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# PDE5 inhibition alleviates functional muscle ischemia in boys with Duchenne muscular dystrophy



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## ABSTRACT

**Objective:** To determine whether phosphodiesterase type 5 (PDE5) inhibition can alleviate exercise-induced skeletal muscle ischemia in boys with Duchenne muscular dystrophy (DMD).

**Methods:** In 10 boys with DMD and 10 healthy age-matched male controls, we assessed exercise-induced attenuation of reflex sympathetic vasoconstriction, i.e., functional sympatholysis, a protective mechanism that matches oxygen delivery to metabolic demand. Reflex vasoconstriction was induced by simulated orthostatic stress, measured as the decrease in forearm muscle oxygenation with near-infrared spectroscopy, and performed when the forearm muscles were rested or lightly exercised with rhythmic handgrip exercise. Then, the patients underwent an open-label, dose-escalation, crossover trial with single oral doses of tadalafil or sildenafil.

**Results:** The major new findings are 2-fold: first, sympatholysis is impaired in boys with DMD—producing functional muscle ischemia—despite contemporary background therapy with corticosteroids alone or in combination with cardioprotective medication. Second, PDE5 inhibition with standard clinical doses of either tadalafil or sildenafil alleviates this ischemia in a dose-dependent manner. Furthermore, PDE5 inhibition also normalizes the exercise-induced increase in skeletal muscle blood flow (measured by Doppler ultrasound), which is markedly blunted in boys with DMD.

**Conclusions:** These data provide in-human proof of concept for PDE5 inhibition as a putative new therapeutic strategy for DMD.

**Classification of evidence:** This study provides Class IV evidence that in patients with DMD, PDE5 inhibition restores functional sympatholysis. *Neurology*® 2014;82:2085–2091

## GLOSSARY

**cGMP** = cyclic guanosine 3',5'-monophosphate; **DMD** = Duchenne muscular dystrophy; **FDA** = US Food and Drug Administration; **HbO<sub>2</sub>** = oxyhemoglobin; **LBNP** = lower-body negative pressure; **MbO<sub>2</sub>** = oxymyoglobin; **MVC** = maximal voluntary contraction; **NIR** = near-infrared; **NO** = nitric oxide; **PDE5** = phosphodiesterase type 5; **TLS** = total labile signal.

Duchenne muscular dystrophy (DMD) is a devastating X-linked muscle wasting disease for which there is no specific treatment.<sup>1,2</sup> Glucocorticoids can prolong ambulation by 2 to 3 years, reduce scoliosis, and temper pulmonary and cardiac decline in the second decade of life.<sup>1,3</sup> However, glucocorticoids cause well-known side effects, which are intolerable in more than 25% of patients.<sup>1,2,4</sup> Thus, a disease-specific treatment is a major unmet need.

The nitric oxide (NO)–cyclic guanosine 3',5'-monophosphate (cGMP) pathway constitutes a putative new drug target for DMD. A compelling body of preclinical research shows that phosphodiesterase type 5 (PDE5) inhibitors, which prolong the half-life of cGMP—the downstream target of NO in vascular smooth muscle—benefit limb, respiratory, and cardiac muscles in *mdx* mice<sup>5–11</sup> and prolong survival in dystrophin-deficient zebrafish.<sup>12</sup> Remarkably, even a single dose of sildenafil or tadalafil, 2 common PDE5 inhibitors, prevents muscle ischemia, as well as injury and fatigue, when *mdx* mice are subjected to a brief bout of exercise.<sup>5</sup>

## Supplemental data at Neurology.org

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Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

The challenge remains to translate this promising preclinical work to actual human patients with DMD. Thus, we first performed a case-control study to establish that blood flow regulation is impaired in boys with DMD despite contemporary background therapy with glucocorticoids and cardiac prophylactic medication. Then, we conducted an acute dose-finding study to test whether tadalafil can eliminate functional muscle ischemia in boys with DMD. Moreover, to test for a drug class effect, we compared tadalafil with sildenafil, another PDE5 inhibitor with a very different chemical structure.<sup>13</sup>

**METHODS Standard protocol approvals, registrations, and patient consents.** The study was approved by the Institutional Review Board at Cedars-Sinai Medical Center. Each subject gave pediatric assent and their parents gave written informed consent to participate. An investigational new drug exemption was granted by the US Food and Drug Administration (FDA) to administer tadalafil and sildenafil for the purposes of this pediatric research study (NCT01580501).

