Title: Tadalafil as an adjuvant therapy for Duchenne muscular dystrophy

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Abstract:
This project seeks to demonstrate the efficacy of a single dose of tadalafil, a common vasodilator drug, to counter sympathetic vasoconstriction (improve blood flow) in leg muscles of boys with Duchenne muscular dystrophy (DMD). This project is the first part of our overall objective which will be to assess the potential of tadalafil as a longer-term intervention to improve muscle pathophysiology and function in DMD. DMD is a rapidly progressive disease, causing muscle deterioration, premature loss of ambulation and death from respiratory or cardiac failure. Emerging disease-modifying gene therapy treatments are expected to partially restore levels of the missing dystrophin protein, lead to improved muscle function and allow boys to engage in greater levels of physical activity[1,2]. However, they are not designed to target the vascular impairment that underlies DMD pathology because they do not restore the dystrophin binding domain for muscle specific neuronal nitric oxide synthase (nNOSμ)[3,4]. The implications of this are that muscle may continue to experience inadequate perfusion and subsequent injury in response to activity despite partial dystrophin replacement. Tadalafil, a phosphodiesterase 5 (PDE5) inhibitor commonly used as a vasodilator drug, has shown to restore blood flow after forearm exercise in boys with DMD[5]. However, a follow up phase 3 clinical trial failed to demonstrate tadalafil impact on ambulatory ability. We postulate that this failure resulted from either down-regulation of PDE5 in leg muscles of patients with more advanced disease, and/or a lack of sufficient daily activity by patients in the trial (since tadalafil is most effective during and after muscle activity). Based on these reasons, dismissing tadalafil due to one failed trial in DMD may be premature, particularly since there is strong rationale that it can be an adjuvant to upcoming therapies for DMD. We have submitted an R21 exploratory clinical trial to assess this idea and our score fell just outside the fundable range with promising review comments suggesting need for pilot data. Hence, this pilot project will determine the leg vascular responsiveness to a single dose of tadalafil using non-invasive techniques of MRI-blood oxygenation level dependent (BOLD) response or pulsed Doppler ultrasonography and cycle exercise in a group of boys with DMD aged 6 to 30 years to seek proof of concept for future studies assessing the hypothesis that if given daily, tadalafil has potential to protect muscle during physical activity by restoring perfusion and thereby slowing disease progression.

Background:

Pathophysiology and treatment: Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular disease caused by a gene mutation causing lack of a functional dystrophin protein. Due to increasing replacement of muscle by fat and fibrotic tissue, boys with DMD experience severe weakness and fatigability, early loss of ambulation and death[6]. Dystrophin plays a key role in stabilizing structural proteins and molecular signaling at the sarcolemma; its deficiency

increases myofiber susceptibility to contraction-induced damage and a perpetuating cascade of abnormalities including mitochondrial pathology and mis-localization of muscle specific *neuronal nitric oxidase synthase* (nNOSμ) from the sarcolemma (a critical position to optimize local muscle perfusion during and after physical activity)[7-8]. Normally, nNOSμ is activated by contraction [9], leading to production of NO and increased levels of guanosine 3',5'monophosphate (cGMP) in vascular smooth muscle causing vasodilation (blunting sympathetic vasoconstriction). This regulatory mechanism increases blood flow to match metabolic demands within active muscle[10]. In dystrophic muscle, nNOS mislocalization results in inadequate blood flow relative to demand during contraction (termed functional ischemia) [7,11], attenuating oxygen and nutrient supply and blunting removal of accumulated metabolites.[12,13] This impaired local vasodilatory response contributes to focal muscle necrosis and accelerates damage in response to high-intensity contractions [10-13]. In addition, loss of nNOSμ causes vascular narrowing and inadequate muscle perfusion *after* stopping exercise in *mdx* mice (DMD model), causing post-exercise fatigue and prolonged inactivity[7]. In DMD patients, post-exercise blood flow is reduced[55,14] and fatigue levels are higher than in healthy boys [15].

