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## Literature Review

# Adaptations to Exercise Training and Contraction-Induced Muscle Injury in Animal Models of Muscular Dystrophy

## ABSTRACT

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This article reviews the current status of exercise training and contraction-induced muscle-injury investigations in animal models of muscular dystrophy. Most exercise-training studies have compared the adaptations of normal and dystrophic muscles with exercise. Adaptation of diseased muscle to exercise occurs at many levels, starting with the extracellular matrix, but also involves cytoskeletal architecture, muscle contractility, repair mechanisms, and gene regulation. The majority of exercise-injury investigations have attempted to determine the susceptibility of dystrophin-deficient muscles to contraction-induced injury. There is some evidence in animal models that diseased muscle can adapt and respond to mechanical stress. However, exercise-injury studies show that dystrophic muscles have an increased susceptibility to high mechanical forces. Most of the studies involving exercise training have shown that muscle adaptations in dystrophic animals were qualitatively similar to the adaptations observed in control muscle. Deteriorous effects of the dystrophy usually occur only in older animals with advanced muscle fiber degeneration or after high-resistive eccentric training. The main limitations in applying these conclusions to humans are the differences in phenotypic expression between humans and genetically homologous animal models and in the significant biomechanical differences between humans and these animal models.

**Key Words:** Neuromuscular Disease, *mdx* Mouse, Rehabilitation

It is well established in both humans and animals that exercise causes muscle injury and that eccentric exercise has a more damaging effect than concentric exercise.<sup>1-7</sup> In humans with neuromuscular disease, there has been considerable controversy regarding the use of exercise, particularly maximal strengthening programs. Therefore, animal studies have been used to investigate the adaptive mechanisms that diseased muscles use in accommodating to exercise training.

One of the problems in studying the effect of exercise in neuromuscular diseases is that muscular dystrophies are a heterogeneous group of disorders that have traditionally been classified by clinical phenotype, including mode of inheritance, age of onset, and overall progression of the disease. However, in the last 10 yr, an increasing number of defects in specific genes have been identified as the underlying cause of different forms of muscular dystrophy. Most of the genes encode for components of the dystrophin-glycoprotein complex, an assembly of transmembrane and membrane-associated proteins that form a structural linkage between the F-actin cytoskeleton and the extracellular matrix in muscle. The proteins that comprise the dystrophin-glycoprotein complex are organized into three subcomponents: the cytoskeletal proteins, the sarcoglycans, and the sarcospan.<sup>8,9</sup> Many of the differ-

ent types of muscular dystrophies arise from primary mutations in genes encoding components of this complex. Mutations in the dystrophin gene that result in a complete loss of dystrophin lead to the Duchenne muscular dystrophy (DMD) phenotype. Mutations that cause reduced amounts of dystrophin or a truncated, dysfunctional form of dystrophin result in the Becker muscular dystrophy phenotype. There are at least four sarcoglycan subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  subunits) in muscle, and mutations can result in four types of autosomal recessive muscular dystrophy.  $\alpha$ -Laminin-2 (LAMA2) is a basement membrane protein and binds to  $\beta$ -dystroglycan. Mutations in the  $\alpha$ -laminin-2 gene result in one type of congenital muscular dystrophy.<sup>8,10</sup> It is thought that mutations in components of the dystrophin-glycoprotein complex lead to a loss of sarcolemmal integrity and render muscle fibers more susceptible to exercise-induced damage.

Long before a genetic understanding of the basis of muscular dystrophies was known, researchers used animal models of muscular dystrophy to examine the effect of exercise on dystrophic muscle. Animal models that have been used in investigations of increased muscle activity include the dystrophic chicken, cardiomyopathic hamster, *dy/dy* mouse, *mdx* mouse, and the *CXMD* dog. These and other animal models have been the subject of several recent reviews.<sup>11-13</sup> Type of in-

heritance, gene location, affected gene product, and the human correlates are reviewed in Table 1. Species with the same genetic abnormality found in humans often have a different phenotypic expression, and even a different gene-chromosome location. That is, the same genetic defect can produce diverse pathologic findings when expressed in different species. It is important to understand gene product function, contractile characteristics, fiber type, and pathologic characteristics of each species at various ages, since these are the criteria used to evaluate responses to an intervention.

The gene location or locations and affected product or products of the autosomal dominant dystrophic chicken are unknown. White, fast-twitch muscle is the most severely affected. Muscle degeneration does not become severe until after 1 yr of age, and all chickens have a normal life span. The muscles also seem to be myotonic.<sup>14,15</sup> The dystrophic chicken is now rarely used as an animal model because there is no known human correlate.

