Chapter 13
Therapeutic Potential of Skeletal Muscle Plasticity and Slow Muscle Programming for Muscular Dystrophy and Related Muscle Conditions

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Abstract Duchenne muscular dystrophy (DMD) is a devastating life-limiting disease causing progressive and severe muscle wasting in boys and young men. It is simply unacceptable that ~30 years after the discovery of the culprit protein, dystrophin, there is still no cure or effective treatment. Dystrophic muscles are fragile, injury prone and compromised in their regenerative capacity. Interestingly, in DMD and in two well-characterised murine models of the disease (*mdx* and *dko* mice), fast muscle fibres are more susceptible to damage and pathological progression than slow muscle fibres, which are resistant to damage and relatively spared. Therefore, therapies that promote a slower, more oxidative phenotype could protect muscles from damage, ameliorate the dystrophic pathology and improve patient quality of life. Muscle plasticity can be achieved through exercise and/or well-described pharmacologic approaches, including activation of AMP-activated protein kinase (AMPK). Exercise has beneficial effects on muscle health, but unfortunately many patients cannot exercise, especially DMD patients confined to wheelchairs. Modulating muscle activity through low-frequency stimulation (LFS) protocols could mimic exercise to promote a slow phenotype, protect muscles from damage and enhance muscle repair. Enhancing these adaptations by combining LFS with pharmacologic modifiers of muscle phenotype potentially represents a novel therapy that could find immediate application to improve the pathology and enhance patient quality of life. Alternative approaches like anabolic agents or myostatin inhibition also have therapeutic potential, but their efficacy occurs through different mechanisms. Better understanding of the mechanisms underlying skeletal muscle

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adaptations to different interventions and stimuli will help optimise novel strategies to address the pathophysiology of DMD and related muscle conditions.

Keywords Muscular dystrophy • Dystrophin • DMD • Skeletal muscle diseases • Muscle wasting • Electrical stimulation • Exercise • Muscle injury • Muscle regeneration • Fibre type • Fast-to-slow • Slow-to-fast • Muscle phenotype • Anabolic agents • AMPK • Muscle plasticity • Muscle adaptation • Neuromuscular • Muscle function • Contractile properties • Muscle contraction

13.1 Introduction

Modifying muscle phenotype to confer protection from injury or pathology has its origins in the study of muscle plasticity. Skeletal muscles are highly plastic and capable of adapting to different perturbing stimuli. Muscle fibre composition can be altered through pharmacologic manipulation of biochemical pathways that regulate contractile and regulatory protein isoform composition as well as the muscle's metabolic machinery. Altering the pattern of neural stimulation to skeletal muscles can similarly alter muscle phenotype. It is theoretically possible to completely alter a muscle's phenotype, from fast-to-slow or vice versa, depending on the nature (frequency, intensity and duration) of the intervening stimuli. In most cases, the nature of interventions like physical activity (exercise) or functional neuromuscular electrical stimulation, like that applied to humans for therapeutic or rehabilitative purposes, means that they are not sufficient to elicit extreme changes in muscle phenotype. Regardless, the therapeutic potential of electrical stimulation for muscle diseases has been identified and represents an exciting field of research. Despite some promising outcomes from early studies on patients with Duchenne muscular dystrophy (DMD), electrical stimulation as a therapy has not found wide application for this condition.

This chapter describes the underlying basis of skeletal muscle programming and its therapeutic potential for DMD and related conditions. It describes how muscle phenotype can be altered by different stimuli, with potentially opposing effects on parameters such as muscle fibre size and fibre composition. It highlights how pharmacologic and electrical stimuli can alter muscle phenotype, to confer beneficial outcomes that could improve muscle structure and function and ultimately enhance quality of life for patients.

13.2 Duchenne Muscular Dystrophy

DMD is the most common of the muscular dystrophies, caused by mutations and deletions in the dystrophin (*dmd*) gene on chromosome Xp21, leading to a lack of expression or a non-functional corresponding protein in muscle. It is a

devastating, life-limiting disease affecting ~1:3500–6000 live male births, resulting in progressive and severe muscle wasting and weakness in boys and young men [10]. Patients become wheelchair dependent before their teens and have only 25% of the muscle mass of healthy children. Eventually all muscles are affected and patients eventually succumb to respiratory or cardiac muscle failure.

Sadly, there is still no cure or effective treatment for DMD.

