

Role of molecular and metabolic defects in impaired performance of dystrophic skeletal muscles

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There occurs a progressive weakness and wastage of skeletal muscle in different types of muscular dystrophy. The loss of muscle fibers in dystrophic muscle with impaired function is associated with leakage of intracellular enzymes, maldistribution of electrolyte content and metabolic defects in myocytes. Marked increases in the sarcolemma (SL) Na^+ - K^+ ATPase and Ca^{2+} / Mg^{2+} -ecto ATPase activities, as well as depressions in the sarcoplasmic reticulum (SR) Ca^{2+} -uptake and Ca^{2+} -pump ATPase activities were seen in dystrophic muscles of a hamster model of myopathy. In addition, impaired mitochondrial oxidative phosphorylation and decrease in the high energy stores as a consequence of mitochondrial Ca^{2+} -overload were observed in these myopathic hamsters. In some forms of muscular dystrophy, it has been shown that deficiency of dystrophin produces marked alterations in the SL permeability and promotes the occurrence of intracellular Ca^{2+} -overload for inducing metabolic defects, activation of proteases and contractile abnormalities in dystrophic muscle. Increases in SR Ca^{2+} -release channels, SL Na^+ - Ca^{2+} exchanger and SL store-operated Ca^{2+} -channels have been reported to induce Ca^{2+} -handling abnormalities in a mouse model of muscular dystrophy. Furthermore, alterations in lipid metabolism and development of oxidative stress have been suggested as mechanisms for subcellular remodeling and cellular damage in dystrophic muscle. Although, several therapeutic interventions including gene therapy are available, these treatments neither fully prevent the course of development of muscular disorder nor fully improve the function of dystrophic muscle. Thus, extensive research work with some novel inhibitors of oxidative stress, SL Ca^{2+} -entry systems such as store-operated Ca^{2+} -channels, Na^+ - Ca^{2+} exchanger and Ca^{2+} / Mg^{2+} -ecto ATPase (Ca^{2+} -gating mechanism), as well as SR Ca^{2+} -release and Ca^{2+} -pump systems needs to be carried out in combination of gene therapy for improved beneficial effects in muscular dystrophy.

Keywords

Dystrophic muscle; Dystrophin deficiency; Sarcolemmal defects; Sarcoplasmic reticular Ca^{2+} -handling; Store-operated Ca^{2+} -channels; Mitochondrial Ca^{2+} -overload

1. Introduction

Muscular dystrophy is a deadly genetic disorder which represents a group of complex muscular diseases. Patients with this disease generally suffer from muscle weakness as well as loss of ambulation, and mostly die in their early twenties due to respiratory and cardiac complications [1, 2]. The first historical account of muscular dystrophy was given in 1830s by Conte and Gioja that this disease causes progressive weakness of multiple muscle groups [3]; however, in 1852, Edward Meryon suggested that a disorder in the cell membrane causes muscular dystrophy but the disease was mistaken for tuberculosis [4]. It was suspected that this disease is genetically transmitted by females and it affects males only, in addition to causing progressive muscle damage which is replaced with connective tissue. Furthermore, it was pointed out that the development of this skeletal muscle disorder is not only age and sex dependent but it also affects cardiac muscle in advanced stages [4]. Later on in 1868, this disease was called Duchenne muscular dystrophy (DMD) after the name of a French neurologist, Guillaume Duchenne, due to his significant contributions in understanding its mechanism [5]. Most of the diagnostic criteria of this disorder, established at that time, are still being used and these include: (a) weakness in legs; (b) hyperlordosis; (c) hypertrophy of weak muscle; (d) reduced muscle contractility on electrical stimulation; (e) absence of bladder or bowel dysfunction; and (f) sensory disturbances and febrile illness. In addition, there occurs cardiomyopathy and mental retardation at advanced stages of this disease and results in death at early age. The progressive nature of these characteristics of muscular dystrophy is depicted in Fig. 1 [6–9]. It is pointed out that in 1878, Gowers was the first to report the genetic basis of muscular dystrophy [10] but it was not until 1891, when the concept of histological alterations in muscle was outlined and the classification of muscular dystrophies such as infantile and juvenile types along with many other subtypes was described [11].

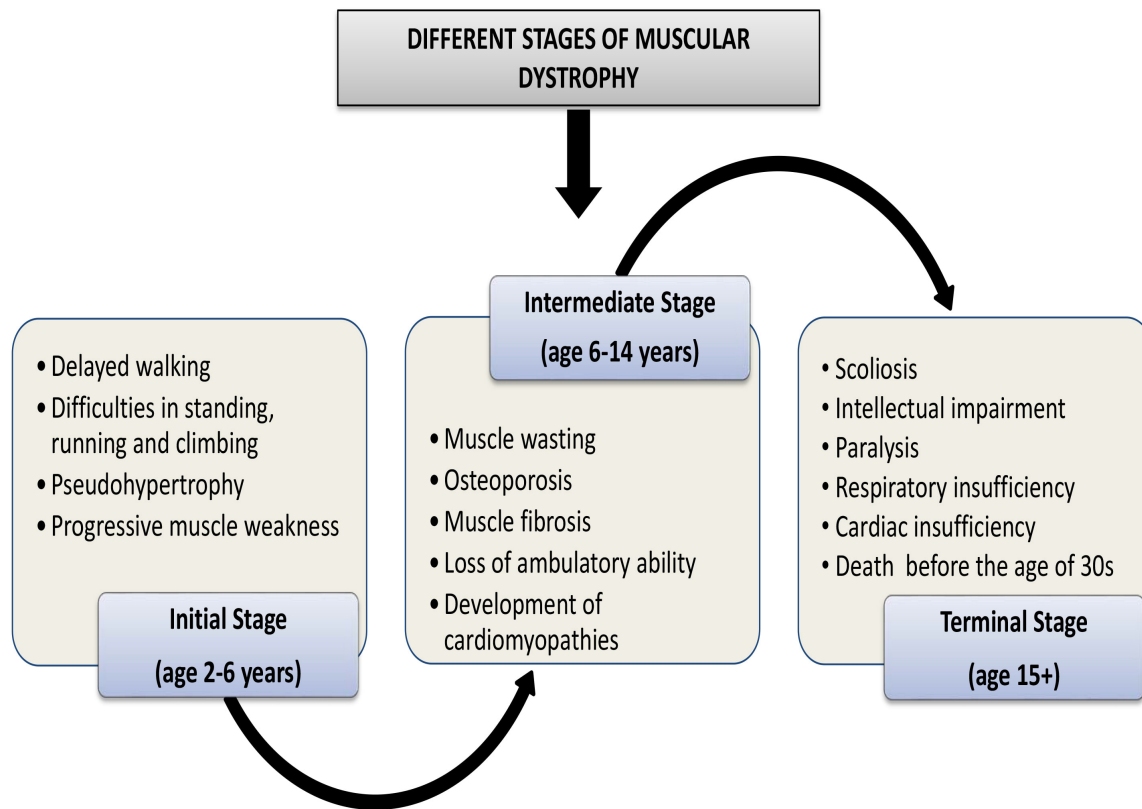


Fig. 1. Schematic representation of different stages of muscular dystrophy.

Currently, there are approximately 50 muscular dystrophy causative genes and more than 40 types and subtypes of these are associated with genetic mutations. These various forms of muscular dystrophies have been characterized by patterns of their inheritance, symptoms, origin of gene mutation, age at onset, rate of progression and level of severity [12]. The spectrum of mutations varies as it ranges from complete deficiency of a gene product to a decrease in gene expression and/or expression of an abnormal molecule with complete or partial loss of functionality. Most of the major types of muscular dystrophies are categorized on the basis of X-linked, autosomal dominant and autosomal recessive genes; the location of altered gene product lies in muscle fiber but is linked to other proteins, enzymes and extracellular matrix [12–14]. While DMD, Becker muscular dystrophy and Limb-Girdle dystrophies are the common forms of this disease, it is noteworthy that DMD is the most prevalent and severe form among various types of muscular dystrophies. In fact, over 80% of cases of muscular dystrophy worldwide are associated with DMD, whereas most of the other types are fairly rare [12]. The global prevalence of DMD is 19.8 per 100,000 male births [15]. However, there are variations in disease etiology; the common physiognomies of muscular dystrophy include primary genetic defects and mechanisms based on repetitive cycles of muscle degeneration, necrosis and impaired regeneration resulting in muscle fibrosis, muscle wastage and mus-

cle dysfunction [8, 16]. The evaluation of family history and physical symptoms such as contracture, muscle stiffness, and weakness are the basic diagnostic procedures. In addition to mutation screening in the predicted gene, the assessment for muscular dystrophies is carried out from the determination of muscle weakness, serum creatine kinase (CK) levels, muscle biopsy examination, muscle magnetic resonance imaging, neurological evaluation, electromyographic and electrocardiographic analysis as well as exercise tolerance examination [16].

