients in Aim 1 will be compared to retrospective data (IRB20170056) from up to 32 age-matched DMD boys on daily GC, who have undergone yearly evaluations

as part of the ImagingDMD (the largest natural history database of MRI assessments in boys with DMD). Analysis of this retrospective data will allow us to generate natural history control data for the expected yearly change in BMI, physical function and FF progression in boys on daily GC. For Aim 2, up to 16 patients already receiving a daily GC regimen will be recruited to undergo 6-months of exercise training, allowing for comparison of the impact and interplay of exercise and GC-dosing paradigm.

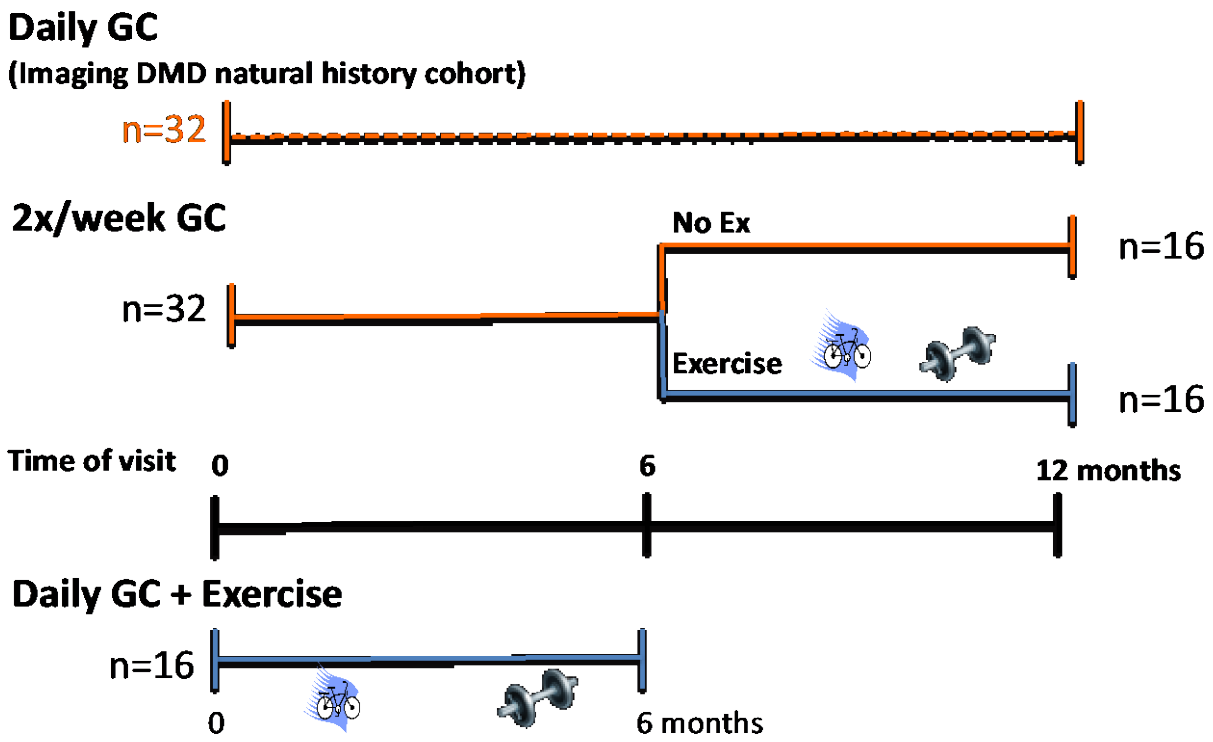


Figure 3: Overall study design. Orange lines indicate comparison of GC dosing regimen (Aim 1). Blue lines indicate comparison of exercise impact and interplay with GC regimen. The 2x/week-GC group will be assessed over 4 visits (baseline, 3, 6 and 12 months). After 6-months, patients will either continue the 2x/week-GC regimen alone (Aim 1) or in combination with exercise (Aim 2). Patients on daily GC recruited into Aim 2 will undergo a total 2 to 3 visits (baseline, mid way and 6 months.

For the majority of patients in Aim 1, the study involves 4 visits (baseline, 3, 6 and 12 months) to the University of Florida. A subset of patients randomized to Aim 1 who are relatively local to the study site may be asked to undergo one additional study visit 4 weeks after starting twice weekly prednisone treatment in order to explore the time course of improvement (Figure 4). The benefits of prednisone initiation in steroid-native individuals are known to be rapid. Previous studies in steroid-naive boys with DMD treated with daily prednisone at a dose of 0.75 mg/Kg show improvements in muscle strength and timed function tests as early as 10 days after starting therapy[49] typically peaking by 3 months [50]. Similarly, corticosteroid benefits on muscle inflammation/edema occur early after initiation in boys with DMD, as suggested by a decrease in MRI-T_2 at 3 months after treatment [44]. Here, in a subset of patients, we will confirm whether 0.75 mg/kg administered twice weekly has an impact on supine to stand, 10 meter walk/run, NSAA as well as MRI-T_2 after the first month of treatment. In the subset of

boys undergoing 6 months of exercise, we may ask the participant to undergo one additional study visit midway during the cycle training (2 to 3 months after starting) to capture adaptations specific to cycle training. This will be based on demonstrating a heart rate response to cycling exercise during the home-based, supervised exercise sessions after starting the intervention (where heart rate is lower at the same level of exercise). Such a heart rate response indicates the exercise training has been done in the right training intensity and heart rate zone, and suggests other physiological adaptations improving muscle and functional performance have occurred and should be quantified at a study visit.

Patients who are on daily GC recruited into Aim 2 will undergo 2 to 3 visits (baseline and 6 months). Patients who demonstrate a heart rate response to cycling exercise during the home-based sessions may be asked to return for a mid-study visit to capture adaptations specific to cycle training (for a total of 3 study visits).

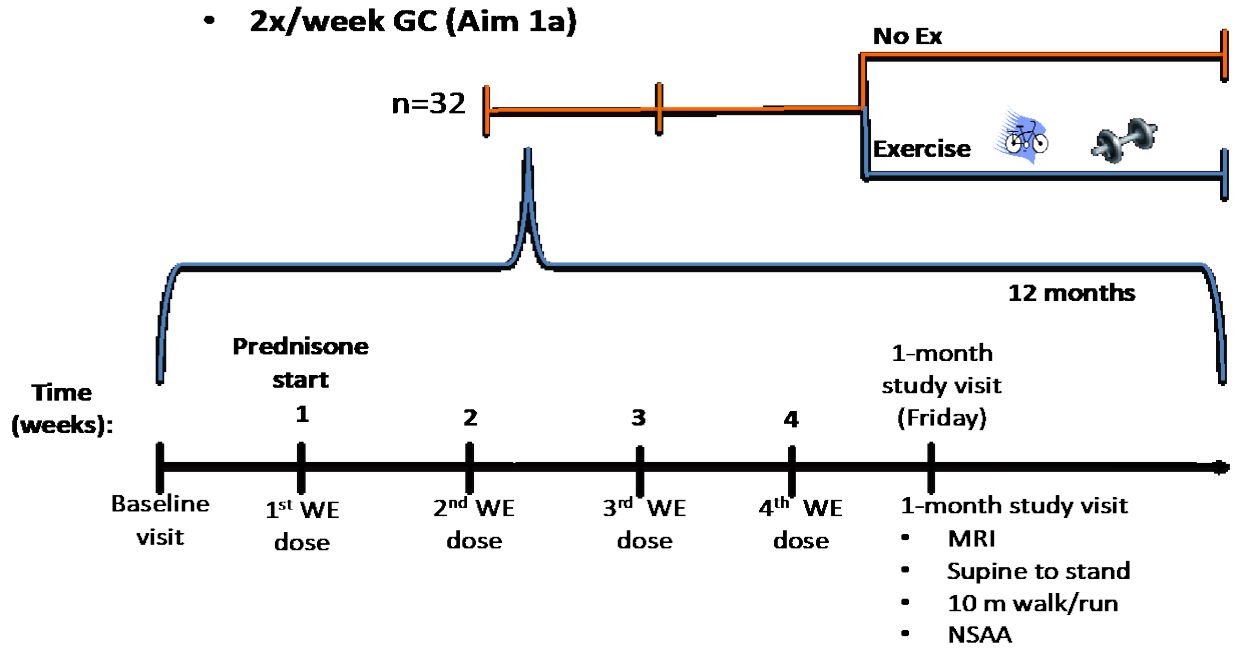


Figure 4: A small group of boys randomized to Aim 1 who live within a 3-hour drive from Gainesville, Florida may be asked to undergo an additional study visit 4 visits after starting twice weekly prednisone treatment to explore the time-course of early prednisone-related improvements. Specifically, on the Friday after the fourth week of dosing, patients may undergo MRI, timed function tests and the NSAA.

Prior to the initiation of the exercise intervention (Aim 2), development and finalization of the therapeutic exercise chair designed specifically for DMD patients as part of this overall study (and conceptualized below in Figure 6) will be done. The design, description and use of this exercise chair by patients with DMD as part of this study was deemed a non-significant risk by the UF IRB-01 review (see letter from IRB dated 10/2/2019). A prototype exercise chair

has been developed in study collaborator Dr. W. Dixon's lab in Mechanical and Aerospace Engineering. The aim of this initial sub-study is to test the components of the prototype as they are being developed (sizing of pedal attachments, ergometer crank, seat height, motor resistance options) and to obtain feedback from a maximum of 10 patients with DMD regarding comfort and ease of use. Patients will be asked to exercise on the chair for no more than 30 minutes while the study team asks for their feedback, ratings of perceived exertion and makes measurements of leg length and heart rate using a standard wrist watch or chest strap monitor. Patients may be invited back for two more visits (total visits to equal 3 within the span of one month) to provide feedback on any adjustments made to the device. To monitor the muscle response to exercise, patients may undergo magnetic resonance imaging and spectroscopy (as described in study procedures below) after exercising on the chair on one of their visits.

