Duchenne Muscular Dystrophy: Experimental Models on Physical Therapy

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1. Introduction

Neuromuscular disorders are a heterogeneous group of genetic diseases. Nowadays, more than 30 genetically defined forms are recognized and, in the last decade, mutations in several genes have been reported to result in the deficiency or loss of function of a variety of important muscle proteins (Shelton & Engvall, 2005). Defects in components of the dystrophin-glycoprotein complex (DGC) are known to be an important cause of different forms of muscular dystrophies (Ervasti & Campbell 1993; Yoshida & Ozawa 1990). Lack of dystrophin protein in muscle cells is characteristic of Duchenne muscular dystrophy (DMD), which is a progressive and fatal X-linked genetic disorder. Many animal models have been studied to identify an efficient treatment for this disease in humans. Two mammalian models of DMD have been widely used in preclinical trials to understand the pathogenesis of the disease and development of efficient therapeutic strategies for humans. Mdx-mouse is the most used animal model for DMD, followed by the Golden Retriever Muscular Dystrophy (GRMD) canine model. Mdx-mouse morphology displays some features of muscle degeneration, but the pathogenesis of the disease is comparatively mild. This model has a slightly shorter life span as compared to wild-type controls (Banks & Chamberlain, 2008) and muscle degeneration is different from the one seen in DMD patients. An important degeneration and regeneration of muscle fibers is observed at a young age in the mdx-mouse (2 to 4 weeks), which results in the muscle morphological changes of centralized nuclei and heterogeneity of fiber size. Necrosis is also observed at this early age but decreases around sixty days. Loss of muscle tissue is slow and muscle weakness is not evident until later in life. Fibrosis, a marked feature of DMD muscle, is less pronounced in mdx-mouse, with the exception of diaphragm muscle (Hueber et al., 2008). Dystrophin deficiency has also been reported in cats as hypertrophic feline muscular dystrophy (HFMD), in which diaphragmatic hypertrophy is often fatal (Shelton & Engvall, 2005). Similar to mdx pathology, the skeletal muscle of the HFMD cats undergoes repeated cycles of degeneration and regeneration but does not develop the debilitating fibrosis that is
characteristic of both DMD and the canine GRMD model (Hoffman & Gorospe, 1991). The GRMD model has been widely studied (Collins & Morgan, 2003) since it presents muscle abnormalities that are closest to the ones seen in humans: increased creatine kinase (CK) activity, muscle hypotrophy associated with contractures, muscle necrosis, degeneration, endomysial and perimysial fibrosis and cardiomyopathy (Howell et al., 1997). This model also presents repeated cycles of muscular necrosis and regeneration, muscle wasting, postural abnormalities, respiratory or heart failure and premature death, as seen in DMD patients (Valentine et al., 1988). Despite the phenotype variability of this model, it has been used due to the strong similarities of body weight and pathological expression of the disease in human (Collins & Morgan, 2003). Since the coding sequence of the dystrophin gene was discovered in 1987, no treatment has been found to stop DMD progression. To improve quality of life and prevent complications of this progressive disease, patients have access to supportive therapies such as motor and respiratory physical therapy, occupational therapy, psychology, nutritional supplements and corticosteroids. Although these therapies cannot cure DMD, they should be well investigated as they intend to lead these patients to a better quality of life and to decrease the complications of their degenerative and progressive illness. Physical therapy (PT) has been used to reduce muscular, cardiac and vascular abnormalities which develop in association with muscle strength loss (Gaiad et al., 2009). The main objective of such motor physical therapy is the prevention of muscle contractures and bone deformities (Strober, 2006). However, motor PT approaches have yielded controversial recommendations (Carter et al., 2002) and there is no consensus regarding the type and intensity of PT (Cup et al., 2007). Just as other muscular dystrophies, DMD is a progressive disorder which causes death by cardiac or respiratory failure. Nevertheless, the absence of dystrophin in the sarcolemma of muscle cells makes DMD a special illness regarding therapeutic exercise prescription. Research using animal models can answer questions regarding which are the best type, frequency, and intensity of therapeutic exercise, as well as highlight a beneficial approach of PT for DMD patients. This chapter aims to detail some actual studies using mdx and GRMD models which reported new insights in this subject contributing to future research designs of therapeutic exercise on dystrophic muscle.