In spite of extensive preclinical and clinical research efforts over the past 50 years, the exact pathogenesis and therapies of muscular dystrophy remain to be poorly understood. It should also be emphasized that muscular dystrophy is not an entity but represents a group of various muscle disorders, which differ from one another with respect to the location of defects in plasma membrane, as well as transmembrane, extracellular matrix, nuclear membrane, nucleus and cytosol proteins. Since clinical phenotype and pathophysiology of muscle degeneration are different in each type of muscular dystrophy, this article is planned to deal with biochemical and metabolic alterations in dystrophic muscles from all forms of this disease in a general way rather than in any specific manner. It is noteworthy that several molecular and biochemical defects have been identified in dystrophic muscle and their modulation has been shown to

slow down the disease progress [12, 17–25]. Abnormalities in dystrophic muscle include increased membrane permeability, Ca^{2+} -handling defects, depressed energy production, oxidative stress and myocyte necrosis as well as apoptosis. Impairment of the blood flow to skeletal muscle is invariably associated with dystrophic muscle dysfunction. It is also pointed out that irrespective of differences in the pathogenesis of different types of muscular dystrophy, dystrophic muscles from all types of diseased subjects show similar metabolic defects and impaired function. This article is therefore intended to provide a comprehensive and updated information about metabolic and biochemical alterations in dystrophic skeletal muscle during the development of muscular disorder in general. Derangements for abnormal Ca^{2+} -entry as well as Ca^{2+} -handling in myocytes from dystrophic muscle will also be described in some details and in particular, the role of store-operated Ca^{2+} -channels in the development of intracellular Ca^{2+} -overload will be outlined. Mechanisms of Ca^{2+} -handling abnormalities and metabolic defects in dystrophic muscles from different experimental models of muscular disease will also be described. Some changes in membrane activities and metabolic status of the hind leg muscle from myopathic hamsters will be discussed to show if there is any relationship with impaired muscle performance. Since dystrophin, an important component for anchoring different proteins and enzymes in the membrane, the function of sarcolemma in several forms of muscular dystrophy will be evaluated [13, 14, 20]. The significance of dystrophin gene mutations leading to dystrophin deficiency in the development of this disease will also be highlighted. In addition, attempts will be made to describe some of the therapeutic strategies, including gene therapy and pharmacologic interventions, which are used for the management or treatment of muscular disorder.

2. Biochemical defects in dystrophic muscle

Different mechanisms including impaired metabolism [8, 26–31], structural and biochemical membrane defects [32–39] and Ca^{2+} regulatory abnormalities [40–52] have been described to understand the pathogenesis of weakness in dystrophic muscle. An increased activity of enzymes, such as CK in the serum is considered to reflect damage to the muscle cell membrane and serves as a sensitive marker for the progression of this disease [53–59]. Recent studies with circulating proteins and metabolites have validated different biomarkers for patients with DMD [60–64]. It was demonstrated that defects of the plasma membrane lead to leakage of several cellular constituents such as myoglobin, glycogen, potassium, ATP and creatine from muscle fibres in addition to producing increased influx of Ca^{2+} in myocytes [33, 43, 65–69]. Ultrastructural examination of dystrophic muscle revealed segmental fiber breakdown and Ca^{2+} -deposits in myocytes with intact basement membrane as significant features [65, 66, 70–72]. Several changes indicating biochemical abnormalities in the sarcolemma (SL) membrane were observed in differ-

ent types of dystrophic muscle [73–76]. Marked alterations in lipid and electrolyte content were also seen in dystrophic muscle indicating abnormal function of the cell for maintaining appropriate levels of intracellular components [73, 77]. Thus, it has become evident that the increased permeability of skeletal muscle is reflected by defective SL membrane during the development of muscular disease [43, 70, 78].

Alterations in the integrity of dystrophic muscle membrane [79, 80] were associated with changes in different SL enzyme systems such as Na^+ - K^+ ATPase, Ca^{2+} / Mg^{2+} ecto-ATPase, and adenylyl cyclase [74–76, 81–85]. Since both cholesterol and phospholipids are known to exert membrane stabilizing effects [86, 87] and the ratio of cholesterol/phospholipids was elevated in dystrophic muscle [88], it has been suggested that the observed changes in enzyme activities are a consequence of alterations in the SL lipid composition. Studies from dystrophic muscle fibroblasts and skin fibroblast cultures have also shown different alterations such as cytoplasmic inclusion bodies, defective collagen incorporation as well as membrane defects [89–93]. Furthermore, derangements in the function of intracellular membrane systems such as the sarcoplasmic reticulum (SR) [40, 76, 88] and mitochondria [41, 76, 94–96] were reported in dystrophic muscle. Although no alterations in myofibrillar ATPase activity and contractile proteins in myopathic hamster muscle were observed [30, 76], some investigators have shown defective myosin in a chicken model of muscular dystrophy [97]. Nonetheless, marked changes in metabolism [26–30, 98] indicating the impaired performance of skeletal muscle in different types of muscle disorders may be associated with subcellular defects and metabolic abnormalities. In addition, maldistribution of electrolytes in dystrophic myocytes [66, 77], there occurs the development of intracellular Ca^{2+} -overload [41–44, 99], which activates different proteolytic enzymes [100, 101] and leads to muscle breakdown as well as structural defects dystrophic muscle of DMD patients [102, 103].

Since the discovery of the DMD gene and identification of the role of dystrophin-deficiency in DMD in 1980s, various genetically engineered animal models have been developed to understand the biology, biochemistry and pathophysiology of different types of muscular dystrophies [104–106]. The most widely used is a dystrophin-deficient mouse (mdx), which is considered to be an excellent model for studying DMD [107–115]. The other animal models of dystrophin-deficiency have employed pigs [116, 117], rats [118, 119], dogs [120–122] and cats [123, 124]. It is pointed out that marked changes in protein, lipids, carbohydrate and energy metabolism have been observed in skeletal muscle from mdx dystrophic mouse [125–129]. Furthermore, impaired mitochondrial oxidative phosphorylation and increased Ca^{2+} content due to dystrophin-deficiency have been reported in mdx mouse skeletal muscle [130–134]. Treatments of mdx dystrophic mouse with Ca^{2+} -antagonists, verapamil and diltiazem, as well as with creatine were observed to re-

duce skeletal muscle degeneration and mitochondrial dysfunction [135, 136]. It is noteworthy that skeletal muscle from DMD patients to have also been found to exhibit mitochondrial dysfunction and sarcolemmal alterations due to dystrophin-deficiency [137, 138]. It is also pointed out that sarcoglycan-deficiency models have also been developed in different animals such as hamsters, mice and chickens to examine metabolic and cellular defects in various muscular disorders [139–145].

3. Metabolic and subcellular defects in skeletal muscle of myopathic hamster

In order to examine the functional significance of metabolic and subcellular alterations for impaired performance of myopathic muscle, hind leg muscles from two experimental models (BIO 14.6 and UM-X7.1) of Syrian hamster (δ -sarcoglycanopathy) at different stages of development were used [30, 40, 74–76]. It may be noted that the clinical signs of muscle impairment in BIO 14.6 strain of hamsters start developing at the age of 100 to 150 days (early stage) whereas moderate and severe stages of myopathy become apparent at 180–210 days and 260–275 days of age, respectively [30, 146]. On the other hand, UM-X7.1 strain of myopathic hamsters develop degenerative lesions as early as 20 to 30 days whereas these animals at the age of about 60 days and about 150 days were considered to be at moderate and severe stages of muscular disorder, respectively [74, 147]. Although different stages of myopathy in both models of hamsters have been categorized on the basis of pathological lesions in the hind leg, it is understood that these stages are arbitrary and reflect the progression of muscular impairment with respect to the age of animals.