Study population: The target population for this study includes ambulatory boys with genetically and clinically confirmed DMD. The subject selection is limited to males as DMD is an X-linked recessive, genetic disorder leading to progressive muscle weakness in males. Although females are carriers, they do not exhibit the same symptoms. Selection is also limited to children since the detrimental effects of DMD begin in early childhood and life expectancy of these boys is shortened to the early to mid-20s. The minimum age for study entry is 4.0 years. This is based on the notion that Study Aim 1 focuses to determine whether low dose 2x/weekly-GC provides a novel, efficacious treatment strategy for DMD. As such, given the fact that clinical decisions for GC treatment typically occur between the ages of 4 to 6 years and to prevent complications related to weaning from daily GC use, only boys who are steroid-naïve upon entry into the study (and 6 months prior) will be recruited. As study participation is expected to be 1 year, we have set the maximum age range as 8 years.

Within Aim 1, boys who are 5 years old and above will undergo randomization to the 2x/week-GC regimen with or without exercise (months 6-12 of study) at baseline to allocate participants to either group in a 1:1 ratio using the randomization feature in RedCap. Patients who are 4 to 5years old enrolled into Aim 1 and patients who are enrolled into Aim 2 (on daily GC) will not be randomized. Because prednisone and deflazacort are the two most commonly prescribed GC in the US, and the current use of deflazacort is reported to be slightly higher than prednisone use (54 vs 46%) [13], boys on a stable regimen of either GC will be included in Aim 2.

For the exercise chair development and feedback sub-study, the age range of boys with DMD will differ. We will recruit a total of 10 boys with confirmed DMD who are between 6 and 12 years of age. The lower limit of 6 years is chosen to be able to ask questions and obtain their feedback. We will recruit up to age 12 years in order to get a range of patient heights as the intent is to make this exercise chair adjustable for future exercise studies. Boys who take part in this sub-study and fit the inclusion criteria below (for Aim 1 and 2) may be eligible to partake in the intervention studies.

Inclusion criteria:

- Diagnosis of DMD confirmed by 1) clinical history with features before the age of five, 2) physical examination, 3) elevated serum creatine kinase level and 4) absence of dystrophin expression, as determined by immunostain or Western blot (<2%) and/or DNA confirmation of dystrophin mutation.
- Aim 1 inclusion is age 4.0 to 8.0 years at enrollment: a lower age limit of 4.0 years is selected as clinicians are prescribing daily steroids at this age.
- Aim 2 inclusion is age 5.0 to 9.0 at enrollment: a lower age limit of 5.0 is selected based on our previous experience with compliance to exercise traiing. An upper age limit of 9 years has been set to allow comparison with boys in Aim 1 who turn 9 years old over the 1-year intervention.
- Ambulatory at the time of the first visit, defined as the ability to walk for at least 100 m without an external assistive device and able to climb four stairs.
- Aim 1 only: GC-naïve at baseline (and prior 6 months)
- Aim 2 only: on stable daily GC regimen for 6 months prior to baseline
- Followed by a doctor or medical professional who coordinates Duchenne care on a regular basis

Exclusion criteria:

- Contraindication to an MR examination (e.g. aneurysm clip, severe claustrophobia, magnetic implants)
- Presence of unstable medical problems, significant concomitant illness including cardiomyopathy or cardiac conduction abnormalities, physical findings, or laboratory abnormality (including but not limited to renal insufficiency or impaired hepatic function) that, in the Investigator's opinion, could adversely affect the safety of the patient, make it unlikely that the course of treatment or follow up would be completed, or impair the assessment of study results
- Prior history of rhabdomyolysis
- Presence of a secondary condition that impacts muscle function or muscle metabolism (e.g. myasthenia gravis, endocrine disorder, mitochondrial disease)
- Presence of a secondary condition leading to developmental delay or impaired motor control (e.g. cerebral palsy)
- Presence of an unstable medical condition (e.g. uncontrolled seizure disorder)
- Behavioral problems causing an inability to cooperate during testing or understand exercise instruction
- Participation in other forms of investigational drug or gene therapy during the period of the study (FDA approved therapies are allowed)
- Use of carnitine, other aminoacids, creatine, glutamine, coenzyme Q10, herbal supplements within 3 months prior to enrollment. Note: Vitamin D, calcium, ranitidine and Tums are allowed
- Use of human growth hormone within 3 months prior to Day 1

Study control subjects: In addition to the recruitment of above-mentioned patients with DMD, we will leverage existing data from the ImagingDMD natural history study (led by Dr.

Vandenborne; R01 AR056973) where patients with clinically and genetically confirmed DMD have undergone yearly assessments (functional testing and MRI/MRS evaluation similar to assessments included in this current proposal). Data from up to 32 patients who are age-matched, ambulatory, on daily GC therapy, and similar exclusion criteria to above will be selected from this database (these individuals have previous enrolled in IRB201700056). Analysis of this retrospective data will allow us to generate natural history data for the expected yearly change in BMI, physical function, MRI and MRS based markers of disease progression.

Subject recruitment: Although DMD is considered a rare muscle disease, it is one of the most frequent genetic conditions affecting approximately 1 in 3,500 live male births. As with any rare disease, having access to the population of interest and the ability to safely implement all experimental procedures are concerns, and a potential barrier to accrual of patients include competing studies for steroid-naïve patients with DMD. The University of Florida has a history of strong recruitment, extensive experience and successful studies in boys with DMD. Patient recruitment will come from various sources, as our group has developed an extensive collaborative network with links to the Muscular Dystrophy Association Clinic at University of Florida as well as nationally and internationally in Canada, Parent Project Muscular Dystrophy, Duchenne Connect as well as close ties with other investigators in the dystrophy community. In addition, we currently have over 180 patients enrolled in our ongoing natural history study (Imaging DMD) from which recruitment may be possible.

Participants will be recruited at the University of Florida using multiple, direct solicitation methods. Flyers with the study information will be distributed in clinics and in strategic places in hospitals (i.e. waiting rooms, physical therapy areas). An invitation letter will be mailed to all families of boys with DMD who may have a sibling or are in the Multi-site study (R01 AR056973), titled “Magnetic Resonance Imaging and Biomarkers for Muscular Dystrophy” (PI Vandenborne). A similar recruitment letter will be mailed to all patients with DMD who meet the age requirements for the study and are registered with the respective regional offices of the Muscular Dystrophy Association, Parent Project Muscular Dystrophy, and DuchenneConnect.

Screening procedures: Prospective participants will be screened by the PI and study physician to ensure they meet the criteria for participation in the study. Parents or guardians who contact the PI and/or the research team will complete a telephone screening interview to assess eligibility. Subjects that meet initial eligibility criteria will be mailed the consent form and a complete description of the study as well as potential dates for the baseline assessment. Informed consent/assent for participation in the study will be obtained on-site during the first visit according to the guidelines of the Institutional Review Board. Oral and written information will be provided that explains in simple terms 1) the criteria for participation, 2) the purpose of the research and the procedures involved, 3) the subject’s right to withdraw at anytime without penalty, 4) potential benefit to the subject, 5) potential risks, and 6) assurance of anonymity. The parent and the subject will be encouraged to ask questions. Written, informed consent will be obtained from the child’s parent or legal guardian and the child will provide written assent. The study will not begin until full approval has been received by the Institutional Review Board.

Study Variables: The primary and secondary endpoints are listed in Table 1. All study procedures will take place at the Clinical Research Center within the Clinical Translational Research Building (CTRB) with the exception of the MRI and MRS which will take place in the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) Facility at the University of Florida. The study drug prednisone will be dispensed by the Investigational Drug Service at UF Shands Hospital. During the study visits, digital photo or video recordings may be made depending on participant consent for educational purposes and to assess the validity of the physical activity bout. More specifically, we will take pictures/videos of each patient on the device at the study visit, and share with them so that they understand proper assembly and use of the the exercise device when used at home. We may also share the photo or video recording with clinicians and other researchers in the DMD community, or families and patients with DMD who inquire about participating in the study. Because no such exercise device currently exists for patients with DMD, and there is little information about the potential benefits of exercise in the DMD community, showing images and videos of the exercise device (in patients who have provided their consent) will be educational and advance the importance of understanding the role of exercise in this patient population.

Aim 1 will consist of 4 to 5 visits (baseline, 3, 6 and 12 months with a possible visit at 8-9 months to assess exercise training progress) and Aim 2 will involve 2 visits (baseline and end-study) with a possible third (mid-exercise) visit at 2-3 months after baseline to assess exercise training progress. The baseline and 3 month visits in Aim 1 will involve testing over 2 days; for patients randomized to no exercise intervention, the 6 and 12-month visits will also involve 2 days of testing. For boys randomized to the exercise intervention (Aim 2), the 6-month visit will require 4 days (and involves familiarization to the exercise device and intervention); the mid-exercise and 12-month visit may involve up to 2 days of testing. For boys on daily GC who will undergo the exercise intervention as part of Aim 2, the baseline visit will require 4 days (familiarization to exercise device and intervention) and the mid-exercise and 6-month visit will require up to 2 days. See table 1 for schedule of procedures.

