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Review

Comparison of Experimental Protocols of Physical Exercise for *mdx* Mice and Duchenne Muscular Dystrophy Patients

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Abstract.

Duchenne Muscular Dystrophy (DMD) is caused by mutations in the gene coding for dystrophin and leads to muscle degeneration, wheelchair dependence and death by cardiac or respiratory failure. Physical exercise has been proposed as a palliative therapy for DMD to maintain muscle strength and prevent contractures for as long as possible. However, its practice remains controversial because the benefits of training may be counteracted by muscle overuse and damage.

The effects of physical exercise have been investigated in muscles of dystrophin-deficient *mdx* mice and in patients with DMD. However, a lack of uniformity among protocols limits comparability between studies and translatability of results from animals to humans. In the present review, we summarize and discuss published protocols used to investigate the effects of physical exercise on *mdx* mice and DMD patients, with the objectives of improving comparability between studies and identifying future research directions.

Keywords: Duchenne muscular dystrophy, mice, *mdx,* exercise

ABBREVIATIONS

ACC	Acetyl-CoA carboxylase
β-HAD	β-Hydroxy acyl-CoA dehydrogenase
DIAPH	Diaphragm
DMD	Duchenne muscular dystrophy
EDL	Extensor digitorum longus
ERK1/2	Extracellular signal-regulated kinase 1/2
GAST	Gastrocnemius muscle
HIF-1	Hypoxia-inducible factor-1
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MHC2a	Myosin heavy chain 2a
NADPH oxidase	Nicotinamide adenine dinucleotide phosphate-oxidase
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1α
PPAR-γ	Peroxisome proliferator-activated receptor gamma
QUAD	Quadriceps muscle
ROS	Reactive oxygen species
SDH	L-Sorbose 1-dehydrogenase
Sirt1	Sirtuin 1

INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked muscular disease caused by mutations in the *DMD* gene, which codes for dystrophin, a cytoskeletal scaffolding protein important in signalling and muscle stability. An absence of dystrophin results in muscle degeneration and death by cardiac or respiratory failure. Symptoms usually appear in boys at 2–5 years of age, manifesting as difficulty standing unaided. As muscle wasting progresses, patients

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experience increasing difficulty in performing daily activities, become wheelchair-dependent between 11 and 13 years of age [1], and die before age 30 [2].

Therapeutic approaches for DMD include reducing inflammatory symptoms with glucocorticoids [3], correcting scoliosis by surgical intervention [4] and aiding respiratory function using mechanical ventilation [5]. Recently, restoration of dystrophin expression has been achieved by ribosomal readthrough of premature stop codons [6] and exon-skipping therapy [7].

Regular physical exercise stimulates muscle protein synthesis and mitochondrial biogenesis [8]. Exercise has therefore been proposed as treatment for DMD, to maintain muscle strength and prevent contractures [9, 10]; however, this recommendation has not been unanimously accepted because exercise might damage dystrophic muscles [11]. The five mechanisms rendering dystrophin-deficient muscles vulnerable to exercise (reviewed elsewhere [12]) are the weakening of the sarcolemma, increased calcium influx and oxidative stress, recurrent muscle ischemia and aberrant signalling to surrounding tissues such as nerves or cells of the immune system. A mechanistic basis for exercise intolerance [13] and recommendations for the management of DMD [9, 14] have also been reviewed. The lack of uniformity between protocols for exercise of dystrophin-deficient muscles, however, has been pointed out [15], but not reviewed.

Here, we summarize and discuss studies addressing physical training in the context of DMD. We focus on articles describing the effects of exercise in dystrophin-deficient *mdx* mice and in patients with DMD (Fig. 1A).

EXPERIMENTAL PROCEDURES FOR THE STUDY OF EXERCISE IN MDX MICE

A murine model for DMD: The mdx mouse

The dystrophin-deficient *mdx* mouse is the most common animal model for DMD. This mutant bears a spontaneous nonsense mutation in exon 23 of the dystrophin gene [16]. Its phenotype is mild compared to the symptoms of DMD in patients. This difference in disease severity between mice and humans arises from differences in size, mechanical loading and lifespan [17, 18]. First, the size difference between mice and humans is 2000–3000 folds. According to the square/cube rule, the mechanical stress experienced by an organism increases with the cube of the linear size; therefore, the difference in mechanical stress experienced by mice and humans is not linear but exponential [18]. Second, humans have a bipedal posture, meaning that the body weight is distributed between the lower limbs and the backbone [17], rather than across four limbs. Third, the difference in lifespan means that humans endure more degeneration–regeneration cycles than mice, resulting in extended muscle deterioration [17].

Compared to DMD patients, *mdx* mice recover from the progressive muscle wasting and show much less accumulation of connective and adipose tissue. The necrotic process persists throughout their life, but the regenerative capacity does not decline until an advanced age (>65 weeks) [19, 20]. These differences must be considered in investigations of physical exercise on dystrophic muscle across species.

Exercise studies in mdx mice

A literature search in PubMed, using the keywords "*mdx* mice" and "exercise" was performed on 25 May 2015. A total of 175 articles were examined, of which 57 investigated the effects of physical exercise, and were selected to form the basis of this part of the review. Of these studies, 37 reported only the negative effects of physical exercise, 15 only beneficial effects, and 5 both negative and positive effects. Studies were classified according to experimental protocol and are listed in Table 1.

The purpose of exercise studies in mdx mice

Physical training of *mdx* mice served three purposes: assessing the physical capacities of the mice; investigating the effects of training on dystrophic muscles; worsening the phenotype before assessing the effects of a drug (Fig. 1B). Depending on the study goals, researchers used acute exercise protocols to reveal immediate effects (Table 1A), or chronic protocols to study long term effects (Table 1B). The mildest methods used included swimming, voluntary wheel running, rotarod or low-speed treadmill (<9 m/min). The hardest ones employed high-speed treadmill (>12 m/min) or downhill running. Further variables included the age of the mice and training duration. Voluntary and brief (<30 min per session) exercise of young mice (<8 weeks old) was defined as low intensity training [30, 59], whereas exercise of older mice, or under intensive or prolonged conditions (>30 min), was defined as high intensity training [63].