By employing the BIO 14.6 hamster model of myopathy, Sulakhe *et al.* [74] were the first to show increases in the activities of SL Mg^{2+} -ATPase and Na^+ - K^+ ATPase in skeletal muscle. This observation showing defect in the SL membrane at the biochemical level was confirmed by Peter and Fiehn [148], who reported increased activities of Na^+ - K^+ ATPase and Ca^{2+} -ATPase in myotonic muscles of rats treated with diazcholesterol. Although no changes in skeletal muscle SL ATPase activities were detected at early stages in BIO 14.6 myopathic hamsters, marked increases in the activities of SL Na^+ - K^+ ATPase, Mg^{2+} -ATPase and Ca^{2+} -ATPase were seen in both moderate and severe stages of muscular disorder (Table 1) [75]. Likewise, marked increases in these SL enzyme activities in skeletal muscle were observed in UM-X7.1 strain of myopathic hamsters at both moderate and severe stages except that Na^+ - K^+ ATPase and Mg^{2+} -ATPase activities were not altered at the moderate stage (Table 1) [76]. Similar increases in the hind leg skeletal muscle SL Na^+ - K^+ ATPase, Mg^{2+} -ATPase and Ca^{2+} -ATPase were seen in rats on vitamin E deficient diet [75], which is considered to be a good model for studying the pathogenesis of muscular weakness [34]. However, preliminary studies with dystrophic skeletal muscles from

human showed that SL Na^+ - K^+ ATPase activity was depressed but both Ca^{2+} -ATPase and Mg^{2+} -ATPase activities were increased [75]. Decreased Na^+ - K^+ ATPase and increased Mg^{2+} -ATPase activities were also observed in SL preparations from dystrophic muscles of Bar Harbor strain of 129/Rej mice [149]. These observations suggest that alterations in SL Na^+ - K^+ ATPase may depend upon the type and stage of muscular disorder; the increased activity of this enzyme may play a compensatory role in maintaining the electrolyte composition of the myopathic muscle whereas the depressed Na^+ - K^+ ATPase may be associated with increased entry of Ca^{2+} through Na^+ - Ca^{2+} exchange system. Furthermore, the increased activities of skeletal muscle SL Ca^{2+} -ATPase and Mg^{2+} -ATPase, which have been shown to represent Ca^{2+} / Mg^{2+} -ecto ATPase [150], may promote Ca^{2+} -influx for the occurrence of intracellular Ca^{2+} overload and thus play a pathogenic role for the impaired performance of myopathic muscle. It is pointed out that SL Ca^{2+} / Mg^{2+} -ecto ATPase, which is activated by millimolar concentrations of Ca^{2+} or Mg^{2+} , has been suggested to serve as a “gating mechanism” for the entry of Ca^{2+} into the cell [150–153]. Although the status of SL Na^+ - Ca^{2+} exchange system was not examined in hamster myopathic muscle, the activity of Na^+ - Ca^{2+} exchange was increased in dystrophic muscle from patients with DMD as well as mdx model of muscular dystrophy in mice [154, 155].

Since the SR plays a critical role in regulating the intracellular Ca^{2+} concentration in myocytes, Ca^{2+} -binding and Ca^{2+} -transport activities of this subcellular organelle were examined in skeletal muscles of both BIO 14.6 and UM-X7.4 strains of myopathic hamsters at moderate and severe stages of muscular disorder [40, 76]. The results in Table 2 show that ATP-dependent Ca^{2+} -binding activities (studied in the absence of oxalate) of SR from skeletal muscles of myopathic animals were not altered at both moderate and severe stages. Although no changes in the ATP-dependent Ca^{2+} -uptake activities (studied in the presence of oxalate) of SR of myopathic muscle were detected at moderate stage, these Ca^{2+} -transport activities were depressed in both models of myopathy at severe stages. It may also be noted from Table 2 that the SR Ca^{2+} -pump ATPase activity was decreased markedly at severe stages of myopathy. Several investigators have also reported defects in the Ca^{2+} -transport activities in the SR preparations from different myopathic animals as well as in DMD patients [156–159]. However, others have denied the occurrence of such abnormalities in muscular disorder [160–162], which may be due to the use of skeletal muscles at early or moderate stage of the disease. Thus, it appears that the reduced ability of SR to accumulate Ca^{2+} at the late stage of muscular disease may be secondary to other mechanisms leading to impaired performance of skeletal muscle.

The data in Table 3 show the status of mitochondrial Ca^{2+} -binding and Ca^{2+} -uptake activities as well as oxidative phosphorylation in UM-X7.1 strain of hamsters at moderate or severe stages of myopathy [76]. No changes in mitochon-

Table 1. ATPase activities of sarcolemma from skeletal muscles of control and myopathic hamsters of different ages.

	Na ⁺ -K ⁺ ATPase (μmoles Pi/mg/hr)	Mg ²⁺ -ATPase (μmoles Pi/mg protein/hr)	Ca ²⁺ -ATPase (μmoles Pi/mg/hr)
A. BIO 14.6 myopathic hamsters			
a. Control: (100–272 days old)	20.4 ± 1.2	33.1 ± 1.6	31.5 ± 2.9
b. Myopathic			
(i) 100–150 days old (Early stage)	19.7 ± 1.9	31.5 ± 2.4	32.4 ± 2.5
(ii) 180–210 days old (Moderate stage)	27.5 ± 1.5*	43.3 ± 2.1*	42.4 ± 1.4*
(iii) 260–275 days old (Late stage)	38.5 ± 3.3*	59.2 ± 4.2*	56.2 ± 3.7*
B. UM-X7.1 myopathic hamsters			
(i) 60 days old			
Control	6.6 ± 0.7	30.6 ± 2.6	10.0 ± 1.3
Myopathic (Moderate stage)	6.5 ± 0.4	28.7 ± 1.7	16.8 ± 1.0*
(ii) 150 days old			
Control	13.8 ± 1.1	25.0 ± 1.9	27.6 ± 2.8
Myopathic (Late stage)	19.1 ± 0.8*	50.5 ± 4.2*	58.0 ± 5.1*

The data for BIO 14.6 strain of myopathic hamsters are taken from our paper, Dhalla *et al.* Res Commun Chem Pathol Pharmacol 6, 643–650, 1973 [75] whereas the data for UM-X7.1 strain of myopathic hamsters are taken from our paper Dhalla *et al.* Clin Sci Mol Med 49, 359–368, 1975 [76].

* Significantly ($P < 0.05$) different from the corresponding control values.

drial Ca²⁺-accumulation and ATPase activities as well as different parameters of oxidative phosphorylation were observed in 150 days old myopathic animals. On the other hand, 60 days old hamster myopathic muscle showed depressions in both mitochondrial Ca²⁺-uptake and oxidative phosphorylation rate, unlike Ca²⁺-binding and ATPase, P:O ratios and RCI values (Table 3). The inability to detect changes in mitochondrial Ca²⁺-transport and oxidative phosphorylation in myopathic muscle at late stages of the disease in UM-X7.1 strain of hamsters was not attributed to the method used for the isolation of mitochondria because the same procedure was employed for obtaining mitochondrial preparation from muscles at moderate stage of muscular disease. Since the mitochondrial defects in myopathic muscle have also been shown to be due to the occurrence of mitochondrial Ca²⁺-overload [41], it is likely that the excessive amount of Ca²⁺ from mitochondria at late stages of myopathy may have been lost during the isolation procedure. Nonetheless, other investigators have shown defects in mitochondrial oxidative phosphorylation activities in myopathic muscles of BIO 14.6 strain of myopathic hamsters [94–96]. Such abnormalities in the mitochondrial oxidative phosphorylation can be seen to impair the ability of myopathic muscle to generate energy for the function of skeletal muscle myocytes. In fact, marked changes in the high energy phosphate content and other metabolic processes showing impaired energy production in skeletal muscles from BIO 14.6 strain of hamsters at late stages of muscular disease have been reported [30]. Some of these data shown in Table 4 indicate that both creatine phosphate and ATP were depressed without any significant changes in ADP and AMP content of dystrophic hamster muscle. Furthermore, lactate, NADH and NADPH were increased indicating marked alterations in muscle metabolism without any changes in pyruvate content of myopathic muscle (Table 4). These observations support the view that re-

duction in the high energy phosphate stores due to impaired process of energy production may play an important role in dysfunction of skeletal muscle in muscular disorder.