**Subjects.** Between 2012 and 2013, we studied ambulatory boys with DMD, 8 to 13 years of age, with normal left ventricular ejection fraction (>50%), and healthy age-matched male controls. All patients had a preexisting clinical diagnosis of DMD based on rate of skeletal muscle disease progression, and all had confirmed DMD mutation.

All subjects were screened for eligibility by clinical examination, 12-lead ECG, and 2-dimensional transthoracic echocardiogram. Potential subjects, both cases and controls, were excluded from study if they had a history of hypertension or measured blood pressure >120/80 mm Hg; diabetes mellitus; heart failure by history, physical examination, or left ventricular ejection fraction <50% by 2-dimensional transthoracic echocardiography; required nocturnal ventilator support; or had any contraindication to PDE5 inhibitors (use of nitrates,  $\alpha$ -adrenergic blockers, other PDE5A inhibitors, or potent inhibitors of cytochrome P450 3A4).

**Hemodynamic measurements.** Subjects were studied in the supine position. Heart rate was measured continuously by ECG and blood pressure by automated oscillometric sphygmomanometry, using a validated monitor (Welch Allyn Vital Signs Monitor 300 Series; Welch Allyn, Inc., Skaneateles Falls, NY).

**Skeletal muscle oxygenation by near-infrared spectroscopy.** Forearm muscle tissue oxygenation was measured with near-infrared (NIR) spectroscopy, as previously described.<sup>14</sup> Briefly, optodes were placed over the flexor digitorum profundus, the main muscle recruited during handgrip. The optodes were housed in a customized flexible rubber casing and secured to the skin with adhesive. The optodes were covered with an optically dense, black vinyl sheet, to minimize interference from external light and loss of NIR transmitted light.

The NIR signal was sampled at a rate of 5 Hz, converted to oxyhemoglobin (HbO<sub>2</sub>) + oxymyoglobin (MbO<sub>2</sub>) concentration with validated algorithms, displayed as the running average of 50 consecutive samples, and stored digitally for analysis (PowerLab; ADInstruments, Boulder, CO). Before each experiment, absorption and scattering coefficients were calibrated against an external standard. After each experiment, a pneumatic cuff was inflated on the upper arm to suprasystolic pressure (200 mm Hg) to establish the total labile signal

(TLS, the difference between the baseline and nadir in muscle tissue oxygenation). Changes in forearm muscle tissue oxygenation were expressed as a percentage of TLS.

**Brachial artery blood flow by Doppler ultrasound.** Brachial artery mean blood velocity was measured using pulsed-Doppler ultrasonography (Siemens iE33). Data were acquired continuously with a 9-MHz probe with a 60° angle of insonation. The ultrasound gate was optimized to ensure complete insonation of the entire vessel cross-section with constant intensity. The Doppler audio signal was converted to a real-time flow velocity signal using a validated Doppler audio converter,<sup>15</sup> and recorded using a PowerLab data acquisition system (ADInstruments). Brachial artery diameter was measured by B-mode imaging. Brachial artery blood flow was calculated as mean blood velocity (cm/s)  $\cdot$   $\pi r^2 \cdot 60$ , where  $r$  is radius of the brachial artery.

**Handgrip exercise.** Handgrip exercise was performed with a dynamometer (Smedley Hand Dynamometer modified by Stoelting). To determine maximal voluntary contraction (MVC), each subject was asked to grip the dynamometer as hard as possible 3 separate times; the greatest force output was taken as MVC. Force output was displayed on a computer screen to provide visual feedback for subjects. Subjects performed intermittent isometric handgrip (20 handgrips per minute, 50% duty cycle) at 20% MVC for 7 minutes. This mild level of handgrip exercise was chosen because it does not activate sympathetic outflow to skeletal muscle but causes sympatholysis in healthy boys.<sup>16,17</sup>