Assessment of the physical capacities of mdx mice

Measuring the running capabilities of *mdx* mice using voluntary wheel or downhill running is a simple

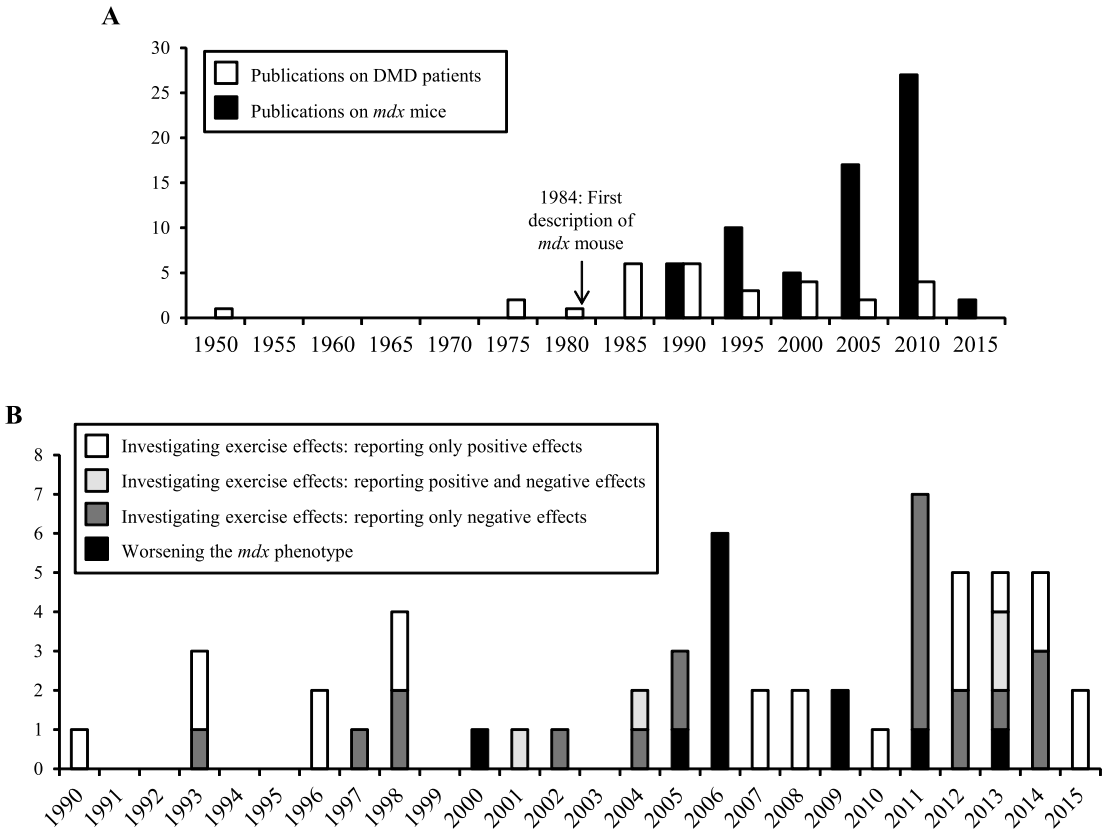

Fig. 1. Frequency of publications reporting the effects of physical exercise in *mdx* mice and DMD patients. (A) Publications describing the effects of physical exercise on *mdx* mice and DMD patients per 5 years. (B) Publications describing the effects of physical exercise on *mdx* mice per year, as a function of research objective.

way to assess their physical abilities. All studies using voluntary wheel running followed the same protocol, namely measuring the total running distance. High inter-individual variability was reported: 4-week-old *mdx* mice ran 0.5 [38] to 9 km [36] per day; 6-week-old mice ran 2 km/day [36], while 10-week-old mice ran 0.03 ± 0.005 to 4.48 ± 0.96 km/day [49]. Performance of *mdx* mice peaked at 8 weeks of age (5.8 [39] to 9 km [36] per day) and decreased to 2.6 km/day at 10 weeks [39] or to 5 km/day at 14 weeks of age [36]. In consequence, a large number of animals should be used when performing experiments with *mdx* mice. The downhill exercise study [76] adapted the 6 minute walking test, used for patients with DMD, to allow comparison of performance between 10-week-old wild type and *mdx* mice. Results show that wild type mice run an average of 500 m in 6 minutes, but *mdx* mice run only 300 m.

Measuring *ex vivo* the properties of specific muscles is another way to assess physical capacity in mice. However, muscle type and choice of protocol varied too much between studies to allow comparison (Table 1). The parameters assessed after voluntary wheel running included tetanic stress and stiffness of the extensor digitorum longus [36], grip strength and specific tetanic force of the soleus [38], maximal isometric torque and fatigue resistance of the plantar flexor [39] or specific and absolute maximal force of the tibialis anterior muscle [41]. Overall experiments reported that phenotype of hind limb and diaphragm of mdx mice improved when training began before 7 weeks old [45], but worsened if exercise began later period [47]. However, worsening of heart phenotype was observed when training started at 4 weeks old mdx mice [41] In spite of these general considerations, important differences in outcome can be observed between the studies, corroborating the need for a common protocol for measurements in individual muscles after exercise.

Different muscles in *mdx* mice, such as the hindlimb muscles or the diaphragm, are not equally affected by an absence of dystrophin; for example, hindlimb muscles show more necrotic events than the diaphragm, but less fibrosis, following regeneration [78]. Conse-