Aim 1

The purpose of this aim is to determine the impact of a low dose, twice weekly regimen of prednisone over the course of 1 year in initially steroid-naïve boys with DMD. Primary and secondary measures for side-effect and efficacy outcomes are listed in Table 1. We hypothesize that compared to the standard daily regimen, DMD boys on 2x/weekly dosing will present with less change in BMI, similar slowing of fat fraction increase, and trend for improvement in physical function over the 1-year. A table of study procedures relating to Aim 1 is shown in Table 2.

Following the consent/assent procedure, each patient will undergo baseline safety measures which will serve as comparison for development of prednisone-related side-effects. First the Study physician will do a clinical exam of the patient which involves assessment of the patient's general appearance; skin; head, ears eyes, nose, throat; lymph nodes; heart; lungs; abdomen; extremities/joints; and neurological and mental status (as needed). The Study physician or research nurse will obtain the patients' vital signs (sitting blood pressure (BP), heart rate (HR) , respiratory rate and temperature) and a 12-lead

electrocardiogram. The 12-lead ECG will be obtained only after the patient is positioned supine, resting, and quiet for a minimum of 15 minutes. After measurement of weight and height, the research nurse will perform a venipuncture in the arm. Blood samples (2 tablespoons) will be analyzed in the Shands hospital to rule out diabetes and in our lab for creatine kinase (CK), myoglobin, troponin I, ALT/AST and inflammatory profile determination. A urine sample will also be collected by the patient using a standard urine collection cup for analysis of metabolites (creatine, creatinine, citrate levels amongst others). If available, a portion of these samples (1 tablespoon blood and 1 tablespoon urine) may be stored for up to 5 years for future analysis of exploratory biomarkers of disease and intervention impact. At the 6 and 12-month time points, patients will under a test of adrenal reserve (low dose ACTH stimulation test) to assess whether the twice weekly steroid regimen has an impact on the normal functioning of the adrenal gland cortisol secretion. This test is done by measuring the cortisol level in blood before and after an injection of ACTH (Cortrosyn). Daily dosing with steroids is known to cause adrenal insufficiency as measured by this test. We are performing this test to demonstrate the additional safety of twice weekly steroids in DMD (and expect a normal cortisol response to this test). The test will involve IV placement with baseline blood sampling for ACTH and cortisol levels, administration of 1 mcg of cortrosyn (in 2-5 ml of 0.9% sodium chloride injection over a 2-minute period), with another blood sample taken at 30 minutes post cortrosyn administration for measurement of cortisol levels. This test involves less than 1 tablespoon of blood.

Thereafter, the patient will have a dual-energy x-ray absorptiometry (DEXA) scan for measurement of bone density and lumbar spine Z score.

Following these clinical measures, patients will undergo quantitative MRI and MRS for determination of efficacy outcomes reflecting disease progression. Characterization of thigh muscles will be performed using a 3T whole body MRI scanner (Philips Elition 3T) within the AMRIS facility. Patients will be positioned supine in the magnet for up to 60 minutes allowing for assessment of 3 measures in muscles of the leg: fat fraction, MR transverse relaxation time (T_2) and cross sectional area (CSA). Specific quality assurance and standardization procedures have been developed for each MR sequence by our group [41,43] for determination of these measures. Determination of FF (FF=lipid / water + lipid) within the vastus lateralis (VL) will be done using single voxel localized ^{1}H spectroscopy (^{1}H-MRS) as previously described [43,51]. ^{1}H-MRS T_2 will be quantified used a spectroscopic relaxometry sequence, and is a measure that is independent of lipid infiltration and used to reflect primarily inflammation and edema in muscle [43]. The VL will serve as the target muscle group for MRS data acquisition, as we have the most longitudinal natural history data on it and it will be targeted for the exercise intervention. MRI 3-point Dixon imaging, which is also increasingly being used to quantify FF in multiple muscles simultaneously, will be used for cross sectional area (CSA) determinations of upper leg muscles. Regions of interest will be drawn around the borders of the knee extensor (KE) and knee flexor (KF) muscles (recruited and targeted by both exercise training modalities) from Dixon images. CSA and contractile area (CSA -(CSA *fat fraction) will be determined as previously reported by our group [52,53]. Determination of CSA (highly reproducible[41]) will allow calculation of specific force (CSA /peak strength) of

KE and KE. MR outcome data analyses will be performed as per standard technique in our laboratory [54, 41,43].

Patients will be given a rest after the MRI/MRS and then undergo pulmonary, muscle and functional performance tests back at the CTRB which will serve as efficacy outcomes. Strength of the respiratory muscles will be measured through standard pulmonary functions testing. Spirometry will be performed using a Carefusion Microlab (San Diego, CA) portable spirometer in the sitting position to assess forced vital capacity (FVC) and peak expiratory flow (PEF). The maximal inspiratory pressure (MIP), and maximal expiratory pressure (MEP) will be performed in sitting using a Carefusion MicroRPM device (San Diego, CA). There is no minimal threshold for FVC and FEV1 in order for the patient to proceed with subsequent exercise; these values are needed to calculate exercise related parameters that elucidate whether the respiratory system limits exercise capacity in a given patient.

The primary outcome measure will be The North Star Ambulatory Assessment (NSAA), an outcome instrument to measure ambulatory function (that range from standing to running) in DMD [55]. 12-month longitudinal data are available in patients with DMD [56] including in our ImagingDMD cohort. The time for the 10-meter walk/run from the NSAA will be used as a secondary measure of functional mobility. The time to climb a standard four stairs and 6MWT, both standard tests to measure functional and ambulatory performance in DMD [58] will also be assessed. As an exploratory measure, we will perform the Neuromuscular Gross Motor Outcome (GRO), which is designed to assess whole body strength, motor development and function for all levels of disability across the lifespan in individuals diagnosed with neuromuscular disease. It includes measures performed as part of the NSAA along with other simple tasks that require between 10-15 minutes to administer. The GRO was recently validated for patients with Spinal Muscular Atrophy {Alfano, 2021 #466} and is being assessed as a clinical tool to quantify function in DMD. During the walking physical activity tests, patients may also be asked to wear a mask that is connected to a portable metabolic cart (the Cosmed K-5 which is like a small backpack strapped to the participant's back that weighs less than 2 pounds). This will allow us to measure the intervention impact on metabolic and breathing variables.

Additionally, peak isometric strength of KE and KF will be tested using a quantitative Biodex dynamometer as previously done by our group reliably in boys with DMD aged 5-14 years [52,57]. Muscle specific force will also be determined by normalization to maximal cross-sectional area (CSA) from MRI.

Peak aerobic capacity will be measured during a cardiopulmonary exercise test performed on a stationary cycle ergometer. Briefly, before the exercise, we will have the patient perform standard breathing tests using spirometry, where we measure forced vital capacity and peak expirotory flow. Following this, patients will do a brief warm-up of up to 5 minutes of unloaded pedaling, and then asked to pedal at moderate intensity for up to 20 minutes. The exercise protocol consists of variable watt/minute ramp increase to volitional exhaustion. Watt increments will vary between 5-20 watts per minute intervals, depending on participant ability

and fitness level. Patients will wear a mask that is connected to a standard metabolic cart to measure different breathing variables that include oxygen consumption, carbon dioxide production, heart rate, respiratory exchange rate (RER), minute ventilation and ventilatory (anaerobic) threshold. A 12-lead ECG and blood pressure are monitored by a research nurse (who is trained in reading ECG during exercise) at baseline, throughout the test, and for 10 minutes of recovery. Throughout the exercise, we will ask the patient to rate his level of perceived exertion by pointing to a number on the OMNI scale of perceived exertion. Exercise will be terminated if the patient reaches 85% of age-predicted heart rate (calculated as 220-age).

Finally, questionnaires will be administered. The Child Behavior Check List (CBCL) will be used to monitor behavioral changes as previously done in boys with DMD [16]. This is a parent-report questionnaire [59] rating various behavioral and emotional problems in children aged 4 through 18 years. The SIDECORT (side effect of corticosteroids), self-report questionnaire on perceived cognitive, behavioral and emotional side effects of GC will also be administered to the patient and parent [60]. As health-related quality of life has become one of the most important goals of disease management, the PedsQL Multidimensional Fatigue Scale, which assesses fatigue with a total of 18 items and has a form specific to young children (5-7 years) will be used [61]. We will also ask 9 questions to the child's caregiver (Caregiver Impression of Change) to obtain their impression of the intervention impact on their child's overall well-being, mood, sleep, etc.

We will use Actigraph accelerometers to quantify physical activity (i.e. steps taken per day, percentage of time spent in varying intensities of activity, etc.). Each subject will be provided an accelerometer and will be asked to wear the accelerometer for all waking hours (except bathing and swimming) for 7 consecutive days as we have previously done[48] at three time points over the course of the study (during the first week of the intervention, prior to the 6-month visit, the last week of the intervention). A self-addressed, postage paid return envelope for the device will be included to enable the subject an easy means to return the accelerometer to the testing site, or they will be asked to return it with them to the study visit. A member of the research team will contact the subject once before and once during the 7 days of wearing the device (and be available for contact should the subject have questions regarding the device), and contact information will be provided to the subject to communicate with the research team should any questions or concerns arise during the time of physical activity monitoring. Data on daily step count and time spent in sedentary behavior as well as varying intensities of activity will be assessed, and changes in any parameter will be correlated with changes in peak aerobic capacity, endurance and other measures obtained from exercise and functional testing. **Aim 2**

The purpose of this aim is to determine the impact of a 6-month in-home exercise training program (involving a combination of aerobic and isometric leg strength exercises) in boys with DMD, who either take low dose twice weekly prednisone or daily GC (prednisone or deflazacort). We hypothesize that boys with DMD undergoing the exercise intervention will show increased physical function, strength and cross sectional area (CSA) of the trained muscles compared to boys who do not exercise, and that exercise will result in greater

attenuation of disease progression and greater muscle remodeling in the 2x/weekly-GC exercise group compared to the daily-GC group.