Although a cure may eventually come from stem cell or corrective gene therapies, limitations of delivery systems, gene carrying capacity, dissemination efficiency, expression persistence and immunological tolerance all pose significant obstacles for clinical application [92]. Until these techniques are perfected, DMD patients will continue to die prematurely. The current mainstays in treating DMD are glucocorticoids (prednisolone or deflazacort) which despite slowing the disease progression have many deleterious side effects [30]. DMD patients also need regular corrective surgeries to relieve stiff joints, correct scoliosis and similar muscle-related interventions [18], which can aggravate the dystrophic pathology and compromise an already defective regenerative process. Clearly, there is a profound, urgent and unmet clinical need for therapies that can ameliorate the pathology, preserve and protect muscles from damage and enhance muscle fibre regeneration.

The most widely used animal model of DMD is the *mdx* dystrophic mouse which has a point mutation in the dystrophin gene and an absence of dystrophin protein expression in muscle [85]. Although sharing the same genetic deficit as DMD, the muscle phenotype of *mdx* mice differs in that the hindlimb muscles undergo severe degeneration at 3–4 weeks of age, but an enhanced regenerative capacity ensures almost complete functional recovery. In *mdx* mice there is compensatory upregulation of the dystrophin-like protein, utrophin, which may account for its more benign phenotype. Unlike *mdx* mice, dystrophin-utrophin double knockout (*dko*) mice exhibit severe wasting and weakness, spinal deformities (kyphosis) from an early age and a shortened lifespan more phenotypically representative of DMD. These models are fundamental for understanding the dystrophic pathophysiology since functional roles for dystrophin and utrophin remain unclear, and much information can be gained from knockout phenotypes [75, 76]. These murine models are essential for understanding how dystrophic skeletal muscles adapt to different interventions or stimuli that could ameliorate the pathophysiology of DMD and related conditions [28].

13.3 Skeletal Muscle Diversity and Adaptability

Skeletal muscle is comprised of functionally diverse fibres ranging in size, metabolism and contractility [4, 9, 37, 81, 82]. Based on myosin heavy chain (MyHC) protein isoforms, which largely dictate the rate of force development, shortening velocity and rate of cross-bridge cycling, mammalian muscle fibres are

broadly classified as slow-twitch (type I) or fast-twitch (type IIa, IId/x and IIb). Type I and IIa fibres primarily generate ATP via oxidative metabolism, whereas type IId/x and IIb fibres generate energy mostly through glycolysis [59, 80].

Muscle fibres are highly plastic and can alter their structural, functional, metabolic and molecular properties in response to altered contractile demands or pharmacologic interventions that manipulate signalling pathways that regulate isoform composition. Altered motor neuron activity can dramatically change muscle fibre composition, a phenomenon first demonstrated through a series of elegant nerve cross-reinnervation studies by Sir John Eccles and colleagues [8]. Together, these studies revealed that when fast muscles were innervated by a slow nerve, the muscle transformed from a fast (glycolytic) to a slower, more oxidative phenotype and contracted more slowly. When slow muscles were innervated by a fast nerve, the muscle transformed from an oxidative to a more glycolytic phenotype and contracted more quickly. Such phenotypic changes were attributed to the specific impulse patterns delivered to the muscle via the motor neuron [7]. Chronic low-frequency (10–15 Hz) stimulation induces transcription of slow oxidative genes in fast muscles through sustained elevations in low-amplitude intracellular [Ca^{2+}] transients, which stimulate downstream signalling pathways and key proteins regulating muscle phenotype, specifically promoting fast to slow changes [52, 68, 94]. Through studies using transgenic mouse lines and specific drug targeting, these key proteins have been identified and include: calcineurin, peroxisome proliferator-activated receptor (PPAR) γ coactivator 1α (PGC-1α), PPARβ/δ, silent mating type information regulator 2 homologue 1 (SIRT1) and AMP-activated protein kinase (AMPK) [14, 42–44, 89]. These studies have contributed significantly to our understanding of the signalling pathways regulating skeletal muscle adaptation and plasticity and have been reviewed elegantly in detail elsewhere [44].