2. Duchenne muscular dystrophy (DMD)

Dystrophin is located beneath the sarcolemma and is part of a large dystroglycan complex termed the dystrophin-glycoprotein complex (DGC), which includes the dystroglican complex (α and β), sarcoglycan complex (α, β, γ and δ) and syntrophin/dystrobrevin subcomplexes (Ervasti & Campbell, 1991). DGC forms a critical link for force transmission between the contractile machinery of the muscle fiber and the extracellular matrix. Where dystrophin is defective or absent, the myofiber is fragile and the sarcolemma is readily damaged in response to exercise, leading to myofiber necrosis (Hoffman et al., 1987; Sharp et al., 1989). The absence of dystrophin in dystrophic muscles leads to altered myofiber integrity, perturbed calcium homeostasis, and activation of the calcium-dependent calpain proteases and necrosis (Straub et al., 1997). The other consequence of the loss of dystrophin is the absence or great reduction of components of the DGC, as described for skeletal muscle fibers from DMD patients and the mdx mouse (Ervasti et al., 1990; Ohlendieck & Campbell, 1991). Loss of dystrophin leads to membrane leakiness as a result of mechanical or hypoosmotic stress. Consequently, Ca^{2+} permeability is increased and various Ca^{2+} dependent proteases such as calpain are activated
under conditions of dystrophin deficiency. It has also been proposed that alteration of the expression or function of plasma membrane proteins associated with dystrophin such as neuronal nitric oxide synthase (nNOS) and various ion channels are involved in the molecular mechanisms of muscle degeneration (Straub et al., 1997). Although the defective gene of dystrophin was identified in 1987, there is still no effective treatment for DMD patients. While cell or gene therapy to replace the defective dystrophin is the ideal solution, the clinical application of such therapies has yet to become a reality (Davies & Grounds, 2006; Foster et al., 2006). The existing treatment for DMD is administration of corticosteroids, broad-based anti-inflammatory drugs that decrease inflammatory cell populations in dystrophic muscle and increase myofiber mass. However, their precise mechanism of action in DMD is not yet known and is under intense investigation (Griggs et al., 1993; Connolly et al., 2002). Disadvantages of steroid treatment include their association with severe adverse side effects such as weight gain and osteoporosis and the variable response by the individuals undergoing treatment (Muntoni et al., 2002; Moxley et al., 2005).

3. Animal models for neuromuscular diseases

Several animal models that have been identified in nature or generated in laboratory present the phenotypes observed in neuromuscular diseases. These models generally present physiological symptoms observed in human patients and can be used as important tools for genetic, clinic, and histopathological studies (Vainzof et al., 2008). Animal models are essential for elucidation of the disease pathogenicity and for assessment of efficacy and toxicity during therapy development. Two mammalian models of DMD have been widely used in preclinical trials to understand the pathogenesis of the disease and to develop efficient therapeutic strategies for humans. *Mdx*-mouse is the most used animal model for DMD, followed by the Golden Retriever Muscular Dystrophy (GRMD) canine model.

3.1 Mouse model (*mdx*-mouse)

A mouse X-chromosome mutant (*mdx*) was first discovered in 1984 due to the observations of elevated plasma levels of muscle creatine kinase and pyruvate kinase enzymes and histological lesions characteristic of muscular dystrophy. In the *mdx* model the mutations cause a premature stop codon in exon 23 due a single base substitution (Sicinski et al., 1989). *Mdx*-mouse shows a mild non-progressive phenotype associated with comparatively moderate muscle pathology and muscle degeneration followed by subsequent significant regeneration (Dangain & Vrbova, 1984). Though the mouse models have slightly shorter life spans compared to wild-type controls (Banks & Chamberlain, 2008), they are indispensable for elucidation of the pathogenic mechanism and for development of therapeutic approaches, since they can be easily and reliably reproduced (Nakamura & Takeda, 2011).

3.1.1 Pathogenesis in the *mdx* mouse

Muscle degeneration in the *mdx* mouse is different from that seen in DMD patients. The progression of pathology in the *mdx* mouse is influenced by growth (Grounds, 2008) and may be divided into three main phases: (1) the pre-weaning phase (0–3 weeks of age), which is strongly influenced by growth and corresponds roughly to the first 6 months of human patients, (2) the post-weaning phase, with an acute onset of pathology around 3 weeks,
followed at about 8 weeks by (3) the adult phase with a reduced low level of chronic damage that persists throughout life (Willmann et al., 2011). An important degeneration and regeneration of muscle fibers is observed at a young age of mdx-mouse (2 to 4 weeks) which results in the muscle morphological changes such as centralized nuclei and heterogeneity of fiber size. In mature limb muscle, the murine model is characterized by successive degeneration/regeneration processes and does not exhibit the progressive muscle wasting and accumulation of connective tissue observed during development of the human disease (Coulton et al., 1988). Necrosis is also observed at this early age but decreases around sixty days. Loss of muscle tissue is slow and muscle weakness is not evident until later in life. Fibrosis, a marked feature of DMD muscle, is less pronounced in mdx-mouse, with the exception of diaphragm muscle (Hueber et al., 2008). For this reason, histological analysis of the diaphragm, one of the most severely affected muscles of the mdx, is often used as a marker of weakness progression, once it reproduces the degenerative changes of muscular dystrophy.

3.2 Feline muscular dystrophy

Dystrophin deficiency has also been reported in cats, called hypertrophic feline muscular dystrophy (HFMD). The HFMD cat has a large deletion of muscle and Purkinje promoters resulting in a lack of dystrophin in the skeletal and cardiac muscle. These animals have a unique phenotypic expression of hypertrophy of the tongue, neck, and shoulder muscles, lingual calcification, excessive salivation, megaesophagus, gait disturbance manifesting as bunny hopping, dilated cardiomyopathy, hepatosplenomegaly, and kidney failure (Winand et al., 1994). Diaphragmatic hypertrophy is the principal cause of death in these animals (Shelton & Engvall, 2005). Similar to mdx pathology, the skeletal muscle of the HFMD cat undergoes repeated cycles of degeneration and regeneration but does not develop the debilitating fibrosis that is characteristic of both DMD and GRMD (Hoffman & Gorospe, 1991). Muscular dystrophy associated with absence of Merosin (laminin α2) was described in cats that presented muscle atrophy and marked weakness or progressive spasticity and contractions, as well as the serum creatine kinase (CK) at moderate levels (O’Brien et al., 2001). Other muscular dystrophies that have been reported are α-dystroglycan deficiency in Sphynx and Devon Rex cats (Martin et al., 2008) and reduced β- sarcoglycan in a shorthair male cat (Salvadori et al., 2009).

