INVITED REVIEW

EXERCISE AND DUCHELLE MUSCULAR DYSTROPHY: TOWARD EVIDENCE-BASED EXERCISE PRESCRIPTION

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ABSTRACT: To develop a rational framework for answering questions about the role of exercise in Duchenne muscular dystrophy (DMD), we focused on five pathophysiological mechanisms and offer brief hypotheses regarding how exercise may beneficially modulate pertinent cellular and molecular pathways. We aimed to provide an integrative overview of mechanisms of DMD pathology that may improve or worsen as a result of exercise. We also sought to stimulate discussion of what outcomes/dependent variables most appropriately measure these mechanisms, with the purpose of defining criteria for well-designed, controlled studies of exercise in DMD. The five mechanisms include pathways that are both intrinsic and extrinsic to the diseased muscle cells.


Effective supportive therapy for Duchenne muscular dystrophy (DMD), let alone a cure using gene therapy, has been a challenge despite identification of the missing protein, dystrophin, over 20 years ago. 1 Exercise, depending on several poorly researched parameters (including frequency, intensity, time, and type), may be either beneficial or detrimental to dystrophic skeletal and cardiac muscle. A recent mini-review of the literature 2 recommended that systematic research studies of exercise training in DMD are warranted to better define exercise prescription for boys afflicted with DMD. However, the most commonly used animal model, the mdx mouse, does not fully recapitulate the disease. Although similar biochemically, mdx mouse muscle and muscle from a DMD patient differ, for example, in terms of regenerative ability 3 and compensatory protein expression (utrophin). 4 Thus, some researchers 5–9 have used exercise in mdx mice to worsen the dystrophic phenotype. The fact that exercise in this instance is detrimental does not necessarily translate categorically to boys with the disease for three reasons: (1) differences exist between the dystrophic mouse and dystrophic human; (2) there is a dearth of data regarding the very wide spectrum of variables to consider prior to exercise prescription; and (3) the adaptability to exercise in animal models of the disease and in DMD boys is unknown. 2,10,11 Although a positive role for exercise is well accepted in non-diseased populations, 12 scientific studies have not yet resolved whether exercise is therapeutic in a dystrophic population. 13,14 Furthermore, although some practical recommendations are available, 15,16 specific guidelines regarding exercise prescription (the type, frequency, and intensity of exercise) do not exist. 17–22 Thus, families of diseased patients are left with some fundamental questions. Does exercise help or hurt my child? What are the specific guidelines for ensuring that the exercise is safe and beneficial and, importantly, not detrimental?

To begin development of a rational framework for answering questions regarding the role of exercise in DMD patients, this review focuses on five proposed pathophysiological mechanisms of the disease and offers brief hypotheses regarding how exercise may modulate the pertinent cellular and molecular pathways of these mechanisms in a beneficial manner. The goals of this review are twofold. First, we sought to provide an integrative overview of mechanisms of DMD pathology that may improve or worsen as a result of exercise training. Second, we sought to expand upon a recent review, 2 stimulating discussion of what outcomes/dependent variables most appropriately measure these mechanisms, with the purpose of defining criteria for well-designed, controlled studies of exercise training in DMD. The five mechanisms, as defined and discussed by Petrof, 23 include: (1) mechanical weakening of the sarcolemma; (2) inappropriate calcium influx; (3) aberrant cell signaling; (4) increased oxidative stress; and (5) recurrent muscle ischemia.

MECHANICAL WEAKENING OF THE SARCOLEMMA

An appropriate starting point for discussion of the issues that affect dystrophin-deficient animals and...
humans with DMD is with the mdx mouse, the most widely studied animal model of the disease, and with mechanical weakening of the sarcolemma, widely considered a key mechanism underlying the pathogenesis of the disease. The mdx mouse is accepted as a biochemical and genetic model of DMD and has been studied since the 1980s. The reader is referred to a recent review and has been studied since the mouse is accepted as a biochemical and genetic model of DMD. Although useful as clinical animal models of DMD, although useful as clinical models of DMD. Although useful as clinical animal models of DMD. Although useful as biochemical and genetic models of the disease, a biochemical and genetic model of DMD and has been studied since the 1980s. The mdx mouse is accepted as a biochemical and genetic model of DMD and has been studied since the 1980s. The reader is referred to a recent review.