13.4 Promoting a Slower, More Oxidative Muscle Phenotype – A Therapeutic Target for DMD

The lack of dystrophin in the muscles of DMD patients and *mdx* and *dko* mice renders muscle fibres fragile and prone to injury. Interestingly, muscles composed of fast fibres are more susceptible to damage and pathological progression than predominantly slow muscles, both in DMD patients and in *mdx* and *dko* mice. Thus, dystrophin deficiency in fast muscle fibres of DMD patients is associated with degenerative changes, while slower muscle fibres are more resistant to damage and relatively spared [99]. Therefore, therapies that can promote a slower, more oxidative muscle phenotype could ameliorate the dystrophic pathology and improve patient quality of life.

That slower, more oxidative muscle fibres express significantly more utrophin-A protein compared with their faster, more glycolytic counterparts, has been suggested as one factor protecting slower fibres against damage. Studies in transgenic*mdx* mice

(overexpressing utrophin) established that utrophin can functionally substitute for dystrophin and ameliorate the dystrophic pathology [5, 20, 34, 95]. Activation of signalling pathways that promote a slower, more oxidative phenotype also promote increases in utrophin-A expression, highlighting the therapeutic relevance of manipulating muscle plasticity to mitigate the dystrophic pathology. For example, calcineurin-NFAT (nuclear factor of activated T cells) signalling plays an important role in regulating fast-to-slow muscle phenotypic adaptations [17]. Calcineurin is a Ca^{2+}/calmodulin-dependent phosphatase that dephosphorylates NFAT, resulting in its nuclear translocation and binding to specific sequences on the promoters of target genes that induce slow oxidative fibre programming [1, 23, 64, 65] with potential benefits for the dystrophic phenotype [13, 14, 87, 88]. For example, muscles of transgenic *mdx* mice expressing an active form of calcineurin exhibited a shift to a slower, more oxidative phenotype, increased utrophin-A expression and an attenuated dystrophic pathology [89, 90]. Inhibition of this signalling exacerbated the dystrophic pathology in skeletal muscles of *mdx* mice [15].

Conversely, promoting a slower, more oxidative muscle phenotype may have beneficial effects on DMD that extend beyond simply increasing utrophin-A expression [93]. Muscles of DMD patients and *mdx* and *dko* mice have impaired oxidative phosphorylation and mitochondrial function, which contributes to the disease aetiology [56]. Therefore, promoting a slower, more oxidative muscle phenotype may rescue normal mitochondrial function and help alleviate the dystrophic pathology.

13.5 Exercise, Low-Frequency Stimulation and DMD

Physical activity, especially endurance training, has many beneficial effects on muscle health including the potential to promote a slow, oxidative phenotype [11, 44, 70]. Whether exercise has beneficial effects for DMD patients remains contentious, with some activities like low-intensity, low-weight bearing exercise shown to have therapeutic effects in some studies, while other exercises involving potentially injurious lengthening (eccentric) contractions can aggravate the pathology [50, 96]. For a comprehensive review on the effects of exercise on dystrophic skeletal muscle, see Markert et al. [49].

Unfortunately many patients are simply unable to exercise, especially boys with DMD who are confined to a wheelchair usually before their teens. Devising contraction/activity protocols that mimic the benefits of exercise to attenuate loss of muscle mass and improve function in these situations could provide a significant improvement in patient quality of life. Low-frequency stimulation (LFS) is a well-established model of muscle training that can promote a slower, more oxidative muscle phenotype [51, 79]. A multitude of studies have shown that LFS mimics the electrical discharge pattern of slow motor neurons innervating slow muscles and

induces downstream signalling pathways that promote transcription of slower, more oxidative, fibre-specific genes [54, 67]. The resultant faster-to-slower adaptations include increased oxidative metabolism and mitochondrial biogenesis concurrent with fibre transitions in the type IIb→type IId/x→type IIa→type I direction, even within 14 d of daily LFS [42, 43]. While LFS challenges a muscle to its full adaptive potential, it does so efficiently and in the absence of injury and regeneration (Pette & Vrbova [53, 54, 71, 72]). Collectively, LFS is an ideal model for investigating the therapeutic potential of promoting a slower, more oxidative muscle phenotype to ameliorate the dystrophic pathology.

Electrical stimulation (especially LFS) to enhance muscle function in health and disease has been studied for nearly a century and remains an intervention with broad therapeutic relevance, for patients in the intensive care unit [86], with spinal cord injury [12, 46], cerebral palsy [73], sarcopenia [2] and as a supplement for sports training [41, 57].