3.3 Canine muscular dystrophy

Numerous sporadic cases of canine muscular dystrophy have been recognized in the last two decades, but the Golden Retriever Muscular Dystrophy (GRMD) has been the most extensively examined and characterized (Cooper et al., 1988). GRMD is a degenerative myopathy homologue to Duchenne muscular dystrophy (DMD) in humans. A frame-shift point mutation in the dystrophin gene is responsible for the GRMD phenotype (Sharp et al., 1992), whereas deletions are the most frequent mutations in DMD patients. Nevertheless, in both DMD and GRMD patients, dystrophin protein is lacking (Hoffman et al., 1987; Cooper et al., 1988). Canine dystrophinopathies have also been reported in many other purebred and mixed breed dogs. In addition to the Golden Retriever (Kornegay et al., 1988), genetic mutations have also been characterized in Labrador Retriever (Bergman et al., 2002; Cosford et al., 2008), Irish Terrier (Wentink et al., 1972), German shorthaired pointer (Schatzberg et al., 1999), Samoieda (Presthus & Nordstoga, 1993), Japanese Spitz (Jones et al., 2004), English Spaniel (Van Ham et al., 1995), Old English Sheepdog (Wieczorek et al., 2006), Schnauzer...
miniature (Paola et al., 1993), Weimaraner (Baltzer et al., 2007), and Boston Terrier (Deitz et al., 2008). GRMD dogs closely resemble DMD patients in terms of both body weight and in the pathological expression of the disease (Collins & Morgan, 2003). However, their phenotype is variable (Banks & Chamberlain, 2008), as some pups survive only for a few days, while others are ambulant for months or even years (Ambrosio et al., 2008). Animal trials employing these dogs have substantial animal welfare implications and high costs associated with both maintenance and treatment (Nakamura & Takeda, 2011).

Researchers have developed a strain of medium sized dystrophic Beagles, designated as canine X-linked muscular dystrophy in Japan (CXMDj). They show atrophy and weakness of limb muscles which appear at 2-3 months, followed by development of macroglossia, dysphasia, gait disturbance, and joint contracture around 4 months of age. These symptoms rapidly progress until around 10 months of age, after which the progression of the disease is retarded (Shimatsu et al., 2005). The GSHPMD (German shorthaired pointer muscular dystrophy) is a spontaneous canine dystrophin ‘knockout’ model with complete lack of dystrophin immune reactivity. These dogs have been useful for dystrophin gene therapy trials, myoblast transfer, and in combination of the two. Any dystrophin transcripts or protein detected in GSHP skeletal muscle after therapeutic intervention could therefore only be produced by the dystrophin delivery vehicle (Schatzberg et al., 1999). Due to the common genetic basis of the disease in dog and human, the GRMD and other inbred dystrophic dog lines descended from animals with spontaneous mutations have been extensively used in preclinical settings, particularly for cell and gene therapy studies. An interesting Becker-like dystrophy with a truncated form of dystrophin was recently identified in a family of Japanese Spitz dogs (Jones et al., 2004). In these dogs, a 70–80 kDa protein on immunoblots that reacted with antibodies to the C-terminal domain of dystrophin was found, but not with antibodies to the rod domain. Canine models with deficient sarcoglycan (SG) proteins have been identified in Boston Terriers and Cocker Spaniels. The phenotype includes failure to thrive and exercise intolerance. Serum CK is highly elevated, and muscle histopathology shows a dystrophic pattern and a variable degree of loss of SG proteins staining (Shelton & Engvall, 2005). A dystrophic myopathy should be considered in any young dog or cat (male or female, mixed breed or purebred) with persistent muscle weakness, muscle atrophy or hypertrophy, gait abnormality, or contractures beginning in the first few months of life.
Muscular Dystrophy

(Shelton & Engvall, 2002). The breed of an affected animal is one of the most useful
distinguishing diagnostic criteria. A DNA-based test is commercially available for the
dystrophin mutation in Golden Retrievers, but not yet for mutations in dystrophin or related
proteins in other breeds. Diagnosis of Muscular Dystrophy (MD) in companion animals has
been based on clinical presentation, markedly elevated serum creatine kinase concentration,
and the presence of a dystrophic phenotype based on histopathological evaluation of muscle
biopsy specimens (Shelton, 2010). Such analysis can be done by immunohistochemical
localizations of dystrophin, dystrophin-associated proteins, laminin and other proteins. This is
a cost-effective and sensitive method which can be performed directly on fresh-frozen biopsy
specimens. Results of immunohistochemical staining using various monoclonal and
polyclonal antibodies against skeletal muscle proteins involved in the muscular dystrophies
can guide the direction of mutational analyses and development of diagnostic tests for specific
mutations (Shelton, 2004). Serum CK activity should be part of every neuromuscular
minimum database, most importantly for preneuter evaluations in young dogs because
increased activity may be an early indicator of underlying muscle disease. Marked or
persistent increases of CK activity may be indicative of a congenital or inherited muscle
disease even if the animal is clinically asymptomatic (Shelton, 2010). The most marked
increases in serum CK activity (420,000 U/L) are associated with necrotizing myopathies or
MD (Kornegay et al., 1988; Bergman et al., 2002).