4. Role of dystrophin deficiency in muscular dystrophy

It is now well known that stability of the cell membrane is very critical for appropriate function of skeletal muscle and thus any defect in the plasma membrane can be seen to induce muscular abnormality [14, 15, 23, 25]. Since dystrophin based cytoskeleton has been shown to anchor various proteins and other constituents of the SL membrane, dystrophin deficiency has been associated with the development of muscular dystrophy [14, 15, 20, 46, 73, 163]. Advances in molecular biology techniques in late 1980's have facilitated the discovery of mutations in dystrophin gene on the chromosome region Xp21, which is the largest known gene, responsible for the expression of dystrophin protein [164, 165]. This research was started with the elucidations of 2300 kb dystrophin gene, which consists of 79 exons and encodes dystrophin protein. Various mutations in this gene identified thus far, have been shown to reduce the level of dystrophin protein and lead to the development of muscular dystrophy as well as cardiomyopathy, respiratory dysfunction and mental retardation [166]. It is also pointed out that the SL dystrophin protein has been reported to occur as a dystrophin-glycoprotein complex consisting of dystroglycans, sarcoglycans, sarcospan, dystrobrevins and syntrophins [167, 168]. Furthermore, this devastating inherited muscular disorder is considered to be caused by mutations in dystrophin gene and is known to affect mainly males due to X-linked recessive mode of inheritance; females are carriers of these gene mutations [169].

A deficiency of dystrophin protein plays a significant role in the pathology of muscular dystrophy, and in this regard,

Table 2. Ca^{2+} -transport activities of sarcolasmic reticulum from skeletal muscles of control and myopathic hamsters of different ages.

	ATP-dependent Ca^{2+} - binding (nmoles/mg protein/5 min)	ATP-dependent Ca^{2+} - uptake (μ moles/mg protein/5 min)	Ca^{2+} -pump ATPase (μ M Pi/mg protein/5 min)
A. BIO 14.6 myopathic hamsters			
(i) 220 days old			
Control	153 \pm 22	3.6 \pm 0.20	–
Myopathic (Moderate stage)	136 \pm 11	3.4 \pm 0.15	–
(ii) 260 days old			
Control	142 \pm 5	3.8 \pm 0.45	2.76 \pm 0.31
Myopathic (Late stage)	135 \pm 7	2.3 \pm 0.62*	1.32 \pm 0.19*
B. UM-X7.1 myopathic hamsters			
(i) 60 days old			
Control	118 \pm 13	3.8 \pm 0.52	1.44 \pm 0.15
Myopathic (Moderate stage)	95 \pm 11	3.6 \pm 0.21	1.07 \pm 0.19
(ii) 150 days old			
Control	145 \pm 12	5.2 \pm 0.39	2.56 \pm 0.27
Myopathic (Late stage)	109 \pm 14	2.8 \pm 0.26*	1.45 \pm 0.21*

The data for BIO 14.6 strain of myopathic hamsters are taken from our paper, Dhalla & Sulakhe. Biochem Med 7, 157–168, 1973 [40] whereas the data for UM-X7.1 myopathic hamsters are taken from our paper Dhalla *et al.* Clin Sci Mol Med 49, 359–368, 1975 [76].

* Significantly ($P < 0.05$) different from the corresponding control values.

Table 3. Mitochondrial Ca^{2+} -transport and oxidative phosphorylation activities of skeletal muscles from UM-X 7.1 strain of myopathic hamsters of different ages.

	60 days old		150 days old	
	Control	Myopathic (Moderate stage)	Control	Myopathic (Late stage)
Ca^{2+} - binding (nmoles/5 min/mg)	84 \pm 7	79 \pm 5	116 \pm 14	113 \pm 11
Ca^{2+} - uptakes (nmoles/5 min/mg)	552 \pm 64	353 \pm 58*	596 \pm 73	571 \pm 49
ATPase activity (μ mol/5 min/mg)	3.9 \pm 0.63	4.3 \pm 0.71	4.5 \pm 0.96	5.2 \pm 1.08
P:O ratio	2.8 \pm 0.07	2.9 \pm 0.05	2.9 \pm 0.04	3.0 \pm 0.06
Phosphorylation rate (μ mol ADP phosphorylated/min/g protein/min)	110 \pm 8.8	81 \pm 5.6*	127 \pm 19.4	112 \pm 26.0
RCI	5.3 \pm 0.52	6.3 \pm 0.21	8.1 \pm 1.4	7.6 \pm 1.1

The data are taken from our paper, Dhalla *et al.* Clin Sci Mol Med 49, 359–368, 1975 [76].

* Significantly ($P < 0.05$) different from the corresponding control values.

it is pointed out that more than 1000 mutations have been identified in dystrophin gene. As a part of an incredibly complex group of proteins, dystrophin allows muscle to function properly as well as aids in anchoring various components within muscle cells and link them to the SL membrane [167]. Thus, dystrophin provides a scaffold for holding several molecules in place near the cell membrane whereas dystrophin deficiency dislocates these molecules and cause disorders in their function [167]. There are several reports on structural, functional, biochemical, molecular and metabolic defects, which are induced by these dystrophin gene mutations [13, 29, 73, 170, 171]. Reduction of dystrophin-glycoprotein complex promotes the occurrence of structurally unstable SL membrane, which is more permeable to extracellular environment and thus contribute to muscle fiber damage and wastage of skeletal muscles [170, 171]. Particularly, repetitive cycles of contraction and relaxation of dystrophin deficient skeletal muscle produce microtears in the SL membrane, and result in cellular instability and progressive leakage of intracellular components including CK. Such

a leakage of CK can be seen to reduce the intracellular level of CK content and thus may impair the storage of energy in skeletal muscle. In addition, the increased permeability of the SL membrane due to dystrophin deficiency will promote an excessive entry of Ca^{2+} into myocytes to result in the development of intracellular Ca^{2+} -overload, which is well known to activate different proteolytic enzymes and produce muscle wastage. Furthermore, dystrophin deficiency has been reported to induce different abnormalities in the SL signal transduction pathways, which impair muscle regeneration and induce weakness in muscle performance [171]. A schematic representation of dystrophin deficiency related events is shown in Fig. 2.

It needs to be emphasized that mutations of genes other than for dystrophin gene have also been reported to induce muscular disorders. For example, mutations in dysferlin gene have been shown to decrease dysferlin content and result in progressive development of muscle wasting and myopathic characteristics [172]. Although deficiency of laminin- α 2, a protein of the extracellular matrix and a component of the

Table 4. High energy phosphate stores, glycolytic intermediates and pyridine nucleotides in skeletal muscle of 215 day old BIO 14.6 strain of myopathic hamsters.

	Control	Myopathic (Late stage)
(i) Creatine phosphate ($\mu\text{mol/g}$ muscle)	13.3 ± 0.26	$6.13 \pm 0.49^*$
(ii) Adenine nucleotides ($\mu\text{mol/g}$ muscle)		
ATP	5.97 ± 0.21	$4.04 \pm 0.27^*$
ADP	0.35 ± 0.04	0.39 ± 0.02
AMP	0.17 ± 0.02	0.21 ± 0.02
(iii) Glycolytic intermediates ($\mu\text{mol/g}$ muscle)		
Lactate	1.19 ± 0.02	$7.18 \pm 0.14^*$
Pyruvate	0.050 ± 0.002	0.051 ± 0.003
(iv) Pyridine nucleotides (mmol/g muscle)		
NADH	139 ± 5.0	$168 \pm 3.1^*$
NADPH	45 ± 3.1	$67 \pm 5.7^*$

The data are taken from our paper, Dhalla *et al.* Can J Biochem 50, 550–556, 1972 [30].