Promising disease-modifying therapies are on the horizon for patients with Duchenne muscular dystrophy (DMD), where gene-based strategies seek to restore partial dystrophin production to yield a milder muscle phenotype [16]. For example, initial results from a microdystrophin gene-transfer trial indicate clinically meaningful improvements in DMD muscle function [1]. With therapeutic advances, improvements in physical function are anticipated, thus allowing boys with DMD to engage in greater levels of physical activity and exercise [1,2]. However important challenges remain[17]. Notably, with only one exception[18], none of the strategies currently approved or in clinical trials restore the nNOS binding domain [11]. Treatment of *mdx* mice with this binding domain improves muscle perfusion during exercise, prevents ischemic injury and enhances exercise performance[11] whereas *mdx* mice expressing microdystrophin lacking nNOS showed restored force but still displayed exaggerated post-exercise fatigue [7]. Clinical support for restoring sarcolemmal nNOS comes from Becker muscular dystrophy patients (milder phenotype compared to DMD) whose mutation disrupts the nNOS domain and have severely impaired NO-mediated vasodilation compared to those with an intact domain who do not[19] and present with milder clinical manifestations overall [20]. Collectively these findings emphasize that restoring nNOS signaling is essential to any dystrophin-replacement therapy[3, 4]. This is particularly relevant given the anticipated improvements in muscle function and subsequent increases in exercise capacity and physical activity afforded by gene-based therapies. Without sufficient nNOS signaling during muscle activity, fibers expressing the gene construct may undergo functional ischemia and injury, resulting in depletion of microdystrophin and reducing efficacy over time [2].

Prior research and rationale for tadalafil use in DMD: Phosphodiesterase 5 inhibitors (PDE5i) are common vasodilatory drugs that amplify nNOS signaling by prolonging the half-life of cGMP improving perfusion of active skeletal muscle. In preclinical studies, PDE5i prevented exercise-induced muscle ischemia and injury and alleviated the exaggerated fatigue response and post-exercise inactivity in *mdx* mice [7,21 9]. This suggests PDE5i imparts a myofiber protective effect by alleviating susceptibility to muscle damage during activity. Of further interest, chronic tadalafil targets mitochondrial abnormalities and induces an oxidative muscle phenotype in *mdx* mice, improving contractile function and reducing fiber injury[22,23]. These and other preclinical findings [24] offer promise for tadalafil as a clinical treatment approach, however findings from human studies are contradictory. While short-term tadalafil markedly attenuates functional ischemia and improves muscle blood flow after forearm exercise in boys with DMD [5]; a phase 3 trial failed to show benefit on the 6-minute walk distance (6MWD)[25]. One important difference between these two studies relates to the muscles targeted for assessment (arm versus legs) and that arms are affected later in the disease course [26]. Boys in the upper age range of that study (14 years) have

significantly less intramuscular fat infiltration in arms compared to legs [27,28], meaning the drug could exert vasodilatory effects to improve arm muscle blood flow but could not exert a meaningful impact in the legs given the high degree of fat infiltration and lower muscle volume. Further to this point, **tadalafil has the greatest impact on blood flow when the muscles are active and during recovery**, accounting for benefits in arm function in the phase 3 trial, as boys rely on greater daily use of arms particularly when more advanced disease progression in legs impacts ambulation. As declines in ambulation begin early in teenage years [29] and daily step activity decreases[30], many patients in the phase 3 trial may not have had enough daily activity (to increase metabolic demand and require increased blood flow) for the drug to exert its target effect. Supporting this, tadalafil had no effect on muscle blood flow in the resting condition [5]. Equally relevant to discussion of the failed phase 3 trial is **use of the 6MWT as the primary outcome** as it is not a suitable determinant of drug efficacy for boys in the upper age range of the study, particularly given the expected degree of fat infiltration in their locomotor muscles. In addition, 6MWT measures central effort and coordination and is influenced by motivation, incentive and training effect, emphasizing the need for alternative clinical trial endpoints; thus denouncing the only phase 3 trial of Tadalafil in DMD because of lack of efficacy on 6MWD may be premature.