3.3.1 Pathogenesis in the GRMD dog

Mutations in the dystrophin protein result in membrane damage allowing massive
infiltration of immune cells, chronic inflammation, necrosis, and severe muscle degeneration
(Valetine et al., 1990b). The histopathological changes in the muscle are similar to the ones
seen in humans and include muscle fiber degeneration and regeneration, fiber splitting,
numerous fibers with centralized myonuclei, and intense connective tissue replacement.
Myofiber hypertrophy and variability in myofiber size are likely to be an early change
associated with dystrophin deficiency rather than a compensatory mechanism related to
muscle impairment (Hoffman & Gorospe, 1991). Dystrophin deficiency in mdx mice and
HFMD cats does not lead to significant muscle weakness (Hoffman & Gorospe, 1991).
GRMD dogs, as well as DMD patients, suffer from repeated cycles of muscular necrosis and
regeneration, muscle wasting and fibrosis, postural abnormalities, respiratory or heart failure,
and premature death (Valetine et al, 1988). The clinical signs in GRMD dogs include the
gradual loss of muscle mass and the development of contractures that often lead to skeletal
deformities (Cooper et al., 1988). A prominent feature in dystrophic dogs is enlargement of the
base of the tongue due to muscle fiber hypertrophy and pseudohypertrophy. Dysphagia,
regurgitation and drooling associated with pharyngeal and esophageal dysfunction can be
observed (Shelton and Engvall, 2005). GRMD dogs can present difficulty in opening the
mouth, exercise intolerance, and atrophy of the trunk, limbs and temporallis muscle (Valetine
et al., 1988). Elevation of serum CK is a feature of both canine and human muscular
dystrophies (Cooper et al., 1998; Valetine et al., 1988). CK values were significantly elevated
in GRMD dogs and increased with exercise. Serum ALT activity was also elevated, a finding
which has been identified in Duchenne-like muscular dystrophy in dogs. The degree of
elevation of the CK and ALT did not correlate with the severity of the clinical signs in any dog
(Valetine et al, 1990a, Ambrosio et al., 2009). Gaiad et al. (2011) have found no correlation
between clinical features or premature death in GRMD dogs and CK levels. Female dogs
present a variety of clinical signs including generalized weakness, muscle wasting, tremors,
exercise intolerance, gait abnormalities, and limb deformity. Elevation of serum creatine kinase activity may vary (Shelton et al., 2001). The gait abnormalities in GRMD dogs during growth and disease progression using an ambulatory gait analyzer (3D-accelerometers) showed that speed, stride length, total power and force had already significantly decreased ($p < 0.01$) at the age of 2 months. The decrease of stride frequency was a later event, secondarily contributing to the reduction of speed (Barthélémy et al., 2011).

### 3.3.2 Therapeutic approaches using canine model of muscular dystrophy

Papers have been recently published using the canine model of muscular dystrophy to develop various therapeutic approaches such as gene therapy, cell therapy, and pharmaceutical agents. As an animal model for DMD therapy, GRMD dogs were used in preclinical trials examining the transfer of dystrophin gene (Howell et al., 1998), utrophin gene (Cerletti et al., 2003) or oligonucleotides (Bartlett et al., 2000). These therapeutic strategies were all applied to a single muscle after local intramuscular injection. However, dystrophin deficiency appears as a generalized muscle defect, therefore achieving clinically relevant improvement may likely require intravascular delivery of genetic material. Gene therapy using viral vectors has been extensively investigated. Adeno-associated virus (AAV) vectors are the most appropriate tools for viral vector gene therapy because they are nonpathogenic due to a replication defect and have low immunogenicity as well as an effective ability to infect non dividing cells (Nakamura & Takeda, 2011). The intravenous administration of a dystrophin cDNA plasmid in the dystrophin-deficient $mdx$ mouse resulted in significant dystrophin expression (Liu et al., 2001). This gene transfer was carried out on 5-week-old $mdx$ mice diaphragm muscles, in which fibrosis is still minimal. It is not certain that similar gene transfer efficiency would be achieved in heavily compromised muscles, such as those occurring in GRMD and DMD. However, the treatment using myoblast or mesenchymal stem cell implantation in GRMD dogs during the early 1990s did not show improvement of muscle condition, even though other studies had demonstrated success in the $mdx$ mouse. Similarly, hematopoietic stem cell transplantation did not restore dystrophin expression in affected dogs despite promising results in $mdx$ mice (Dell’Agnola et al., 2004). In older GRMD dogs, fibrosis seems to be the major factor influencing microvascular architecture in skeletal muscles. Increasing extent of connective tissue correlated with lower microvessel density and longer intercapillary distance. The fibrosis might create a physical barrier between the capillary contour and the myofiber membrane. Thus, endomysial fibrosis, the hallmark of muscle pathology in DMD patients and GRMD dogs, may compromise intravascular therapeutic trials performed in the late stage of the dystrophic process. Antifibrotic treatment may be a necessary prerequisite to systemic genetic transfer in dystrophin-deficient canine and human muscles (Nguyen et al., 2005). Among these therapeutic strategies, exon skipping using antisense oligonucleotides (AOs) is considered to be one of the most promising therapies for the restoration of dystrophin expression at the sarcolemma in dystrophin-deficient muscle. The therapy involves a multiexon-skipping technique for targeting exons 6 and 8 to convert an out-of-frame mutation into an in-frame mutation using PMOs (Yokota et al., 2009). Kerkis et al. (2008) have reported the transplantation of human immature dental pulp stem cells from baby teeth to GRMD dogs by local and systemic via. Moreover, they have analyzed the efficiency of single and consecutive early transplantation of these cells. Their results show that Human Immature Dental Pulp Stem Cells (hIDPSC) presented significant engraftment in GRMD dog muscles, although human dystrophin
expression was modest and limited to several muscle fibers. Better clinical condition was also observed in the dog which received monthly arterial injections and was still clinically stable at 25 months of age. Systemic myostatin inhibition in GRMD dogs by liver directed gene transfer of a vector designed to express a secreted negative myostatin peptide showed increase in muscle mass in these dogs assessed by MRI (Magnetic Resonance Imaging) and confirmed by muscle histology (Bish et al., 2011).