* Significantly ($P < 0.05$) different from the corresponding control values.

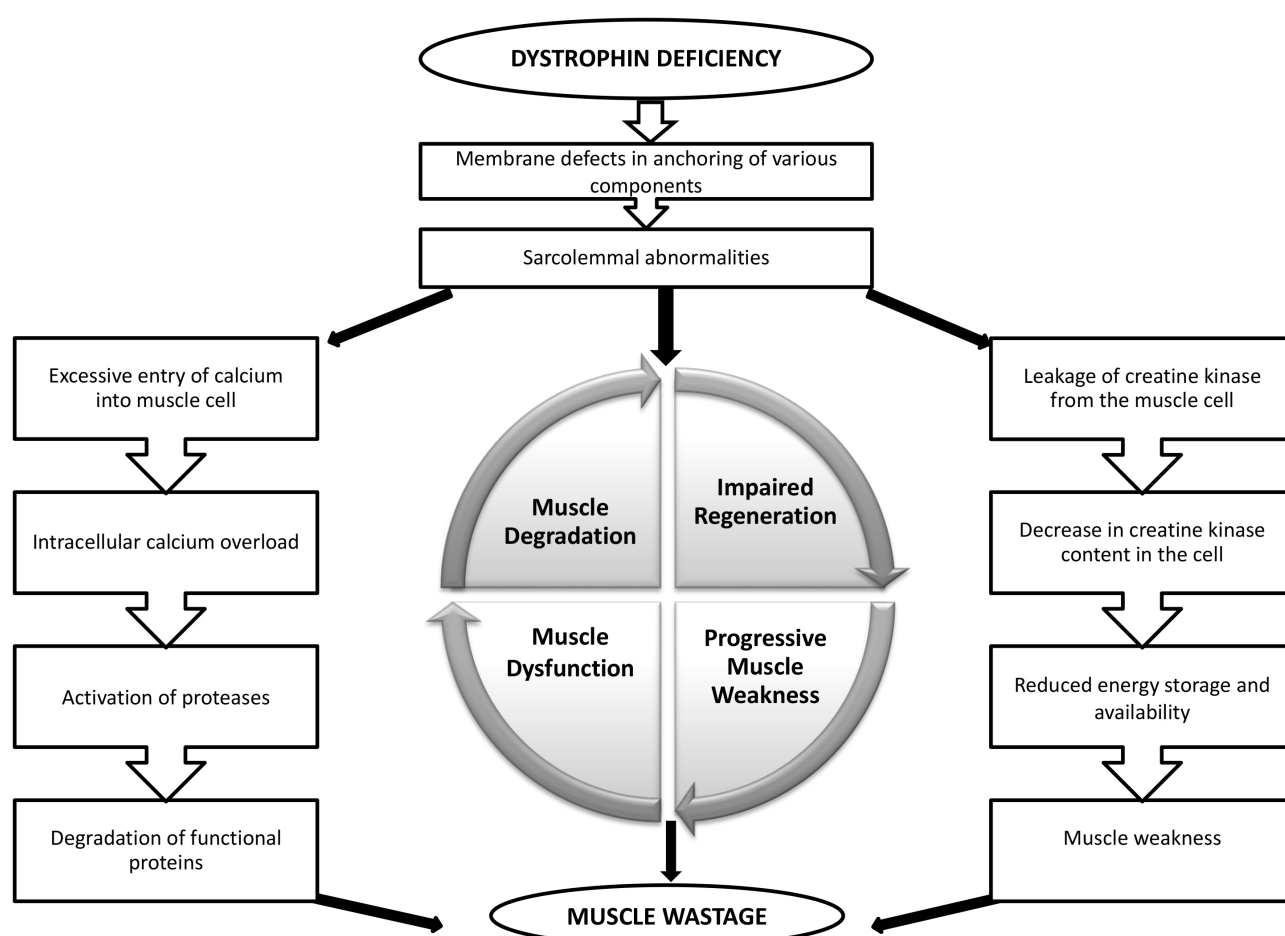


Fig. 2. A schematic representation of dystrophin deficiency related events.

basal membrane, has also been reported to induce the SL instability and result in the development of muscular disorder [23, 24], dystrophin mutations have been recognized as the primary cause in the development of muscular dystrophy because of a significant decrease in the expression of dystrophin associated complex proteins. In fact, changes in dystrophin associated proteins such as sarcoglycan complex as

transmembrane proteins have been shown to promote the production of oxidative stress and lead to muscle cell death as well as stiffness in myopathic muscle [171]. It should be mentioned that neuronal nitric oxide synthase, which interacts indirectly with dystrophin protein, is also influenced markedly due to dystrophin deficiency and thus affects the performance of dystrophic muscle [173]. In addition, some studies have

indicated that the membrane impairment occurs frequently in the absence of dystrophin, leading to localized cell damage and leakage of intracellular constituents [174]. However, a significant increase in total Ca^{2+} content is consistent with excessive Ca^{2+} entry into the cell through damaged membranes due to dystrophin deficiency. Although other pathways of Ca^{2+} entry such as Na^{+} - Ca^{2+} exchange [155] and Ca^{2+} -gating system (Ca^{2+} / Mg^{2+} -ecto-ATPase) [153] may also be enhanced in muscular dystrophy as a consequence of dystrophin deficiency, extensive experiments need to be carried out to make any meaningful conclusion. Nonetheless, it is evident that dystrophin deficiency may play a critical role in the pathogenesis of muscular dystrophy due to the occurrence of intracellular Ca^{2+} -overload [171, 173, 175].

5. Role of store-operated Ca^{2+} entry in muscular dystrophy

The store-operated Ca^{2+} entry (SOCE) is one of the important mechanism for extracellular Ca^{2+} influx and is activated by depletion of intracellular Ca^{2+} stores [176, 177]. Several studies have been carried out to explore the status of SOCE regulation in normal and dystrophic skeletal muscles [48, 178, 179]. In fact, SOCE it was found to play an essential role in some physiological functions including skeletal muscle development, contractile activity and metabolism. Furthermore, it prevents muscle weakness and serves as a signaling pool of Ca^{2+} required to modulate muscle-specific gene expression in myopathic muscle [180–183]. Abnormal increases in the SOCE channel activity result in muscle dysfunction including the activation of Ca^{2+} signaling pathways leading to metabolic disorders, irregular protein handling, and detrimental remodeling phenotype in the pathogenesis of muscular disease [179, 180, 184–186]. The disruption of SOCE channels has been suggested to cause an imbalance in the level of intracellular Ca^{2+} and subsequent Ca^{2+} -dependent activation of proteases and muscle degeneration due to dystrophin deficiency in dystrophic muscle [40, 187, 188]. It has been reported that the occurrence of an excessive SOCE occurrence is an early event in the pathology of muscular dystrophy and the altered Ca^{2+} dynamics in myotubes result in continued cytosolic Ca^{2+} transients and increased Ca^{2+} uptake by mitochondria [187, 189]. The phospholipase A_2 product, lysophosphatidylcholine, was found to trigger Ca^{2+} entry through SL channels, and acts as an intracellular messenger responsible for the opening of store-operated channels in dystrophic fibers [190]. Elevation of intracellular Ca^{2+} was found to increase phospholipase A_2 activity and the overexpression activity of NADPH oxidase and excessive production of reactive oxygen species (ROS) were observed to contribute to the pathology in dystrophin deficient dystrophic muscle [191, 192].