Table 1
Effects of physical exercise on *mdx* mice

1.A Acute exercise				
Age	Period	Protocol[a]	Effects	Reference
Swimming exercise				
4 weeks	1 time	20 min	**Mol.** ↑ *Membrane breakdown in TA*	Bouchentouf et al., 2006 [21]
Voluntary running				
4 weeks	24 hours	At will	**Mol.** ↑ *Membrane leak in QUA, GAST, TA and DIA*	Archer et al., 2006 [22]
12 weeks	16 hours	At will	**Mol.** ↑ *Myofiber apoptotic nuclei;* ↑ *Apoptosis of endothelial cells;* ↓ *Expression of Bcl-2;* ↑ Expression of Bax, Fas, ICE-family and ubiquitin in TA	Podhorska-Okolow et al., 1998 [23]
Treadmill running				
12 weeks	1 time	30 min, **12m/min**	**Clin.** ↑ Serum Creatine Kinase **Mol.** ↑ Necrosis; ↑ *Thiol oxidation;* ↑ *IL-6 mRNA in QUA*	Terrill et al., 2011 [24]
Downhill running				
7 weeks	1 time	10°: 90 min, **8–16 m/min**	**Mol.** ↑ *Membrane breakdown in recto femoris*	Quinlan et al., 2006 [25]
7 – 10 weeks	1 time	17°: 45 min, **10 m/min**	**Clin.** ↓ *Isometric force of EDL* **Mol.** ↑ *Membrane breakdown in EDL*	Whitehead et al., 2006 [26]
12 weeks	1 time	16°: 5 min, **0,6 m/min**	**Mol.** ↓ *Expression of FGF in triceps muscle*	Clarke et al., 1993 [27]
32 – 56 weeks	1 time	16°: 5 min, **10 m/min**	**Clin.** ↑ *Serum Creatine kinase level 1 hour after exercise*	Vilquin et al., 1998 [28]
60 weeks	1 time	14°: 45 min, **10 m/min**	**Clin.** ↑ *Transverse relaxation time constant (T2) in lower hind limbs*	Mathur et al., 2011 [29]
1.B Chronic exercise				
Age	Period	Protocol[a]	Effects	Reference
Swimming exercise				
4 weeks	4 weeks	30 min	**Clin.** ↑ Grip strength **Mol.** ↓ Carbonylation and ↑ Expression of proteins of contraction and energy metabolism; ↑ Expression of slow and fast type Troponin T and Myosin binding protein C in GAST	Hyzewicz et., 2015 [30]
4 weeks	56 weeks	30 min	**Clin.** ↓ Fatiguability of EDL	Wineinger et al., 1998 [31]
5 weeks	15 weeks	5 min +5 min/day to 2 hour	**Mol.** ↓ Sensitivity of soleus to Ca^{2+} and Sr^{2+} in EDL	Lynch et al., 1993 [32]
5 weeks	15 weeks	5 min +5 min/day to 2 hour	**Clin.** ↑ Tension, relaxation and fatigue resistance of soleus and EDL **Mol.** ↑ Fiber I type in EDL	Hayes et al., 1993 [33]
6 – 8 weeks	1 week	30 min	**Mol.** ↑ *Muscle hypoxia in GAST and TA*	Matsakas et al., 2013 [34]
96 weeks	10 weeks	Until exhaustion	**Clin.** ↑ Relative tetanic tension of soleus and EDL	Hayes et al., 1998 [35]
Age	Period	Protocol[a]	Effects	Reference

Table 1
(continued)

Voluntary running				
3 weeks	3 weeks	At will	**Clin.** ↑ Tetanic stress; ↑ Stiffness of EDL **Mol.** ↑ I and IIa fiber type; ↓ IIb fiber type; ↑ Total contractile proteins in EDL; ↑ Anti-oxidant capacities; ↑Activity citrate synthase in heart; ↑ Activity β-hydroxy acyl-CoA dehydrogenase (β HAD) in QUAD and heart	Call et al., 2008 [36]
4 weeks	4 weeks	At will	**Clin.** ↑ Soleus muscle mass **Mol.** ↑ I and IIa fiber type; ↓ IIb fiber type	Landisch et al., 2008 [37]
4 weeks	12 weeks	At will	**Clin.** ↑ Grip strength; ↑ Specific tetanic force of soleus **Mol.** ↑ Expression of vinculin in soleus; ↑ Expression of β-dystroglycan in GAST	Call et al., 2010 [38]
4 weeks	12 weeks	At will	**Clin.** ↑ *Heart mass;* ↑ Maximal isometric torque and fatigue resistance of planta flexor **Mol.** ↑ Activity of citrate synthase and β-HAD; ↑ Expression of COX IV in GAST	Baltgalvis et al., 2012 [39]
4 weeks	12 weeks	At will	**Mol.** ↑ *Expression of utrophin in QUAD*	Gordon et al., 2014 [40]
4 weeks	16 weeks	At will	**Clin.** ↑ Specific and absolute maximal force of TA; ↓ *Left ventricular function, ejection and shortening fractions in heart* **Mol.** ↑ mRNA expression of MHC2a in TA	Hourdé et al., 2013 [41]
4 weeks	16 weeks	At will	**Clin.** ↑ Force output of soleus and plantaris; ↑ EDL fatigue resistance **Mol.** ↓ Fiber type IIa; ↑ Fiber type I in EDL	Hayes et al., 1996 [42]
4 weeks	52 weeks	At will	**Clin.** ↑ Absolute force of plantar flexor; ↑ Mass of GAST and soleus; ↑ Tetanic force of soleus; ↑ Left ventricular functions, end-diastolic, systolic volume in heart; ↓ *Specific tension of DIAPH*	Selsby et al., 2013 [43]
4 weeks	52 weeks	At will	**Clin.** ↑ Normalized active tension in DIAPH	[44] Dupont-Versteegden, 1996
6 -7 weeks	7 weeks	At will	**Mol.** ↑ Expression of PGC1-α, LC3; ↑Activity of citrate synthase, SDH, cytochrome C in GAST	Hulmi et al., 2013 [45]

Table 1
(continued)