A table of study procedures relating to Aim 2 is shown in Table 3. In addition to all the testing described above for Aim 1, patients will undergo an additional MR assessment after the other measures (fat fraction, T_2 and CSA) have been obtained. This involves ^{31}P MRS to measure the recovery kinetics of phosphocreatine following a brief bout of leg exercise in the magnet, as this reflects the mitochondrial capacity for oxidative phosphorylation and will serve as a marker of energetics. ^{31}P MRS is done using a surface coil secured over the VL and after appropriate localization and shimming of the muscle, unlocalized spectra will be collected at rest, and after a brief ($\leq$ 2-minutes) bout of moderate intensity exercise using the KE muscles. We will also conduct an interview with the caregiver(s) to obtain insight on the exercise training and device used for the study at the 6-month visit.

As part of Aim 2, baseline testing will be done over the course of 4 days to allow for familiarization to the exercise intervention and the therapeutic exercise device, as well as to develop the individualized exercise prescription for the at-home training intervention.

On day 2, a 6-minute cycle test similar to what was used by Jansen et al (2010) will be performed to assess muscle endurance on the custom-built exercise device. Heart rate will be monitored at rest and throughout cycle exercise using standard non-invasive monitoring techniques (6 to 10 lead electrodes or chest strap and wrist watch). The patient will be asked to cycle at his maximal intensity level for 6 minutes and the rating of perceived exertion on the OMNI scale and distance covered will be noted. After a 5-10 minute rest period, the patient will be asked to cycle again for 5 minutes, at an intensity of between 3 and 6 on the OMNI scale and this will correspond to the exercise intensity the patient will undergo at home. For the strengthening, the maximal voluntary contraction for KE and KF will be determined and then patients will perform ~4 sets of 6 reps of isometric KE, KF at an intensity of 50% of MVC.

On day 3, patients will not undergo any testing. On day 4 the patient will undergo another exercise familiarization session, where he will do cycle exercise for up to 10 minutes at an OMNI intensity of 3 to 6. This will be followed by a strength exercise session. Following this, patients and their families will receive detailed instructions relating to the exercise protocol and device. We will also provide detailed instructions and video of how to operate the exercise device on the laptop computer that will be provided to the patient. A final blood draw will be obtained 4 hours after the exercise sessions on day 4 to monitor serum CK.

Table 1: Outcome measures

		Side-effect outcome	**Efficacy outcome**
AIM 1	Primary	BMI (weight and linear height)	NSAA and 10 m walk/run time
			MRS FF (VL)
	Secondary	Bone density, Questionnaire on steroid side effects (Sidecort),	Muscle strength, 4-stair climb, 6MWT, peak aerobic

		behavior (Child behavior check list), quality of life (PedsQL), ACTH stimulation test	capacity, pulmonary function, blood and urine biomarkers
			MRS T_2
	Exploratory		GRO, change in physical activity levels
AIM 2	Primary	MRI T_2	NSAA and 10 m walk/run time, Peak aerobic capacity
			Muscle CSA
	Secondary	BMI	Similar to Aim 1, muscle endurance
		Aim 1 questionnaires Caregiver Impression of change, Caregiver interview	MRS FF (VL) ^{31}P MRS
	Exploratory		GRO, change in physical activity levels

Table 2: Events for AIM 1:

AIM 1	Visit 1	Visit 2	Visit 3	Visit 4 (for patients in exercise group only)	Visit 5
Consent	X				
Medical history & exam	X				
Vital signs, ECG	X	X	X	X	X
ACTH stimulation test			X		X
MRI/MRS	X		X	X	X
Dexa scan	X				X
Muscle strength	X		X	X	X
Functional testing	X	X	X	X	X
Pulmonary function testing	X		X	X	X
Cardiopulmonary exercise test	X[a]	X[a]	X	X	X
Questionaires	X	X	X	X	X
Prednisone dispensation	X	X	X		X

[a] = Cardiopulmonary exercise testing will be done on one of these 2 visits (baseline OR 3 months) and used as a familiarization test prior to the pre-exercise training visit (6 months).

Table 3: Events for AIM 2:

AIM 2	Visit 1	Visit 2	Visit 3
Consent	X		

Medical history & exam	X			
Vital signs, ECG	X	X	X	
MRI/MRS	X	X	X	
Dexa scan	X		X	
Muscle strength	X	X	X	
Functional testing	X	X	X	
Pulmonary function testing	X	X	X	
Cardiopulmonary exercise test	X	X	X	
Exercise practice	X			
Questionaires	X	X	X	

Study drug prescription and administration:

The Investigational Drug Service (IDS) at the University of Florida will oversee study drug related matters. The lead pharmacist is Irin Reji will be the direct contact, but all communication should be sent to IDS@shands.ufl.edu.

Prednisone tablets (Manufacturer: multiple FDA-approved generic options) will be prescribed by the study physician at a dose of 0.75 mg/Kg and dispensed by IDS or Outpatient pharmacy at UF Shands Hospital to the patient. Tablets are available as 2.5, 5 or 10 mg strengths.

Doses for prednisone will be according to patient weight using dosing tables. Throughout the study, patients and their parents/caregivers will complete a diary documenting prednisone administration and diet compliance. Specifically, parents/patients will be asked to log drug administration and diet history that is checked every two weeks. Upon completion of data entry, the database will undergo a quality review to ensure acceptable accuracy and completeness. In addition, site calls, which will occur throughout the study will include a compliance check on prednisone and diet.

Exercise prescription for boys with DMD:

Exercise prescription in clinical populations advocates for a combination of endurance and strength training for optimal benefits [62]. We have taken consideration of previous exercise-based studies in DMD [3,4,29] in establishing an effective yet safe exercise intervention, which will involve a sequential 3-phase combination of aerobic training followed by strengthening followed by a mixed combination of both aerobic and strengthening exercise (**Figure 4**). The rationale is based on the notion that aerobic exercise first targets pathophysiological mechanisms to induce adaptation within recruited muscle that will be protective to subsequent strengthening exercise, and thereafter a combination of the two for maintenance of adaptations gained. In the "No Use is Disuse" study[4], assisted bicycle training was compared to usual care in late ambulatory or wheelchair dependent boys with DMD, demonstrating the safety and feasibility of dynamic exercise using parameters of: 5x/week, 15 min/day for 6 months using the OMNI scale ($\geq$ 3 and $\leq$ 6) for patient rating of perceived exertion (RPE) to guide exercise intensity [63]. As this was the first study to ever use dynamic exercise in DMD and the boys were older (mean age 10.8 years), exercise was performed with electrical motor support. Our study will use similar exercise parameters but also

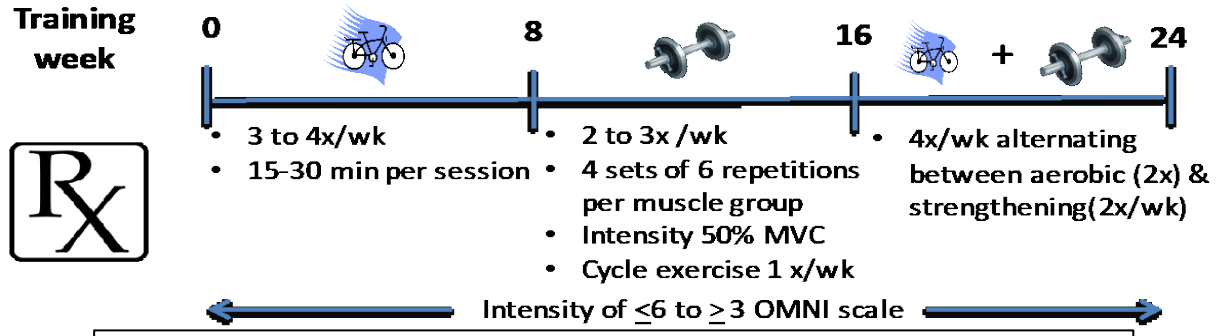


Figure 5: Exercise prescription parameters for the 6-month training program.

proposes a combination of isometric strengthening with cycle ergometry, which is expected to lead to functional gains not detected in the No Use study. The strength training paradigm proposed herein is based on our pilot data (shown in Figure 1), indicating that 3 times/week of 4 sets/6 repetitions at 50% MVC over 12 weeks was safe and significantly improved muscle strength and functional ability. In the current proposal, we will have patients do bilateral KE and KF exercise after 8 weeks of initial aerobic cycling, and then in combination on alternate days to maximize potential gains.

The schedule for exercise (days of the week and time of session) will be discussed at the baseline visit and the study investigators will provide a tentative monthly training calendar to the patient/parent based on their feedback and availability. The study investigator will confirm the weekly schedule every Friday prior to the scheduled training week. Additionally, at the end of every exercise session, the study team will confirm the date and time of the subsequent exercise session. The patient and parent will be provided with the contact information of the study team members responsible for leading the training session.