### Methodological considerations

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Study title</th>
<th>Methodological considerations that potentially confound this interpretation</th>
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</thead>
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<tr>
<td>Karpali et al. (1986)</td>
<td>Small-caliber skeletal muscle fibers do not suffer deleterious consequences of dystrophic gene expression</td>
<td>a,d</td>
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<td>Bansal et al. (2003)</td>
<td>Defective membrane repair in dysferlin-deficient muscular dystrophy</td>
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*Exercise was studied in mouse not human, or was not studied.
*Exercise was not voluntary.
*Exercise was biased toward pliometric muscle action (e.g., see refs. 20, 30, 35, and 37) have focused on some aspects of the DGC, dystrophin appears to have a role in cellular signaling (see Aberrant Cell Signaling section).

The 427-kDa dystrophin protein can be isolated from an oligomeric complex of proteins often referred to as the dystrophin–glycoprotein complex (DGC). The literature that has hinted toward re-envisioning the role of exercise in DMD has been reviewed. In this section, particular focus is placed on the underlying mechanism of sarcolemma weakening and on studies that provide counterpoints to the assertion that “...Both sarcolemmal permeability and necrosis of dystrophin-deficient muscle are exacerbated by physical exercise and improved by muscle immobilization.”

Components of the DGC include the dystroglycans, sarcoglycans, sarcospan, dystrobrevins, syntrophins, and neural nitric oxide (NO) synthase, which regulates NO signaling. Especially when considered as part of the DGC, dystrophin appears to have a role in cellular signaling (see Aberrant Cell Signaling section). However, many studies (e.g., see refs. 20, 30, 35, and 37) have focused on dystrophin’s structural contribution, and these studies have suggested that it performs a mechanical role in stabilizing the sarcolemma during shortening, lengthening, or isometric muscle actions.

In each of these contraction types, the goal is transmission of force. In both skeletal and cardiac
As it is relayed from muscle to external load. Positive work is the product of muscle force and shortening, whereas negative work is the product of muscle force and muscle shortening (i.e., a lengthening action). Costameres are protein assemblies that interact intimately with the sarcolemma and are believed first to couple the force generation of sarcomeres to the sarcolemma and to other fibers, and second to convert a mechanical stimulus to cellular signaling or gene expression response. In muscles that lack dystrophin, costameres are disrupted, and sarcolemmal fragility (commonly measured by release of creatine kinase and pyruvate kinase) ensues. Of note, increased membrane permeability and membrane mechanical weakness are not necessarily the same. A certain threshold of work (most often negative work—i.e., a lengthening action) is capable of producing contraction-induced injury. This threshold is diminished in muscle that lacks dystrophin. The work-overload theory proposed that a muscle, such as diaphragm, which is continuously activated throughout life, would deteriorate faster than a less frequently activated muscle. In the context of limited exercise studies available at that time, the work-overload theory made sense and provided an explanation of cardiorespiratory failure in dystrophic deficiency. The theory was challenged in 1994 by observations in mdx mice that voluntary wheel-running exercise improved, rather than worsened, the contractile function of the diaphragm.

Since that time several more murine studies have concluded that exercise may be beneficial. There is a conspicuous similarity between studies, because the exercise was voluntary. Human studies focused on inspiratory muscle training in DMD patients have shown that the function of respiratory muscles improves with training. Importantly, respiratory muscle training is most effective when initiated in the early stages of DMD, suggesting an age-dependent effect. Thus, it is reasonable to presume that, based on their own volition, dystrophic animals (and boys) may self-regulate the amount of force transmitted to their costameres by limiting their external work. Stated more simply, dystrophic boys might self-impose limits to their physical activity. Although few studies have investigated sensation of intramuscular fatigue and central fatigue in patients with DMD, it has been observed that cage activity of mdx mice is diminished relative to controls and that DMD boys cannot keep up with their peers. Whether this outcome is volitionally or pathophysiologically imposed, work is performed at the subject’s discretion. We pose the question: Do DMD patients develop an awareness and memory of a given workload/physical activity if significant muscle pain or myoglobinuria follows the activity? If so, does this affect their choices regarding participation in physical activities? Do they self-regulate? In addition, we reiterate the call for increased partnership between DMD families and patient registries and/or clinical trials. Data collected through this partnership might improve our understanding of the amount and type of daily self-selected physical activity performed by the patients in relation to the progression of the pathology that could guide future studies to quantitatively define appropriate exercise.