4. Pre-clinical therapeutic studies using animal models

The availability of standardized operating procedures (SOPs) to unify experimental protocols used to test the effects of new treatment in animal models is a step that will undoubtedly improve the comparability of studies from different laboratories (http://www.treat-nmd.eu/research/preclinical/dmd-sops/). To date, the main attempt to evaluate the relative translational benefit from an animal species to human subjects has focused on the minimal levels of dystrophin protein required for functional stabilization of dystrophic myofibers. Many factors need to be considered. This protective effect will depend not only on the amount of dystrophin protein within an individual myofibers, but also on the extent of distribution within all myofibers, the size of the nuclear domain (how far dystrophin protein extends along the sarcolemma from the myonuclei where the mRNA is generated) and on when during development the dystrophin must be produced (Willmann et al., 2011). This situation was considered by Chamberlain (1997) who concluded from analysis of mosaic transgenic mice and viral vector delivery with suboptimal doses into mdx mice, that >50% of myofibers need to express dystrophin, and that these must accumulate approximately 20% of wild-type levels of dystrophin for a significant correction of the muscle pathology in mice. Factors to consider in the selection of outcome measurements (Determination and evaluation of the results of an activity, plan, process, or program and their comparison with the intended or projected results) for pre-clinical therapeutic studies using mouse model include reproducibility, objectivity, blind assessment, relevance to disease biology (e.g. muscle histology), and similarity of measures in the mdx mice (e.g. locomotion and in vivo muscle strength) to human clinical trials endpoints (e.g. ability to walk and muscle strength testing). Depending on the presumed mechanism of action and the intended target of the experimental agent, additional outcome measures (e.g. to assess cardiac function) may be appropriate (Willmann et al., 2011). Standardized protocols for the assessment of most of the recommended parameters have already been produced by specialized working groups of experts and are reported in brackets (PDFs available http://www.treat-nmd.eu/research/preclinical/dmd-models/). Based on the mechanism of action, different drugs may be more or less effective depending on the age at which treatment is initiated and the time period over which the drug is administered (i.e. treatment duration). Due to the ultimate translational aim of the pre-clinical experiments, it is important to consider the relationship between the age of the mdx mice and possible equivalence in DMD patients. A comparison of developmental stages in mice and humans is described in details elsewhere (Grounds et al., 2008). The recovery score is a tool that can be used to compare different therapies applied to mice or results obtained by different laboratories with the same therapy.

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\text{Recovery score (\%)} = \left( \frac{[\text{wild type}] - [\text{untreated} \text{mdx}]}{[\text{wild type}] - [\text{untreated} \text{mdx}]} \right) \times 100
\]
A score of 100% indicates that the parameter in treated \(mdx\) mice is equal to that of control wild type mice, and 0% indicates that no improvement was obtained relative to untreated \(mdx\) mice (Gillis et al., 2002). Therefore, this calculation represents a tool to evaluate the effective recovery achieved by the treatment tested. Although this implies the need to include a wild type group of mice in any pre-clinical therapeutic study, we encourage the calculation of the recovery score in all studies where this effort is feasible (Willmann et al., 2011). Measurements of muscle strength, joint contractures, and timed function tests were made in dogs to evaluate recovery of muscular function after drugs or gene therapy. The evaluation of muscle by magnetic resonance imaging (MRI) was performed by Kornegay et al. (2010) after single intravenous injection of an AAV9 vector (1.5 \(\times\) 1014 vector genomes/kg) carrying a human codon-optimized human mini-dystrophin gene under control of the cytomegalovirus (CMV) promoter. Earlier, the same research group performed analysis of muscle strength by measuring isometric force decrement after eccentric contraction (Childers et al., 2002) and by measuring the titanic isometric force at the tibiotarsal joint in vivo (Kornegay et al., 1994). The MRI evaluation has several strengths that include studying distribution of pathology, pathophysiology, monitoring of therapies, assessment of heart and diaphragm, and morphometry (Bish et al., 2011).