7 weeks	4 weeks	At will	**Clin.** ↑ *Dilatation of ventricles;* ↓ *Size lateral ventricular walls;* ↑ *Sign of dystrophin-related cardiomyopathy and cardiac fibrosis in heart*	Costas et al., 2010 [46]
8 weeks	4 weeks	At will	**Clin.** ↓ Interstitium space; ↑ Cross sectional area in triceps bracialis **Mol.** ↑ *Ubiquitinated proteins;* ↑ p-AMPK α and p-ACC/ACC ratios in triceps bracialis	Bueno Júnior et al., 2012 [47]
10 weeks	2 weeks	At will	**Mol.** ↑ *Necrosis in QUA*	Hunt et al., 2011 [48]
10 – 12 weeks	1 weeks	At will	**Mol.** ↑ *Necrosis in QUA and GAST;* Insuffisant resting increases damages	Smythe et al., 2011 [49]
12 weeks	4 weeks	At will	**Clin.** ↑ *Kyphosis* **Mol.** ↑ *Fibrosis in erector spinae*	Brereton et al., 2012 [50]
24 weeks	12 weeks	At will	**Clin.** ↑ Cross sectional area of soleus	Mangner et al., 2012 [51]
28 weeks	4 weeks	At will	**Clin.** ↑ Absolute maximal force of female *mdx* mice; No sign of cardiomyopathy	Ferry et al., 2015 [52]
Age	Period	Protocol[a]	Effects	Reference
Treadmill running				
4 weeks	4 – 8 weeks	30 min, **12m/min**	**Clin.** ↓ *Forelimb strength;* ↑ *Degenerative area in GAS*	De Luca et al., 2005 [53]
4 weeks	4 – 8 weeks	30 min, **12m/min**	**Clin.** ↓ *Forelimb strength* **Mol.** ↓ Cl^- *conductance of DIA and EDL;* ↓ *voltage threshold for contraction of EDL;* ↑ *necrotic fibers in TA*	De Luca et al., 2003 [54]
4 weeks	4 – 8 weeks	30 min, **12m/min**	**Clin.** ↓ *Strength of EDL* **Mol.** ↓ Cl^- *conductance*	Burdi et al., 2006 [55]
4 weeks	4 – 8 weeks	30 min, **12m/min**	**Clin.** ↑ *Plasma ROS*	Burdi et al., 2009 [56]
4 weeks	4 – 8 weeks	30 min, **12m/min**	**Mol.** ↑ *Resting cytoplasmic* $[Ca^{2+}]$; ↑ *Sarcolemmal permeability in EDL*	Fraysse et al., 2004 [57]
4 weeks	6 weeks	30 min, **9m/min**	**Mol.** ↓ *Mitochondrial oxygen consumption;* ↑ *Lipid peroxydation;* ↑ *Lipofusin deposition;* ↓ *Quantity of vitamin E;* ↑ Activity glutathione peroxidase in QUA and GAS	Faist et al., 2001 [58]
4 weeks	8 weeks	30 min, **9m/min**	**Mol.** ↓ Malondialdehyde level; ↓ Total protein carbonylation in GAS	Kaczor et al., 2007 [59]
4 weeks	8 weeks	30 min, **9m/min**	**Clin.** ↓ Creatine kinase level	Hall et 2007 [60]
4 weeks	12 weeks	30 min, **12m/min**	**Mol.** ↑ *Fibrosis in QUAD*	van Putten et al., 2012 [61]
4 weeks	12 weeks	30 min, **12m/min**	**Clin.** ↓ *Forelimb strength* **Mol.** ↓ *mRNA of PGC1-α, Sirt1, PPARγ, Bnip3, HDAC5, SERCA2, FST, MYOG in GAS*	Camerino et al., 2014 [62]

Table 1
(continued)

8 weeks	4 weeks	30 min, **12m/min**	**Clin.** ↓ *Forelimb strength* **Mol.** ↑ Necrosis; ↑ *Il-1β, Il-6 mRNA; ↑ Thiol oxidation in QUA, triceps, DIA and TA*	Radley-Crabb et al., 2011 [63]
8 weeks	5 weeks	30 min, **12m/min**	**Mol.** ↑ *Collagen III, fibronectin deposition in GAS, pectoralis, TA, DIA, QUA and triceps*	Rocco et al., 2014 [64]
8 weeks	24 weeks	30 min, **12m/min**	**Clin.** ↓ *Net force* **Mol.** ↑ *Collagen, fibronectin deposition in GAS*	Morales et al., 2013 [65]
10 – 77 weeks	10 weeks	60 min, **9m/min**	**Mol.** ↑ *Necrosis in plantaris*	Zeman et al., 2000 [66]
12 weeks	4 weeks	30 min, **12m/min**	**Clin.** ↓ *Forelimbs tetanic force; ↑ Serum Creatine Kinase level* **Mol.** ↑ Necrosis in QUA, Triceps, DIA and TA; ↑ *Thiol oxidation; ↓ TNF-α mRNA in QUA*	Terrill et al., 2011 [24]
12 – 20 weeks	4 – 12 weeks	30 min, **12m/min**	**Mol.** ↑ *Fibrosis; ↑ CollagenIII, fibronectin deposition; ↑ Expression of P-Smad2/3; ↑ TGFβ1, CTGF mRNA in TA*	Pessina et al., 2014 [67]
Rota-rod training				
8 weeks	6 weeks	–	**Clin.** ↓ Necrotic area in GAS and QUA **Mol.**↓ Expression connexin 39	Frinchi et al., 2014 [68]
Age	Period	Protocol[a]	Effects	Reference
Downhill running				
3 weeks	3 weeks	18°, 25 min, **4 m/min**	**Clin.** ↑ Twitch tension, tension development and relaxation of soleus **Mol.** ↓ Necrosis; ↓ Centrally nucleated fibers in soleus and EDL	Fowler et al., 1990 [69]
4 weeks	3 days	10°, 10 min, **10 m/min**	**Mol.** ↑ *Muscle damages in TA*	Anderson et al., 2006 [70]
4 weeks	6 weeks	16°: 20 min, **12 m/min**	**Clin.** ↓ *Grip strength*	Bizario et al., 2009 [71]
8 weeks	3 days	15°: 10 min, **10 m/min**	**Mol.** ↑ *Membrane breakdown in lower limb and DIAPH*	Brussee et al., 1997 [72]
6 weeks	10 weeks	7°: 60 min, **23 m/min**	**Mol.** ↓ *IGF1 mRNA in soleus, GAS, TA and QUA*	Okano et al., 2006 [73]
6 weeks	10 weeks	7°: 60 min, **23 m/min**	**Mol.** ↑ *Phosphorylation of ERK1/2, p38 and JNK2 in GAST*	Nakamura et al., 2004 [74]
6 weeks	10 weeks	7°: 60 min, **23 m/min**	**Clin.** ↑ *Heart weight* **Mol.** ↑ Infiltration of inflammatory cells; ↑ Fibrosis and adipose tissues; ↑ *ERK1/2 and calcineurin expression;* ↑ *Phosphorylation of p38 MAPK in heart*	Nakamura et al., 2002 [75]

Table 1
(continued)

10 weeks	2 weeks	15°: 10 min, **15 m/min**	**Clin.** ↓ *Strength of EDL* **Mol.** ↑ *Exercise-induced myoglobinuria;* ↑ *Oedema and inflammation in GAS and QUA*	Kobayashi et al., 2011 [76]
24 weeks	7 weeks	15°: 60 min, **17 m/min**	**Clin.** ↓ *Grip strength* **Mol.** ↑ Fibrosis in DIA and biceps brachii; ↑ *Expression of TGFβ1 in biceps brachii and heart*	Taniguti et al., 2011 [77]