How can we best determine appropriate exercise to minimize negative effects on the dystrophic membrane and maximize positive adaptations to improve muscle performance? A recent literature review of the biology of the DGC included several references that demonstrated exercise was contraindicated in DMD (Table 1). What several of these studies reported was that enforced exercise or lengthening contractions exacerbated the muscle pathophysiology, whereas immobilization did not. A reasonable conclusion on the basis of these studies is that inappropriate exercise can indeed be detrimental. However, the benefits or detriments of exercise would be defined more clearly if future studies focused on dose-response relationships in specific exercise protocols. Of interest is the question of whether appropriate exercise can improve the structural and signaling characteristics of the myofibers that compose dystrophic muscle despite the absence of the DGC. Future studies should address this issue by varying the key elements of exercise prescription, such as type, intensity, frequency, and duration, to determine whether there are ideal values (the right “prescription”) by which dystrophic muscles can positively adapt to improve function. Such studies could stimulate new hypotheses regarding the role of dystrophin as a determinant of the mechanical properties of the sarcolemma.

INAPPROPRIATE CALCIUM INFUX

Maintenance of calcium homeostasis within skeletal muscle fibers is important both to confer functionality and to prevent excessive calcium-activated protease (calpain) activity. Other ions, such as Na⁺ and Cl⁻, clearly contribute to function and health/disease of skeletal muscle fibers. Although the importance of other ionic changes is not to be downplayed, this section focuses on mechanisms whereby exercise may modify intracellular Ca²⁺ changes in dystrophic muscle.

There exist different schools of thought on the hierarchy of calcium-dependent events leading to...
myofiber degradation in muscular dystrophy. One contends that the mechanism of cell death in damaged dystrophic myofibers is mediated by calpains, which exert well-documented calcium-activated proteolytic activity in dystrophic muscle.\(^{58,59}\) This activity is increased following disruption of the sarcolemma, mechanical or otherwise. Skeletal muscle contains both of the ubiquitous forms of calpain, termed \(\mu\)-calpain and \(\mu\)-calpain, to indicate, respectively, the millimolar and micromolar \(Ca^{2+}\) concentrations required for their activation. Skeletal muscle also contains a third muscle-specific calpain, called calpain-3. In healthy muscle, calpain activity is antagonized by calpastatin. Because calpastatin concentration is generally in excess, calpain activity is effectively regulated. Little information is available regarding regulation of calpain activity in dystrophic human muscle. Muscles of \(mdx\) mice have increased calpain concentration and activity as compared with wild-type counterparts\(^{60,61}\); dystrophic symptoms are diminished in \(mdx\) mice that overexpress calpastatin.\(^{62}\) Symptoms in \(mdx\) mice are also ameliorated after treatment with BN 82270, a calpain inhibitor\(^{63}\); however, it is unclear whether calpain-inhibitory or antioxidant properties of this compound were responsible.

A second school of thought contends that the mechanism of cell death in damaged dystrophic myofibers is mediated by the mitochondria, which are sensitive to the abnormally high levels of intra-

Accelerated 

channel. Treatment of \(mdx\) mice with an inhibitor of cyclophilin D, Debio-25, provided mitochondrial protection.\(^{65}\) Thus, interventions that either reduce dysregulation of intracellular \(Ca^{2+}\) or offer mitochondrial protection might be expected to ameliorate dystrophic pathology.\(^{66}\) Both of the above neglect the evidence that suggests sarcolemmal tearing is not necessarily a prerequisite for inappropriate \(Ca^{2+}\) influx.\(^{67}\) For example, one investigation found differences between dystrophic and control muscle \(Ca^{2+}\) channels or intracellular calcium stores.\(^{68}\) These mechanisms appear to foreshadow pathology without overt membrane damage. Further reviews are available.\(^{69-71}\)

In studies of dystrophic \(mdx\) muscle fibers, it has been shown that, without the membrane stabilization and mechanical coupling function normally mediated by the dystrophin protein, extracellular \(Ca^{2+}\) influx occurs with catastrophic consequences.\(^{72}\) This influx may occur either as a result of sarcolemmal tearing\(^{73}\) and/or as dysfunction of stretch-activated cation channels.\(^{74}\) Mislocalization or loss of \(Ca^{2+}\) channels may occur in dystrophic muscle cells,\(^{75}\) reminiscent of the loss of the proteins of the DGC. Furthermore, membrane ruptures lead to increased calcium leak channel activity.\(^{67}\) Thus, inappropriate \(Ca^{2+}\) influx may have a structural basis related only indirectly to mechanical weakening of the sarcolemma. The question arises whether exercise may modulate the expression or leakiness of \(Ca^{2+}\) channels, but few studies\(^{76,77}\) have directly considered this. Of note, two studies\(^{28,78}\) have permitted a clear comparison of calcium influx in response to the same exercise regime in healthy and dystrophic animals. These studies showed that a 4–6-week exercise protocol, both in wild-type and \(mdx\) mice, significantly increases the calcium influx via specific channels. It was also shown that the basal and exercise-induced increases in calcium permeability are significantly higher in \(mdx\) than in wild-type mice. Further study of mechanosensitive ion channels has been recommended.\(^{56,79}\)