Both STIM1 and Orai1 proteins have been identified as essential components of SOCE channel in the plasma membrane [193, 194]. Studies from genetic mouse models with deletion of these components have provided evidence of im-

paired skeletal muscle growth, suggesting the physiologic role for SOCE in skeletal muscle development and contractile function [195–199]. These molecular components which skeletal muscle expresses in abundance serve as the main channel constituents involving SOCE for contributing to the mitochondrial Ca^{2+} homeostasis and a range of downstream signaling pathways as well as in the regulation of several transcription factors [144, 200–204]. STIM1 is a multipurpose stress transducer initiated by various stimuli such as oxidation, temperature, hypoxia, and acidification and may regulate varied downstream targets including different ion channels, pumps/exchangers, adaptor proteins, endoplasmic reticulum (ER) chaperones, signaling enzymes, and ER stress/remodeling proteins. On the other hand, Orai1 serves as a SOCE channel in skeletal muscle [205]. Furthermore, fast kinetics of the SOCE activation in the skeletal muscle, depends on the pre-formation of STIM1-protein complexes with the plasma-membrane, whereas Orai1-mediated Ca^{2+} influx appears essential to control the resting Ca^{2+} concentration and proper SR Ca^{2+} filling. Ca^{2+} influx through STIM1-dependent activation of SOCE from the T-tubule system may recycle the extracellular Ca^{2+} loss during muscle stimulation and thereby maintain proper filling of the SR Ca^{2+} stores and muscle function [206, 207]. Various studies have demonstrated that STIM1/Orai1-dependent signals promote muscle fiber maturation, growth, oxidative process, fatigue-resistant fibers, and muscle development [195, 207–209]. There is evidence to suggest that the STIM1/Orai1-dependent SOCE promotes sustained force generation during periods of prolonged activity as well as resistance to muscle fatigue [210–213].

Altered function of essential proteins regulating the SOCE activity, contributes to or amplifies the pathogenesis of muscle disorders including muscular dystrophy. A number of studies have provided evidence for a modulatory contribution of the STIM/Orai1-dependent SOCE in animal models of muscular dystrophy [178, 214–216]. Increased STIM1/Orai1 expression as well as SOCE functionality, including a shift in the threshold for SOCE activation and deactivation to SR luminal Ca^{2+} concentrations have been observed in muscle fibers from dystrophic mice [178, 217]. In another study, although STIM1 levels were unchanged in muscles from dystrophic mice, an increase was found in both Orai1 mRNA and protein levels corresponding to the enhanced SOCE activity and SR Ca^{2+} storage. Since these augmented activities were reduced by either shRNA-mediated Orai1 knockdown or treatment of animals with BTP-2 (a potent CRAC channel inhibitor), it was proposed that increased function of the STIM1/Orai1dependent SOCE contributes to Ca^{2+} -mediated muscle fiber degeneration in dystrophic mice [218]. Enhanced SOCE and increased muscle inflammation, fibrosis, necrosis, mitochondrial swelling, and serum CK levels, were noticed due to the muscle-specific STIM1 overexpression in dystrophic mice [214]. The role of STIM1-mediated Ca^{2+} signaling for skeletal muscle hy-

peritrophic growth has also been demonstrated in the skeletal muscle STIM1 knockout mice [195]. Furthermore, a correlation of the overstimulation of SOCE in dystrophic cells with increased STIM1 protein content has been reported [188, 207, 208]. Since overexpression of STIM1 stimulates muscle cell differentiation whereas silencing inhibits this process [208], it is evident that high SOCE and elevated STIM1 levels in muscular dystrophy are associated with greater differentiation of dystrophic myoblasts [198].

Increased rate of SOCE activity with high STIM1 protein levels in dystrophic mice myoblasts has revealed that mutation of dystrophin gene may significantly impact the cellular calcium response to metabotropic stimulation. It is pointed out that an aberrant response to extracellular stimuli may contribute to the pathogenesis of muscular dystrophy and inhibition of such responses might modify the progression of this deadly disease [188]. Overactivation of SOCE upon dystrophic cell stimulation may lead to intracellular Ca^{2+} -overload and increased susceptibility to cell death as well as progressive muscle degeneration [219]. It has been demonstrated that Ca^{2+} -influx across an unstable SL due to increased activity of the STIM1/Orai1 complex is a major determinant in muscular dystrophy [214] and STIM1/Orai1 along with TRPC1 are involved in increasing SOCE in the dystrophin deficient myotubes in both dystrophic patients and dystrophic mice. Thus, the participation of a specific Ca^{2+} /PKC/PLC pathway in increasing the SOCE activity may be due to STIM1/Orai1/TRPC1 protein interactions, which are regulated by the dystrophin scaffold [215]. These findings support the functional role of STIM1/Orai1-dependent SOCE in the pathophysiology of muscular dystrophy and are considered to represent a potential therapeutic target [216].

6. Mechanisms of subcellular Ca^{2+} -handling abnormalities in dystrophic muscle

An excessive amount of Ca^{2+} entering dystrophic muscle is considered to play a critical role in raising the intracellular level of Ca^{2+} as well as inducing metabolic defects, subcellular remodeling, Ca^{2+} -handling abnormalities and impairment of muscular function [15, 20, 25, 47, 155, 171, 180, 185, 217]. Increases in Ca^{2+} -entry and SL permeability have been shown to be associated with dystrophin deficiency in muscular dystrophy; such defects in some other types of this disease are linked to deficiencies of different proteins including dysferlin and α -sarcoglycan [47, 171, 172, 180, 185, 220]. The role of intracellular Ca^{2+} -overload in the pathogenesis of muscular disorder is supported by observations that exercise in dystrophic mdx mice enhanced Ca^{2+} -influx, impaired Ca^{2+} -homeostasis and aggravated this disease [221]. It should be pointed out that the increased Ca^{2+} -influx in myopathic muscle may be occurring through SOCE channels [192, 216, 222], voltage-independent Ca^{2+} -leakage channels [223], voltage-dependent Ca^{2+} -channels [189] and Na^+/Ca^+ exchange system [154, 155, 224, 225] in the SL

membrane. Since the SL phospholipase A_2 [190] and adenylylate cyclase activities [226] are increased in dystrophic muscles, these signal transduction systems have also been suggested to participate in enhancing Ca^{2+} -entry into muscle fiber. The involvement purinoceptor associated mechanisms has also been reported to induce increased Ca^{2+} -influx because the expressions and activities of P2X receptors are increased in dystrophic muscle [227–229]. The increased activities of both Mg^{2+} -ATPase and Ca^{2+} -ATPase in the surface membrane of dystrophic muscle were decreased with age of the animal [230]. Likewise, some investigators have shown an increase [231] whereas others have observed a decrease in the Na^+/K^+ ATPase activity in dystrophic muscle [232]. Such variable changes in the SL enzyme activities may be due to the type or age of dystrophic animals. In fact, a variety of changes in other SL components have been identified in dystrophic muscle [233, 234]. Taken together, all these observations support the view that increased Ca^{2+} -influx through the plasma membrane of dystrophic muscle may be a consequence of SL remodeling during the development of muscular dystrophy.

The impaired function of dystrophic muscle is generally considered to be due to Ca^{2+} -handling abnormalities in myocytes. Since the SR is intimately involved in maintaining the intracellular Ca^{2+} -homeostasis, different defects in this intercellular organelle have been identified in muscular dystrophy. Marked alterations in the structure and function of Ca^{2+} -release channel or ryanodine receptor in the SR have been demonstrated in dystrophic muscle [235–237]. A progressive increase in the expression of ryanodine receptor and ryanodine receptor binding in the SR has been shown to occur during the development of muscular dystrophy [238]. The leaky ryanodine receptors in dystrophic muscle have been indicated to limit the activation of SOCE channels and produce changes in Ca^{2+} -homeostasis [239]. It should also be noted that drastic reductions in both sarcoplasmic reticulum and calsequestrin have been reported in dystrophic muscle to depress Ca^{2+} -binding in the lumen of SR and are considered to play a role in abnormal Ca^{2+} -handling in muscular dystrophy [240, 241]. Some investigators have shown marked depressions in the SR ATP-dependent Ca^{2+} -uptake and Ca^{2+} -pump ATPase activities in dystrophic muscle [242–245], whereas others have failed to observe such defects [246–248]. Such conflicting results may be due to the stage or type of muscular dystrophy. However, the depression in the Ca^{2+} -transport in the SR can be seen to occur because of the increased expression of sarcolipin, a known endogenous inhibitor of Ca^{2+} -pump ATPase, in dystrophic muscle [249–251]. Although calmodulin mRNA and content in dystrophic muscle are increased, the stimulation of the SR Ca^{2+} -pump ATPase by calmodulin is markedly depressed [252]. Thus, a reduction of Ca^{2+} -transport in the SR may also contribute to eliciting Ca^{2+} -handling abnormalities in dystrophic muscle. In this regard, it is noteworthy that an overexpression of the SR Ca^{2+} -pump ATPase has been

demonstrated to alter intracellular Ca^{2+} levels in dystrophic muscle and mitigate muscular dystrophy [181].