It is also possible that the tadalafil target, PDE5, is down-regulated as disease progresses, rendering the drug ineffective. Lack of PDE5 efficacy has been reported in two clinical studies[31,32], both using sildenafil (a different PDE5i) in patients with BMD. Witting et al [33], attributed the lack of benefit on exercising muscle blood flow to down-regulation of the drug target in leg biopsies of 5 patients. However this finding may be explained by known reductions in PDE5 levels with age in dystrophic muscle[33]. The mean age of the BMD cohort was 38 yrs (range 25-62). The other study reporting lack of sildenafil efficacy was also in adults with BMD and advanced DMD (mean age 25)[32]. Based on a report that muscle PDE activity is higher in DMD boys aged 4-6 yrs versus control subjects aged 20-65 yrs[34] and that tadalafil restores blood flow in exercising DMD muscles[5], we postulate that the PDE5 target exists in muscle of younger boys with DMD. Moreover we will use tadalafil, shown to have greater specificity compared to sildenafil for cGMP, a longer half-life and a better safety profile[35]. Taken together, the **prior failed studies emphasize the need to assess tadalafil in patients an earlier disease stages, with sufficient muscle and engagement in prescribed regular activity**. Since the effects of tadalafil are immediate[5] and we don't know when PDE5 is down-regulated in the progression of DMD, we will first assess the acute response to a single dose at baseline in a group of boys with DMD and determine the extent of vascular responsiveness. We expect these results to lay the foundation for a subsequent study where we aim to demonstrate that acute benefits of tadalafil translate to meaningful clinical benefits over time when combined with exercise.

Specific Aim:
To assess vascular responsiveness in leg muscles to one dose of tadalafil in a group of boys with DMD.

Research Plan:
This project will target up to 25 ambulatory boys with DMD aged 6-30 years. Because the vascular impact of tadalafil is immediate [5], we will confirm that PDE5 is a valid target in lower extremities by assessing the change in post-exercise microvascular perfusion using Blood Oxygen Level-Dependent (BOLD) MRI or change in post-exercise hyperemia using Doppler ultrasonography. Previous studies have shown MRI and Doppler techniques to be sensitive to detecting PDE5 inhibition [5,36] such that we are expecting improvement of the vascular response after one dose. This study will require 2 visits where the patient will be randomized to tadalafil on Day 1 or Day 2 and undergo MRI or Doppler and cycle exercise on each day.

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 immunostaining or Western blot (<2%) and/or DNA confirmation of dystrophin mutation.
- Minimum entry age of 6.0 years
- Ambulatory

Exclusion criteria:

- Older than 30 years of age
- Contraindication to an MR examination (e.g. aneurysm clip, severe claustrophobia, magnetic implants)
- Presence of unstable medical problems, significant concomitant illness including cardiomyopathy, uncontrolled seizure disorder, uncontrolled hypo or hypertension
- Presence of a secondary condition that impacts muscle function or muscle metabolism (e.g. myasthenia gravis, endocrine disorder, mitochondrial disease)
- Contraindications to Tadalafil (use of nitrates, alpha-adrenergic blockers, other PDE5A inhibitors) anti-hypertensive drugs or other medications known to modulate blood flow or muscle metabolism or inhibit or induce CYP3A4)

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.

Patients will be recruited to 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 participated in previous research trials for the PI. 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 other patient organizations.

Screening procedures: Prospective participants will be screened by the PI and study physician to ensure they meet the criteria for participation in the study. This includes asking about all current medications taken by the patient. 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 assessments. 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 any time 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 Design and procedures:
The study will take place within the Clinical Research Unit of the Clinical Translational Research Building and the Advanced Magnetic Resonance Imaging and Spectroscopy center of the McKnight Brain Institute, all at the University of Florida. Participation in this study involves 2 visits to the University of Florida. Visit 2 will be conducted within 3 weeks (± 1 week) of Visit 1. All procedures will be performed by the trained investigative team.

Consent, medical history and vital signs: Following the consent/assent procedure, medical history and detailed log of current medications will be obtained to ensure inclusion/exclusion criteria. Vital signs (sitting blood pressure (BP), heart rate (HR), respiratory rate and temperature) will be obtained.