Again, exercise, appropriately tailored to the DMD patient, may constitute a useful intervention. For example, it is logical that pliometric\(^{38}\) (lengthening) muscle actions expose susceptible regions of titin, resulting in their increased vulnerability to calpain proteolysis.\(^{68}\) This molecular mechanism of contractile protein degeneration is complemented by organismal observations such as the widely accepted notion that lengthening muscle actions are excessively degenerative, and therefore contraindicated, for DMD patients. Thus, at the organismal level, exercise must be carefully prescribed based on our knowledge of deleterious molecular mechanisms of degeneration. Once a tailored exercise prescription is considered, then questions regarding its efficacy at both the organismal and molecular levels may be posed; for example, does the exercise intervention improve muscle function via increased calpastatin? Finally, exercise may also mediate a fast-to-slow myosin heavy chain (MHC) shift and concomitant alterations in \(Ca^{2+}\) handling\(^{38}\); however, the intensity sufficient to induce a functionally significant MHC shift\(^{38}\) is likely inappropriate for DMD patients.

**ABERRANT CELL SIGNALING**

Aberrant cell signaling is a key mechanism underlying DMD pathophysiology. For the purposes of this review, we discuss aberrant cell signaling involving two cell types: differentiated cells and stem/progenitor cells.

**Differentiated Cells.** The interaction of differentiated dystrophic muscle cells with differentiated cells composing microvessels, nerves, muscle cell
neighbors, and the extracellular matrix are considered in what follows.

**Muscle–Microvessel Interactions.** Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis, and it has been shown that peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1α) orchestrates not only VEGF expression but also oxidative metabolism, as is predicted by its transactivator status. Evidence of concerted cellular cues was provided by angiomyogenic studies using green fluorescent protein (GFP)-positive bone marrow–derived cells transplanted into mice with satellite cells that had been depleted by irradiation. Transplanted cells took up residence in both perivascular and satellite cell niches, and the investigators concluded that the endothelial cells of the microvasculature secreted VEGF and a variety of other growth factors essential for trophic support of the transplanted (and host) cells. The capillary-to-fiber ratio is another example of vessel–muscle interaction, and this ratio can be manipulated with exercise. In addition, well-documented changes in fiber type—conferring, for example, a switch from oxidative to glycolytic metabolism, that is, slow- to fast-twitch fiber transformation—are correlated with vascular glycolytic metabolism, for example, a switch from oxidative to glycolytic metabolism, that is, slow- to fast-twitch fiber transformation—are correlated with vascular glycolytic metabolism, that is, slow- to fast-twitch fiber transformation—are correlated with vascular glycolytic metabolism. Thus, expression profiling and immunohistochemistry provide means for further study of exercise-induced modifications to neuromuscular biological cascades.

**Muscle–Muscle Interactions.** Satellite cells are the myogenic progenitors responsible for postnatal muscle growth, repair, and regeneration. Several experiments have shown that physical association of muscle satellite cells with mature myofibers has an antiproliferative effect on the satellite cells. However, it is unclear whether the ability of satellite cells to proliferate on basal lamina void of myofibers (decellularized extracellular matrix) occurs because antiproliferative cues are not present (e.g., cell–cell inhibition is absent), or whether proliferation is actually a function of factors secreted by the basal lamina. Nonetheless, it is clear that the muscle progenitor cell niche has profound effects on the potential for regeneration.

In addition, it has been suggested that muscle progenitor cells in an aged environment, characterized by oxidant stress, are more likely to differentiate into adipocytes. Some similarities between DMD and aging suggest further exploration of common pathological mechanisms, such as the five considered here, and how exercise interventions might ameliorate the pathology.