The development of Ca^{2+} -handling abnormalities in dystrophic muscle has been considered to affect other subcellular organelles such as mitochondria and myofibrils. In fact, mitochondrial dysfunction has been shown to occur before the onset of myofiber necrosis, muscle wasting and myofibrillar defects [253]. Impaired substrate utilization and ATP synthesis have been reported in dystrophic mitochondria [254, 255]. There occurs an uncoupling in the process of mitochondrial oxidative phosphorylation during the progression of muscular dystrophy [256, 257]. Although the development of mitochondrial Ca^{2+} -overload is considered to induce defects in energy production in dystrophic muscle [41], abnormalities in ATP synthesis by dystrophic mitochondria have also been shown to be caused by complex I insufficiency [258], disruption of mitochondrial protein Mss51 [259] and decreased CK content [260]. It is pointed out that the impaired performance of dystrophic muscle is not only a consequence of depressed energy stores but defects in the process of energy utilization have also been observed during the development of muscular dystrophy. In this regard, myofibrillar Ca^{2+} -stimulated ATPase and myosin ATPase activities have been shown to be decreased in dystrophic muscle [261, 262]. A shift in myosin heavy chain from alpha to beta isoform as well as changes in troponin-tropomyosin have been reported to cause alterations in dystrophic myofibrillar ATPase activities [263, 264]. Increased Ca^{2+} -activated protease activity in dystrophic muscle has also been shown to induce myofibrillar defects [100, 101, 265, 266].

It is becoming clear that Ca^{2+} -handling abnormalities in dystrophic muscle are associated with various defects in the subcellular organelles. Such alterations have been suggested to be caused by abnormalities of lipid metabolism in dystrophic muscle [78–88, 267, 268]. It may be mentioned that marked changes in cholesterol, triglycerides, unsaturated fatty acids and phospholipid content have been observed in dystrophic muscle [269–272]. Furthermore, increased oxidative stress in dystrophic muscle [172, 191, 273, 274] has been suggested to account for inducing remodeling and Ca^{2+} -handling abnormalities. It is pointed out that the expression of NADPH oxidase, which generates ROS, is markedly increased in addition to mitochondrial oxyradical production in dystrophic muscle [275, 276]. Furthermore, synergistic interactions of nitrosative/oxidative stresses due to increased neural nitric oxide synthase in dystrophic muscle [277, 278], have been indicated to be involved in Ca^{2+} -handling abnormalities during the development of muscular dystrophy.

7. Strategies for the treatment of muscular disorder

Corticosteroids therapy was reported for the first time to delay the progression of muscular disorder and was considered as the gold standard for its treatment [279]. Because almost all types of muscular dystrophies are caused by a single-

gene mutation [280], gene therapy was also introduced as a potential intervention to replace, repair or bypass the mutated gene [281, 281–283]. In order to restore an appropriate level of dystrophin in skeletal muscle, different gene strategies have been based on implantation and delivery of naked plasmid DNA for the dystrophin gene [284–288], by adeno-associated virus (AAV) [289] as well as exon-skipping with antisense oligonucleotides [290–292]. Due to challenges like targeted delivery of therapeutic molecules, lysosomal setup, enzymatic degradation as well as low intracellular uptake, successful working with gene therapy has stimulated the search for different drug delivery systems such as polymersomes [293, 294], liposomes and lipid-nucleic acid complexes [295–297], PMMA nanoparticles [296, 298], and cell-penetrating peptides [290, 298, 299]. While a great progress has been made with gene therapy [12, 300], other interventions such as injections of myoblasts (derived from healthy donors) did not induce beneficial effects due to low survivability, migration and immune rejection of the transplanted cells; thus stem cells based therapies have been suggested for the treatment of diverse muscular disorders [301, 302]. Furthermore, various molecular pathways have been shown to be most befitting for the development of novel medicinal products to prevent muscle degeneration and fibrosis. The genetic modifiers including LTBP4, Jagged1 and osteopontin, which regulate the disease progression by interfering with pro-fibrotic and pro-regenerative pathways (TGF- β , myostatin and Notch signaling), have also offered a platform to identify novel pharmacological targets for the therapy of muscular weakness [303, 304]. Thus, several approaches (Fig. 3) are considered worthwhile for not only preventing the progression of this disorder but also the improvement of dystrophic muscle performance.

It is noteworthy that, recent data have shown better efficiency of gene therapy when high doses are administered [287]. Although high doses are fairly tolerated and achieve adequate transgene expression, these lack adequate balance of safety and efficacy for the success of gene therapy [305]. Clinical trials have shown a slight risk of liver toxicity represented by transient elevation in liver enzymes and bilirubin as side effects in humans subjected to such a gene therapy. Furthermore, the major obstacle for gene therapy is the pre-existing immune reaction to AAV, which is used as vehicle; but the use of plasmapheresis was found to evade the immune reaction to AAV and this was considered to allow safe administration of gene therapy to patients with impaired muscular function [306]. Although, there is some optimism for utilizing plasmapheresis or T-cell suppression by agents such as rituximab and rapamycin to overcome the pre-existing immunity, the success of these approaches remains to be clearly demonstrated [307]. One of the shortcomings for the use of AAV is its limited packaging capacity for dystrophin molecule, which necessitated the development of a shorter protein, mini-dystrophin [308]. The construction of mini-dystrophin was based on the shortened dystrophin pro-

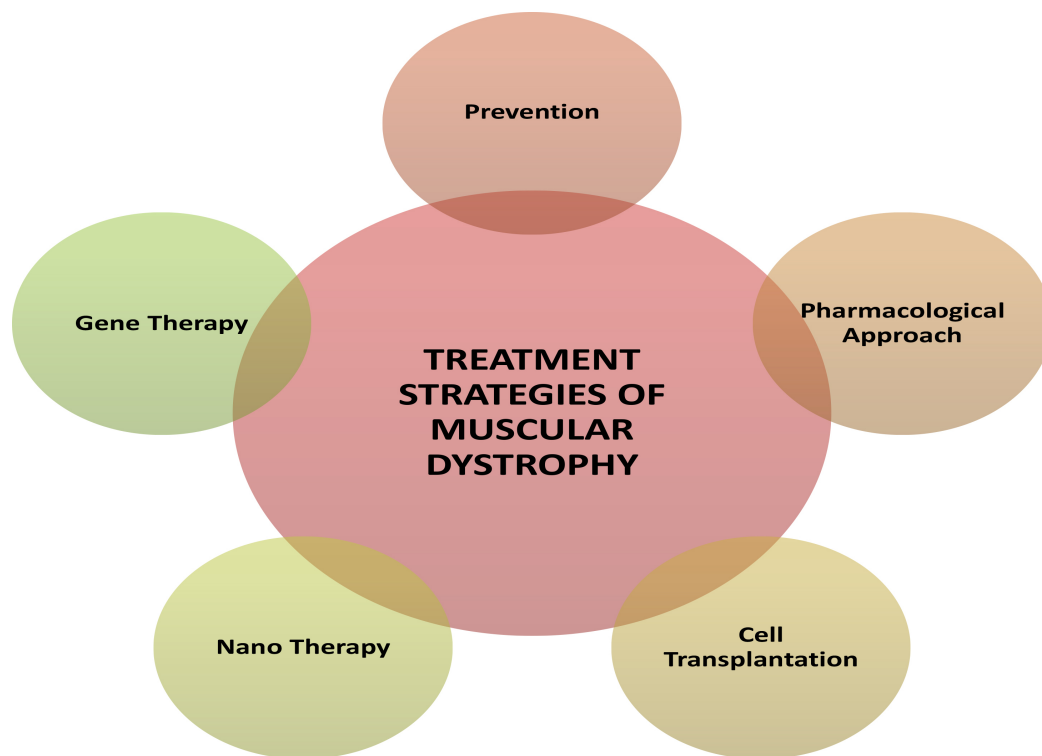


Fig. 3. Current strategies for the treatment of muscular dystrophy.

tein as expressed in dystrophic muscle and found to be highly functional [309]. Although mini-dystrophin gene therapy was shown to improve the systemic muscle function at early stages, it was only effective partially in advanced cases of muscular disease [308]. Such observations suggest that restoring muscle function to near-normal levels with gene therapy will probably necessitate additional research to improve and enhance the muscle strength in dystrophic muscle.