**Functional sympatholysis.** The subjects' lower body was enclosed in a negative pressure chamber to the level of the iliac crest as previously described.<sup>16</sup> The pressure in the chamber was measured by a Statham transducer (Gould Inc., Oxnard, CA). Lower-body negative pressure (LBNP) at  $-20$  to  $-30$  mm Hg simulates mild orthostatic stress caused by transition from the supine to the seated position. This technique mainly unloads the low-pressure cardiopulmonary baroreceptors, producing highly reproducible reflex increases in sympathetic vasoconstrictor drive to the skeletal muscle vasculature without changing systemic blood pressure.<sup>16</sup> To measure exercise-induced attenuation of reflex vasoconstriction (i.e., functional sympatholysis), the LBNP was (1) applied at rest, and then (2) superimposed on mild rhythmic handgrip at 20% MVC. Reflex vasoconstriction was measured as the LBNP-induced decrease in forearm muscle oxygenation by NIR spectroscopy. Using the pressure inside the LBNP chamber as a trigger, the PowerLab software was programmed to mean the HbO<sub>2</sub> + MbO<sub>2</sub> signal for 20 seconds before the onset of LBNP and for 20 seconds before the offset of LBNP; the difference between these mean values was taken as the LBNP-induced change in forearm muscle tissue oxygenation.<sup>18</sup> Blood pressure, heart rate, and handgrip force were also recorded in response to 2 minutes of LBNP applied at rest and during the third to fifth minutes of the 7-minute exercise protocol.

**Exercise-induced hyperemia.** Brachial artery blood flow was measured at rest and for 60 seconds postexercise to evaluate exercise-induced changes in skeletal muscle blood flow, defined as the percentage increase in brachial artery blood flow from rest to postexercise. Vascular conductance was calculated as forearm blood flow divided by mean arterial pressure.

**Specific protocols. Case-control study.** Functional sympatholysis (and exercise-induced hyperemia) was compared in 12 boys with DMD and 10 age-matched healthy male controls. The screening visit included a practice session to familiarize the boys with the protocol. Baseline data were acquired the next day. Background therapy of DMD with glucocorticoids and/or prophylactic cardiac medication was continued throughout the study.

**Table 1** Baseline characteristics of patients with Duchenne muscular dystrophy

Patient no.	Exon mutation	Age at study, y	Maximal voluntary contraction (grip strength)	Dominant hand	Creatine kinase, IU/L	Frequency of wheelchair/scooter use	Medications
1	Deletion, 38-43	9	6	Right	6,672	Rarely	Deflazacort, Ca <sup>2+</sup> -carbonate, CoQ10, MV, VD <sub>3</sub>
2	Deletion, 46-52	10	6	Right	10,038	Never	Deflazacort, Pepcid, Ca <sup>2+</sup> -carbonate
3	Deletion, 45	8	5	Right	6,828	Never	Prednisone, Prevacid
4	Deletion, 34-35	8	5	Right	11,474	Never	Prednisone, CoQ10, creatine, L-glutamine, fish oil, VD <sub>3</sub>
5	Deletion, 45	11	9	Right	6,183	Rarely	Deflazacort, Ca <sup>2+</sup> -carbonate
6	Duplication, 3-44	12	9	Right	1,557	Frequently	Prednisone, lisinopril, Ca <sup>2+</sup> -carbonate
7	Duplication, 3-44	12	10	Left	2,246	Frequently	Prednisone, lisinopril, Ca <sup>2+</sup> -carbonate
8	Deletion, 43	11	9	Right	9,493	Rarely	Deflazacort, losartan, hGH, Protandim, Ca <sup>2+</sup> -carbonate, potassium, pamidronate
9	Deletion, 36	8	10	Right	12,659	Rarely	Deflazacort, lisinopril, VD <sub>3</sub> , CoQ10, creatine monohydrate, glutamine, MV
10	Deletion, 3-6	13	12	Right	2,422	Rarely	Deflazacort, lisinopril, Singulair, Prevacid, MV

Abbreviations: Ca<sup>2+</sup>-carbonate = calcium carbonate; CoQ10 = coenzyme Q<sub>10</sub>; hGH = human growth hormone; MV = multivitamin; VD<sub>3</sub> = vitamin D<sub>3</sub> (cholecalciferol).

**Acute dose-finding study.** Boys with DMD (but not healthy controls) then participated in the PDE5 inhibitor dose-finding study. Patients received either single doses of sildenafil or tadalafil in an open-label crossover design with a 2-week washout period before crossover (accounting for the 17.5-hour elimination half-life of tadalafil). The order of sildenafil or tadalafil was random. Patients received 0.5 mg/kg (not to exceed 20 mg) of either oral sildenafil or oral tadalafil on day 1, followed by 1.0 mg/kg of sildenafil or tadalafil (not to exceed 40 mg) on day 2. Experiments were performed 1 hour after administration of sildenafil or 3 hours after administration of tadalafil to match the expected peak blood levels. Patients were queried about potential side effects throughout the study visits.