**Muscle–Extracellular Matrix Interactions.** The elasticity of the extracellular matrix (ECM) is a variable that directs stem cell fate. Softer substrates promoted adipogenic differentiation of mesenchymal stem cells, whereas increasingly stiffer substrates promoted myogenic and osteogenic lineage specification. Sensing the elasticity of the microenvironment was ascribed to non-muscle myosin; primary cilia may also play a role. The basal lamina is the ECM of muscle cells. Besides the elasticity of the matrix, the composition is of importance. The basal lamina is formed from collagen type IV, laminin, fibronectin, and assorted glycoproteins and proteoglycans. Evidence indicates that these components serve dual structural and regulatory roles. For example, the basal lamina serves as a scaffold for regeneration of muscle fibers, but its components also bind growth factors and growth inhibitors, such as hepatocyte growth factor (HGF) and TGF-β, respectively. The nuclear factor–kappaB (NF-κB) pathway sits at the center of immune-mediated and inflammatory-mediated cell signaling networks. Accordingly, NF-κB regulates the expression of many pro-inflammatory genes, in cells as diverse as lymphocytes, macrophages, and muscle fibers. As a result of NF-κB upregulation, a given lymphocyte or dystrophic muscle cell is stimulated to secrete cytokines and chemokines, the molecules that are the front-line mediators of inflammation. These molecules...
appear to amplify the inflammatory response by attracting additional immune cells to the dystrophic area\textsuperscript{106} and also control the time course of degeneration–regeneration of the affected muscle\textsuperscript{107,108} A further review has been published\textsuperscript{109}.

**Muscle (Mitochondria)–Immune System Interactions.** An additional layer of complexity can be added to the previous discussion of calcium-signaling aberrations in the mitochondria by studies that described mitochondrial damage–associated molecular patterns (DAMPs) as attractants of polymorphonuclear neutrophils (PMNs).\textsuperscript{109} The phenomenon may explain, in part, the excessive inflammation and fibrosis seen in dystrophic, injured muscles. Briefly, if the sarcolemma of a given dystrophic fiber is damaged and the mitochondrial DAMPs contained within are no longer “protected” from surveillance by the immune system, then an inflammatory response follows. Although previous studies have briefly addressed mitochondrial–immune system interactions,\textsuperscript{110–112} considering the inflammation in DMD as a mitochondrial-mediated process deserves further study. Interaction of histones\textsuperscript{113} and adenosine triphosphate (ATP)\textsuperscript{114,115} with neutrophils may also explain the hyperinflammation observed in dystrophic muscle. Further details on inflammation are presented in the Increased Oxidative Stress section.

**Involving Stem Cells.** The previous subsections discussed the weakening of the sarcolemma and the subsequent myofiber degradation that occurs in response to muscle loading/external work. We now focus on events after myofiber degradation, with an emphasis on the regenerative processes.

Following myofiber degradation, myofiber necrosis and initiation of the inflammatory response results in the secretion of cytokines and growth factors, such as insulin-like growth factor-1 (IGF-1), HGF, epidermal growth factor (EGF), and VEGF, to name a few.\textsuperscript{116} These cytokines and growth factors are required for the activation and recruitment of muscle satellite cells. Satellite cells, located between the sarcolemma and the basal lamina, fuse together, or with damaged myofibers, to form regenerated myofibers.\textsuperscript{117} In the absence of satellite cell activation, muscle regeneration cannot occur. A further review has been published.\textsuperscript{118}

Due to the repeated and continuous rounds of myofiber degeneration–regeneration of dystrophic muscle, the number of satellite cells significantly decreases over time.\textsuperscript{119} Moreover, satellite cells from dystrophic muscle demonstrate a markedly diminished doubling time\textsuperscript{120} and a decreased capacity for myogenic differentiation (unpublished results). Unpublished findings from our laboratory have demonstrated that muscle-derived precursor cells isolated from the hindlimb skeletal muscle of mdx mice demonstrate significantly reduced myotube formation, in vitro, when compared with precursor cells isolated from wild-type control muscles, a phenomenon that also characterizes aged skeletal muscle. In addition, accelerated telomere shortening occurs in boys with DMD.\textsuperscript{3,121}

As in skeletal muscle models of aging, in the absence of effective myofiber regeneration, degenerating dystrophic myofibers become replaced by connective or fatty tissues, severely limiting force-producing capacity. In aged muscle models, this shift from effective myogenic regeneration to fibrosis after skeletal muscle injury is partly a result of a lineage conversion of muscle precursor cells.\textsuperscript{97} With time, muscle precursor cells shift in their ability to restore the original structure of the injured muscle to a default quick-fix in which the injured muscle is rapidly patched with a fibrotic framework.\textsuperscript{73} This is due to fibrogenic differentiation of muscle stem cells, perhaps mediated by increased matrix tension and/or cell shape.\textsuperscript{98,122} This myogenic-to-fibrogenic conversion of muscle precursor cells has been recently shown to be under control of the canonical Wingless int (Wnt) signaling pathway.\textsuperscript{97} With increasing age, there is increased Wnt signaling activation, which impairs the myogenic regeneration of muscle stem cells. This raises the question as to whether decreased inhibition of the Wnt signaling pathway may similarly play a role in the increased fibrosis formation that characterizes dystrophic muscle. A delayed inflammatory response may be responsible for the delayed reparative regeneration in aged muscle,\textsuperscript{123} and future studies should aim to address this in dystrophic muscle. Testing whether differential shifts in the macrophage phenotype in healthy vs. DMD samples occurs\textsuperscript{124} and whether exercise plays a regulatory role is another promising avenue for research. The TGF-β pathway has also been implicated.\textsuperscript{92,125} The fibrotic default program and its underlying mechanisms should be the topic of future studies. In-depth reviews of mechanisms of mesenchymal stem cell therapy\textsuperscript{126} and the role for stem cells in DMD therapy have been published.\textsuperscript{127,128}

