

# Pathophysiology of Duchenne Muscular Dystrophy: Current Hypotheses

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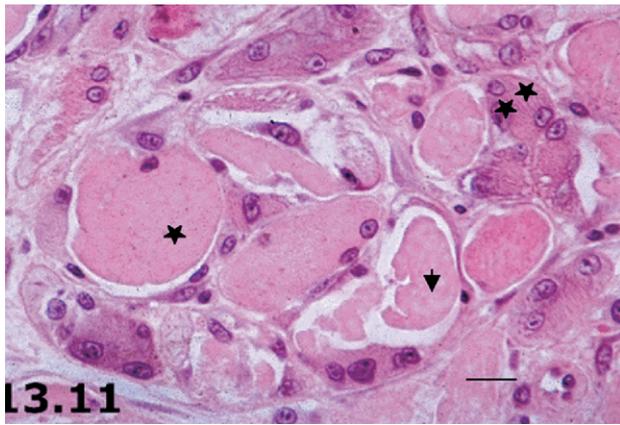
Duchenne muscular dystrophy is a devastating inherited neuromuscular disorder that affects one in 3300 live male births. Although the responsible gene and its product, dystrophin, have been characterized for more than 15 years, and a mouse model (*mdx*) has been developed, comprehensive understanding of the mechanism leading from the absence of dystrophin to the muscular degeneration is still debated. First, dystrophin is considered a key structural element in the muscle fiber, and the primary function of the dystrophin-associated protein complex is to stabilize plasma membrane, although a role of signaling is still possible. Mechanically induced damage through eccentric contractions puts a high stress on fragile membranes and provokes micro-lesions that could eventually lead to loss of calcium homeostasis, and cell death. Altered regeneration, inflammation, impaired vascular adaptation, and fibrosis are probably downstream events that take part in the muscular dystrophy and that probably vary a lot along species (i.e., *mdx* mice), probands within families, stressing the importance of epigenic factors. Because no etiologic therapy is available for Duchenne muscular dystrophy, a better understanding of the primary and downstream mechanisms could prove useful for producing new adjuvant treatments. All pathophysiologic mechanisms are reviewed together with perspectives on management. © 2007 by Elsevier Inc. All rights reserved.

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## Introduction

Duchenne muscular dystrophy (OMIM#310200) is a devastating inherited neuromuscular disorder with an incidence of 1 in 3300 live male births. Although the responsible gene and its product, dystrophin, have been characterized for more than 15 years, and a mouse model (*mdx*) has been developed and extensively studied, comprehensive understanding of the mechanism leading from the absence of dystrophin to the muscular degeneration is still lacking. In patients with Duchenne muscular dystrophy, muscle biopsy characteristically demonstrates necrotic or degenerating muscle fibers, often observed in clusters. These necrotic fibers are surrounded by macrophages and CD4+ lymphocytes. Small immature centrally nucleated fibers are also observed, reflecting muscle regeneration from myoblasts [1,2] that results in a balance between necrotic and regenerative processes in the early phase of the disease. Later, the regenerative capacity of the muscles appears to be exhausted and muscle fibers are gradually replaced by connective and adipose tissue (Fig 1). Therefore the manifestations of Duchenne muscular dystrophy are considered to result from imbalance between muscle fiber necrosis and myoblast regeneration, the primary pathologic feature being necrosis, although animal evidence suggests that regenerative capacity per se may decrease with age [3].

Although the dystrophin gene is homologous in human and mouse and the functions of both encoded proteins are supposed to be similar, *mdx* mice have a milder phenotype than Duchenne muscular dystrophy; this has been ascribed to better regenerative capacities in dystrophic mice. The localization of dystrophin in the vicinity of a large number of proteins leads to the formation of the dystrophin-associated protein complex [4]. The best-studied roles of the dystrophin-associated protein complex involve struc-



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 Figure 1. Duchenne muscular dystrophy patient muscle biopsy characteristically revealing necrotic or degenerating muscle fibers (arrow), often observed in clusters, surrounded by macrophages and CD4<sup>+</sup> lymphocytes (star). Small immature centrally nucleated fibers are also present (double star), reflecting muscle regeneration from myoblasts (bar = 200  $\mu$ m).

tural stabilization of the sarcolemma. Mutations of other dystrophin-associated protein complex components also cause muscular dystrophy by disassembling the complex and compromising the linkage between the extracellular matrix of the fibers to the cytoskeleton. However, marked differences between these muscular dystrophies may be related to specific consequences of dystrophin-associated protein complex disruption. This review will revisit the main current pathophysiologic hypotheses suggested in Duchenne muscular dystrophy, namely the mechanical hypothesis and the impaired calcium homeostasis. In addition, hypotheses that involve inflammation, apoptosis, regeneration, or other mechanisms are discussed. Currently there is no effective therapy for Duchenne muscular dystrophy. Although gene therapy seems to be a promising strategy, there has been no evidence for diffuse, persistent, and nontoxic production of dystrophin in human muscle to date. There is an urgent need to fully understand the pathophysiology underlying the deficits resulting from the absence of dystrophin in order to develop new avenues for the treatment of Duchenne muscular dystrophy.