For each exercise session, a parent or guardian must be in the home during the time of the exercise and in close enough proximity to hear the study investigator over the video call/computer. They may be needed to help set up the patient on the exercise device and/or laptop computer. This will be discussed at the baseline visit and factored into the training

schedule to ensure adult supervision is available at the scheduled times. The study team will also interact with the parent/guardian to discuss the exercise experience.

To allow for both exercise modalities to be performed in the home-setting, the Department of Engineering (Co-investigator Dr. Dixon) will modify and repurpose components from the exercise device used in Dr. Lott's previous IRB approved study (IRB201400874). As shown in Figure 5, this will consist of a stable chair for postural support, a motorized-assisted cycling ergometer, a cuff and strain gauge cable system, and a live computer monitoring system. Similar to the previous study by Jansen and colleagues (2013), the ergometer will allow cycling to be assisted with motor support. However, because our patient cohort will be less clinically affected compared to the Jansen study, the ergometer will also have options of no load or low resistance to pedaling once muscles become accustomed to the exercise. Customizable, set thresholds of workload and 3 levels of safety checks are built into the device. As standard in commercially available devices, at all times the participant will have access to an emergency stop button that will immediately halt the motor should it become at all uncomfortable. Additionally, hardware and software limits will be imposed that ensure the minimal risks of the session are mitigated. The device will be fairly compact for in-home use and can be easily configured for exercise modality. For strengthening exercise, the lower leg will be secured to a padded bar and the patient will perform kicking of pulling type exercise isometrically with the hip at ~90˚ of flexion and the knee positioned at 60˚ of flexion recruiting the KE and KF muscles as in our previous study. For the cycling, the padded bar will swivel away and the ergometer will slide towards the patient. The intensity of exercise will be guided by the level of patient exertion using the OMNI scale of rating of perceived exertion (RPE). Heart rate will be monitored and recorded on a wrist watch provided to the patient We will use the real-time video feature (Skype or Zoom) on the laptop to monitor all training sessions and provide encouragement and verbal feedback regarding any concerns. The computer will be equipped with custom-written software that will provide visual and auditory guidance and instructions for the subject and his parent(s) to

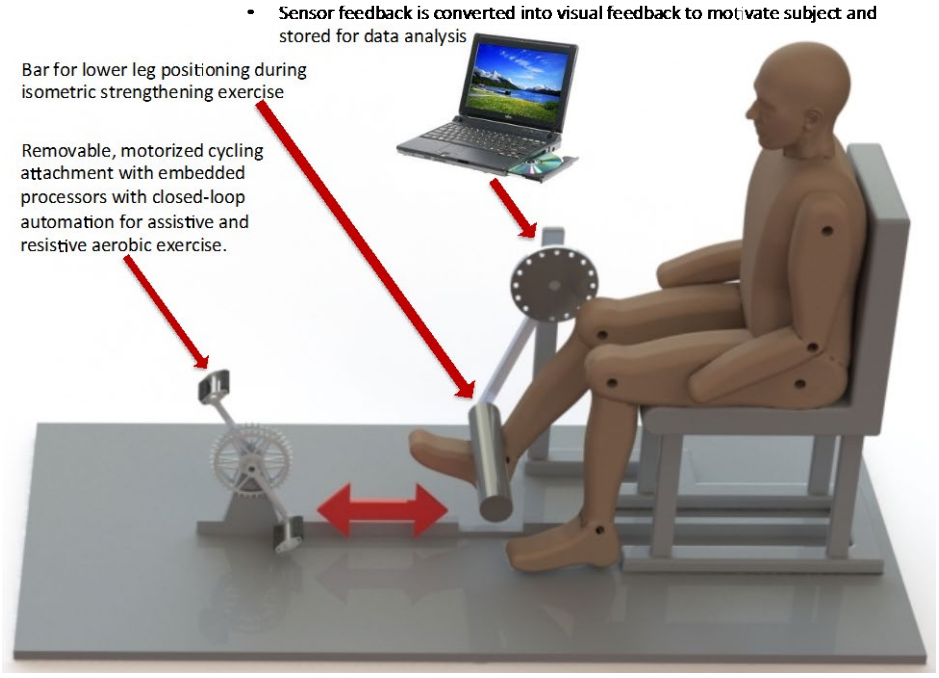


Figure 6: Exercise modalities used for leg cycle and isometric strength training in boys with DMD.

complete the exercise sessions at home. Patients and their families will be familiarized to the exercise device and instruction during the baseline visit of Aim 2. We will use and repurpose 7 exercise chairs from our previous study for this current project.

Data Analysis

After the aforementioned procedures are completed, the collected data will be compiled for analysis. The participants will provide data on primary and secondary outcome measures specifically for research purposes. Only the PI and research staff involved with subject recruitment and data collection will have access to the individual, identifiable private data collected during the study. The participants of this study are children and as such, written informed consent will be obtained from the parent or legal guardian and assent will be obtained from the child. Subject data will be coded to ensure confidentiality and no personal health information will be released. All electronic data will be secured on password protected, encrypted computers and hard copies will be kept in locked filing cabinets. In case of any adverse events from the study protocol that result in injury, medical attention will be sought. Both adverse events and/or breeches of confidentiality will be reported immediately to the PI, who will immediately report the event to the IRB. If an adverse event occurs, the investigators will reassess the risk/benefit ratio of the study and submit any modifications deemed necessary to the IRB for approval. Dr. Taivassalo will inform the funding agency of any actions taken by the IRB in response to any adverse event reports.

Access to linked identifiers is limited to research personnel intimately involved with the human participants. All data and records acquired from participants is for research purposes only and will be kept confidential and maintained in a secure database identifiable only by participant code. The results of the study may be published for scientific purposes; however, participants' identities will not be revealed and data will not be traceable to any individuals in any resultant publications. The information gathered during this study will be kept confidential to the extent permitted by law.

Given the similarities in testing protocols between this study and PI Dr. Taivassalo's study (IRB202301491), data collected from participants will be shared, integrated, and analyzed as part of the Vaso Rex study, contingent upon participants enrolling and providing consent/assent. This integration will apply only to participants who have consented to the Vaso Rex study and have completed identical tests related to this project within three months prior to their enrollment in IRB202301491. This approach aims to streamline testing protocols, enabling the research team to reduce the schedule for the Vaso Rex study while still leveraging available data from this study within the specified timeframe. Ultimately, this will help minimize testing and alleviate the burden on participants.

7. Possible Discomforts and Risks:

As the safety of the boys with DMD is paramount, we are in the unique position to have the personnel, experience, and the necessary equipment to monitor the proposed interventions and conduct all aspects of the proposed protocol. Members of this proposed research team have working on projects involving boys with DMD over the past 10 years, with more than 180 boys enrolled in our ongoing studies. With regards to safely implementing the methodology proposed in this study, we have performed MRI/MRS, strength, and functional assessments hundreds of times on boys with DMD without incident and with high confidence and subject compliance. We have also implemented the proposed isometric strength training portion of the exercise intervention in a previous study (Dr. Lott, IRB201400874) in 8 boys with DMD with no adverse events. As such, the research team has extensive experience to conduct the assessments following special precautions to minimize risks or hazards. The potential risks for each assessment and intervention proposed in this study are described, along with the measures we undertake to protect the patient against the risk.

Study parameters will be assessed at baseline, 3, 6 and 12 months and include physical examinations, vital signs, clinical laboratory tests (blood and urine collection), ECGs, concomitant medication monitoring and treatment-emergent adverse events (AEs) within in the Clinical Research Unit at every study visit. If clinically significant deterioration is noted in vital signs or physical examination, the change will be documented as a treatment-emergent adverse event (AEs). Clinical significance is defined as any finding that has medical relevance that results in an alteration in medical care. The Study physician will continue to monitor the patient until the parameter returns to its Baseline status. The Study Physician will review the results of the ECG report and designate the findings as normal or abnormal; if abnormal, the Study Physician will indicate whether the ECG tracing is not clinically significant or clinically significant. Clinically significant deterioration should be reported as an AE. Any indication of heart abnormality will be followed up by a cardiologist.

Adverse events will be recorded from when the Informed Consent Form (ICF) has been signed until the End-of Study visit. AEs before study drug first intake will be considered as “unrelated”. The study investigators are responsible for reporting all serious and unexpected adverse events (regardless of relatedness) to the UF-IRB within 5 working days after learning of the event. Any AE or other unwanted response which occurs in the course of the study will be monitored and followed up until: it has resolved or stabilized according to the judgment of the study physician; there is a return to normal or baseline values, it has been shown to be unrelated to the study intervention.

MRI/MRS: Current guidelines from the US Food and Drug Administration (FDA) indicate that there are no known risks for human exposure to magnetic fields of 3.0 Tesla, unless metallic implants or cardiac pacemakers are introduced into the magnetic field or fringe field. The participants and those individuals assisting during the MRI will be screened using standardized procedures for metal or magnetic implants and other incompatible devices prior to entering the MRI scanner area. Anyone with a contraindication to MRI will not be permitted to enter the MRI scanner area. The MRI produces a loud noise, which has the potential to produce hearing loss in a very small number of patients when hearing protection is not used.

All persons present in the MRI during the scan will be provided with ear protection (ear plugs and/or insulated headphones) and instructed to wear these for the entire duration of the scan, which nearly eliminates the risk of hearing loss.