**Pharmacokinetics.** Serum blood samples for pharmacokinetic determinations were collected from patients with DMD at time 0, 0.25, 0.5, 1, 2, 4, 8, and 24 hours after each dose of both sildenafil and tadalafil. Blood levels were measured using high-performance liquid chromatography–tandem mass spectrometry (NMS Labs, Willow Grove, PA).

**Statistics.** The prespecified primary outcome was a 50% restoration of functional sympatholysis (i.e., exercise-induced attenuation of reflex vasoconstriction). Based on recent work from our laboratory,<sup>18</sup> the sample size was chosen to achieve a power of 80% to detect an effect size of 0.9, based on a 2-sided *t* test.

Baseline characteristics of patients and controls were compared using Student *t* test. Exercise-induced attenuation of reflex vasoconstriction (functional sympatholysis) was assessed by

comparing the LBNP-induced  $\Delta\text{HbO}_2 + \text{MbO}_2$  (%TLS) at rest vs the LBNP-induced decrease in  $\Delta\text{HbO}_2 + \text{MbO}_2$  during handgrip. Student *t* test was used to test the following: group differences in  $\Delta\text{HbO}_2 + \text{MbO}_2$ ; drug treatment effect on LBNP-induced  $\Delta\text{HbO}_2 + \text{MbO}_2$  (%TLS) at rest vs the LBNP-induced decrease in  $\Delta\text{HbO}_2 + \text{MbO}_2$  during handgrip; group difference in the % $\Delta$  in brachial artery blood flow; and drug treatment effect on the % $\Delta$  in brachial artery blood flow. The apparent elimination half-life ( $t_{1/2}$ ) was determined using linear regression of  $\geq 3$  natural logarithm-transformed data points in the terminal phase. Data are expressed as mean  $\pm$  SEM, unless otherwise specified.

#### Primary research question and classification of evidence.

Does PDE5 inhibition alleviate functional muscle ischemia in boys with DMD? This study provides Class IV evidence that in patients with DMD, PDE5 inhibition restores functional (i.e., exercise-induced) sympatholysis.

**RESULTS** Data are presented for 10 boys with DMD and 10 healthy controls; movement artifacts precluded data analysis in 2 boys with DMD. Baseline characteristics and indices of disease severity of these 10 patients are shown in table 1. All patients were ambulatory, although some often used a wheelchair or scooter. All patients with DMD were receiving background therapy with glucocorticoids (either