In rodents and in other animals with dystrophy, such as the dog, fast-twitch muscles and type 2B fibers are the earliest and most severely affected.<sup>11</sup> However, there is a marked difference between species in pathology, gene location, and gene product. The major organ system involved in the hamster is the heart,<sup>11</sup> whereas the diaphragm is more severely affected than skeletal muscle in the *mdx* mouse.<sup>16,17</sup> The peripheral nerves are also involved

**TABLE 1**

*Animal models used in exercise investigations*

Animal Model	Inheritance	Human Gene Location	Affected Gene Product	Human Correlate
<i>mdx</i> mouse and <i>CXMD</i> dog	XR	Xp21.2	Dystrophin and associated glycoproteins	DMD/BMD
<i>dy/dy</i> mouse	AR	6q2	Laminin- $\alpha$ 2 (merosin)	Congenital muscular dystrophy
Hamster	AR	5q33-q34	$\delta$ -sarcoglycan	SCARMD (limb girdle MD2F)
Chicken	AD	Unknown	Unknown	None

XR, x-linked recessive; AR, autosomal recessive; AD, autosomal dominant; DMD/BMD, Duchenne and Becker muscular dystrophy; SCARMD (LGMD2F), severe childhood autosomal recessive muscular dystrophy.

in the *dy/dy* mouse, which is the most severely impaired and has the most rapid disease progression of any of the rodent models of muscular dystrophy, although this may be due in part to the neuropathy.<sup>18</sup>

The X-linked recessive *mdx* mouse lacks dystrophin, similar to boys with DMD. A lack of dystrophin affects the entire dystrophin-glycoprotein complex. The life span of the *mdx* mouse is normal, and it has minimal clinical muscular weakness. After the initial bout of extensive degeneration at 2–4 wk of age, the muscles almost completely regenerate and exhibit subsequent hypertrophy.<sup>19</sup> However, after this, their muscles undergo a continuous low level of degeneration and regeneration. Although their functional performance, as determined by contractile studies, progressively declines with age, the changes are relatively mild, even at 2 yr of age.<sup>20</sup> Whereas skeletal muscle has mild progression, the diaphragm exhibits a more severe degeneration and fibrotic infiltration similar to that seen in DMD.<sup>16</sup> There is no satisfactory explanation as to why the *mdx* mouse diaphragm shows so much evidence of dystrophy, whereas mouse limb muscles do not. Gillis<sup>21</sup> proposed that forced lengthening, induced by the elastic recoil of the thorax as the diaphragm contracts during the first half of expiration, induces an eccentric contraction injury. It is likely that other mechanisms also play a role. During exercise, the mouse can spare the hind limb muscles by avoiding activity, yet the diaphragm must continually contract to do the work of breathing. Compared with larger mammals, mice have more muscle power per body weight. During normal activity, mouse limb muscles are not significantly strained.

The *CXMD* dog, which also lacks dystrophin, shows overt clinical signs of the dystrophy at 6–9 wk of age. These signs include weakness, stiff gait, bunny-hopping, and increased

serum creatine kinase levels.<sup>22</sup> Morphologically, necrosis and regeneration are observed with marked endomysial fibrosis and fiber size variation.<sup>23</sup> The *CXMD* dog has a clinical progression closer to that of DMD than does the *mdx* mouse. The dog, being a larger mammal, has a relative biomechanical disadvantage compared with the mouse. Thus, for larger mammals with a dystrophin-deficient condition, locomotion and other less intense forms of activity may place a greater burden on skeletal muscle and induce injury and subsequent necrosis.

The autosomal recessive *dy/dy* mutant mouse (129 ReJ) and the 129B6F<sub>1</sub>/J hybrid have a deficiency in the laminin- $\alpha$  2 chain (merosin) and are considered models for one type of congenital muscular dystrophy.<sup>8</sup> There are several other murine models for laminin- $\alpha$  2 deficient congenital muscular dystrophy. Skeletal muscle fiber degeneration occurs early and progresses very rapidly in the 129 Re J mutant and only slightly less so in the hybrid. The life spans vary but are much shorter than normal. Hindlimb paralysis, progressive ataxia and twitching, and scoliosis are apparent as early as 2 wk of age. There is normal development during the first week postnatal, followed by a severe progression of the disease during the second week of life. During the third week, there is rapid degeneration followed by a relatively stable period between the fourth and eighth weeks of age. Thereafter, there is general fiber atrophy and dedifferentiation of the histochemical profile of the fiber. The phenotype of DMD is more like that of the *dy/dy* mouse than of the *mdx* mouse.<sup>11–13</sup>

The autosomal recessive BIO cardiomyopathic hamster has a mutation in the  $\delta$ -sarcoglycan gene and is considered to be a model for one type of autosomal recessive limb-girdle muscular dystrophy. The life span of the cardiomyopathic hamster is about 12 mo, and it has no obvious

physical disability. In the early phase (1 mo) and midphase (4–6 mo), the fundamental pathologic events are repeated cycles of segmental necrosis of groups of fibers, followed by vigorous regeneration.<sup>11–13</sup>

Many of the exercise experiments on animal models of muscular dystrophy were performed before there was an understanding of the underlying genetic defects. These experiments were typically conducted to simulate exercise training or to study contraction-induced injury. The objective of the exercise-training studies was primarily to compare the adaptations to exercise of normal and dystrophic muscle. To determine whether dystrophin-deficient muscles were more susceptible to injury than normal muscles, studies of contraction-induced injury utilized vigorous short-term exercise testing or simulated exercise.