Normal words signal a positive effect of exercise. Underscored words signal a neutral effect of exercise. *Italic words signal a negative effect of exercise.* **Clin.** signals an observation at the physiological/clinical level. **Mol.** signals an observation at the molecular level. [a]Description of experimental protocol with duration of exercise, speed or slope value if appropriate. Abbreviations: *DIA Diaphragm; EDL Extensor Digitorum Longus; FGF Fibroblast Grown Factor; GAST Gastrocnemius; QUA Quadriceps; ROS Reactive Oxygen Species; TA Tibialis Anterior.*

quently, studies should investigate different muscles simultaneously. However, most studies focused on the effects of exercise on hindlimbs; others investigated the diaphragm [43, 44, 77] or the heart [38, 39, 41, 43, 46, 75]. The hindlimbs and the diaphragm of 4-week-old *mdx* mice tolerate the effects of voluntary running well (Table 1B), whereas necrosis and fibrosis occur after 10 weeks of age. Conversely, cardiac complications appear after voluntary running in 4-week-old *mdx* mice, with increased cardiac mass [39] and impaired function [41, 43]. Effects of swimming on cardiac function have only been investigated preliminarily. Our own results have shown that 30 minutes daily swimming from 8 to 16 weeks of age had no influence on cardiac weight (Hyzewicz, unpublished data), but further investigations are necessary.

The studies cited above were performed using male *mdx* mice. Studies using female *mdx* mice at 24–28 weeks of age did not reveal signs of cardiomyopathy after voluntary running [52]. Interestingly, female *mdx* mice were more susceptible than males to develop cardiac problems [79]. Further studies must be conducted to determine whether voluntary running can protect female hearts from complications.

Investigations of physical exercise on mdx mouse muscle

Acute exercise (Table 1A) leads to membrane leakiness, even under mild conditions such as swimming using 4-week-old mice [21]. Voluntary running in 10-week-old *mdx* mice also causes apoptotic events in the tibialis anterior muscle [23]. Necrosis, thiol oxidation and increased expression of interleukin (IL)-6 mRNA have been reported in quadriceps muscle of 12-week-old mice after 30 minutes of treadmill running at 12 m/min [24]. These results show that even single bouts of exercise can cause muscle damage in *mdx* mice.

In wild type mice, adaptation to chronic exercise leads to large changes in signal transduction mechanisms [8], including subfamilies of the mitogen-activated protein kinase (MAPK) signalling pathways, namely: extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal kinase (JNK), and p38 MAPK [80]. These signalling pathways are activated by reactive oxygen species and lead to activation of genes involved in mitochondrial adaptation, such as PGC1-α, and muscle differentiation [81]. In *mdx* skeletal muscle, production of such species is abnormally elevated, owing to either mitochondrial Ca^{2+} overload [82] or over-activation of membrane-bound NADPH oxidase 2 [83]. Chronic exercise studies using 6–8-week-old *mdx* mice that performed 10 weeks of downhill running on a 7° slope at 23 m/min for 1 hour showed that proteins downstream of MAPK were over-phosphorylated [74, 75]. This protocol also resulted in infiltration of immune cells, fibrosis, and deposits of adipose tissues in skeletal muscle and heart. Chronic treadmill running at 12 m/min for 30 min caused downregulation of Sirt1, PGC1-α, PPARγ and myogenin in 4-week-old animals [62].

In contrast, low intensity training is beneficial. Expression of mitochondrial [39, 45] and muscle differentiation [41] genes was increased after voluntary wheel running in 4–8-week-old animals (Table 1B). Low intensity swimming and running in young *mdx* mice also stimulated a switch from fast glycolytic muscle (type IIb) to oxidative (type IIa) and slow (type I) muscle [34, 37]. Despite this switch, protein expression in slow and fast skeletal muscle increased after low intensity swimming in 4-week-old mice [30].

Comparison of wild type and *mdx* muscle following low intensity swimming also pointed to higher protein expression levels in *mdx* fibres [30]. This observation suggests that dystrophic muscles could benefit from smaller quantities of training than wild type muscles. The fact that hypoxia is more severe in muscles of *mdx* mice than in wild types could explain this phenomenon, since stimulation of HIF-1 initiates adaptation to training via the MAPK signalling pathway [34].

Exercise as a means to worsen the dystrophic phenotype

The mild disease phenotype in *mdx* mice causes a bias when assessing effectiveness of potential drugs for DMD therapy. To worsen the *mdx* phenotype, researchers increase the mechanical stress using voluntary wheel running [50], treadmill [24, 53–56, 65, 66] or downhill running [70, 71, 73, 77] (Table 1). We compared these protocols to determine which types of exercise are likely to make *mdx* muscles become more like those in patients with DMD.

Voluntary running in 10–12-week-old animals caused a suitable worsening of the *mdx* phenotype, showing fibrosis, kyphosis [50] and necrosis of quadriceps muscle [48]. Similar results were obtained with treadmill running in 4-week-old mice for 4 weeks at 12 m/min, leading to fibrosis [61], gastrocnemius degeneration [59], decreased forelimb strength [24, 55] and elevated levels of reactive oxygen species in plasma [56]. Twelve weeks of training also caused downregulation of Sirt1, PGC1-α and PPARγ mRNA expression [62]. However, the acute damaging effects of exercise tended to disappear 96 hours after training, as shown by decreased levels of mRNA coding for pro-inflammatory IL-1β and IL-6 [63].

Downhill running was reported to result in increased muscle damage and decreased grip strength [70, 71], but no information about fibrosis or inflammation was available in these reports. The most complete studies on downhill running showed evidence of fibrosis, adipose tissue and infiltration of immune cells in the hearts of 18-week-old mice after 10 weeks of running on a 7° slope at 23 m/min [75], or decreased muscle strength, increased myoglobinuria and inflammation in 12-week-old mice after 2 weeks of training on a 15° slope at 15 m/min [76].

Based on these observations, we conclude that to worsen the phenotype of *mdx* mice, a minimum of 4 weeks of voluntary exercise from 10 weeks of age, or at least 4 weeks of treadmill running at 12 m/min from 4 weeks of age, is required. Furthermore, in order to avoid the bias due to acute exercise effects, the ability of drugs to prevent exercise-induced damage should be ideally measured with a proper lag time (around 2-3 days) after last exercise bout [54].

TREAT-NMD protocols for exercise in mdx mice

TREAT-NMD is a network for research on neuromuscular diseases that proposes standard operating procedures (SOPs) for experiments with the aim of improving comparability between studies [84]. Two exercise protocols were proposed for *mdx* mice: one to worsen the phenotype [85] and the other to assess the progression of the dystrophic state [86]. Both are based on previous publications on wheel [21, 32, 35, 36] or treadmill [53, 54, 56] running.