## Muscle Fiber Necrosis

### *Mechanical Hypothesis*

Early description of muscle dystrophy (as “delta lesions”) and raised levels of muscle enzymes in Duchenne muscular dystrophy patients have long been interpreted as reflecting excessive fragility of the muscle fibers [5,6]. The discovery of dystrophin and other members of the dystrophin-associated protein complex scaffolding supported the view that the absence of one of these proteins could compromise the muscle membrane integrity of the fibers, particularly after sustained contractions, as ability to sustain eccentric contraction (i.e., contraction with forced lengthening) appears to be dramatically reduced in Duchenne muscular dystrophy [7-9]. This research has led

to the emergence of the notion of exercise-induced damage, which has important implications for management [10,11].

The absence of dystrophin results in striking alteration in membrane structure related to delocalization of the dystrophin-associated proteins from the membrane. Dystrophin-associated protein complex and additional proteins (e.g., vinculin, desmin, and spectrin) normally form rib-like lattices on the cytoplasmic face of the sarcolemma known as costameres [12]; these anchor the cytoskeleton to the extracellular matrix [13,14]. Costameres act as mechanical couplers to distribute contractile forces generated in the sarcomere laterally through the sarcolemma to the basal lamina and thereby maintain uniform sarcomere length along the fiber [15]. Absence of expressed dystrophin leads to complete loss of the dystrophin-associated protein complex and disruption of costameric lattice, thought to underlie membrane fragility [14,16].

Membrane fragility in Duchenne muscular dystrophy patients and *mdx* mice has been demonstrated by various methods. Cytoplasmic accumulation of proteins that are normally not present in muscle fibers, such as albumin and immunoglobulins, suggests increased membrane permeability. Such permeability was confirmed by incorporation of a dye *in vivo*, *ex vivo*, and *in vitro*, after immersion of the muscle in a physiologic bath containing the dye (orange Procion and Evans Blue) [10,17-20]. In contrast to *mdx* mice, disruption in the dystrophin-associated protein complex at the extracellular level, as observed in *dy/dy* mice, is not associated with high levels of dye incorporation, although these animals are grossly more affected than *mdx* mice. Sustained exercise is known to increase permeability to such dyes, both in normal and *mdx* mice, though incorporation remains much higher in the latter (Fig 2), with evidence of membrane resealing in normal mice [11,21-23]. Hence, the idea that exercise could provoke greater damage in dystrophin-deficient muscles than in controls.

*Mdx* mouse muscles immobilized by toxin injection or hind limb immobilization were found to develop markedly reduced signs of dystrophy [24,25]. These studies were conducted in 3-week-old mice, i.e. during the first phase of degeneration, suggesting that this phase could be related to the increased muscle activity after weaning [25,26]. These findings are not specific to absence of dystrophin, as *dy/dy* mice manifest the same “protective” effect of immobilization though not if initiated in adult mice. Furthermore, they may be related to the smaller diameters of immobilized fibers, as *mdx* mouse small-caliber muscle fibers tend to exhibit less necrosis [26-28]. Relative sparing of small caliber fibers has also been documented in Duchenne muscular dystrophy, for example, in extraocular, esophageal striated, or distal foot muscles [29]. In contrast, proximal muscle groups, which contain larger fibers and bear more weight, are affected first.