From a clinical perspective, there was significant interest in LFS as a therapy for DMD during the late 1970s to early 1990s, but this waned as the field shifted to tackle the dystrophic pathophysiology through molecular biochemistry, especially after the discovery of dystrophin in 1987 [31]. These early studies conducted on DMD patients led by Vrbová, Dubowitz, Salmons, Zupan and colleagues were highly encouraging (including conferring a preservation of strength in some studies), but generally they were preliminary in nature (consisting of relatively few patients and usually of limited duration) with a resulting lack of scientific and statistical clarity [22, 83, 84, 101, 102]. There remains a dearth of information regarding the application of such a well-described and utilised intervention like LFS (with its current broad applications in rehabilitation medicine and physical therapy) for ameliorating the dystrophic pathology. Until recently, the only previous studies of LFS performed on dystrophic mice had not been conducted on mouse models of DMD [19, 97]. These studies had shown LFS to have beneficial effects on diseased (laminin-deficient) muscles of C57Bl/6J–dy2J (*dy/dy*) mice (a model of congenital muscular dystrophy). These effects were different from those observed in normal muscle and were not strictly relevant to DMD [97]. Only one study [41] has looked at short-term (2 week), 20 min/day LFS in dystrophic *mdx* mice, and this very preliminary proof-of-concept study was of too short a duration to have any therapeutic relevance for the dystrophic pathophysiology [41]. Longer-term studies evaluating the therapeutic merit of LFS have yet to be performed on the accepted mouse models of DMD nor have studies been undertaken to determine whether muscle wasting can be attenuated or reversed using different protocols of electrical stimulation. As to the therapeutic merit of LFS for DMD, my contention is that the jury is still out! Understanding how dystrophin-deficient muscles (in *mdx* and *dko* mice) adapt to LFS is critical for informing best clinical practice for any strategy that might be applied for DMD and related muscle conditions.

13.6 Pharmacologic Activation to Promote Slow Muscle Programming

There is considerable evidence that upregulating key proteins like calcineurin, PPARγ, PGC-1α, SIRT1 and AMPK (among others) can exert fast to slow changes within muscles, including in *mdx* mice. Ljubicic et al. [44] comprehensively and elegantly evaluated the merits of different pharmacologic and transgenic approaches to exert fast to slow phenotypic changes within dystrophic muscles. AMPK activation is among the best studied and effective approaches, with the AMPK activator, AICAR, conferring significant slow oxidative myogenic programming and improving the dystrophic pathology in *mdx* mice (Ljubicic et al. [42–44]. A critical discovery by Ljubicic et al. [43] revealed that prior pharmacologic conditioning with an AMPK activator was a salient determinant in how dystrophic muscles adapted to complementary, acute physiological stress stimuli, like treadmill running. Therefore, pharmacologic AMPK activation could potentially enhance the LFS-mediated favourable phenotypic adaptations in dystrophic muscles. Since DMD patients cannot exercise, one therapeutic option could be to combine AMPK activation with LFS, ideally to amplify the favourable effects of either intervention alone. LFS could confer 'exercise-like' contraction-mediated benefits on muscle fibres that pharmacological exercise mimetics are simply unable to elicit – producing not only local muscle effects but potentially amplifying systemic benefits. The therapeutic merit of this seemingly straightforward approach should be evaluated as a priority. There are other powerful and effective activators of AMPK signalling that could be employed to promote a slow, oxidative muscle phenotype that could potentially ameliorate the dystrophic pathology, and these are therapeutic targets for muscular dystrophy and related conditions.

It should be noted that adaptations within skeletal muscle fibres might differ between those mediated by contraction or pharmacologic stimuli. For example, physical activity or electrical stimulation may induce release of myokines from activated muscles that regulate mitochondrial biogenesis [21, 24, 91] through different mechanisms than what may be achieved through pharmacologic activation [35]. Load-bearing exercise may confer different cellular adaptations than pharmacologic activation. Although the loading on skeletal muscle fibres would be less during electrical stimulation than with physical activity, even that level of cellular (mechanical) stress could induce different adaptive programming than with pharmacologic activation. This issue remains speculative until hypotheses regarding comparative adaptations and plasticity are tested rigorously.