The first protocol advised that 3–4-week-old mice perform voluntary wheel running 1–7 days/week, or treadmill exercise at 12 m/min for 30 minutes twice per week. Based on our review (section 2.3.3), treadmill exercise in mice aged 3–4 weeks is suitable for worsening the *mdx* phenotype, but voluntary wheel running requires the mice to be at least 10 weeks old; in younger mice, the benefits of exercise counteract the aggravation of the dystrophic phenotype [36–38].

The second protocol also suggested voluntary wheel running 1–7 days/week, or treadmill exercise at 9 or 12 m/min for 30 minutes twice a week, in young mice. Authors recommended avoiding downhill running since *mdx* mice barely tolerate this exercise. They also pointed out that all mice should perform the same amount of exercise, especially during voluntary wheel running. We agree with these recommendations.

SOPs are an important tool for harmonizing experiments between laboratories. We suggest adding protocols for swimming training based on previous publications [21, 30–35].

EXPERIMENTAL PROCEDURES FOR EXERCISE IN PATIENTS WITH DMD

Literature search for exercise studies in patients with DMD

We performed a literature search in PubMed, using the keywords “DMD” and “exercise”, which was completed on 25 May 2015. A total of 167 articles were examined. Twenty-five of them reported effects of exercise in patients with DMD and described the protocol or results; these formed the basis of this part of the review. They were classified according to the type of muscle performing the exercise and are listed in Table 2.

Table 2
Effects of physical exercise on Duchenne Muscular Dystrophy patients

2.A Acute exercise				
Age (years)	Period	Protocol[a]	Effects	Reference
Exercise of upper and lower limbs				
8.4	1 time	Bicycle ergometer and isokinetic limb strength measurements	**Clin.** Exercise in DMD is limited by reduced cardiorespiratory capacities, leg strength and peripheral oxygen utilization	Sockolov et al., 1977 [87]
6 – 10	1 time	15 minutes of exercise in water	**Clin.** ↑ *Myoglobunuria;* ↑ *Serum creatine kinase*	Pöche et al., 1987 [88]
or 11 – 16				
Early stage (<12)	1 time	50–80 lengthening contractions of calf muscle	**Clin.** ↓ Muscle injury at the end of exer. **Mol.** ↓ *Inorganic phosphate recovery;* ↓ *intracellular pH resting value recovery*	Barbiroli et al., 1993 [89]
Early stage (<12)	1 time	Aerobic exercise on forearm flexor digitorum superficialis	**Mol.** ↑ *Intracellular pH at the end of exercise*	Kemp et al., 1993 [90]
5 – 10	1 time	Maximum voluntary contraction of tibialis anterior for 4 min	**Clin.** Less central fatigue of TA	Sharma et al., 1995 [91]
10.8 ± 0.5	1 time	20 handgrips/min for 5 min	**Mol.** No vasoconstrictor response to exercise	Sander et al., 2000 [92]
6 to 8	1 time	Playing football or running	**Clin.** ↑ *Myoglobinuria*	Garrood et al., 2008 [93]
$8{,}2 \pm 2.6$	1 time	20 steps on a 20 cm high-bench	**Mol.** ↑ *Contrast enhancement in TA*	Garrood et al., 2009 [94]
2.B Chronic exercise				
Age (years)	Period	Protocol[a]	Effects	Reference
Exercise of upper and lower limbs				
Early stage (<12)	8 weeks	Training with arm ergometer	**Clin.** ↑ Ambulation scores; ↑ Endurance and arm functions; ↑ Proximal muscle strength	Alemdaroğlu et al., 2014 [95]
Late stage (>12)	28 weeks	?	**Clin.** ↑ Muscle strength; ↓ Contractures; ↑ Performance of daily activities	Abramson, Rogoff, 1952 [96]
Early stage (<12)	48 weeks	Full extension of knee using Cybex isokinetic exerciser 5 days/week	**Clin.** ↑ Isokinetic strength	de Lateur, Giaconi, 1979 [97]
10 ± 3	96 weeks	Bicycle training of arm and legs	**Clin.** Stabilisation of motor function for the duration of the training	Jansen et al., 2013 [98]
Exercise of masticatory muscles				
16 – 24	24 weeks	5 minutes jaw clench. Open jaw 5 times. Move tongue 5 times.	**Clin.** ↑ Biting force; ↑ Latency of jaw-jerk reflex; ↑ Masticatory performance of masseter	Kawazoe et al., 1982 [99]

Table 2
(continued)

20	24 weeks	Massage of masseter 10 min and jaw training 5 min per day	**Clin.** ↑ Greatest occlusal force; ↑ Satisfaction to eat	Nozaki et al., 2010 [100]
Age	Period	Protocol[a]	Effects	Reference
Exercise of respiratory muscles				
≈ 11.4	2.5 weeks	Triflow II inspirometer, 20 inspirations/day	**Clin.** No benefit of exercise	Rodillo et al., 1989 [101]
Late stage (>12)	5 weeks	Video game ajusted to respiratory efforts, 10 min/day	**Clin.**↑ Maximum voluntary respiration; ↑ Maximal achieved respiration ↑ Duration of progressive isocapnic hyperventilation manœuvre	Vilozni et al., 1994 [102]
14.4 ± 5	6 weeks	Inspiratory resistance 15 min, twice/day	**Clin.** ↑ Maximum resistance and maximum duration of ventilation	DiMarco et al., 1985 [103]
18	6.5 weeks	Inspiratory muscle training 5 to 30 min/day	**Clin.** ↑ Vital capacity; ↑ Maximal inspiratory airway pressure	Aldrich, Uhrlass, 1987 [104]
14.7 ± 4.5	6 weeks	Breathing through a valve 10 min, twice/day	**Clin.** ↑ Endurance of respiratory muscles	Topin et al., 2002 [105]
14.5 ± 3.8	24 weeks	Breathing through a valve 10 min, twice/day	**Clin.** ↑ Maximal sniff assessed esophageal and transdiaphragmatic pressure; ↑ Inspiratory muscle endurance	Wanke et al., 1994 [106]
12	24 weeks	Resistive inspiration and expiratory loads	**Clin.** ↑ Maximal static inspiratory and expiratory pressures; ↓ Decreased respiratory load perception	Gozal, Thiriet, 1999 [107]
8 – 29	36 weeks	Force training then endurance training 10 times, twice/day	**Clin.** ↑ Maximal inspiratory mouth pressure; ↑ 12 s-maximum voluntary ventilation	Winkler et al., 2000 [108]
9.5 ± 2.3	40 weeks	Yoga training: [fast pelvic contractions], [forced apnea after expiration] and [maximal contraction followed by apnea]	**Clin.** ↑ Increased of force vital capacity; ↑ Forced expiratory volume in 1 second	Rodrigues et al., 2014 [109]
12.5 ± 2.3	96 weeks (2 years)	Breathing through a valve 10 min, twice/day	**Clin.** ↑ Maximal inspiratory mouth pressure; ↑ 12 s-maximum voluntary ventilation	Koessler et al., 2001 [110]
16.5 ± 4				
19.9 ± 5				