### Exercise Training

Regardless of differences in methodology, the objective of most of the following investigations was to evaluate the effects of exercise training on functional performance, muscle contractile characteristics, and muscle pathology. Animal models included the dystrophic chicken, cardiomyopathic hamster, *dy/dy* mouse, and *mdx* mouse. With the exception of one study, exercise training consisted of high-repetition, aerobic-type activity: swimming, treadmill running, and voluntary-wheel running. Exercise intensity ranged from submaximal exercise to maximal exhaustive exercise. The exercise protocols ranged from 1 wk to several months. The duration and type of the exercise-training protocols ranged from 2 to 60 min of swimming each day or 15 min to several hours of treadmill running per day. Many of the studies allowed the animals to exercise ad libitum on an exercise wheel. In these ad libitum studies, the *mdx* mice ran approximately 4–5 km/day, whereas the control mice ran anywhere from

5 to 7 km/day. The animals would usually run a total time of anywhere from 2 to 4 hr/day. There was also marked variability in the age of the animals at the initiation of the studies and therefore variability in the degree of muscle degeneration and regeneration at baseline. In most investigations, the number of animals was adequate, and nonexercised dystrophic animals, normal animals, and exercised normal animals served as controls.

**Dystrophic Chickens.** In the investigations using dystrophic chickens, rotating cage exercise or righting practice were reported to improve functional ability but did not retard the muscle atrophy.<sup>14,15,24</sup> This was measured by the number of times the animal would immediately right itself after being placed on its back.

**Dystrophic Hamster.** There have been three swimming studies, one weightlifting exercise study, and three treadmill studies of the cardiomyopathic hamster. Homburger et al.<sup>25</sup> reported that swimming to exhaustion for 8 days accelerated cardiac and skeletal necrosis. Ho et al.<sup>26</sup> examined the effect of swimming on the histopathology of the heart using tail weights for 30–60 min/day for 8 wk. They found greater calcium necrosis in the heart of the exercised hamsters than in the heart of the nonexercised controls. Sembrowich et al.<sup>27</sup> examined the effect of 18 wk of vigorous treadmill exercise. They reported a beneficial contractile response in the heart as measure by cardiac output, but found no differences in the skeletal muscle weight or severity of skeletal muscle necrosis after exercise training.

In young, developing hamsters, vigorous treadmill running for 2 hr/day for 1 mo had no effect, whereas running for 4 hr/day for 1 mo resulted in significant increases in muscle tension and less muscle necrosis in both fast- and slow-twitch

muscles. There was an increase in the oxidative capacity of the skeletal muscles, with hypertrophy of type 1 fibers, an increase in the percentage of type 1 fibers in slow-twitch muscles, and an increase in the number and area of type 1 fibers of the plantaris muscles.<sup>28</sup>

In the only animal investigation using resistive exercise training, Howells and Goldspink<sup>29–31</sup> subjected hamsters (9, 21, and 45 wk old) to a progressive weightlifting program for 5 wk. Whereas nonexercised hamsters had decreased fiber size and area, the exercised hamsters had an increase in fiber size and area and an increase in succinic dehydrogenase activity in fast- and slow-twitch muscles. None of the 21-wk-old hamsters exhibited these changes, whereas 45-wk-old hamsters exhibited a decrease in fiber size, fiber area, and succinic dehydrogenase activity.

After 4 wk of vigorous treadmill running, the cardiomyopathic hamsters failed to increase their muscle myoglobin concentration in the same manner as normal hamsters.<sup>32</sup> Tate et al.<sup>33</sup> found that one bout of swimming to exhaustion had no effect on cardiac or skeletal muscle, calcium uptake, or ATPase activity.

**Laminin-Deficient dy/dy Mouse.** There have been three swimming, one voluntary-wheel running, and two treadmill exercise investigations in the *dy/dy* mouse. Ages at the start of the exercise programs ranged from 3 to 14 wk, and the exercise programs lasted from 6 days to 5 wk.