**Table 2** Baseline characteristics of patients with DMD compared with healthy controls

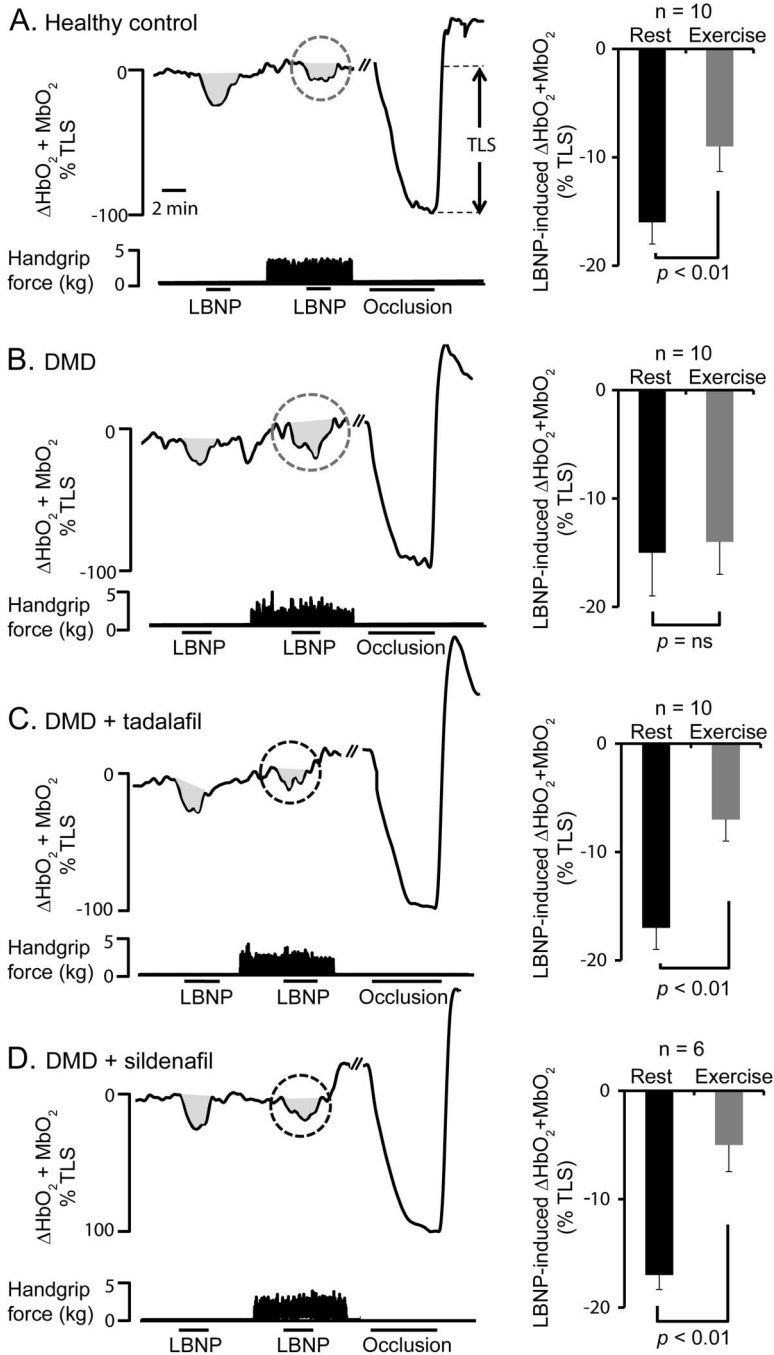
Group	No.	Age, y	BMI, kg/m	MVC, kg	SBP, mm Hg	DBP, mm Hg	HR, beats/min	LVEF, %
Patients with DMD	10	10 $\pm$ 1	28 $\pm$ 3	8 $\pm$ 1 <sup>a</sup>	106 $\pm$ 3	57 $\pm$ 3	91 $\pm$ 5 <sup>a</sup>	62 $\pm$ 2
Healthy controls	10	11 $\pm$ 1	27 $\pm$ 2	19 $\pm$ 2	105 $\pm$ 3	56 $\pm$ 2	76 $\pm$ 2	64 $\pm$ 1

Abbreviations: BMI = body mass index; DBP = diastolic blood pressure; DMD = Duchenne muscular dystrophy; HR = heart rate; LVEF = left ventricular ejection fraction; MVC = maximal voluntary contraction; SBP = systolic blood pressure.

Data reported as mean  $\pm$  SEM.

<sup>a</sup>Indicates significantly different from healthy control (*p* < 0.05).

**Figure 1** Functional sympatholysis is impaired in patients with DMD and rescued by acute PDE5 inhibition



(A) Left panel shows a representative tracing from a healthy control subject, showing that the lower-body negative pressure (LBNP)-induced decrease in forearm muscle oxygenation ( $\text{HbO}_2 + \text{MbO}_2$ ) is greatly attenuated during mild handgrip exercise (gray dashed circle), demonstrating functional sympatholysis. At the end of each experiment, an arm cuff was inflated to supra-systolic pressure to occlude the forearm circulation, producing a maximal decrease in muscle oxygenation to calculate the total labile signal (TLS). Right panel shows summary forearm muscle oxygenation data from the 10 healthy control subjects studied at rest and during exercise expressed as a percentage of TLS. (B) Representative tracings from a patient with Duchenne muscular dystrophy (DMD) at baseline (pretreatment), showing that sympatholysis is impaired because handgrip fails to attenuate the LBNP response (gray dashed circle). Summary data are shown in the right panel for 10 boys with DMD. (C) Representative tracing from a subject with DMD treated with tadalafil, showing that the LBNP-induced decrease in forearm muscle oxygenation is greatly attenuated during mild handgrip exercise (black dashed circle). Summary data are shown in the right panel for 10 patients with DMD. (D) Representative tracing from a subject with DMD treated with sildenafil, showing that the LBNP-induced

decrease in forearm muscle oxygenation is greatly attenuated during mild handgrip exercise (black dashed circle). Summary data are shown in the right panel for 6 patients with DMD. Data are reported as mean  $\pm$  SEM. ns = not significant.

deflazacort or prednisone); 5 of the 10 patients were also receiving prophylactic cardiac medication (either lisinopril or losartan).

Patients and controls were well matched for age, body mass index, blood pressure, and left ventricular ejection fraction (table 2). As expected, resting heart rate was higher in the boys with DMD ( $p < 0.05$  vs healthy controls), and MVC (i.e., grip strength) was much lower ( $p < 0.05$ ).