**Benefits of Muscle Loading (Exercise) on Myofiber Regeneration.** Methods such as gene therapy\textsuperscript{129,130} and direct growth factor injection\textsuperscript{131} have been investigated in the laboratory to enhance the regenerative capacity of dystrophic muscle. However, drawbacks, such as cost, feasibility, and safety issues, may limit their clinical application. A more practical approach to manipulation of the microenvironment with mechanical loading, by exercise or electrical stimulation, for example, provides a...
A non-invasive method that could be widely used to promote the secretion of myogenically favorable growth factors and enhance skeletal muscle regenerative capacity. In both animals and humans, it has been shown that mechanical overloading is beneficial for improving muscle quality in healthy skeletal muscle. This occurs via activation and proliferation of satellite cells, skeletal muscle angiogenesis, and release of growth factors such as IGF-1 and VEGF.132,133 In vitro, just 24 hours of cyclical mechanical stimulation induces a significant increase in muscle precursor cell (MPC) proliferation134 and increased VEGF secretion by MPCs.135 Not only has mechanical loading been linked to stimulation of critical factors that control myofiber regeneration, but it has also been associated with inhibition of myostatin, a negative regulator of skeletal muscle growth.136–138 We have previously demonstrated that addition of a muscle loading protocol enhances myofiber regeneration by transplanted MPCs139 and decreases the formation of fibrosis after a severe contusion injury.140 This suggests that mechanotransductive pathways may play a role in determination of the fate of muscle precursor cells responsible for skeletal muscle regeneration. Accordingly, administration of a running protocol in mice is associated with decreased genetic expression of Wnt signaling pathways.140 Using recent studies of exercise-induced satellite cell proliferation and activity in aging skeletal muscle141 as a model, future studies should investigate further how mechanical stimulation may enhance myofiber repair and decrease fibrosis in dystrophic muscle models.

**INCREASED OXIDATIVE STRESS**

Oxidative stress is the accumulation of destructive reactive oxygen and nitrogen species (RONS) such as superoxide, the hydroxyl radical, and peroxynitrite (i.e., \( \text{O}_2^-/\text{H}_2\text{O}_2 \), \( \cdot \text{OH}^- \), and \( \cdot \text{ONOO}^- \), respectively; Fig. 1A). When oxidative stress is severe and/or prolonged, antioxidants, the body’s natural defense system, can be overwhelmed, which can lead to subsequent oxidative damage of lipids, proteins, and DNA.

Lipids that comprise the sarcolemma, such as polyunsaturated fatty acids, are preferentially attacked by RONS142 in a process commonly referred to as lipid peroxidation. An oxidized polyunsaturated fatty acid (PUFA) is readily oxidized by RONS such as superoxide anion to form a peroxydienyl radical (·). Peroxydienyl radicals can oxidize nearby DNA, PUFA, cysteine residues on transmembrane proteins, and glycogens found on glycoproteins. These processes, left unchecked, can lead to destruction of biomolecules.

FIGURE 1. (A) Production of RONS in muscle cell mitochondria and cytosol. SODmit and SODcyt, mitochondrial and cytosolic superoxide dismutase; Cat, catalase; Gpx, glutathione peroxidase; \( \text{O}_2^- \), superoxide; NO, nitric oxide; \( \text{H}_2\text{O}_2 \), hydrogen peroxide; \( \cdot \text{OH} \), hydroxyl radical; \( \text{H}_2\text{O} \), water; ONOO\(^- \), peroxynitrite; (adapted from ref. 181). (B) A polyunsaturated fatty acid (PUFA) is readily oxidized by RONS such as superoxide anion to form a peroxydienyl radical (·). Peroxydienyl radicals can oxidize nearby DNA, PUFA, cysteine residues on transmembrane proteins, and glycogens found on glycoproteins. These processes, left unchecked, can lead to destruction of biomolecules.
levels may increase the production of RONS to sufficiently to overwhelm endogenous antioxidant enzyme defenses. This overt oxidative stress leads to protein degradation and altered contractile protein function.