In functional performance studies, *dy/dy* mice produced less work and power and had lower levels of fatigue resistance<sup>34</sup> than normal mice. The *dy/dy* mice also swam or ran more slowly and developed a higher percentage of muscle fibers with large diameter.<sup>35,36</sup>

In a voluntary wheel running study, Hayes et al.<sup>36</sup> compared 4-wk-old, exercise-trained *dy/dy*, *mdx*, and C57 mice with their sedentary con-

trols. The *mdx* and C57 mice ran for 16 wk, and the *dy/dy* mice ran for 4 wk. The difference in performance between species was marked. The C57 mice ran 6.5 km/day, *mdx* mice ran 1.6 km/day, and *dy/dy* mice ran 0.5 km/day. The trained *mdx* and *dy/dy* mice produced increased tension and exhibited less fatigue resistance in fast-twitch muscle as compared with their sedentary controls. There were no significant changes in slow-twitch muscle. Several *dy/dy* mice with extremely reduced hindlimb function died before the end of the experiment. The results from these animals were not included in the study.

In a study by Taylor et al.,<sup>37</sup> 2 wk of maximal treadmill running resulted in a marked decrease in slow-twitch muscle tension as compared with sedentary controls. Maximal here is defined as the distance and time the animals would run on a treadmill until they would no longer run even when prodded by an electrical stimulus. There was high mortality in the mice with severe impairment. In a related study from this same group, Fowler et al.<sup>38</sup> reported that 3 wk of submaximal treadmill running resulted in increased slow muscle fiber twitch tension, increased rate of twitch tension development, and increased rate of twitch tension relaxation. There was no change in the contractile properties of the fast-twitch muscle. However, exercise significantly retarded the histopathologic progression of the disease in both fast- and slow-twitch muscles. There was a reduction in the variability of the fiber sizes in the type 1 fibers of the slow-twitch soleus muscle and the type 2B fibers of the fast-twitch extensor digitorum longus (EDL) muscle. The data indicate that submaximal training is better than exhaustive or maximal exercise in terms of retarding evidence of dystrophy or causing increased impairment.

In a simulated exercise study, Luthert et al.<sup>39</sup> subjected *dy/dy* mice to slow-frequency (10 Hz) electrical

stimulation for 1 wk. This stimulation resulted in increased tensions of slow- and fast-twitch muscle when compared with the control contralateral muscles. Succinic dehydrogenase activity of the stimulated muscles also was increased. A similar electrical stimulation study by Dangain and Vrbova<sup>40</sup> resulted in a speeding of the time course of the muscle contraction and an increase in the fatigue resistance of skeletal muscles. There was an increase in the force output of weak dystrophic muscles with smaller fibers but a slight decrease in force output of the relatively strong dystrophic muscles. The authors suggested that stimulation promotes growth and development of small regenerating fibers and induces an increase in the mitochondrial content of the muscle fibers.

#### *Dystrophin-Deficient mdx Mice.*

There have been four voluntary wheel-running studies and three swimming investigations of the *mdx* mouse. Ages at the start of the exercise programs ranged from 3 wk to 2 yr, and the exercise periods lasted from 4 wk to 52 wk. There were also several drug-exercise studies that are not included in this review<sup>41–44</sup>

Hayes et al.,<sup>45</sup> in a 15-wk endurance swimming program that started with mice at 5 wk of age, observed increased tension, increased fatigue resistance, and a greater percent of type 1 fibers in the soleus of the exercised *mdx* mice. The EDL muscles exhibited a longer half-relaxation time and an increase in the number of type 2A fibers. Lynch et al.,<sup>46</sup> using the same swimming exercise protocol, measured the contractile properties of skinned fibers. They found that type 2B fibers from the EDL and type 2A fibers from the soleus of trained animals were less sensitive to  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  when compared with fibers of the same type from sedentary mice. In a 1998 study, Hayes and Williams<sup>47</sup> examined the effects of a 10-wk, once-a-day endurance swimming

protocol using adult (8–10 mo) and old (24 mo) *mdx* and C57 mice. The trained mice had increased tension per cross-sectional area in both the soleus and the EDL, but there were no effects on body mass, fatigue properties, muscle mass, or fiber-type profiles in either muscle.

Ten to 12 mo of voluntary wheel running ad libitum, usually averaging 7 km/day, increased the maximal tetanic tension and the contraction time of the *mdx* diaphragm, but it had no effect on the slow-twitch soleus muscle.<sup>17</sup> Voluntary wheel running increased the maximal tetanic tension of the *mdx* diaphragm by 33% and increased the contraction time by 14%, but it had no effect on the slow-twitch soleus muscle. The *mdx* mice ran approximately 5 km/day, whereas the controls ran approximately 7 km/day. Carter et al.<sup>48</sup> examined the effect of 4 wk of voluntary wheel running in *mdx* and C57 mice using two different age groups: young (4 wk of age) and adult (6 mo of age). The young *mdx* mice ran 78% of the distance run by the C57 mice, whereas the adult *mdx* mice ran only 31% of the distance run by the C57 mice. After exercise, the slow-twitch soleus muscle of both the young and the old *mdx* mice exhibited hypertrophy with no change in tension per cross-sectional area and no change in fatigue resistance. The *mdx* EDL exhibited slight hypertrophy and loss of tension in the adult, whereas the young exhibited no changes. There was a significant increase in serum levels of creatine kinase in the adult exercised *mdx* mice when compared with their sedentary controls. Hayes and Williams<sup>36</sup> reported that 16 wk of voluntary wheel running, beginning at 4 wk of age, resulted in hypertrophy of the soleus and an increase in soleus tension, fatigue resistance, and proportion of type 1 fibers. In the EDL, there was no effect on tension, but there was an increase in fatigue resistance and a conversion to oxidative fiber types.