Normal words signal a positive effect of exercise. Underscored words signal a neutral effect of exercise. *Italic words signal a negative effect of exercise.* **Clin.** signals an observation at the physiological/clinical level. **Mol.** signals an observation at the molecular level. [a]Description of experimental protocol with duration of exercise, speed and other parameters. Abbreviations: *TA Tibialis Anterior.*

The purpose of exercise studies in DMD patients

Physical training was mainly used to assess therapeutic methods for improving dystrophic muscle capacity in wheelchair-dependent patients with DMD, measuring the effects of exercise on respiratory or masticatory muscles. Several studies investigated the effects of acute exercise (Table 2A). Studies assessing therapeutic exercises at early stages of the disease (before 10 years of age) were rare, and mainly used chronic training of upper and lower limbs (Table 2B).

Therapeutic training in patients with DMD before wheelchair dependence

Only three studies focused on improving ambulation in young patients by arm and leg training [95, 97, 98] (Table 1B). Interestingly, documentation for parents of DMD patients recommends physical exercise during the early stages of the disease [10, 111] based on observations in *mdx* mice [111]. Moderate exercise is recommended, without pushing the child, and stopping before the threshold of exhaustion, switching to cycling or swimming when difficulties become apparent.

Bicycle [98] or ergometer [95, 97] training in young DMD patients confirmed the benefit of long-term physical exercise from early stages of the disease. However, two studies showed that running or step exercise damaged the tibialis anterior muscle and caused myoglobinuria immediately after training in patients aged 6–10 years [93, 94]. Theoretically, damage induced by short-term exercise does not prevent long-term improvement in muscle status. But the benefits of training have been demonstrated on bicycle and ergometer, whereas studies reporting short-term negative effects have involved running or step exercises without equipment. The long-term benefits of non-assisted leg training remain to be demonstrated.

Swimming is often recommended for DMD patients [98, 112], but only one study has investigated its effects, and found that myoglobin and creatine kinase levels were elevated after training [88].

Therapeutic training in patients with DMD after wheelchair dependence

The first demonstration that muscle training could improve the daily life of late-stage DMD patients was in 1952 [98], but used no control, and was therefore hard to interpret [113]. Subsequent studies demonstrated that appropriate training could improve the capacity of masticatory and respiratory muscles in wheelchair-dependent patients with DMD.

For masticatory muscles, two studies showed that jaw and tongue training for 24 weeks, accompanied by massage of the masseter muscle, could improve jaw performance and ease of eating [99, 100] (Table 2B).

For respiratory muscles, two approaches were followed: non-assisted or assisted training. Non-assisted training involved resistive inspiratory muscle training [103, 104] or yoga [109]. Assisted training involved the use of special apparatus [101, 108], video games [102], breathing through a valve [105, 106, 110] or resistance to a load [107] (Table 2B).

All studies except one [101] reported an improvement in patients' respiratory capacity. Non-assisted training improved maximal resistance, duration of ventilation [103], inspiratory airway pressure [104], forced expiratory volume in 1 s [109], and vital capacity [104, 109]. Assisted training improved maximal voluntary respiration, maximal achieved respiration [102], maximal sniff assessed oesophageal and transdiaphragmatic pressure [105], static inspiratory/expiratory pressures [107] and inspiratory mouth pressure, as well as 12 s maximal voluntary ventilation [108, 110], duration of progressive isocapnic hyperventilation manoeuvre [102] and respiratory muscle endurance [105, 106].

Non-assisted and assisted respiratory training data are not comparable because different parameters were measured. Only one study compared the effects of non-assisted and load-assisted training and concluded that non-assisted training had no effect [107].

In conclusion, training of respiratory muscles successfully delays the need for mechanical ventilation [104], but there is a lack of studies comparing non-assisted and assisted respiratory training to determine whether training equipment is useful for therapeutic purposes or could be replaced by non-assisted inspiratory training.

Investigating acute exercise in patients with DMD

An early ergometer study showed that DMD patients have limited adaptation to exercise owing to reduced cardiorespiratory capacity, weaker leg strength and limited use of peripheral oxygen [87] (Table 2A). Arm muscle training studies revealed that the intracellular pH at the end of the exercise was higher in DMD muscle fibres than in healthy patients and that inorganic phosphate and pH recovery rates were lower [89, 90]. These differences were explained in part by the fact that the vasoconstrictor response of dystrophic muscles is not blunted in response to exercise [92]. In contrast, DMD patients feel less fatigue and have less muscle injury at the end of most types of exercise [89, 91].

DIFFERENCES IN RESEARCH APPROACHES BETWEEN MICE AND HUMANS

Research in animal models is commonly the first step before clinical trials in patients. However, because the *mdx* mouse was discovered only in 1984 [16], much after the first report in humans in 1868 [114], experiments investigating the effect of physical exercise on dystrophic muscles began in DMD patients 40 years before the first studies in *mdx* mice. Here, we have reviewed 80 articles and found none reporting results from both the murine model and patients. The consequence is a large number of differences between results of studies in mice and humans.

Research focusing on respiratory function in patients with DMD

The majority of investigations in patients with DMD aimed to improve respiratory function alone, and recruited mainly wheelchair-dependent patients [101–110]. In comparison, only three studies document results of investigations of respiratory function in exercised *mdx* mice [43, 44, 77]. However, limb and diaphragm muscles were investigated together in *mdx* mice, but separately in patients with DMD.