This notion of mechanical processes as a link between absence of dystrophin and muscle fiber death has influ-

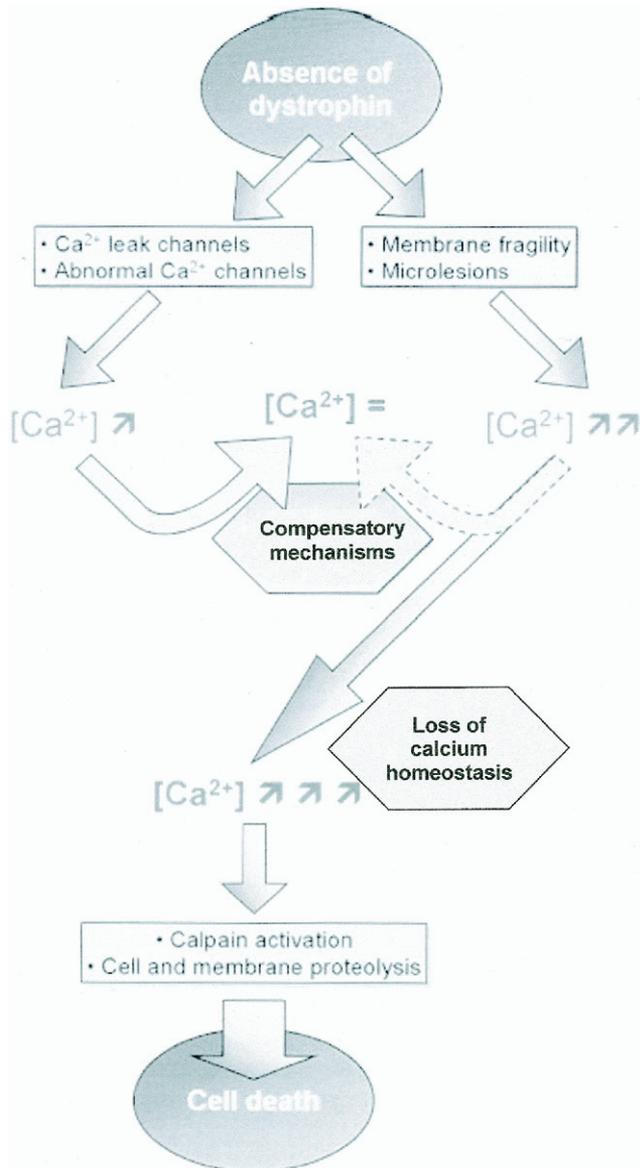


Figure 2. Proposed pathophysiology of dystrophinopathies. Compensating mechanisms are efficient in dystrophin-deficient fibers and, unless there is irreversible membrane damage, calcium homeostasis is preserved. When  $\text{Ca}^{2+}$  influx is too high to be counterbalanced, activation of calcium-dependent proteases enlarges the membranous defect, leading to irreversible cell death.

enced management as on one hand, physical therapy appears mandatory to improve or stabilize muscle function, whereas on the other hand, excessive or otherwise inappropriate activity may be harmful [30].

### Calcium Hypothesis

Calcium homeostasis is critical to many aspects of muscle function [31]. Documentation of calcium accumulation and of hypercontracted fibers in muscle biopsies of Duchenne muscular dystrophy patients has led to the investigation of the possible role of calcium in the pathophysiology of Duchenne muscular dystrophy [32-34]. Increased influx through a dystrophin-deficient membrane

has been demonstrated [35-38]. This influx seems to occur mostly through mechanosensitive voltage-independent calcium channel [39]. However, despite increased influx, low to normal calcium concentration can be maintained within the fiber cytosol, reflecting the robustness of the calcium homeostatic mechanisms in *mdx* mice [38,40,41]. Abnormal increase in submembranous concentration may occur, but needs to be confirmed at physiologic values of membrane potential. Nevertheless, if mechanical stress induces microlesions in the fiber membrane, high influx of extracellular calcium inevitably occurs, overriding the capacity to maintain physiologic cytosolic  $\text{Ca}^{2+}$  concentration [11,21]. Sustained increase in cytosolic calcium concentration leads to activation of proteases, particularly calpains, resulting in the destruction of membrane constituents which, in turn, will increase calcium entry. Excessive calcium may then lead to cell death (Fig 2) [42,43].

In line with the calcium hypothesis, several treatment trials with different calcium-blockers (e.g., diltiazem) have been tested in Duchenne muscular dystrophy, but have demonstrated almost no clinical benefit [44,45]. In accordance with the calcium-dependent activation of proteases, overexpression of calpastatin (endogenous inhibitor of calpains) has been demonstrated to reduce necrosis in *mdx* muscle [46,47].