Physical function and muscle strength assessment: Safety precautions will be taken during the muscle strength and functional assessments. The 10-meter walk/run and six-minute walk tests will be administered in an unobstructed hallway. There is a minimal risk of falls with the stair climbing test. To minimize this risk, the child will be asked to wear suitable footwear and will be supervised at all times by a physical therapist. During the six minute walk test, safety precautions will be taken during the test by applying standardized stopping criteria. If the participant reports pain, tightness or pressure in the chest, significant shortness of breath, feeling faint, lightheaded or dizzy, or significant other medical problems the test will be stopped. Blood pressure will be assessed before and after this test. This measurement is non-invasive, however, cuff inflations may pose momentary discomfort. Research nurses are immediately available in the CRC if needed. The exercise assessment (both on cycle ergometer and determination of peak muscle strength may cause muscle fatigue and/or soreness in some children. If the participant becomes tired during testing, he/she may request a break to rest. To decrease the risk of these undesirable effects, participants will be attended to at all times while participating at UF.

Blood draws: There is a slight risk of minimal bleeding or infection (< 1% chance) during the blood draw. To minimize the risk, a qualified nurse in the CRC will perform the blood draw at UF, and only nurses/physicians will perform the blood draw in the subjects' own community. The blood draw is done using sterile techniques to reduce the risk of infection. For the ACTH stimulation test, other than venipuncture, there are no additional risks.

Study drug interventions:
Upon initiation of prednisone, the study physician and/or PI will be in contact with the patient and family by phone or videoconference once every 2 weeks for the first 3 months and monthly thereafter to ask the patient about any new signs or symptoms, review medication and diet history and inquire about any specific concerns they may have. If the patient has evidence of developing any side effects, we may require the patient to see his primary care physician at home for follow up. If these changes are persistent and/or the patient has non-manageable side-effects, the dose will be adjusted appropriately by the study physician. Also, during the entire course of the study, the patients and their parents will be encouraged to contact the investigator at any time to report any safety concerns that may have emerged between the scheduled visits or phone communications.

The study visits are 3 months apart in the first half of the intervention. This time frame was based on standard clinical practice whereby steroid naïve patients are typically seen by their physician 3 months after initiating of glucocorticoids. For Aim 2, study visits are 6 months apart. Safety parameters for both study Aims include physical examinations, growth parameters, vital signs, clinical laboratory tests (including chemistry, hematology, urinalysis, and tests for adrenal function), ECGs, and concomitant medication monitoring and AEs.

Prednisone:

There are several well-established risks associated with chronic daily use of glucocorticoids. Despite this, glucocorticoid treatment is standard of care for DMD and prednisone has been used to treat boys with DMD off-label for the past 30 years. Prednisone has shown to improve or stabilize muscle strength and delay the loss of ambulation compared to boys with DMD who remain steroid-naïve. The evidence-based dose recommendation for prednisone is 0.75mg/kg/day [13]. Because of the numerous risks/side effects associated with this daily dose, the search for an optimum dosing regimen exists. In patients with DMD, the most commonly reported risks are weight gain (mean of 3.2 kg), central obesity, cushingoid features, behavioral changes, growth delay and fractures [12,64]. In boys with DMD treated on an alternate high-dose weekend regimen (weekend dosing using 5 mg/kg/2 days on weekend for 6 to 12 months) compared to untreated boys with DMD, linear growth was maintained and obesity rates did not differ from untreated patients [17] Furthermore, Cushinoid features such as hirsuitism, acne, and hypertension did not occur in the weekend dose patients. In a subsequent study comparing the weekend dosing regimen described above to daily dosing of prednisone over 12 months [16], increases in body mass index were detected with both regimens (although tended to be more with the daily dosing). The weekend dosing was associated with greater linear growth than daily dosing. Although femoral bone density was note assessed in this study, lumbar spine Z score showed small changes with both dosing regimens. The aim of the current study is to demonstrate that low dose (0.75 mg/kg) twice weekly use will lessen the side effects all together.

We submitted an Investigational New Drug application to the Food and Drug Administration for the use of prednisone in boys with DMD for this specific study and received an exemption (IND 144718) in June 2019. The current 'standard of care' prednisone regimen commonly prescribed to patients with DMD poses a greater risk than the low-dose twice weekly regimen proposed in this study. Therefore, given the dosing regimen, we do not expect adverse side effects but will take precautions to safely monitor each patient. At the same time, we will also take precautions to monitor any potential inadequacy of the low-dose prednisone to delay onset of muscle pathology. Based on the preclinical data presented by Quattrocelli et al (2017) in the *mdx* mouse showing that weekly prednisone dosing enhanced sarcolemmal repair and reduced fibrosis and immune cell infiltration to a comparable extent as daily dosing, we expect the low-dose twice weekly prednisone administration to delay the onset of disease in boys with DMD similar to the established daily regimen. This, along with the notion that the twice weekly dosing will lessen the side effects associated with chronic daily dosing while also inducing ergogenic remodeling is the main premise being studied in Aim 1.

To monitor the risk of steroid inadequacy, muscle pathology (fat accumulation) and strength will be assessed at the 3 and 6-month visits. Based on data from our natural history cohort within ImagingDMD who were corticosteroid naïve, we can expect a 5% increase in MRS-measured vastus lateralis fat fraction in a 6-month time frame. Therefore, we will take a 5% or greater increase in muscle fat fraction in a boy with DMD on low-dose twice weekly prednisone dosing after 6 months as an indication that the glucocorticoid is delaying benefit. In addition, if we detect a decrement in strength measured in the knee extensor or knee flexor muscles at this time point (along with an increase in MRS fat fraction), we will assume the dosing paradigm is inadequate and will adjust the dose. The current recommended weekend dose based on work done by Ann Connolly's group is 5 mg/Kg on each of two weekend days.

As this dose is often associated with irritability and sleep disturbances that are time stamped to the days following this high-dose administration, we will use a dose of 2 mg/Kg (which is in between our current dose of 0.75 mg/Kg and 5 mg/Kg) twice weekly for the remaining 6 months of the study. Any adjustments to the dose and potential findings of drug 'inadequacy' will be reviewed by our local Safety Monitoring Committee.

Exercise intervention:

Based on the previous studies assessing exercise in DMD demonstrating safety [3,4] along with our pilot data on strength training in boys with DMD (see above), we do not expect our proposed exercise intervention to pose any risks, induce any muscle damage or cause significant pain. Long-standing clinical concerns of exercise in DMD are largely based on preclinical data using eccentric muscle actions, which are known to be damaging to muscle. We have designed the proposed exercise intervention to minimize any risks or potential for muscle damage in boys with DMD. The proposed exercise intervention involves a combination of aerobic cycling and isometric strength exercise modalities, performed at a low to moderate intensity level. As such, there is no potential for the boys to perform an eccentric muscle action. Dynamic forward pedaling will be performed during the cycling with assistance from the motor or low intensity resistance. As part of daily life, boys with DMD in this age group (5-8 years of age) typically engage in play and physical activity that involve repeated eccentric contractions. Therefore, the risks to the boys with DMD from the muscle contractions and exercise protocols for this study are less than the risks posed from eccentric contractions routinely encountered in their daily lives.

To ensure safety of the patient during exercise, we will take certain precautions. First, throughout the exercise intervention, our study team will supervise every exercise session remotely over the real-time video on the laptop computer (provided by us to the patient) and adjust the exercise guidelines accordingly. During the exercise sessions, procedures to minimize discomfort of exercise include a warm-up and cool-down. The participants will also be introduced to the exercise in a structured way, such that they begin with lighter intensity and gradually increase over the course of the intervention. Prior to starting each exercise session, the patient will be asked to rate any pain they may be experiencing at the time of assessment using a Wong-Baker FACES Pain Rating Scale with faces and corresponding numbers ranging from 0 (No Hurt) to 10 (Hurts Worst) [65]. Subjects will be asked to select one of the faces with its corresponding numerical rating and pain description. An increase in pain $\geq$4 will be considered the threshold for muscle damage and patient will not be allowed to exercise. We will also ask the patient if he has noticed dark urine. This will be noted and discussed with Study Physician. To ensure the patient exercises at low to moderate intensity, we will monitor the patients rating of perceived exertion using the OMNI scale, an assessment of perceived exertion using pictorial and verbal cues suited for healthy and clinically affected children [63]. Patients will be instructed to exercise at an intensity corresponding to $\geq$ 3 and $\leq$ 6 on the OMNI scale. If the patient rating is > 6 on the OMNI scale, the bicycle resistance may be lowered accordingly and noted by the investigator, or a rest break will be taken. Heart rate at rest and during exercise will also be monitored to gain information on perceived and physical exertion of exercise. Some muscle soreness may be expected initially when the patient starts the exercise program (given that the muscle has been unaccustomed to exercise) and initially if workload is increased. Patients will be asked to rate any pain they

may experience before, during or after exercise using a Wong-Baker FACES Pain Rating Scale with faces and corresponding numbers ranging from 0 (No Hurt) to 10 (Hurts Worst). Patients will be asked to select one of the faces with its corresponding numerical rating and pain description. An increase in pain $\geq$4 will be considered the threshold for muscle pain and exercise will be stopped. The research investigator will also ask the patient and parent if there are any other concerns or experiences of uncomfortable feelings during or after the exercise. Based on the response, the exercise intensity may be lowered accordingly and noted by the investigator. Some muscle soreness may be expected initially when the patient starts the exercise program (given that the muscle has been unaccustomed to exercise) and initially if workload is increased. Rest breaks will be provided as needed.