5. What’s new on exercise training that can guide physical therapy for DMD related to \(mdx\) and GRMD models?

The coding sequence of the dystrophin gene in DMD was discovered and deciphered in 1987 (Koenig et al., 1987). Its discovery has brought hope for a cure of DMD through gene therapy, although it has not happened yet. Several therapeutic strategies are being investigated in developing a cure for this disease. Nowadays, DMD patients have access to therapeutic and supportive care aiming to prevent complications and improve their quality of life. Among them, drug therapy with corticosteroids has been widely studied in DMD and there is some controversy in its use mainly due to its multiple side effects. Nutritional supplements, psychology, occupational and physical therapy are the most used supportive therapies.

Although these therapies cannot lead to a cure of DMD, they should be well investigated because they intend to lead these patients to a better quality of life and to decrease complications of their degenerative and progressive illness. Physical therapy (PT) has been used to reduce muscular, skeletal, cardiac, and vascular abnormalities which develop in association with muscle strength loss (Gaiad et al., 2009). The main objective of such motor therapy is the prevention of muscle contractures and bone deformities (Strober, 2006). However, motor PT approaches have yielded controversial recommendations (Carter et al., 2002) and there is no consensus regarding the type and intensity of physical therapy (Cup et al., 2007). The recommendations often include exercise therapy to improve or preserve muscle strength or endurance and aerobic capacity to prevent the secondary problems of contractures, pain and fatigue. According to the review published by Grange & Call (2007) the same exercise used to increase muscle strength and endurance in normal individuals can exacerbate muscle damage in a dystrophic muscle. The authors suggested that a threshold must be defined to guide suitable exercise prescription for DMD patients (Grange & Call, 2007; Cup et al., 2007). Kimura et al. (2006) showed in a case report that immobility could
reduce muscle fiber necrosis in muscular dystrophy cases. They reported a case of a three-year-old boy with a diagnosis of spina bifida and DMD. A muscle biopsy on this patient showed that necrosis and regeneration of muscle fiber was more prevalent in the biceps brachii (with normal movement) than in the gastrocnemius muscle (without movement). The authors suggest that immobility reduces muscle fiber necrosis in dystrophin-deficient muscle and attribute this characteristic to the movement restriction in the lower extremity of this patient. Reduced physical activity by mdx mice could theoretically be a muscle sparing strategy (Landisch et al., 2008). On the other hand, authors suggest that patients should undergo some physical activity in order to avoid muscle disuse associated with the intrinsic loss of muscle mass related to the disease progression (Ansved, 2005; Eagle, 2002, McDonald, 2002 and Caromano, 1999). Once physical therapies display an important role on DMD patients’ quality of life, the prescription of its parameters must be evidence-based and well documented. In the last few years, some researchers have brought some insights into the subject of therapeutic exercise using experimental animal models for DMD. There are many publications on gene, cellular and pharmacological therapies using the DMD animal models, mdx-mice and GRMD dogs, but these models also have much remaining to contribute to the therapeutic exercise approach (Mercuri et al., 2008). The contribution of animal models, mainly the GRMD model, on prescription of type, frequency and modality of PT was also suggested by Grange & Call (2007).

According to Markert et al. (2011) the effect of exercise on DMD has poorly researched parameters (frequency, intensity, time and type) and until now it is unknown whether therapeutic exercise is beneficial or detrimental to dystrophic skeletal muscle. Despite the difference between mdx-mice and humans DMD patients in terms of regenerative ability and compensatory protein expression, this model is still the most used one to investigate exercise prescription for this population. Reasons for the wide use of mdx-mouse, despite its limitations, are well detailed in the first topic of this chapter.

In 2002, Eagle published a consensus about exercise recommendations for patients with neuromuscular disorders. Among them, they suggest maintenance or improvement of muscle stretch, improvement of functional ability and use of nocturnal orthesis to avoid contractures. More recently, Bushby et al. (2010) have brought some recommendations for management of rehabilitation of DMD patients. Regarding stretching, authors suggested that during ambulatory and non-ambulatory phases a regular active, active-assisted and/or passive stretching to prevent or minimize contractures should be performed a minimum of 4–6 days per week for any specific joint or muscle group. The authors agree that only limited research has been carried out on type, frequency and intensity of exercise for DMD. Although, their recommendations are in accordance with the known pathophysiology and animal studies which show contraction-induced muscle injury in dystrophinopathies. According to these authors, PT should avoid high-resistance strength training and eccentric exercise due to the knowing contraction-induced muscle fiber injury. They recommend that patients should undergo regular submaximum functional strengthening activity, including a combination of swimming-pool exercises and recreation-based exercises in the community. Based on these questions and recommendations of exercise prescription on DMD, some recent publications using animal models will be detailed in order to highlight the contribution of these models to PT prescription and recommendations.
5.1 PT exercise prescription