### Vascular Hypothesis

Because necrotic fibers are often observed in clusters in affected Duchenne muscular dystrophy, early pathophysiologic hypotheses postulated a role of the muscle vasculature. However, structural studies have revealed no blood vessel abnormalities [48-50]. More recent insights into the local vasodilator role of nitric oxide (NO) in skeletal muscle may, however, be relevant to Duchenne muscular dystrophy pathophysiology [51]. NO is produced in muscle cells by the neuronal isoform of NO synthase (n-NOS) that is normally bound to dystrobrevin and syntrophin. In dystrophin-deficient muscles, n-NOS is delocalized from its subsarcolemmal anchorage, floating freely in cytoplasm, and its content is reduced [52,53]. During exercise, when need in oxygen is increased, muscle ischemia may occur in Duchenne muscular dystrophy [54,55]. However, *n-NOS* knockout mice do not develop muscle disease and *n-NOS/mdx* double mutant mice have a phenotype different from simple *mdx* knockout mice [56,57]. This suggests that n-NOS does not play a direct role in Duchenne muscular dystrophy. Nevertheless, it could contribute to the extent of damage, as suggested by intracellular pH dysregulation found *in vivo* in repetitively stimulated *mdx* mouse muscles, and its lack of expression could contribute to the amount of inflammation present in muscle [58,59].

### Gene Regulation Hypothesis

In addition to its role in membrane stabilization, dystrophin-associated protein complex is involved in other

processes, such as mechanotransduction, i.e. muscle activity-related gene expression. Disruption of dystrophin-associated protein complex related to absence of dystrophin results in selective regulation of various genes [60]. Interestingly, injection of stem cells into dystrophin-deficient muscle not only partially restores dystrophin and dystrophin-associated protein complex, but also restores physiologic gene expression [61]. However, it is not clear if the affected genes identified to date have a role in the dystrophic changes.

Several genes involved in intracellular signaling molecules, such as calcineurin, p38 mitogen-activated protein kinase, c-Jun N-terminal kinases, and other protein kinases, have been demonstrated to be upregulated by mechanical stress specifically in the hearts of dystrophin-deficient mice [62]. Moreover, recent studies have demonstrated similar patterns of muscle gene expression in Duchenne muscular dystrophy and in healthy subjects undergoing endurance exercise training [63]. This suggests that the Duchenne muscular dystrophy gene upregulation profile might reflect a compensatory mechanism mainly involving the integrin signaling pathway. Given the lack of further documentation, potential implications for management are not clear.

### ***Glycosylation Hypothesis***

Although posttranslational processes, such as glycosylation, are important for correct assembly and function of muscle proteins, these processes had not been implicated in the pathogenesis of congenital muscular dystrophy until recently. Glycosylation of components of the dystrophin-associated protein complex, such as alpha-dystroglycan, control interaction with extracellular matrix components. Aberrant glycosylation of alpha-dystroglycan is a common feature in four different forms of inherited muscular dystrophy that are caused by mutations in genes encoding glycosyltransferases. It results in uncoupling of the muscle fiber from the extracellular matrix and the loss of these interactions probably causes progressive muscle degeneration and often neuronal migration disorders, as it is observed in muscle-eye-brain disease and Fukuyama congenital muscular dystrophy [64]. However, to date, no evidence of a direct role of aberrant glycosylation has been demonstrated in dystrophinopathies.

### **Tissue Remodeling**

Several observations emphasize that secondary features of dystrophin deficiency may be of great importance in determining the severity of the disease. For example, null mutations of dystrophin produce early-onset, progressive disease in humans and dogs but cause a late-onset progressive pathology in mice with hypertrophic muscles, emphasizing the potential importance of epigenetic factors in Duchenne muscular dystrophy, and their potential role as a therapeutic target [65]. Conserving muscle mass in

Duchenne muscular dystrophy patients would slow functional decline. Treatment of Duchenne muscular dystrophy with anabolic steroids has provided an obvious, and explored approach for protecting muscle mass. Norethandrolone and methandrostenolone were found to cause initial modest improvements but were accompanied by androgenic side effects. More recent studies have indicated greater promise for oxandrolone, producing improvements in quantitative muscle testing but not in functional testing (Table 1) [66]. An alternative approach for shifting dystrophic muscle toward a positive protein balance resides in the use of growth factors. Insulin growth factor 1 delivery into *mdx* muscle increased muscle mass, increased specific force, and induced muscle hyperplasia (Table 1) [67,68].