Wineinger et al.<sup>49</sup> reported that 12 mo of voluntary wheel running slightly increased the fatigue resistance of fast-twitch EDL muscles, but had no effect on their tension development.

All of the investigations used repetitive exercise-training protocols, usually submaximal wheel running or swimming, with the exception of one investigation that used a resistive strengthening training protocol. Regardless of species and differences in methodology, all of these investigations reported a relatively normal and beneficial adaptation to aerobic exercise. The benefits of the high-repetition, low-impact training, whether by running, swimming or electrical stimulation, typically has a greater effect on the more oxidative fibers and may reduce the risk for further mechanical damage to these fibers. This risk reduction may be related to better cell management of free radical through enzyme induction or improved oxidative capacity through an increase in the number of mitochondria. The possibility of increases in number of mitochondria providing the protective effect is supported by the fact that this type of training had little impact on fast-twitch fibers.

Three major factors determining the beneficial or deleterious effect of exercise training in animals with muscular dystrophy are: (1) the age of the animal at the start of the study, (2) the severity of the dystrophy at the start of the study, (3) the intensity of the training (submaximal or maximal), (4) the duration of training and total training time, and (5) the type of training (repetitive aerobic or resistive strength). These factors are similar to those reported in humans with muscular dystrophy.

#### **Contraction-Induced Injury**

A large number of studies have shown that eccentric exercise causes injury in normal humans and animals.<sup>1–3,8,11,12</sup> Several studies have been performed to help elucidate the

mechanisms and subsequent adaptation of skeletal muscle to contraction-induced injury in animal models of muscular dystrophy. This information, theoretically, would help assess what types of training would be either beneficial or deleterious to muscle. Regardless of methodology, the objectives of these investigations were to: (1) determine if there was an increased susceptibility of dystrophic muscle to contraction-induced injury as compared with normal muscle and (2) identify the reasons for any increased susceptibility.

Preliminary studies examined the role of the sarcolemma in muscle injury. McNeil and Ito<sup>50</sup> proposed that microinjuries to the sarcolemma and their subsequent repair may be an important mechanism that regulates muscle atrophy, homeostasis, and hypertrophy. In studies to determine whether plasma membrane injury and rapid repair occurred in skeletal muscles, they examined the medial head of eccentrically exercised rat triceps and used labeled antibodies to demonstrate the presence of rat serum albumin within the muscle. Even when no disruption of the sarcolemma was detectable by ordinary light microscopy, the presence of albumin within the muscle was an indication of microinjury to the sarcolemma.<sup>51</sup> They also found that the sarcolemma is so vulnerable to injury that even "unexercised" caged laboratory rats incur transient sarcolemmal disruptions, as demonstrated by the presence of albumin within their muscles. They proposed that these resealable focal membrane breaks are an early form of exercise-induced injury. This injury could be repaired or, if severe, progress to fiber necrosis. They also hypothesized that the absence of exercise would prevent microinjuries to the muscle and would lead to muscle atrophy. Mokhtarian et al.<sup>52</sup> provided further evidence to support this hypothesis. After a 14-day period of immobilization of the soleus and EDL of 3-wk-old *mdx*

mice, the striking feature was the low percentage of regenerated myofibers, regardless of the length at which they were fixed. This study provided evidence that limb immobilization prevents the occurrence of the first round of myofiber necrosis in *mdx* mice and suggests that muscle contractions play a role in the degeneration of dystrophin-deficient *mdx* mouse skeletal muscle.