**Functional sympatholysis is impaired in DMD and restored by PDE5 inhibition.** In resting forearm muscle, LBNP evoked comparable decreases in forearm muscle oxygenation ( $\text{HbO}_2 + \text{MbO}_2$ ) in patients and controls (figure 1). When LBNP was superimposed on handgrip in healthy controls, the reflex decrease in muscle oxygenation was attenuated by  $54\% \pm 8\%$  ( $\Delta\text{HbO}_2 + \text{MbO}_2$ :  $-18\% \pm 3\%$  at rest vs  $-9\% \pm 2\%$  during handgrip,  $p < 0.01$ ), indicating functional sympatholysis. In contrast, no such attenuation was observed in the patients with DMD ( $\Delta\text{HbO}_2 + \text{MbO}_2$ :  $-14\% \pm 2\%$  at rest vs  $-13\% \pm 2\%$  during handgrip), indicating functional muscle ischemia (figure 1). In boys with DMD, sympatholysis was similarly impaired in those treated ( $n = 5$ ) or not treated ( $n = 5$ ) with prophylactic cardiac medication (figure e-1 on the *Neurology*<sup>®</sup> Web site at Neurology.org).

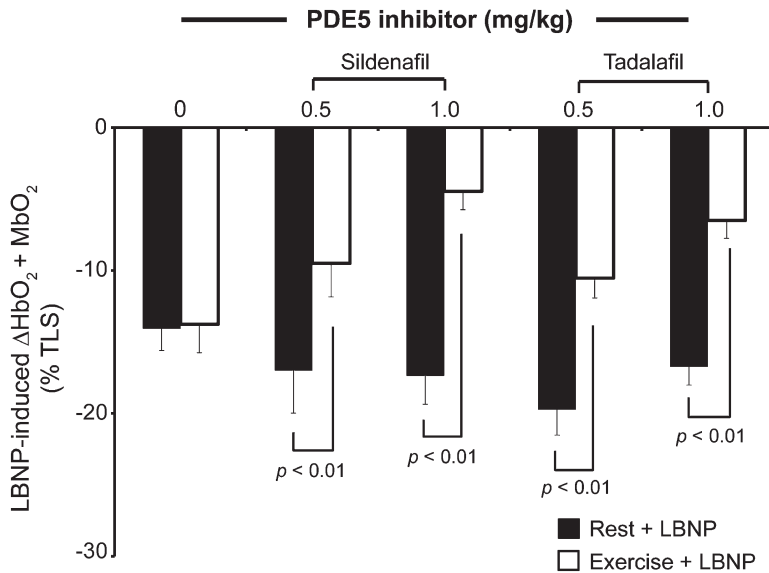
Tadalafil restored functional sympatholysis in boys with DMD (figure 1) in a dose-dependent manner. With the 0.5 mg/kg dose of tadalafil, the reflex decrease in muscle oxygenation was attenuated by  $45\% \pm 8\%$  ( $\Delta\text{HbO}_2 + \text{MbO}_2$ :  $-20\% \pm 2\%$  at rest vs  $-11\% \pm 1\%$  during handgrip,  $p < 0.01$ ), a response that is indistinguishable from normal ( $p =$  not significant vs healthy controls). The 1.0 mg/kg dose caused a super-normal  $63\% \pm 5\%$  attenuation ( $\Delta\text{HbO}_2 + \text{MbO}_2$ :  $-17\% \pm 5\%$  at rest vs  $-7\% \pm 1\%$  during handgrip,  $p < 0.01$ ). In a subset of patients ( $n = 6$ ), we confirmed these results with sildenafil (figures 1 and 2). Individual data are illustrated in figure e-2.

**Exercise-induced hyperemia is blunted in boys with DMD and restored by tadalafil.** Handgrip exercise increased brachial artery blood flow by  $78\% \pm 12\%$  over baseline in healthy controls but only by  $32\% \pm 5\%$  in boys with DMD ( $p < 0.05$ , figure 3). Remarkably, in DMD, tadalafil restored exercise-induced hyperemia in a dose-dependent manner (figure 3, table e-1). A similar tendency was seen with sildenafil, but the treatment effect was not statistically significant (figure e-3).

**Pharmacokinetic data.** Nine boys with DMD completed the pharmacokinetic study. The average group

decrease in forearm muscle oxygenation is greatly attenuated during mild handgrip exercise (black dashed circle). Summary data are shown in the right panel for 6 patients with DMD. Data are reported as mean  $\pm$  SEM. ns = not significant.