### ***Inflammatory Hypothesis***

Muscles of patients with Duchenne muscular dystrophy consistently exhibit inflammatory changes, though to a lesser extent than in other muscular dystrophies, such as facio-scapulo-humeral muscular dystrophy. Data from genome profiling studies provide evidence for coordinated activity of numerous components of a chronic inflammatory response, including cytokine and chemokine signaling, leukocyte adhesion and diapedesis, invasive cell type-specific markers, and complement system activation [69]. In vivo depletions of CD4+ or CD8+ T cells or macrophages significantly reduced the pathology in *mdx* mice, illustrating the role of those cell types in aggravating the disease [70]. In particular, selective chemokine upregulation may be a key determinant in the inflammatory response in dystrophic muscle. Conjugate immune response signals and local overexpression of extracellular matrix genes were evident in Duchenne muscular dystrophy muscle [70]. Because the muscles of *mdx* mice exhibit little fibrosis, in contrast with Duchenne muscular dystrophy, these findings suggest that collagen regulation at posttranscriptional stages mediates extensive fibrosis in Duchenne muscular dystrophy. However, the relationship between the immune response and extracellular matrix gene upregulation is yet to be clarified. Moreover, these studies have provided no direct insights into the mechanisms implicated in cell death.

Corticosteroids, which have potent anti-inflammatory effects, are the most commonly used drugs in Duchenne muscular dystrophy. Prednisolone treated Duchenne muscular dystrophy patients experience significant delay in the disease progression, prolongation of ambulation, and prevention of the development of scoliosis [71,72]. Deflazacort, an oxazoline derivative of prednisolone, produces similar effects but with less weight gain [73].

### **Conclusions**

The pathophysiologic changes following the loss of dystrophin are still speculative. Several hypotheses coex-

**Table 1. Therapeutic approaches/strategies for Duchenne muscular dystrophy treatment**

Therapeutic Strategy	Treatment Target		Outcome	Safety in Human	References
<i>Decreasing muscle fiber necrosis</i>					
Decreasing muscle membrane damage	Restore functional dystrophin	Full length, mini-dystrophin or utrophin gene delivery	In <i>mdx</i> mice: restores DAPC proteins, prevents fiber necrosis, maintains muscle force	Stable protein expression, Immune reaction	[10,11]
	Avoid eccentric contractions during exercise	Adapted physiotherapy	Lack of consistent data		
Stabilize intracellular Ca <sup>2+</sup> concentration	Inhibition of membrane calcium entry	Calcium blockers	No clinical benefit in DMD		[44,45]
Decrease proteolysis	Inhibition of calcium-dependent proteases	Calpastatin (endogenous inhibitor of calpains)	Necrosis reduction in <i>mdx</i> muscle	NA	[47]
<i>Increasing muscle mass</i>					
	Use of anabolic steroids	Oxandrolone	Mildly improves strength in DMD	Some androgenic side effects	[74]
	Use of growth factors	IGF1	Muscle mass increase, stimulates regeneration in <i>mdx</i> mice	Potential carcinogenic effect	[68]
		Anti-myostatin antibodies	Increase of muscle mass and strength in <i>mdx</i> mice	NA	[75]
<i>Decreasing the inflammatory response</i>					
Immunosuppressants	Use of glucosteroids	Prednisolone	Delay in disease progression (muscle strength, ambulation, scoliosis development)	Bone loss, weight gain	[71, 72]
		Deflazacort	Similar as prednisolone	Less weight gain than prednisolone	[73]
Cytokine modulation	TNF- $\alpha$		Conflicting results in <i>mdx</i> mice	NA	[76]
	Normalizing nitric oxide production	Nitric oxide synthase transgene	Decrease of muscle ischemia and inflammation in <i>mdx</i> mice	NA	[55]

## Abbreviations:

DAPC = Dystrophin-associated protein complex

DMD = Duchenne muscular dystrophy

IGF1 = Insulin growth factor 1

NA = Not applicable

TNF  $\alpha$  = Tumor necrosis factor  $\alpha$

ist: Dystrophin is considered a key structural element in the muscle fiber, and the primary function of the dystrophin-associated protein complex is to stabilize plasma membrane, although a role of signaling is still possible. Mechanically induced damage seems particularly harmful to dystrophin-deficient fibers. Eccentric contractions put a high stress on fragilized membranes and provoke microlesions that could eventually lead to massive calcium entry, loss of calcium homeostasis, activation of Ca<sup>2+</sup>-dependent proteases, and finally to cell death. As we have reviewed, altered regeneration, inflammation, apoptosis, impaired vascular adaptation, and fibrosis are probably secondary events that take part in the muscle dystrophic degeneration. It is unpredictable when gene therapy strategies will be clinically available; a successful strategy remains to be discovered. Improved therapeutics to reduce secondary features of the disease will be of great importance too. A better understanding of these mechanisms could prove useful for producing new adjuvant treatments.

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