Since the intricacy of numerous mutations of dystrophin gene causes several challenges for the dystrophin gene therapy, a great deal of experimental work is needed for having an effective treatment of muscular disorder. Furthermore, it should be recognized that deficiency of laminin—alpha 2 protein has also been reported in dystrophic muscle and this was shown to be attenuated by omigapil, an antiplatelet agent [23]. Thus, it is likely that some combination gene therapy may prove more beneficial in delaying the course of this disease progression. It is also pointed out that pathological lesions in dystrophic muscle were found to be associated with marked increase in Ca^{2+} content but these changes were not prevented with verapamil treatment, a well known antagonist of voltage sensitive Ca^{2+} -channels in the SL membrane [147]. On the other hand, reduction of Ca^{2+} -influx through SOCE channels was found to improve function in diseased muscle [185, 189]. Reintroduction of mini-dystrophin in dystrophic muscle was also reported to reduce Ca^{2+} transients as a consequence of its effect on SOCE channels [192]. In addition, inhibition of phospholipase A_2 as well as lysophosphatidylcholine production was shown to de-

press Ca^{2+} -entry and prevent the degeneration of dystrophic muscle [190]. It should be mentioned that diapocynin, an inhibitor of NADPH oxidase, which reduces the production of ROS, was observed to depress the phospholipase A_2 activity as well as Ca^{2+} -influx through SOCE channels in dystrophic muscle. Accordingly, it is suggested that gene therapy in combination with some inhibitors of Ca^{2+} -entry and oxidative stress may be more effective for the treatment of muscular disorder.

8. Conclusions and future perspectives

From the foregoing discussion, it is evident that muscular dystrophy is a group of complex diseases, which results in skeletal muscle degeneration, loss of muscle fibers and impaired muscle function. Some forms of muscular dystrophy are considered to be a consequence of a genetic defect leading to deficiency of dystrophin for inducing abnormalities in the SL membrane. The progression of this disease is associated with leakage of intracellular enzymes and other constituents, maldistribution of electrolyte content, marked metabolic alterations and development of necrosis, apoptosis as well as fibrosis in dystrophic skeletal muscle. The depression in the high energy stores as a consequence of the mitochondrial Ca^{2+} -overload, and abnormalities in the SR due to defects in Ca^{2+} -release channel and Ca^{2+} -pump ATPase are considered to explain the impaired muscle performance. On the other hand, the activation of different proteolytic enzymes due to the occurrence of intracellular Ca^{2+} -overload is responsible for dystrophic muscle degeneration and wastage.

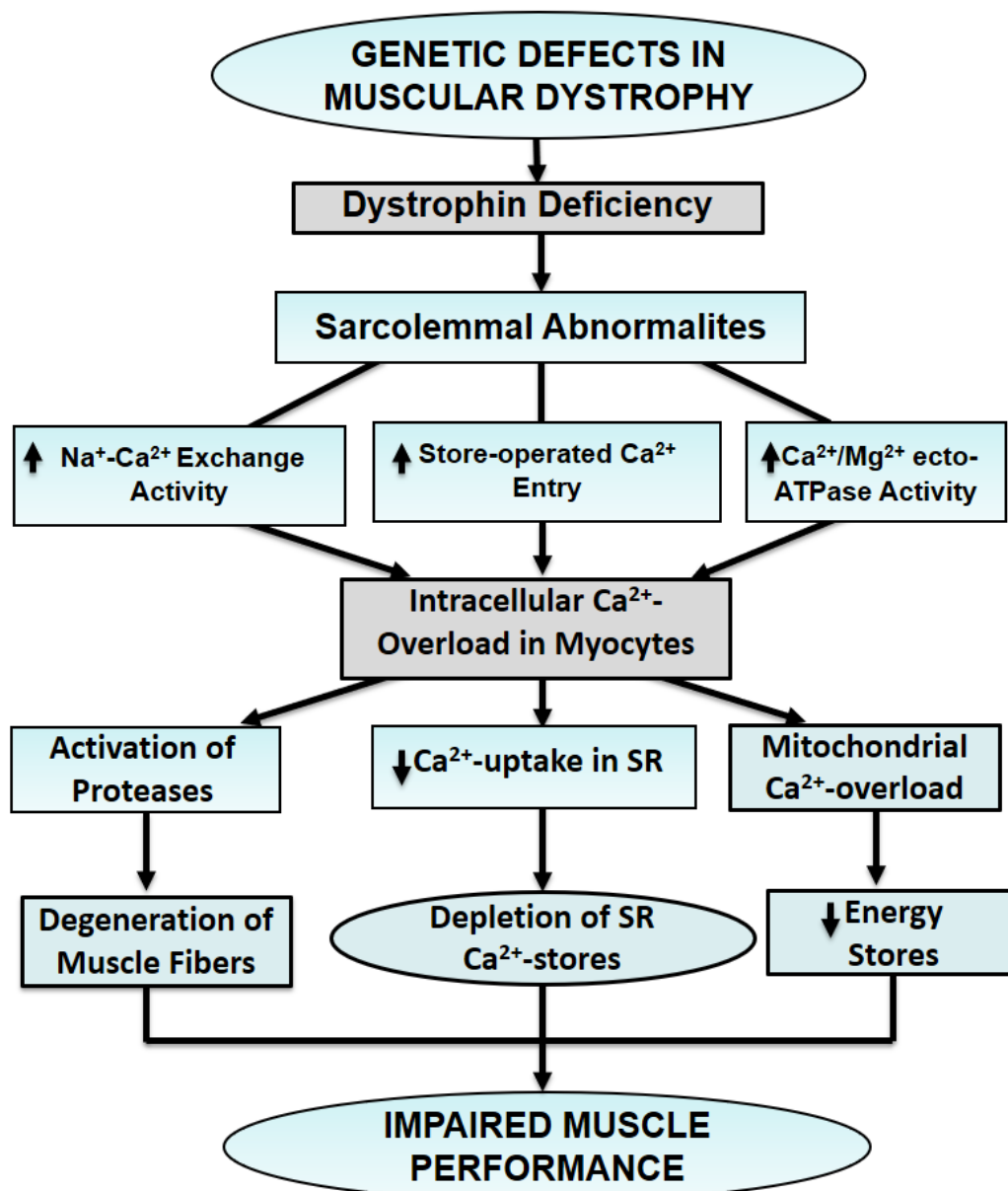


Fig. 4. A schematic representation of some main events associated with the development as well as consequence of intracellular Ca^{2+} -overload in dystrophic muscle.

Some studies concerning defects in the SL membrane and other subcellular organelles as well as metabolic status of skeletal muscle have been conducted by employing a myopathic hamster model of muscular weakness. Other studies by using genetic mouse and chicken models of muscular dystrophy indicate that dystrophin deficiency induce the activation of SOCE channels, Na^+ - Ca^{2+} exchanger (forward mode) and $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase (Ca^{2+} -gating system) in the SL membrane and promote the occurrence of intracellular Ca^{2+} and subsequent abnormalities in dystrophic muscle. A schematic representation of some main events associated with the development as well as consequence of intracellular Ca^{2+} -overload due to dystrophin-deficiency is shown in Fig. 4. Various, treatments including gene therapy with dys-

trophin and some pharmacological interventions such as antioxidants are being attempted for delaying the progression of this disease as well as improving the performance of dystrophic muscle. It is suggested that a combination therapy, by employing dystrophin gene and some drugs, which reduce the development of intracellular Ca^{2+} -overload, may prove more beneficial for the treatment of impaired muscular performance.

Author contributions

NSD conceived, designed and edited the article; SKB searched the literature, analyzed the data and wrote the first draft; AKS and MN analyzed the data and wrote the manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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