13.7 Slow Muscle Programming and Protecting Against Muscle Damage

Slow muscle programming may also confer protective effects within muscles that promote better functional outcomes after surgeries, especially where concomitant ischemia-reperfusion damage is unavoidable. This is relevant not just for DMD

patients who must undergo regular surgical procedures, but for millions of otherwise healthy patients worldwide who experience long-term disability and functional deficits after such traumatic surgeries. Muscle injury is a significant public health problem contributing to the large burden of musculoskeletal disability and suffering worldwide [27]. Muscles can be injured in many ways including ischaemia-reperfusion (I-R), contusion, crush, strains, laceration, extremes of temperature, chemical (myotoxic) and metabolic injury. While regeneration usually occurs spontaneously after damage, the process can be slow, incomplete and accompanied by fibrosis (scarring) that compromises the restoration of function. This is especially the case when the muscle blood supply has been occluded or when blood vessels, nerves, basal laminae and other supporting structures have been compromised.

I-R injury concomitant with tourniquet application is common during many surgeries, especially those involving muscle transfers and microsurgical procedures. I-R injury can also occur in muscles that have been crushed, with compartment syndromes, in limbs that have been broken or traumatised and with the replantation of amputated limbs. After injury induction, there is currently no effective treatment [25]. I-R occurs when the blood/oxygen supply to a muscle is occluded (ischemia) but later restored (reperfusion). Muscle fibres can be damaged in two ways during I-R: during the ischemia when blood flow is occluded or during reperfusion where free radical production accompanies a 'second wave' of injury [27]. This damage can impact negatively on the outcome of surgical interventions and so protecting muscles from I-R has the potential to improve tissue repair and enhance functional restoration. Antioxidants to attenuate I-R damage have largely proved unsuccessful [58] or produced only modest beneficial outcomes [6], and so novel and effective approaches that better protect muscles during these surgeries are needed.

The fact that slow muscles are better protected than fast muscles from I-R injury [98] provides the key rationale for advocating slow muscle programming to confer protection from this type of damage. Successful repair is vital for restoring mobility and patient quality of life, and there is an important medical need for therapies that can attenuate muscle damage, promote regeneration, reduce fibrosis and enhance function [27]. There has been renewed interest in LFS, primarily at a cellular and subcellular level, with demonstrated increases in stem cell proliferation, differentiation, matrix formation and migration, important for tissue regeneration [40]. The potential for LFS to stimulate regeneration remains a hypothesis worthy of rigorous testing in appropriate models of muscle health and disease. Furthermore, since it has been argued that AMPK activators could 'prime' muscle for complementary interventions, it is important to determine whether co-treatment of an AMPK activator with LFS might confer greater improvements in regeneration than either intervention alone. If such interventions hasten restoration of muscle function post-trauma, they could be rapidly applied in rehabilitation medicine to optimise recovery in a wide range of affected patients with muscle injuries.

13.8 Muscle Plasticity in the Other Direction – Are Slow-to-Fast Muscle Fibre Modifications Contraindicated in Muscular Dystrophy?

Although conferring fast-to-slow muscle fibre modifications has therapeutic relevance for muscular dystrophy, whether modifications in the opposite (i.e. slow-to-fast) direction exacerbate the dystrophic pathophysiology is not definitive. Another consideration is whether making muscles smaller (an adaptation with an intervention like chronic LFS) has greater protective effects for the dystrophic pathology than making muscles larger, such as with chronic administration of anabolic agents such as β-adrenoceptor agonists (β-agonists). Are there therapeutic interventions that preserve or increase muscle strength while simultaneously conferring fast-to-slow muscle fibre modifications in muscle phenotype?

Slow-to-fast muscle fibre modifications are possible through high-frequency electrical stimulation (HFS) as demonstrated in studies on rat skeletal muscle [29] and in studies using variable (often higher) frequency protocols for potential therapeutic and sports applications [3]. It is also well established that there are transcriptional activators or repressors that control genes that regulate or alter fibre composition (towards the fast, glycolytic myogenic programme) to ultimately affect muscle performance and metabolism [74]. These include RIP140, NCoR1, Ets-2 repressor factor (ERF), E2F1 and Baf60c and their roles in myogenic programming and skeletal muscle metabolism and phenotype have been reviewed in detail elsewhere [44].