Kumar & Boriek (2003) studied the effects of passive mechanical stretch on the activation of nuclear factor-kappaB (NF-kB) pathways in skeletal muscles from normal and mdx mouse. Nuclear factor-kappaB (NF-kB) is a transcription factor which regulates genes involved in the inflammatory and acute stress response. They found that this factor in the diaphragm muscle was increased by the application of mechanical stress in a time-dependent manner. Their results show that one of the stretch exercises, mechanical stretch, activates the classical NF-kB pathway and it seems to be more active in DMD muscle than control muscle. Another study investigated the morphological effect of two different protocols of passive stretch on the immobilized soleus muscle of healthy rats (Gomes et al., 2007). They have analyzed the morphology and the proportion of fibers types (I, II and C) of four groups: control, immobilized, immobilized and stretched every 3 days, and immobilized and stretched every 7 days. The passive stretch was 40 minutes long. They found that signs of cell degeneration were more intense in the group immobilized and stretched three times a week. The authors suggest that the passive stretching applied to the soleus muscle during immobilization induce muscle fiber injury, suggesting that this therapeutic tool should be applied carefully to disused muscles, such as dystrophic ones. Even if stretch exercises are widely used in PT, its real implication on muscle structure and morphology must be better investigated, especially on dystrophic muscle. Exercise-induced muscle injury in healthy humans occurs mainly after unaccustomed exercise, particularly if the exercise involves a large amount of eccentric (muscle lengthening) contractions (Clarkson & Hubal, 2002). The exact mechanism of this injury remains unknown, but it has been ascribed to mechanical disruption of the fiber, and subsequent damage is linked to inflammatory processes and to changes in excitation-contraction coupling within the muscle. According to Childers et al. (2002) a cycle of weakness, stretch, damage, and further weakness might explain observations of selective involvement of eccentric-contraction in dystrophic muscles. This mode of exercise has not been widely recommended for DMD patients (de Araujo Leitão et al., 1995; Eagle, 2002; Ansved, 2005; Cup et al., 2007; Bushby et al., 2010). This exercise-induced dystrophic muscle damage due to eccentric contraction was also attested in the mdx-mouse and GRMD models (Childers et al., 2002; Tegeler et al., 2010; Mathur et al., 2010) and humans DMD patients (Marqueste et al., 2008). Childers et al. (2002) have investigated the eccentric injury in dystrophic GRMD dogs. They have found that dystrophic canine flexor muscles of hindlimbs are more susceptible than normal ones to eccentric contraction-induced injury analyzing muscle torque three days after the eccentric contraction. Clinical implications of this study show that dystrophic muscle is preferentially injured by mechanical stress. Another study by the same group of Childers and co-authors (Tegeler et al., 2010) has shown that dystrophic muscles of GRMD dogs undergo damage immediately after the eccentric contraction. Mathur et al. (2010) studied the effects of downhill and horizontal running on the magnetic resonance imaging (MRI) in mdx-mouse. A higher percentage of pixels with elevated $T_2$ were observed in mdx-mouse compared with controls pre-exercise, which suggest muscle damage. Moreover, downhill running which is dependent on lengthening muscle contraction induced acute changes in mdx-mouse muscle after exercise. Also using the MRI technique, Marqueste et al. (2008) investigated the effect of acute and successive bouts of downhill running on muscle performance of healthy rats. Their results show less muscle injury effect due to repetition of exercise bouts at a low frequency (one session per week) probably due to muscle adaptation and to the
inflammatory phase occurring for a week after a single eccentric exercise bout. Another interesting result of this study is the specificity of the stimulated muscle. Soleus and gastrocnemius muscle have shown different responses to lengthening contractions on MRI analysis. The author suggested that this muscle specificity might be linked to different anatomical properties, such as fiber pennation angles, typology and/or exhausting nature of the downhill running sessions. This last result is quite interesting because in PT sessions it is more difficult to isolate a single muscle as it is possible in an experimental model. So, care must be taken when translating experimental data to human therapy. On the other hand, it is important to keep in mind that a single therapeutic exercise can influence different muscles of the same limb in different manners. In agreement with the results presented by Kimura et al. (2006) that immobility can lead to preservation of dystrophic muscle in humans DMD Mokhtarian et al. (1999) investigated whether immobilization of the hindlimbs of the mdx-mice would prevent the occurrence of muscle degeneration. The authors clarify that dystrophin-deficient skeletal muscle of mdx-mice undergo their first rounds of degeneration-regeneration at the age of 14-28 days. They have mechanically immobilized the hindlimbs of 3 week old mdx-mice to restrain the Soleus and Extensor digitorum longus muscles in the stretched or shortened position. The position had no influence in the final result that showed low percentage of regenerated myofibers in Soleus and Extensor digitorum longus muscles when compared to the same muscles of the contralateral limb. Regenerated myofibers was attested by the presence of central nuclei in dystrophic fibers. According to these authors (Mokhtarian et al. 1999), limb immobilization prevents the occurrence of the first round of myofibers necrosis in mdx-mice and reinforces the idea that muscle contractions play a role in the skeletal muscle degeneration of dystrophin-deficient muscles. Even though some authors have suggested that restriction of movement prevent cycles of degeneration and regeneration in dystrophic muscle, we should consider that restriction of movement leads to muscle disuse and has drastic consequences to the patients, e.g. contractures, bone deformities, cardiovascular disease, obesity, and osteoporosis over time. It is imperative that a balanced threshold of therapeutic exercise must be well-defined and that more research on this subject is necessary. Over the last ten years, the number of papers aiming to define the threshold of PT prescription has grown. Outcome measurements of these investigations generally use morphological features of dystrophic muscle, enzymatic, protein localization and quantification, and/or biomechanical analyses. Podhorska-Okolow et al. (1998) have studied apoptosis of myofibers and the presence of satellite cells in skeletal muscle of healthy mice after spontaneous exercise wheel running. Exercised mice have run for sixteen hours and were sacrificed after a period of 6 or 96 hours. For analysis, the authors have counted the numbers of myofibers with central nuclei, (indicative of regenerated myofibers), performed immune histochemistry, quantified by Western blot proteins related to cell death, and used electron microscopy to find satellite cells. Their results show that spontaneous running in sedentary mice increases the number of apoptotic nuclei in adult muscle fibers and in endothelial cells. These results suggested that voluntary exercise plays an important and detrimental role on disused muscle, which can be applied to dystrophic muscle. In general, any study that used voluntary wheel running detrimentaly affected the hindlimbs of mdx mice (Landisch et al., 2008). Studies that have investigated non-voluntary exercise have shown muscle injury in mdx model mainly when animals are running downhill. Landisch et al. (2008) investigated whether a voluntary endurance type of exercise could be beneficial to dystrophic muscle;
assessing cellular adaptations that typically occur in response to endurance exercise. They hypothesized that a voluntary endurance type of exercise would improve mdx mouse muscle to the same extent that exercise improves healthy muscle. They analyzed the histological features by counting the central nuclei, fiber types, capillarity and mitochondrial enzymes activity. In part their hypothesis was true, except that mitochondrial adaptations did not occur in mdx mouse muscles. They suggest that this type of exercise can improve skeletal muscle weakness and fatigue as well as prevent secondary consequences of the inactivity. In 2009, Gaiad et al. reported the effect of the free walking activity during 24 weeks/3 times per week during 45 minutes in GRMD dogs. Muscle collagen area was quantified by histomorphometry and collagen types I, III and IV were localized by immunohistochemistry. Passive joint range of motion (ROM) was measured to investigate the secondary consequences of the exercise on the muscle skeletal system. There was an improvement on tarsal ROM in dogs of the treated group. Muscle collagen area was different between the groups after treatment, and an increasing trend in these values was observed in non-treated group. This suggests a higher muscle fibrosis in dogs that have not undergone exercise. Collagen types I and III were observed in both groups. The authors suggest that the modality of free walking activity can improve ROM without increasing muscle fibrosis in dystrophic dogs. Studying markers of oxidative stress in skeletal muscle of mdx-mouse, Kacsor et al. (2007) applied low intensity training through treadmill running 30 minutes per day, 2 times per week during 8 weeks. They considered 9 meters per minute as a training of low intensity based on previous studies with the same animal model. This intensity of training has not been able to provoke any adaptation on healthy mice (wild type). In mdx-mouse, low intensity training has lead to physiologic adaptations evidenced by decrease of markers of oxidative stress. New studies should be conducted following this same intensity of training for new analyses. The investigation of creatine quinase (CK) enzyme, muscle fibrosis and morphology, as well as clinical and behavioral features of these animals can elucidate aspects of the best threshold of exercise for DMD patients. With this focus, van Putten and co-authors (2010) have investigated some functional tools on the disease progression of 4-week-old mdx mice using CK analysis and muscle morphology (measuring percentage of fibrotic/necrotic area). They suggest four functional tests (forelimb grip strength, rotarod analysis, and two and four limb hanging wire) that may be suitable for short-term functional evaluation of therapeutic approaches in the mdx mouse without affecting dystrophy progression. Aiming to validate parameters of functional evaluation on pre-clinical trials for DMD, Gaiad et al. (2011) investigated the use of PT assessment tools to evaluate disease progression and phenotype variability in GRMD dogs. In this study the outcome measurements were passive joint range of motion, limb and thorax circumferences, weight, CK analysis, and physical features of each of the dogs using a physical exam score previously described by Thibaud et al. (2007). The author have described the physical and behavioral features of 11 dystrophic dogs during 9 months and these PT measurements tools were considered reliable and useful to evaluate disease progression in GRMD dogs.