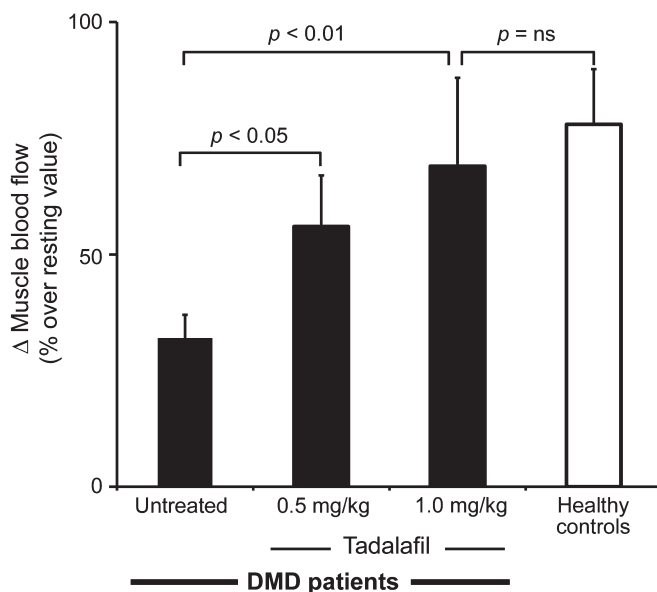
**Figure 2** Sildenafil equally restores functional sympatholysis compared with tadalafil



Lower-body negative pressure (LBNP)-induced decreases on forearm muscle oxygenation by near-infrared spectroscopy at rest and during mild rhythmic handgrip exercise (n = 6). Data expressed as a percentage of the total labile signal (TLS). Data reported as mean ± SEM. PDE5 = phosphodiesterase type 5.

response curves are presented in figure e-4. Blood levels peaked approximately 1 hour after oral sildenafil administration, with an elimination half-life of  $2.4 \pm 0.09$  hours for the 0.5 mg/kg dose and  $3.2 \pm 0.5$  hours for the 1.0 mg/kg dose. In contrast, blood levels peaked 2 to 4 hours after oral

**Figure 3** Postexercise hyperemia is impaired in DMD and rescued by tadalafil



Exercise-induced change in skeletal muscle blood flow, from rest to postexercise, in 10 patients with Duchenne muscular dystrophy (DMD) and 10 healthy controls. Data expressed as mean ± SEM. ns = not significant.

tadalafil administration, with an elimination half-life of  $24.9 \pm 3.1$  hours for the 0.5 mg/kg dose and  $39.5 \pm 6.6$  hours for the 1.0 mg/kg dose.

**Safety data.** Facial flushing occurred in all boys with both doses of either PDE5 inhibitor. Blood pressure was unaffected by either drug. One patient developed a penile erection lasting 6 hours after low-dose tadalafil and was not escalated to the higher dose. A second boy experienced erection lasting 3 hours after low-dose tadalafil and 4 hours after high-dose tadalafil. In both cases, erections were nonpainful and resolved spontaneously.

**DISCUSSION** In the dystrophin-deficient *mdx* mouse model of DMD, PDE5 inhibitors alleviate many features of the dystrophic phenotype, including spasm of skeletal muscle microvessels that can lead to muscle hypoperfusion, injury, and fatigue.<sup>5</sup> However, the *mdx* mouse is an important but imperfect milder model of the virulent human disease. Thus, the challenge is to determine whether the compelling body of preclinical mouse research can be translated to boys with DMD. The present study directly addresses this challenge with 3 major new findings: (1) functional sympatholysis is impaired in pediatric patients with DMD, producing functional muscle ischemia, despite contemporary background therapy with corticosteroids alone or in combination with cardioprotective medication; (2) tadalafil alleviates this ischemia in a dose-dependent manner; and (3) sildenafil replicates the effect of tadalafil, strongly supporting PDE5 inhibition as the mechanism of action.