Magnetic Resonance Imaging (MRI): MR investigation will be done on all patients to assess muscle disease severity. Our colleagues at UF (ImagingDMD) have developed standardized procedures to measure fat fraction and transverse relaxation time (T_2), markers of fat infiltration and inflammation/damage respectively, in leg muscles shown to be highly reproducible and sensitive to DMD disease severity[27,37]. Values will be compared to those previously published by our group in a large data set obtained from a contemporary natural history cohort of 5 to 13 year old boys with DMD[38].In addition to these standard measures, we will use another technique, post-contractile Blood oxygenation level dependent (BOLD) response, to measure degree of vascular impairment. BOLD measures changes in small vessel oxygen saturation and blood volume and reflects a balance of blood flow and oxygen consumption kinetics within exercising muscle[39-41]. An increase in post-contractile BOLD signal indicates oxygen delivery is in excess of oxygen consumption. We have recently shown that compared to a marked increase in the BOLD signal in healthy boys, the signal is markedly blunted in DMD, consistent with the notion of impaired microvascular function after muscle contraction[42]. Test-to-test reproducibility for the peak BOLD response was demonstrated (with the magnitude of the difference for the repeated tests at 0.8±0.4%). We will assess the BOLD response before and after the single dose of tadalafil. We expect the BOLD results to mirror the Doppler findings, and therefore provide another non-invasive, quantitative index to detect improvements in vascular function in DMD.

Quantitative MRI will be performed using a 3T whole body MRI scanner within the AMRIS facility. Patients will be positioned supine in the magnet for up to 60 minutes. MRI T_2 and MRS FF will be measured using procedures developed by our team[27,43]. Dixon imaging will identify the leg muscles and echo planar BOLD sequences will be obtained at rest and after brief (~1 second) maximal voluntary contractions [44] using leg extensions. The peak post-contractile response will be determined as a change in signal intensity relative to rest, and the peak force produced will be used to normalize signal. The BOLD response will be assessed a second time after dosing with tadalafil where an increase in BOLD response relative to pre-dosing will identify whether acute tadalafil improves microvascular function.

Doppler ultrasound: Determination of vascular responsiveness to tadalafil will be done using non-invasive pulsed-Doppler ultrasonography[45]. This standard technique will be performed on the superficial femoral artery as previously in kids[46] in a reclined position to assess mean blood velocity at rest and for 60 seconds post-leg cycling exercise[47] using a standardized protocol (20% peak torque)[5] and appropriate probes and angle of insonation. Femoral artery diameter will be measured by B-mode imaging, allowing calculation of femoral artery blood flow (mean blood velocity (cm/s)* Pi r2*60, where r is radius of the artery). Measures will be taken at rest and after leg exercise performed on a stationary bicycle or Biodex. We will follow the same protocol used

by Nelson and colleagues in a prior study assessing the efficacy of tadalafil in DMD [5]. Briefly, patients will perform intermittent leg exercise at a submaximal intensity for approximately 7 minutes. The change in femoral artery blood flow from rest to post-exercise will reflect exercise-induced hyperemia. This test will be done before and approximately 2 hours after administration of tadalafil (time frame expected to match peak blood levels[5,48]. A patient is considered responsive to PDE5 inhibition (and tadalafil) if the post-exercise hyperemic response (post-exercise–resting blood flow) is greater (by approximately double) after tadalafil dosing compared to before dosing[5].

Data Analysis

After the procedures are completed, the collected data will be compiled for analysis. The participants will provide data on 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 breaches 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.

1. Possible Discomforts and Risks:

Study parameters include vital signs 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. The study investigators are responsible for reporting all serious and unexpected adverse events 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 (i.e. non-drug trial procedures).

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. The magnet is segregated in a shielded area and no one may enter this room until he/she has been screened to be safe. 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. Some people may become claustrophobic while inside the magnet, and in these cases the subject will be removed. Patients will be required to do a short bout of exercise in the magnet to assess vascular and mitochondrial function. To minimize muscle fatigue, the exercise will be no more than 2 minutes. Also, dystrophic muscles are more susceptible to muscle damage, particular when performing eccentric contractions. Therefore, to minimize the risk of muscle damage, the protocol will use only isometric contractions in the magnet, which are the least likely to induce muscle damage in boys with DMD. For patient comfort, we allow one parent and study member to accompany the child in the MRI scanner area.

Use of Tadalafil*:* Tadalafil is extensively used, and FDA approved to treat erectile dysfunction (ED) and benign prostatic hyperplasia in adult men. Tadalafil is also approved for use to treat pulmonary arterial hypertension (PAH) and to improve exercise capacity in men and women[49]. Much is known regarding its toxicology, potential side effects and tolerance to long term use. In children, there are an increasing number of studies assessing the safety and efficacy of tadalafil in pediatric patients with PAH [50-53]. They all suggest that tadalafil improves clinical symptoms and is well tolerated in children and adolescents with PAH, and may even prevent disease progression. A population pharmacokinetic model for tadalafil in pediatric patients with PAH was recently reported [54], with results suggesting clinical trials use doses similar to adults (40 mg) once daily for pediatric patients with a body-weight of 40 kg or higher, and a dose of 20 mg once daily in patients with a body weight of less than 40 kg.