If the patient does experience muscle soreness and/or joint stiffness, these symptoms should not persist more than a few days and are comparable to typical post-workout soreness. The forces used during exercise for this study are well within safety limits, and the exercise device has numerous hardware and software constraints that limit the exercise to safe levels. For example, both hardware and software constraints are built in to limit the power output of the motor to avoid lower-limb rotations at excessive speeds. This speed is constantly measured so that excessive speeds will cause the test to automatically stop. The electric motor torque will be continuously monitored to prevent torque that may lead to muscle and/or tissue tear.

There are also other risks that relate to soft tissue injury, falls and fractures, post-exercise hypotension, and cardiovascular events. Although the patient will be seated throughout thee exercise, there is a risk that a participant may trip, stumble, or fall getting on or off the device. The patient may experience shortness of breath, dizziness, rapid or irregular heartbeat, chest pain or discomfort, heartburn, light headedness, or feeling about to faint. If the patient becomes tired during exercise, he may request a break to rest. During the exercises, the patient will have immediate access to a stop button that can be pressed at any time for any reason that will halt the exercise. We will require a parent or guardian be present at home during every exercise session. The risks to patients participating in this study are lower than risks if the patient were to exercise alone and without our instruction since we are monitoring the exercise and providing safety precautions. If any of the above-mentioned conditions occur during exercise or persist after rest, the participant's primary physician may be called and referred for evaluation. If the participant complains of angina at rest, loss of consciousness occurs, or cardiac arrest, the investigator will instruct the parent/guardian to contact emergency medical services immediately.

For exercise performed within the CTRB at UF, research staff will be present and available throughout and emergency treatment will be available if it becomes necessary. In the event of an adverse medical event, standard facility emergency procedures will be followed and proper personnel notified. In the event that a cardiac event is suspected the exercise session will be stopped immediately, and local emergency medical technicians (EMTs) will be called and the participant will be taken to an emergency medical center, if recommended by the EMTs.

To confirm the safety of the exercise intervention, blood for analysis of serum creatine kinase (CK) levels will be obtained at baseline and after 6 months on exercise. Serum CK is

commonly elevated and variable in DMD independent of any intervention. A previous study using whole-body vibration used an increase in CK level by >40,000 U/L to indicate muscle damage relating to their intervention [66]. We will use a more conservative threshold (>20,000 U/L), and we will also assess MRI-T_2 within the exercised quadriceps muscles at the study visits, where an elevation (>2SD) in the proportion of pixels within a muscle is considered the threshold for muscle damage. If at the 6-month assessment the patient has elevated serum CK (>20,000 UL from baseline) and elevated MRI T_2, the exercise intervention will be reported as a serious adverse event.

Adverse events: definitions and action plan

An adverse event (AD) is any untoward medical occurrence in a clinical investigation patient, which does not necessarily have a causal relationship with the investigational product (active or placebo drug, biologic, or device). An AE can, therefore, be any unfavorable and unintended symptom, sign, disease or condition, or test abnormality, whether considered related to the investigational product.

Adverse events include:

- Symptoms described by the patient or signs observed by the Investigator or medical staff
- Test abnormalities (laboratory tests, ECG, etc.) that result in an alteration in medical care (diagnostic or therapeutic)

Adverse events do not include:

- Changes that are characteristic of disease progression in DMD

Abnormalities present at Baseline are considered AEs only if they reoccur after resolution or they worsen during the study.

Seriousness of an AE serves as a guide for defining regulatory reporting obligations. An SAE is any AE that results in any of the following: death, life-threatening event, required or prolonged inpatient hospitalization, 30ersistent or significant disability/incapacity, important medical events

Evaluation of Adverse Events/Serious Adverse Events: Assessment of the association between the AE and study drug exposure is important for regulatory reporting. For each AE/SAE, the Investigator and Study Physician will determine whether there is a reasonable possibility that the AE may have been caused by the study drug according to the following categories:

- Not Related: The AE is clearly not related to study drug.
- Unlikely Related: There is no evidence of a causal relationship between study drug and the AE; although such relationship cannot be ruled out.
- Possibly Related: There is some evidence supporting the possibility of a causal relationship between study drug and the AE.
- Related: There is strong evidence that there is a causal relationship between study drug and the AE.

- A relationship to the investigational product must be given for each AE/SAE recorded, even if there is only limited information at the time. The Investigator may change his/her opinion of causality in light of follow-up information, amending the AE/SAE report accordingly.

Severity of an AE (Note that severity is not the same as "seriousness,": The Investigator will grade the severity of all AEs/SAEs as mild, moderate, or severe, based on the following definitions:

- Mild: An AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Moderate: An AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort, but poses no significant or permanent risk of harm to the research participant.
- Severe: An AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

The Investigator will provide information regarding the patient outcome of each AE. Possible results of an AE outcome are defined as follows:

- Fatal: The termination of life as a result of an AE.
- Not recovered/not resolved: The patient has not recuperated or the AE has not improved.
- Recovering/resolving: The patient is recuperating or the AE is improving.
- Recovered/resolved: The patient has recuperated or the AE has resolved.
- Recovered with sequelae/resolved with sequelae: The AE has resolved, but the patient has been left with symptoms or pathology.
- Unknown: Not known, not observed, not recorded, or refused.

The Investigator will be required to provide the action taken regarding prednisone in response to the AE according to the following categories:

- Dose not changed: No change in administration of the investigational product.
- Dose reduced: Reduction in the frequency, strength or amount of investigational product administered.
- Drug interrupted: Temporary interruption (termination) in administration of the investigational product.
- Drug withdrawn: Administration of the investigational product terminated (no further dosing).

- Not applicable: Determination of a value is not relevant in the current context.
- Unknown: Not known, not observed, not recorded, or refused.

All AEs/SAEs experienced by the patient will be recorded on a Study adverse event log form. The following information will be recorded: a concise description of the event; date and time of event onset and resolution; determination of seriousness, severity, corrective treatment, outcome, and relationship to investigational product; and, action taken regarding the investigational product. Abnormal vital signs, laboratory results, or other abnormal safety assessments will be recorded as an AE if they meet the definition of an AE. When possible, a diagnosis should be recorded as an AE rather than symptoms or isolated laboratory abnormalities related to that diagnosis. A medical or surgical procedure is not an AE; rather the condition leading to the procedure should be recorded as the AE. If the condition is not known, the procedure must be reported as an AE instead. Similarly, death is not an AE, but rather is the outcome of the AE(s) that resulted in death. If the AE(s) leading to death are not known, then death must be reported as an AE.

The study investigators are responsible for reporting all serious and unexpected adverse events (regardless of relatedness) to the UF-IRB within 5 working days after learning of the event. Any AE or other unwanted response which occurs in the course of the study will be monitored and followed up until: it has resolved or stabilized according to the judgment of the study physician; there is a return to normal or baseline values, it has been shown to be unrelated to the study intervention.

All Serious Adverse Events that are a suspected adverse reaction, serious **and** unexpected must be submitted by the investigator to the FDA using form 3500A (MedWatch). Note: events must meet all criteria to be submitted to the FDA.

All AEs/SAEs documented at a previous visit/contact that are designated as not recovered/not resolved or recovering/resolving will be reviewed by the Investigator at subsequent visits/contacts.

All AEs will be followed until the parameter returns to its Baseline status, resolution of AE, completion of the patient's participation, agreement is reached between the Investigator and Sponsor that the event no longer requires follow-up, or study termination, whichever occurs first. SAEs will be followed until resolution, the condition stabilizes, or the Investigator notes that follow-up is no longer necessary. Rules for AE/SAE follow-up apply to all patients, including those withdrawn prematurely to the extent allowed by the patient's consent. The Investigator will ensure that follow-up includes further investigations consistent with appropriate medical management and patient consent to elucidate the nature and/or causality of the AE/SAE.

Temporary or Persistent Dose Reductions: Temporary or persistent dose reductions may be made for safety or tolerability reasons in the judgment of the Study Physician and Principal Investigator. If at any time after initiation of treatment the patient experiences a clinically significant AE indicative of drug intolerance, the patient will undergo clinical assessment as soon as reasonably possible.

Monitoring of Abnormal Laboratory Values: During the study, liver function tests will be monitored, including ALT, AST, GGT, alkaline phosphatase, glutamate dehydrogenase, and bilirubin. If increases in ALT or AST are noted, the relationship of these to CK will be determined, as well as to GGT and measures of liver synthetic function. Trends in these parameters will be monitored. The Investigator may stop dosing in individual patients based on tolerability or AEs.

8. Possible Benefits:

Boys with DMD who participate in this study may experience direct, tangible benefits, although any benefit cannot be guaranteed. Anticipated benefits of taking the low dose twice weekly prednisone alone or in combination with exercise training include improved muscle strength and slowing of disease progression whereby patients may experience a stabilization or improvement in performance of daily activities including walking and stair climbing. The patient may experience improved endurance and resistance to fatigue. Together these improvements may be reflected as a benefit in their daily mood and/or quality of life (measured using the Pediatric Quality of life inventory (PedsQL). Boys who undergo low dose twice weekly prednisone alone(without exercise training) may also benefit from the anti-inflammatory and ergogenic effects of prednisone without experiencing the side effects (weight gain, central adiposity, behavioral issues) associated with daily use.