Pharmacologic stimuli can also affect muscle fibre composition and muscle metabolism. Chronic administration of β-agonists to rats and mice can exert significant anabolic effects (increasing muscle mass through increases in muscle fibre cross-sectional area) and shift muscles from an oxidative to a more glycolytic phenotype, depending on the type of β-agonist, dose, mode of administration and duration of treatment [47, 69, 77, 100]. In some studies, chronic β-agonist (clenbuterol or formoterol) administration to rats transformed muscle fibre composition in the soleus muscle from predominantly slow-twitch to a more mixed fast and slow fibre composition, as well as increasing cross-sectional area of both of the main fibre types [78]. The implications of a shift in muscle phenotype from slow to fast, as well as an increase in muscle fibre size, are potentially significant for the aetiology of muscular dystrophy. Therapeutic strategies in DMD to increase muscle mass may well produce larger and stronger muscle fibres, but are they contraindicated by increasing muscle susceptibility to contraction-induced injury and so aggravating the dystrophic pathology? Large, fast type II fibres produce higher forces than smaller, slow type I fibres and can be more susceptible to damage after lengthening contractions [45, 48]. Fast muscle fibres are preferentially affected in DMD [16, 66, 99], whereas smaller calibre fibres are relatively spared in DMD and in animal models of muscular dystrophy [32, 33]. But the relationship between muscle fibre size and susceptibility to damage in muscular dystrophy is not always

clear. In one study, tibialis anterior muscles of *mdx* mice were not more susceptible to contraction-induced injury if the mice had been treated with formoterol (100 μM for 4 weeks). In fact, despite formoterol treatment increasing muscle mass and force production, the cumulative force deficit was actually lower in TA muscles of treated than untreated *mdx* mice [26]. This relatively low dose of formoterol did not change fibre type or oxidative capacity (i.e. no slow-to-fast fibre changes) but was sufficient to elicit a hypertrophic response in type IIb fibres that conferred protection from contraction-mediated injury [26]. These findings support the contention that anabolic agents also have therapeutic potential for DMD and related conditions.

13.9 Inhibiting Myostatin Signalling

Are there therapeutic interventions that preserve or increase muscle strength while simultaneously conferring fast-to-slow muscle fibre modifications in muscle phenotype? Such an attractive combination of phenotypic changes has therapeutic relevance for muscle wasting disorders including DMD. One approach that can confer these effects is myostatin inhibition. Myostatin, originally termed growth and differentiation factor-8 (GDF-8), is a member of the transforming growth factor-β (TGF-β) superfamily. Described as a negative regulator of skeletal muscle mass because it inhibits myoblast proliferation and differentiation [38, 55], inhibiting myostatin through genetic deletion or pharmacologic inactivation increases muscle mass and strength [39]. In a series of studies examining the therapeutic applications of myostatin inhibition, our laboratory showed that the myostatin inhibitory antibody PF-354 (developed by Pfizer Inc.) conferred favourable effects in mouse models of cancer cachexia, muscular dystrophy (*mdx*), aging (sarcopenia) and disuse atrophy with plaster casting (Murphy et al. [60–63]). In the Lewis lung carcinoma (LLC) mouse model of cancer cachexia, PF-354 attenuated muscle atrophy and loss of force production with improvements in muscle mass and fatigue (force during repeated stimulation of tibialis anterior muscles in situ), accompanied by increases in succinate dehydrogenase (SDH) activity and the proportion of oxidative muscle fibres [62]. PF-354 conferred similar improvements in these parameters in aged mice [61] and improved diaphragm structure-function in young *mdx* mice [60] and in mice with unilateral plaster caster casting PF-354 attenuated muscle atrophy and loss of force [63]. There is still much to be learned regarding the therapeutic potential of this and similar approaches for manipulating myostatin/activin signalling in skeletal muscle. Conferring changes in muscle phenotype (fast-to-slow) to improve muscle fatigue resistance while increasing muscle mass and strength is an attractive combination of effects relevant to multiple muscle wasting conditions, especially DMD. There is still considerable interest in developing novel strategies to manipulate TGF-β signalling for therapeutic application in skeletal muscle conditions [36].

13.10 Conclusion

Altering muscle phenotype can have dramatic effects on skeletal muscle structure, function and metabolism. Muscle plasticity can be achieved through various means including physical activity, electrical stimulation and pharmacologic activation, and each approach has potential therapeutic merit for muscular dystrophy. Better understanding the mechanisms underlying skeletal muscle adaptations to different interventions and stimuli will help optimise novel strategies to address the pathophysiology of DMD and related muscle conditions.

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