Effect of exercise on cardiac function in patients with DMD

Since the development of mechanical ventilation, cardiac failure has become the primary cause of death of DMD patients. Experiments in exercised *mdx* mice demonstrated a vulnerability of cardiac muscle with running, even with low intensity training [39, 41, 43]. However, no study in patients with DMD has ever investigated the impact of exercise on the heart. The limits of exercise for DMD patients should be adjusted by taking into account the limits of the cardiac muscle, especially because experiments in mice have shown that voluntary training can also damage the heart. This aspect is important because it suggests that children with DMD might exercise over their limit, without considering the damage occurring in their heart.

Swimming is recommended for patients, but without evidences

Swimming exercise appears intuitively to be beneficial for DMD patients, because water supports a large part of the patient's weight and thus reduces mechanical stress. However, benefits of water training have never been assessed in patients and scarcely investigated in mice. Vulnerability of the *mdx* heart has been observed with running [39, 41, 43], but results from preliminary studies of swimming (Hyzewicz, unpublished data) suggest that this exercise might spare the cardiac muscle. If further studies confirm the harmlessness of swimming, then it should become a research priority in patients with DMD.

CONCLUSIONS

We have reviewed here the present state of research into the effects of physical exercise on dystrophic muscles, and suggested further investigations to establish evidence-based recommendations regarding optimal training modes. The main conclusions we have drawn are summarized in Fig. 2 and outlined below.

Studies in *mdx* mice have demonstrated that voluntary running exercise in 4-week-old animals improves hindlimb and diaphragm capacity but is harmful for the heart. The effects of swimming on cardiac tissue have not yet been studied. Forced treadmill running of at least 4-week-old mice at 12 m/min for 4 weeks renders the *mdx* phenotype closer to that of DMD patients.

Studies in human have shown that respiratory and masticatory muscle training successfully improves functional capacity in patients aged 12 years or older. However, further comparisons between machine-assisted respiratory training and non-assisted training are necessary. Bicycle training can also delay the impairment of motor function in young patients, although the effects of exercise on cardiac function have not yet been investigated. There is also a lack of studies investigating the effect of running and swimming in DMD.

In order to fill these knowledge gaps, we suggest the following approaches for future research regarding the effects of physical exercise on *mdx* mice and DMD patients:

1. As DMD causes degeneration of respiratory, cardiac and limb muscles, future studies should assess the effects of exercise on these three types of muscle simultaneously.
2. Running has a negative effect on the heart in *mdx* mice. It is crucial to determine whether a similar effect occurs in the hearts of patients with DMD after running.
3. Effects of exercise on young DMD patients (4–12 years old) should be investigated.

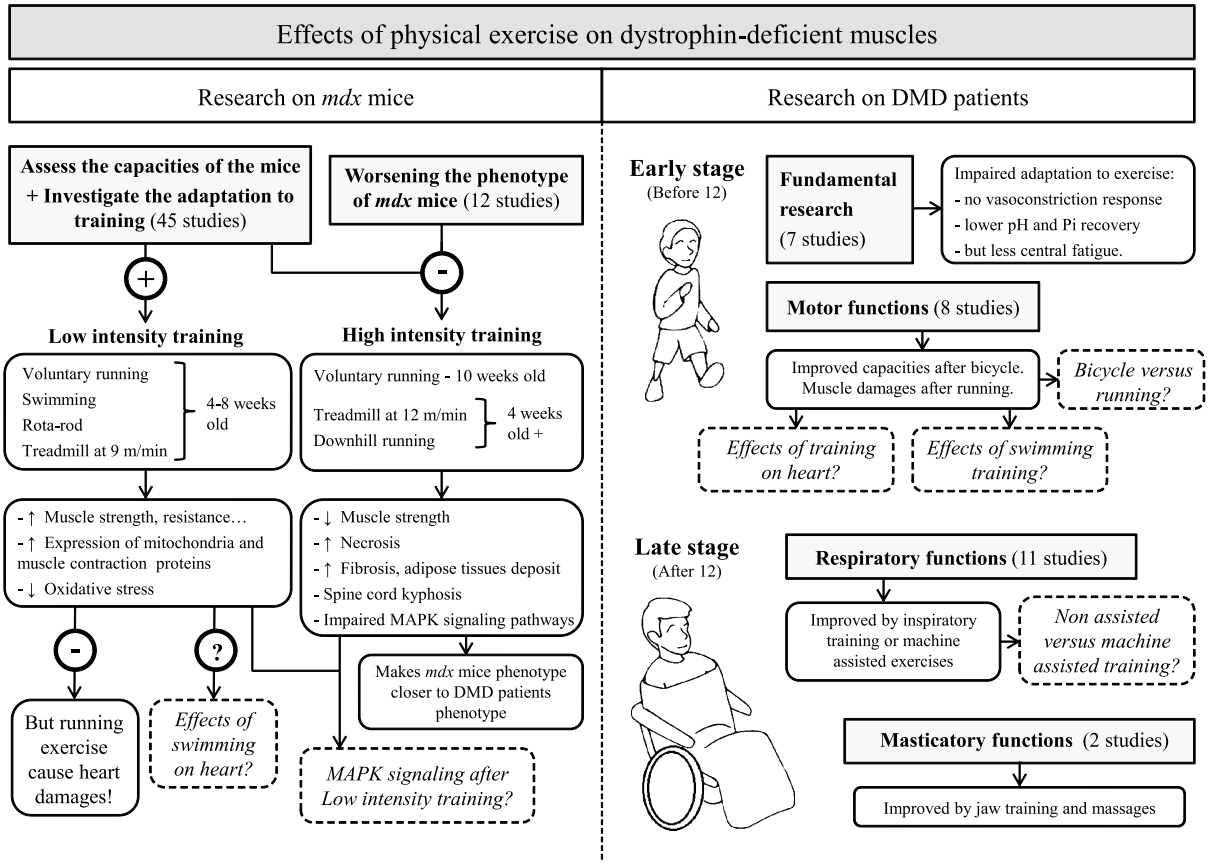

Fig. 2. Schematic summarizing the effects of physical exercise on dystrophin-deficient muscles. Results from studies investigating the effects of physical exercise on muscles in *mdx* mice (left panel) and DMD patients (right panel). Dotted lines represent open questions.

4. Even though swimming is recommended, its cardiac consequences have not been studied in *mdx* mice or patients. This should be performed using appropriate technology, such as MRI and biomarker measurement.
5. Studies should compare non-assisted respiratory training with machine-assisted training.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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