Other potential benefits for boys with DMD and their families include the educational experience during their interaction with investigators who are highly interested in muscular dystrophy and treatment. The boys and families may gain information on their condition from the functional assessments and the MR studies, as well as information on disease progression within their lower extremity muscles and the intervention effect. Individual data obtained in the study will be summarized for each participant and shared with him and his family. All study participants will be encouraged to communicate the results from the study to their primary care providers. The risks of participating in this study are low especially with the careful monitoring that will be done throughout the intervention and study visits. Therefore, the benefits both to the study participants and to the wider muscular dystrophy community outweigh the potential risks. There are no alternative approaches to the procedures outlined in the study protocol; the subject may withdraw (or his parents may withdraw him) at any time without any consequences if he no longer wishes to participate, as will be indicated in the informed consent form.

This study has significant promise to benefit scientific knowledge and have both short-term and long-term impact in the field of DMD. The knowledge gained on low dose twice weekly prednisone dosing has far reaching implications particularly given that more than 50% of boys treated with daily glucocorticoids develop significant enough side effects that medication is decreased or discontinued. The development and validation of exercise paradigm guidelines that may be beneficial dystrophic muscle remains an ever-present need in the field. This study strives to develop exercise prescription parameters that will have important implications for future comprehensive clinical care and management of patients with DMD.

Short-term impact: This study provides fundamental new knowledge relating to an alternate

GC regimen and impact of exercise in DMD. It will be the first to reveal whether a low-dose twice weekly regimen represents a more optimal frequency and dosage to maximize benefit and minimize risk than the current standard, and is expected to have less impact on BMI compared to standard daily dosing, along with few behavioral disturbances. The exercise training is anticipated to provide additive benefit to the GC regimen by improving muscle strength and physical function, and potentially delaying the progression of disease that characterizes this population of patients. Collectively, these novel interventions are expected to markedly improve quality of life and physical function in our cohort of young boys with DMD.

Long-term impact: By providing information on an alternate GC strategy in DMD, this study has real potential to impact clinical practice and patient care for DMD as well as other muscular dystrophies for whom GC are currently part of patient management. New knowledge on the interplay between GC regimen and exercise is expected to form a comprehensive clinical care approach, which needs to be confirmed in older patients. Through the use of non-invasive imaging, our study will provide new understanding regarding the impact of exercise on muscle pathophysiology in dystrophic muscle, thereby helping to shift the current paradigm in the field to promote rather than avoid exercise for patients with DMD. This proposed research is expected to add to the growing recognition that appropriate exercise can be amongst the most potent therapies for a variety of diseases and may work synergistically with pharmacological agents, extending the health span of a boy with DMD. Particularly as molecular and pharmacological treatments advance to allow patients to live a more active lifestyle, the role of exercise as an adjuvant therapy needs to be considered [67], and our findings provide a foundation for these future trial considerations. Finally, the exercise intervention proposed herein may be relevant to patients with other muscular dystrophies.

9. Data Safety Plan:

A local safety committee will be formed consisting of the PI, Study Physician Dr. Sladky, Co-investigator Dr. Sweeney, and a paediatric neurologist. The committee will review the progress of each patient enrolled in the study, review source documents including concomitant medications, compliance to intervention and adverse events. The adverse events include mood changes, hyperglycemia, hypertension, muscle soreness, weight gain and chest pain, all of which have a low likelihood of occurring. The committee will have a predetermined plan to deal with each event if it occurs. The committee will meet at least once every 3 months. In the case of adverse events, an ad hoc meeting may be called by the PI and/or Study physician. The Principal Investigator and study physician, with the assistance of study staff, will treat any adverse events to the extent that they are able and is necessary. If adverse events are serious enough and warrant emergency medical services, 911 will be called and the Principal Investigator and the study staff will ensure maximal comfort and safety of the subject until emergency medical services arrive. If adverse events occur at the patients' home, the PI and study staff will request the parent of the patient to seek medical attention.

A safety endpoint has been established for this study. With respect to the study drug prednisone, the side effects of chronic daily steroids have been acknowledged and although not expected given the low dose twice weekly use of prednisone, they will be monitored in

this study. Any adverse events associated with steroid side effects will be discussed by the local safety committee and a decision will be made to discontinue the patient from the study (described above under “Adverse events”.

There may also be a risk of inadequacy of the low-dose twice weekly prednisone administration, as chronic daily use is known to result in an apparent improvement in muscle strength and ambulation in most boys for 6-12 months, followed by stabilization for about 2 years and long-term slower deterioration than untreated patients. Based on the preclinical data presented by Quattrocelli et al (2017) showing that weekly prednisone dosing enhanced sarcolemmal repair and reduced fibrosis and immune cell infiltration to a comparable extent as daily dosing, we expect the low-dose twice weekly prednisone administration to delay the onset of disease in boys with DMD similar to the established daily regimen. This, along with the notion that the twice weekly dosing will lessen the side effects associated with chronic daily dosing while also inducing ergogenic remodeling is the main premise being studied. However, we will monitor the risk of drug inadequacy at the 3 and 6-month visits when patients return to the University of Florida by assessing muscle pathology (using magnetic resonance spectroscopy, MRS, to measure fat accumulation within the vastus lateralis muscle) and muscle strength (using Biodex dynamometry). Based on data from our natural history cohort within ImagingDMD who were corticosteroid naïve, we can expect a 5% increase in MRS-measured vastus lateralis fat fraction in a 6-month time frame. Therefore, we will take a 5% or greater increase in muscle fat fraction in a boy with DMD on low-dose twice weekly prednisone dosing after 6 months as an indication that the glucocorticoid is delaying benefit. In addition, if we detect a decrement in strength measured in the knee extensor or knee flexor muscles at this time point (along with an increase in MRS fat fraction), we will assume the dosing paradigm is inadequate and will adjust the dose and revise the IRB. The current recommended weekend dose based on work done by Ann Connolly’s group is 5 mg/Kg on each of two weekend days. As this dose is often associated with irritability and sleep disturbances that are time stamped to the days following this high-dose administration, we would plan to use a dose of 2 mg/Kg (which is in between our current dose of 0.75 mg/Kg and 5 mg/Kg) for the remaining 6 months of the study.

An efficacy endpoint has also been established. Similar to above description, if the low-dose (0.75 mg/kg) twice weekly prednisone appears to be inadequate (based on increases in muscle fat fraction and decreases in strength over 6 months) in terms of inducing the benefits normally associated with daily prednisone, we will seek permission (amendment to IRB) to increase the dose to 2 mg/Kg (which is in between our current dose of 0.75 mg/Kg and the high dose 5 mg/Kg previously used on weekends and shown to induce irritability) for the remaining 6 months of the study.

The safety and monitoring plan also includes close supervision of every patient throughout the study. At the study visits, vital signs will be monitored. If the participant displays signs of altered vital signs, procedures will be adjusted or halted and the participant will be closely monitored. The study physician will be contacted if the participant's condition persists. After initiation of the study drug (Prednisone) when the patient is back home, the study physician will be in contact with the patient and family by phone once every 2 weeks for the first 3

months and as needed thereafter. The patients and their parents will be encouraged to contact the investigator at any time to report any safety concerns that may have emerged between the scheduled visits or phone communications. Regular monitoring includes asking about changes in medications, pain ratings, overall function.

To ensure safety of exercise, we will take certain precautions. First, throughout the exercise intervention, our study team will supervise every exercise session remotely over the real-time video on the laptop computer and adjust the exercise guidelines accordingly. We will also be monitoring the patients rating of perceived exertion using the OMNI scale (assessment of perceived exertion using pictorial and verbal cues suited for healthy and clinically affected children, where patients will be encouraged to exercise at an intensity corresponding to > 3 and < 6 on the OMNI scale. Patients will be asked to rate any pain they are experiencing during or after exercise using a Wong-Baker FACES Pain Rating Scale with faces and corresponding numbers ranging from 0 (No Hurt) to 10 (Hurts Worst). They will select one of the faces with its corresponding numerical rating and pain description during the real-time Skype monitoring for the research investigator to note. The research investigator will also ask the patient and parent if there are any other concerns or experiences of excessive pain/ uncomfortable feelings during or after the exercise. If the patient rating is > 4 on the Pain Rating scale, the exercise intensity may be lowered accordingly and noted by the investigator. Some muscle soreness may be expected initially when the patient starts the exercise program (given that the muscle has been unaccustomed to exercise) and initially if workload is increased. Rest breaks will be provided as needed.

The accuracy and quality of data will be checked. All data collection will be supervised by the Principal Investigator or a physical therapist or exercise physiologist who is a study staff member. Accuracy and quality of the physiological and functional data will be checked for accuracy by the PI. Demographic and functional data will be entered via the secure, web-based application Research Electronic Data Capture (REDCap). Heart rate will be obtained from a pulse oximeter. Heart rate may also be assessed manually by study staff with experience assessing heart rate using this method to double-check for accuracy. Blood pressure will be assessed manually using a blood pressure cuff by study staff with experience taking blood pressures. Blood pressure may also be monitored with an automatic blood pressure cuff in order to double-check for accuracy. For the MRI/MRS data, Dr. Mueller will serve as the Informatics Manager for the MRI related projects in the ImagingDMD laboratory. He will be responsible for MR data transfer, data management and data security as per protocol standards set forth by ImagingDMD. He will maintain the patient data base, check data for integrity and establish quality control procedures, produce regular reports summarizing the status of data acquisition and subject enrollment, and convert the data base for entry into the statistical analysis package. He is also responsible for reviewing the master database and flagging any missing or questionable data.Standard, validated assessments of pain will be used. Study staff have experience with boys with DMD and these assessments.

10. Conflict of Interest:

There is no conflict of interest relating to any of the investigators and this protocol beyond the

professional benefit from academic publication or presentation of the results.

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