Dystrophin, which is absent in muscles of the *mdx* mouse, is thought to stabilize the sarcolemma during contractions of normal muscle.<sup>12</sup> This is supported by studies that have shown transient localized plasma membrane instability in dystrophin-deficient fibers. Carter et al.<sup>53</sup> investigated the use of intravenously injected fluorescent dextran molecules as a histologic marker of sarcolemmal injury in dystrophin-deficient *mdx* and control mice. Using fluorescent microscopy, uptake of fluorescent dextran molecules was assessed in sections of quadriceps muscles from three models: nonexercised normal mice, normal mice run downhill (0, 3, and 7 days postexercise), and nonexercised *mdx* mice. In nonexercised normal muscles, strong intercellular fluorescence was observed between fibers. In normal mice run downhill, only small amounts of intracellular fluorescent dextran molecules were observed within cells of the quadriceps from days 0 and 3 postexercise, but not at day 7. On hematoxylin and eosin staining, no muscle pathology was observed at day 0, slight pathology was observed at day 3, and regeneration was observed at day 7. In contrast, extensive intracellular fluorescent dextran molecules were observed within the muscles of nonexercised *mdx* mice, particularly in fibers that appeared pre-necrotic on hematoxylin and eosin-stained sections. Straub et al.<sup>54</sup> used noninvasive albumin-targeted contrast-agent-enhanced magnetic resonance imaging to study muscle membrane disruption in *mdx* and sarcoglycan-deficient mice. They demonstrated that a significant accumula-

tion of contrast material within skeletal muscle reflected the localization and extent of fiber damage in *mdx* mice. These studies indicate an increase in the permeability at the sarcolemmal membrane of the *mdx* mice.

To examine the effect of this increased permeability on muscle injury, several studies simulating contraction-induced injury have been performed on dystrophic and normal animals. In an in vitro study using a servomotor to create various degrees of muscle contraction, fluorescent dye uptake to assess sarcolemmal damage, Petrof et al.<sup>55</sup> concluded that the magnitude of sarcolemmal breakage was greater in *mdx* diaphragm and EDL muscle than in the same muscles of normal mice. There was a linear relationship between peak force and dye-positive fibers in both normal and *mdx* mice. This indicates greater force resulted in more sarcolemmal damage. However, for any level of force, there was a higher percentage of damaged fibers in the *mdx* mice. Using a servomotor, Brooks<sup>56</sup> subjected EDL muscles from 8-wk-old *mdx* and control mice to a series of repeated stretches. The effect on the maximally contracting muscle and the time-course of injury and recovery was determined by measurements of force production. The initial injury, as assessed by the decrease in force production, was significantly greater for the *mdx* EDL than the control EDL, which indicates an increased susceptibility to contraction-induced injury in the *mdx* muscles. However, there was a complete recovery of force in *mdx* muscle within 3 days after injury, whereas the control muscle produced a force that was only 80% of its initial value. The rapid recovery of force observed in the *mdx* mice suggested a rapid and accelerated rate of recovery, although the mechanism remains unclear. Histologic and electron microscopic analysis also revealed a more rapid rate of recovery and increased muscle regenerative capacity in the young

*mdx* mice as compared with the control mice. Moens et al.<sup>57</sup> compared static and eccentric contractions in slow- and fast-twitch muscle and correlated force changes with intracellular accumulation of Procion red, an indicator of cell damage. In both normal and *mdx* mice, there was essentially no force drop or intracellular Procion red dye accumulation with static contractions. With eccentric lengthening, there was a slight drop in force and slight Procion red dye accumulation in the soleus muscles of *mdx* mice and in both the EDL and soleus muscles of control mice. In contrast, there was an irreversible 40–60% force drop in EDL tension in the *mdx* mice, with associated membrane damage, as evidenced by Procion red dye accumulation. Head et al.<sup>58</sup> also reported that muscle stretched during a tetanic contraction resulted in a reduction of the maximal tetanic force produced by the *mdx* EDL, whereas the force in normal EDL and soleus, and *mdx* soleus, was not altered.

Several investigators have examined the effect of different types of running exercise on the susceptibility to injury in various animal models of muscular dystrophy. In the canine model (*CXMD*), Valentine et al.<sup>23</sup> showed that even mild exercise can cause significant elevation of serum creatine kinase, which reached a peak at 4 hr postexercise and returned to baseline values at 24 hr postexercise. Sandri et al.<sup>59,60</sup> demonstrated that the apoptotic index in *mdx* mice dramatically increased after short-term wheel running, whereas in the skeletal muscle of control mice, only a minor increase in apoptotic cells was detected. This was the first evidence to suggest that exercise may trigger apoptosis in dystrophin-deficient mice. Using fluorescent dye, Clarke et al.<sup>61</sup> examined triceps muscles of *mdx* mice after a single bout of downhill running. Seventy-five percent of the fibers of the *mdx* triceps exhibited transient membrane disruptions,

which was seven-fold greater than in control mice. Furthermore, no difference was observed between muscles of control mice after running and those of nonexercised control mice. Brussee et al.<sup>62</sup> examined the effect of downhill running for 3 days on the histology of *mdx* mice. They reported a 31% increase in positive staining for Evans blue muscle in young *mdx* mice, compared with a 0–3% increase in staining in normal muscle. Stained fibers were also present in 2–15% of nonexercised *mdx* mice. Vilquin et al.<sup>63</sup> showed further evidence of injury and repair in skeletal muscle fibers of transgenic dystrophinless mice expressing  $\beta$ -galactosidase. Adult *mdx*/ $\beta$ -galactosidase (dystrophin-negative) and normal/ $\beta$ -galactosidase (dystrophin-positive) mice were subjected to one short session of eccentric, downhill running exercise. The leakage of creatine kinase and  $\beta$ -galactosidase was investigated before, 1 hr after, and 3 days after the running session. A significant and transient rise in the level of these enzymes was noted in the serum of *mdx* mice after the exercise session. The peak leakage was transient, suggesting that muscle fiber lesions were rapidly repaired after this short, eccentric running session. All of these running experiments revealed increased susceptibility to injury in dystrophic animals as compared with controls.