That sympatholysis is impaired in contemporary patients with DMD confirms and extends our earlier observations made well over a decade ago before patients with DMD were treated with any medication.<sup>19</sup> While glucocorticoids represent the first significant advance in medical management of DMD, our new data in glucocorticoid-treated patients show that chronic treatment with neither prednisone nor deflazacort protects dystrophic muscle from functional ischemia. While angiotensin-converting enzyme inhibitors (lisinopril) and angiotensin receptor blockers (losartan) are being used increasingly in boys with DMD as preemptive therapy to delay onset of heart failure,<sup>1,18,20,21</sup> our data show that chronic treatment with neither lisinopril nor losartan restores sympatholysis in DMD, even though these drugs can reduce reactive oxygen species that destroy NO and thereby rescue sympatholysis in common acquired adult cardiovascular diseases such as hypertension and heart failure.<sup>22-26</sup>

In contrast, the seminal finding of this study is that tadalafil rescues sympatholysis in boys with DMD, providing added putative benefit beyond that afforded by the current standard of care. The effect is marked, immediate, and dose-dependent. The lower

dose used in our study is approximately equivalent to an adult dose of 20 mg, which was shown previously to alleviate functional ischemia in adults with Becker muscular dystrophy<sup>14</sup> and is the highest dose approved by the FDA to treat erectile dysfunction. Our higher dose is approximately equivalent to the 40-mg dose approved by the FDA to treat adult pulmonary hypertension and the same dose most frequently used to treat pediatric pulmonary hypertension.<sup>27</sup>

That sildenafil, which was the main PDE5 inhibitor used in the preclinical studies, and tadalafil were virtually identical in their ability to restore sympatholysis in boys with DMD—despite having different chemical structures—strongly implicates PDE5 inhibition as the mechanism of action. Because PDE5 is a cGMP-specific phosphodiesterase, our data support the hypothesis that PDE5 inhibition boosts a residual NO-cGMP signal arising from cytosolic neuronal NO synthase, which, in the absence of dystrophin, is misplaced from the sarcolemma.<sup>5</sup> That neither PDE5 inhibitor affected reflex vasoconstriction or blood flow in resting DMD skeletal muscle nor systemic blood pressure suggests that the exercise-specific action involves neither endothelial NOS-derived NO nor central inhibition of sympathetic outflow.

Moreover, that tadalafil normalized not only sympatholysis but also exercise-induced hyperemia, which was markedly blunted in the boys with DMD, provides further evidence for remarkably close clinical translation of seminal *mdx* mouse studies.<sup>5</sup> This novel finding also suggests that NO released from exercising human skeletal muscle normally exerts a vasodilatory effect even in the absence of  $\alpha$ -adrenergic stimulation. The blood flow data further suggest that a higher degree of PDE5 inhibition may be required to fully restore exercise-induced hyperemia than sympatholysis. Whereas sympatholysis reflects attenuation of a neurogenic vasoconstrictor response specifically in the most distal skeletal muscle microvessels, exercise-induced hyperemia involves multiple vascular segments and multiple active vasodilator mechanisms that remain incompletely understood.

Our study has several limitations. While this was an open-label study, impaired sympatholysis was a robust finding in DMD and its correction with both PDE5 inhibitors was steeply dose-dependent. Because this was a single-dose study, we do not know whether the improved blood flow regulation will be sustained with chronic administration. However, “nitrate tolerance,” which is a common limitation of organic nitrates, simply is not a feature of PDE5 inhibition. This proof-of-concept study does not address the crucial question of whether restoring normal blood flow regulation will preserve dystrophic skeletal muscle and slow disease progression. Despite these limitations, the data herein, together with a compelling body of preclinical research, have informed the design of a pivotal multicenter clinical

trial to determine whether chronic daily tadalafil can preserve muscle function in boys with DMD (NCT01865084).

## AUTHOR CONTRIBUTIONS

All authors contributed significantly to the present manuscript and have approved the final version. Dr. M. Nelson was involved in the conception and design, data collection, data analysis, interpretation of the results, writing the first draft of the manuscript, and editing/revising of the manuscript. Dr. Rader was involved in data collection and editing/revising the manuscript. Ms. Tang had an integral role in data collection, data analysis, and data interpretation. Dr. Tavyev was involved in the conception and design, data collection, and editing/revising the manuscript. Dr. S. Nelson and Dr. Miceli were involved in the conception and design, interpretation of the results, and editing/revising the manuscript. Dr. Elashoff was involved in the conception and design, data/statistical analysis, and editing/revising the manuscript. Dr. Sweeney was involved in the conception and design, interpretation of the results, and editing/revising the manuscript. Dr. Victor obtained the research funding, and was involved in the conception and design, interpretation of the results, drafting the manuscript, and editing/revising the manuscript.

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## DISCLOSURE

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