6. Conclusion

In the last ten years, research on exercise prescription using mdx mouse and GRMD dogs has increased and much of the research were discussed here. We have much to discover about the effects of type, frequency, and intensity of therapeutic exercise on DMD.
Regarding the type of exercise, it is possible to say that eccentric/lengthening contraction has no beneficial effects on dystrophic muscle, and that concentric or aerobic training should be better investigated. Free or voluntary activities seem to prevent secondary consequences of disuse while not leading to detrimental effects. The morphological and clinical effects of the intensity of exercise must be well investigated once it seems near the threshold that must still be defined. Low intensity training leads to beneficial effects though the parameters of this intensity must also be well defined and afterwards, translated to human patients. The harmonization of assessment tools for exercise research with both animal models is another important point on this subject. The definition of assessment tools to pre-clinical trials on animal models will enable the advancement of research on this subject and bring knowledge to the prescription of beneficial therapeutic exercise to DMD patients.

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With more than 30 different types and subtypes known and many more yet to be classified and characterized, muscular dystrophy is a highly heterogeneous group of inherited neuromuscular disorders. This book provides a comprehensive overview of the various types of muscular dystrophies, genes associated with each subtype, disease diagnosis, management as well as available treatment options. Though each different type and subtype of muscular dystrophy is associated with a different causative gene, the majority of them have overlapping clinical presentations, making molecular diagnosis inevitable for both disease diagnosis as well as patient management. This book discusses the currently available diagnostic approaches that have revolutionized clinical research. Pathophysiology of the different muscular dystrophies, multifaceted functions of the involved genes as well as efforts towards diagnosis and effective patient management, are also discussed. Adding value to the book are the included reports on ongoing studies that show a promise for future therapeutic strategies.

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