The contribution of oxidative stress to the pathophysiology and onset of DMD is largely unexplored. The parallel presence of oxidative stress byproducts and elevated antioxidant levels has been observed in many models of DMD, thus supporting the axiom that oxidative stress contributes to DMD pathology (Table 2). Oxidative stress was investigated in the pre-necrotic state, that is, the time preceding the first wave of fiber degeneration/regeneration, in mdx mice (age 10–21 days). Despite no signs of muscle pathology, investigators found increases in lipid peroxidation along with increases in the endogenous antioxidant enzymes. Similar findings (i.e., increased lipid peroxidation) have been recapitulated in studies of patients with DMD, suggesting that oxidative stress and damage contribute to the dystrophic process (Table 2).

Therapies for DMD ideally would prevent muscle wasting and oxidative stress and improve overall muscle function. Exercise may appear to be contraindicated for treatment of oxidative stress because it increases oxygen consumption and can be followed by a short inflammatory response, both of which can lead to increased RONS production. However, a key benefit of exercise arises from the overshoot in RNA transcription and subsequent mRNA translation of antioxidant enzymes that can protect against oxidative damage even after the exercise has ceased.

The protective effects of exercise against oxidative stress have been best documented in healthy muscle. Exhaustive and even moderate exercise activity in an untrained individual can increase mitochondrial lipid peroxidation and protein carbonylation, but these negative effects are blunted subsequent to appropriate exercise training. Indeed, protection from oxidative stress–induced damage after exercise has been observed after both short- and long-term endurance exercise training regimens, as indicated by decreased lipid peroxidation and increased antioxidant activities that can remain elevated up to 72 hours post-exercise. The sustained activity of antioxidants beyond the initial oxidative stress induced by the exercise activity may represent the greatest potential for the longer lasting benefits of exercise training. Hence, one of the benefits of regular exercise is to counter oxidative stresses in the rest periods between exercise activities.

Unfortunately, evidence for similar responses to exercise in DMD patients is limited. No studies

### Table 2. Muscular dystrophy and oxidative stress.

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<th>Model</th>
<th>Marker of oxidative stress</th>
<th>Consensus references</th>
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<td>Dystrophic chickens</td>
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<td>SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GR, glutathione reductase.</td>
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<td><em>Chemiluminescence of urinary markers of oxidative damage.</em></td>
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*References: first author (year).

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**Table 2.** Muscular dystrophy and oxidative stress.
have directly assessed the oxidative stress and antioxidant response to exercise in DMD patients, although there have been a few studies using mdx mice. Voluntary wheel running has been shown to elicit beneficial adaptations in dystrophic muscle, such as increased resistance to fatigue, a shift toward smaller type IIa fibers, and increased muscle strength, but its is still unclear whether exercise training can elicit adaptations to improve oxidative stress and antioxidant capacity. We reported a 22% increase in total antioxidant capacity in male mdx mice after 3 weeks of voluntary wheel running. Similarly, a 38% decrease in lipid peroxidation and a 44% decrease in protein carbonylation in white gastrocnemius muscle from mdx mice were reported after an 8-week, low-intensity treadmill training regimen. Contradicting these findings, a 6-week treadmill training regimen increased lipid peroxidation in mdx mice, and a 4–8-week treadmill training regimen yielded no significant changes in antioxidant activity. Indeed, dihydroethidium (DHE) staining of sections of tibialis anterior muscle, used as a marker of exercise-induced oxidative stress, increased. These differences could reflect voluntary running on a wheel vs. enforced running on a treadmill, suggesting this possibility be tested. Studies that investigated exercise-induced attenuation of oxidative stress in mdx mice are highlighted in Table 3.

Potential exercise-based therapies for DMD patients must be rigorously tested and have defined parameters to assure maximal benefits and avoid exacerbation of the disease. Beneficial outcomes of exercise training on oxidative stress in healthy individuals are easily achievable because exercise prescription and training responses are well defined. Establishing an exercise prescription to limit oxidative stress for patients with DMD will require well-designed studies that address the following criteria:

1. Establish the degree to which dystrophic muscle adapts to exercise.
2. Demonstrate that antioxidant signaling pathways are not perturbed in dystrophic muscle.
3. Confirm antioxidant activity levels remain elevated after physical activity in patients with DMD.