Tadalafil has been previously used in research studies of patients with neuromuscular disease, both adults (aged 18-55 years) with Becker muscular dystrophy[55] and boys with DMD (aged 7 to 14 years)[5,25]. Both the short term and long term (48 weeks) effects were assessed at doses ranging from 0.5 mk/kg to 1.0 mg/kg. These doses were used to approximate maximum adult doses for erectile dysfunction and PAH respectively, with a 30% dose reduction given the once daily dosing and the long elimination half-life of tadalafil. The authors reported that the improvements in functional ischemia and post-exercise hyperemia were dose dependent (with 1.0 mg/kg showing super-normal attenuation). The pharmacokinetic profile was reported, with blood levels peaking 2-4 hours after tadalafil administration, with an elimination half-life of 25 hours for the 0.5 ml/kg dose and 40 hours for the 1.0 mg/kg dose. In all three studies, the known safety profile of tadalafil was confirmed with no unusual side effects (other than those consistent with the known profile of tadalafil as per below). In addition, all three studies reported efficacy of tadalafil in restoring post-exercise blood flow to normal levels.

In the two trials using Tadalafil in boys with DMD (consisting of ~350 boys with DMD), headache and facial flushing were reported, with penile erection reported in 15% of patients, noting that they were nonpainful and resolved spontaneously. Side effects will be closely monitored. To minimize risk of side effects we have chosen a lower dose of (0.6 mg/kg) which has been shown to still

benefit vasodilatory limitation in boys with DMD. The pharmacokinetic data of Tadalafil at this proposed dosage in DMD is known.

Overall, adverse events relating to tadalafil use in the general population are typically mild-to-moderate in intensity, and discontinuation rates are usually low[49]. Commonly reported side effects with tadalafil use in pediatric populations include: headache, flushing (warmth, redness or tingly feeling), nausea/upset stomach, runny nose and nasal congestion, myalgia, allergic reactions along with the expected increase in penile erections without priapism. Concomitant administration of tadalafil with medications that lower systemic blood pressure can result in potentiation of hypotension. Coadministration of tadalafil with nitrates is contraindicated due to the risk of significant hypotension, and, because of the long half-time of tadalafil, nitrates should not be administered for 48 hours following administration. Tadalafil is primarily metabolized by the CYP3A enzyme subfamily to an inactive metabolite. As such, concomitant administration with medications that inhibit or induce CYP3A4 will increase and decrease tadalafil exposure respectively. The use of anti-hypertensive medications and medications that alter CYP3A activity is a study exclusion criteria, thus we do not anticipate these risks. Daily use of corticosteroids (prednisone or deflazacort) is the current standard of care for DMD; no interactions between corticosteroids and tadalafil have been reported to our knowledge.

We will be careful to monitor side effects and drug-associated adverse events in this study. Patients and their families will be advised about the potential side effects prior to study initiation. The study physician will council the patient and parent regarding these effects and be available for consultation at any time during the study. Side effects will be discussed with the study physician as needed.

2. Possible Benefits:

The subject may experience a direct, short-lived benefit from participation in this study, as the single dose of tadalafil is expected to increase muscle blood flow and perfusion during muscle activity, thus protecting the muscle from functional ischemia up to 24 hours. By taking part in this study, the subject will learn whether his leg muscles are responsive to the drug, and this may be important information for participation in a future interventional trial on tadalafil in DMD. A potential benefit of this study to the general community is the advancement of scientific knowledge. This study focuses on the potential for tadalafil, a common vasodilatory drug, to protect muscle from functional ischemia during activity and slow disease progression, and hence be an additive therapy to upcoming gene or other therapies.

3. Data Safety Plan:

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.

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). For the MRI/MRS data, data transfer, data

management and data security will be performed as per protocol standards set forth by ImagingDMD. Study staff have experience with boys with DMD and these assessments.

4. 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.

Referred Citations:

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