Other studies that simulated increased exercise used electrical stimulation and synergistic tenotomies. The tenotomy studies simulate high-resistive exercise by removing one of two synergistic muscles, which places increased load on the remaining synergistic muscle. In a study comparing electrical stimulation and synergistic tenotomy, Weller et al.<sup>64</sup> found a higher rate of fiber necrosis after electrical stimulation in *mdx* tibialis anterior muscle than after tenotomy. They reported that electrically stimulated lengthening contractions result in a significantly greater

number of necrotic fibers, as indicated by the presence of immunoglobulin G within the *mdx* tibialis anterior, than normal muscle. Dick and Vrbova<sup>65</sup> removed the tibialis anterior at 9–12 days of age and examined the contractile and histologic response of young (2–3 mo) and adult (5–8 mo) *mdx* and control EDL muscles. Whereas the tension produced by the normal EDL increased with age, the tension produced by the muscles from the adult *mdx* mice decreased with age, and no change in tension production was observed in the EDL muscles of the young *mdx* mice. In addition, there was little or no fiber degeneration in the normal EDL, whereas the EDL of the adult *mdx* mice exhibited significant fiber degeneration. Skeletal muscle fiber degeneration in *mdx* mice has also been induced by electrically stimulating bundles of EDL fibers.<sup>66</sup> The loss of dystrophin was thought to cause membrane instability, resulting in an influx of  $\text{Ca}^{2+}$ , the activation of proteases, and the subsequent degeneration of skeletal muscle fibers. These results suggest that excess  $\text{Ca}^{2+}$  channels, assessed by iontophoresis, produce an accumulation of  $\text{Ca}^{2+}$ , mainly through L-type channels, and result in premature degeneration of fibers.

One metabolic study has also been carried out in the *CXMD* canine.<sup>67</sup> As with the *mdx* mouse, there were no differences from control dogs in ATP, pH, and PME + PDE in resting muscle, whereas the Pi/PCr ratio was higher. The mean Pi/(Pi + PCr) ratio and pH were, however, normal during stimulation, but 2–3 days after exercise, the resting ratios were elevated, with a 50-fold increase in serum creatine kinase. Regardless of the mechanisms, all of these studies reported an increased susceptibility of dystrophic muscles to injury.

Not all evidence supports the theory that the sarcolemma of dystrophin-deficient is more susceptible to injury. In eccentrically stimulated

muscle, *mdx* and normal tibialis anterior muscles had similar loss of force and rates of recovery.<sup>68</sup> Using the *mdx* mouse model, McArdle et al.<sup>69</sup> studied release of creatine kinase and prostaglandin E<sub>2</sub> in stretched or electrically stimulated fast-twitch EDL muscle. For 2 hr after 30 min of contractions in vitro, muscles of *mdx* and control mice showed elevations in both the efflux of intracellular enzymes<sup>69</sup> and in the influx of calcium,<sup>70</sup> but the increases were not different between *mdx* mice and controls. They concluded that dystrophin-deficient EDL muscles are not more susceptible to damage than the EDL of normal mice.

The reasons for discrepancies between studies might be attributed to the method of exercise, types of muscles examined, age of the mice used, and criteria used to determine injury. Muscle membranes, however, are more easily damaged in vitro. Mechanisms of contraction-induced damage are apparently different when studied in vitro than when studied in situ or in vivo.<sup>56</sup>

Studies have demonstrated that mechanical weakness and contraction-induced muscle injury are not always required for muscle degeneration and the dystrophic process. Evans blue dye, which does not cross into skeletal muscle fibers in normal mice, showed marked accumulation in the muscles of *mdx* mice and in muscles of transgenic mice with different dystrophin mutations. However, the muscles of the *dy/dy* mouse, which have defects in the laminin- $\alpha$  2 chain, an extracellular ligand of dystrophin-glycoprotein complex, showed little dye accumulation.<sup>8</sup> There was no evidence for contraction-induced injury in mice lacking  $\gamma$ -sarcoglycan that were subjected to an extended, rigorous exercise regimen.<sup>10</sup> Isolated muscles lacking  $\gamma$ -sarcoglycan have shown normal resistance to mechanical strain induced by eccentric muscle contraction with normal peak static force generation.