### Recurrent Muscle Ischemia

Throughout this review we have discussed the evidence that the five pathophysiological mechanisms outlined by Petrof are not mutually exclusive. Studies in 1998 and 2000 were among the first to provide mechanistic evidence for recurrent muscle ischemia in dystrophic muscle. These studies...
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Ca\(^{2+}\), calcium; CK, creatine kinase; DAMP, damage-associated molecular pattern; degen-regen, degeneration–regeneration; IHC, immunohistochemistry; iNOS, inducible nitric oxide synthase; Mt, mitochondrial; SOD, superoxide dismutase.
showed that myofibers without pathology are supported by increased blood flow during exercise despite exercise-dependent adrenergic signals for vasocostriction. They do this by increased neural nitric oxide synthase (nNOS) expression and subsequent NO release. However, DMD muscle does not display increased nNOS expression. Thus, the reflex sympathetic vasoconstriction that accompanies contraction of dystrophic muscles is unopposed by NO-mediated vasodilation, and functional muscle ischemia follows. Of note, these studies in DMD patients were performed using near-infrared (NIR) spectroscopy of the flexor digitorum profundus, a muscle used preferentially in handgrip exercise and in pushing a wheelchair. Since these studies, it has become increasingly clear that deficient oxygenation of DMD tissue is only one of many pathophysiological consequences of impaired vascular control. Due to either absence or mislocalization of skeletal muscle nNOS, fatigue experienced by DMD patients after mild exercise is explained by the failure of normal contraction-induced cGMP-dependent attenuation of local vasoconstriction, and this failure causes vascular narrowing in muscles after exercise. Improved anchoring of nNOS to the sarcolemma and subsequent improved muscle perfusion during exercise together provide further evidence of a role for nNOS in the prevention of recurrent muscle ischemia. Importantly, utrophin appears to be unable to recruit nNOS to the sarcolemma.

NO pathway involvement also affects gastric smooth muscle and thus may partially explain gastrointestinal (GI) issues in DMD patients, who have decreased serum concentrations of NO. Mechanical loading regulates NOS expression, but further study in dystrophic muscle is needed. These proposed focused studies may find targets on the NO pathway, such as phosphodiesterase inhibitors, to ameliorate both GI and skeletal muscle pathology, and may contribute to an interdisciplinary management plan. Clearly, polytherapy is indicated because, in the absence of an appropriate contraction-induced vasodilation, excessive exercise may exacerbate the ischemia.

Capillary–myofiber contacts are increased in exercised muscles in healthy individuals, particularly the young. Because an improved phenotype of mdx mice is observed by developmentally increasing the vasculature, future studies of physical activity in DMD patients might help to determine whether the response of exercised muscles in dystrophic individuals is similar. Of note, the microvasculature is dynamic, and changes might be expected in DMD boys as they age, and as recruitment of fibers from various muscle groups changes. The interaction of these changes with the muscle progenitor cell niche might be studied in arm muscles of DMD boys before and after use of a wheelchair.

Observations of skeletal muscle ischemia in DMD are difficult to reconcile with observations of enhanced arteriogenesis and wound repair in mdx mice. This difficulty may be due to general species differences, or there may be differences in the specific dystrophic pathology (such as sarcolemmal nNOS anchoring) between the species. Or, myofibroblast precursor/progenitor cells may receive signals inductive of differentiation into blood vessels and epithelial tissues. Differentiation into these types of tissues may occur preferentially, because these tissues require little if any dystrophin expression.

CONCLUSIONS

A sense of urgency permeates research into the pathophysiological mechanisms underlying DMD. Improved understanding of the pathophysiology is critical, and the incorporation of exercise into experimental designs could help to mechanistically define the pathophysiology. At present, informed exercise prescription for DMD patients is challenging due to lack of inquiry and lack of evidence. The cumulative effect of further inquiry—informed exercise prescription—might improve the quality of life of DMD patients by improving their mobility. Table 4 summarizes methods and outcome measures that might be used in studying the role of exercise with respect to its effects on the five underlying mechanisms of DMD. In the vast majority of preclinical studies in mdx mice, exercise has been used as a means to increase the severity of the damage, to better understand the pathological cascade, or to better evaluate the effects of various therapeutics. For this reason and others discussed in this review, we consider the ability of exercise to improve quality of life for DMD patients to be an issue that requires further study. However, recently published standard operating procedures and clinical trial protocols are clear steps in the right direction. Defining criteria for well-designed, controlled studies of exercise training in DMD may lead not only to informed exercise prescription, but also to enhanced understanding of the pathophysiology of the disease.

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