Cohn and Campbell<sup>71</sup> suggest that some signaling pathway may play an important role in the pathogenesis of muscular dystrophy of  $\gamma$ -sarcoglycan-deficient mice.

Several experiments suggest that different sarcoglycans may have different functional roles. Mice lacking  $\alpha$ -sarcoglycan,  $\beta$ -sarcoglycan, and  $\delta$ -sarcoglycan also develop dystrophy. Membrane integrity is disrupted in most of these models. Pathogenesis of these limb-girdle muscular dystrophies might be related to impaired membrane repair and resealing of damaged muscle, instead of increased occurrence of damage per se.<sup>71</sup> This concept is supported by evidence that  $\alpha$ -sarcoglycan-deficient mice may have an ecto-adenosine triphosphate activity and that the subsequent elevation of ATP may contribute to calcium overload and cell death.<sup>72</sup>

## DISCUSSION

Regardless of species and differences in methodology, the majority of these investigations reported that dystrophic animals had a normal and beneficial adaptation to mild, submaximal aerobic exercise, whereas maximal exhaustive exercise had a deleterious effect, especially to fast-twitch muscle. The beneficial adaptations to mild aerobic exercise training included an increase in muscle strength per cross-sectional area and a reduction in muscle degeneration. The beneficial effect of increased strength was likely due, in part, to some palliation of the muscle disease. There was a hypertrophic response in the muscles that were not severely affected by the dystrophy. High-repetition, low-impact exercise increased the oxidative capacity and the proportion of oxidative fibers, especially in slow-twitch muscles. Younger animals tend to benefit more from exercise studies than do older animals. However, high-repetition exercise typically had no effect, or it had a deleterious effect, in fast-twitch mus-

cles that were more severely affected by disease, which makes them more vulnerable to damage by eccentric exercise. There are not enough reports examining the effect of high-resistive strength training exercises in dystrophic animals to allow any conclusions to be drawn.

The results from studies on animals are similar to those reported in humans with muscular dystrophies. In humans, adaptations to exercise did not occur in very weak muscles.<sup>73</sup> People with slowly progressive muscular dystrophy also had a beneficial adaptation to submaximal, high-repetition aerobic exercise<sup>74</sup> in a manner similar to that observed in animal studies. High maximal resistive exercise training resulted in increased weakness, whereas moderate submaximal resistive exercise increased strength.<sup>75,76</sup>

Studies performed in both normal and dystrophic animals have shown that unaccustomed eccentric exercise (lengthening of the muscle during contraction) may injure the contractile and cytoskeletal components of the muscle fibers. Concentric exercise, which involves shortening of the muscle during contraction, does not have the deleterious effects observed in eccentric exercise. During eccentric exercise, sarcomeres are stretched and the actin and myosin filaments are pulled apart, leading to disruption of the thick and thin filament array and damage to cytoskeletal proteins. Structural damage is observed by the appearance of z-line streaming and myofibrillar disruptions. Mechanical strain, the contributing factor that induces muscle injury, causes an immediate loss of force-generating capacity and initiates a cascade of processes that result in skeletal muscle damage. The inability to quickly repair a disruption of the membrane causes an elevation in intracellular calcium concentration, which triggers calcium-activated degradation pathways and further ultrastructural damage. This

damage results in fiber degeneration followed by inflammation and, eventually, fiber regeneration.

A majority of studies shows that dystrophic muscles have an increased susceptibility to high mechanical force. Eccentric exercise reduces the force-generating capacity of dystrophic muscles to a greater extent than in normal muscles. There is also evidence of greater mechanical disruption of the sarcolemma, increased fiber degeneration, and necrosis. However, the muscles of young dystrophic *mdx* mice have a more rapid rate of recovery of force production than those of normal mice. Histologic and contractile studies suggest that this is due to an increased regenerative capacity in young dystrophin-deficient *mdx* mice. This increased regenerative capacity is lost in older *mdx* mice.

Most of the exercise studies using animal models of muscular dystrophy were performed with the assumption that they were models of DMD. We now know that some of these animal models were more similar to other muscular dystrophies. The *dy/dy* mouse is a model of one of the congenital muscular dystrophies and the BIO hamster is a model of one of the limb-girdle muscular dystrophies, whereas the *mdx* mouse and *CXMD* dog are models of DMD.

With advances in molecular genetics, we now study animal models with the same genetic defects as are found in humans. As we develop a greater understanding of the genes that encode the different muscular dystrophies and the biomechanical factors underlying the different clinical phenotypes, efforts to develop physical, genetic, and pharmacologic therapeutic interventions will improve. For the future, the matrix of findings that relate gene defects of muscle to functional capacity offers a promising area of basic and applied research for the development of clinical tools that stretch from individu-

alized exercise interventions to corrective gene therapy.

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