# Abnormal Calcium Handling in Muscular Dystrophy

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**Abstract**

Duchenne muscular dystrophy, primarily caused by a deficiency in the membrane cytoskeletal protein dystrophin, is the most frequent neuromuscular disorder in humans and is characterized by progressive muscle weakness and wasting. This review outlines pathophysiological mechanisms of abnormal calcium handling by the sarcolemma, sarcoplasmic reticulum and mitochondria that may contribute to muscle fibre destruction. Dystrophin-deficient skeletal muscle membranes succumb to exercise-induced membrane ruptures more frequently than those of normal fibres. These transient micro-ruptures allow for the insertion of calcium leak channels into the sarcolemma during the natural processes of cell membrane resealing. Ion leak channels give rise to localized calcium elevations, contributing to a cycle of enhanced protease activity and leak channel activation. Within the sarcoplasmic reticulum, loss of the calsequestrin-like proteins may contribute to decreased luminal calcium buffering. This might indirectly amplify elevated free cytosolic calcium concentrations. Mitochondria proximal to ryanodine receptor calcium release channel complexes, although thought to buffer channel leakiness, may give rise to an increase in pro-apoptotic signals, ultimately giving rise to muscle cell death. Here, we summarise the potential effects of abnormal calcium homeostasis on fibre degeneration in dystrophic skeletal muscle.

**Key words**: calcium homeostasis, calcium leak channel, calsequestrin, Duchenne muscular dystrophy, dystrophin-glycoprotein complex.

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**D**uchenne muscular dystrophy (DMD) is the most common and severe form of a group of muscle wasting diseases termed the muscular dystrophies. Affecting about 1:3,500 live male births [35], the disease is characterized by progressive muscle weakness and wasting. Myopathic changes include endomysial connective tissue proliferation, scattered degenerating and regenerating myofibres, centralized nuclei, foci of mononuclear inflammatory cell infiltrates as a reaction to muscle fibre necrosis, mild architectural changes in still functional muscle, and many dense fibres [84].

## Dystrophin and Associated Proteins

The primary defect in DMD is the loss of the cytoskeletal protein dystrophin, normally found on the subsarcolemmal surface of the skeletal muscle plasma membrane [2]. However, the mechanism by which absence of dystrophin leads to progressive muscle fibre degeneration is poorly understood. The dystrophin gene, localized to chromosome Xp21 [58], is one of the largest genes known to date, spanning roughly 2.5 Mb of genomic sequence [21, 82], giving rise to a 14kb transcript producing 79 exons. The protein product of 3685 amino acid full-length dystrophin was found to be predominantly expressed in skeletal and cardiac muscle [2]. A rod-shaped cytoskeletal protein [59], dystrophin functions to bind cortical actin [44] through actin-binding domains in the N-terminus and part of the rod domain [5, 64, 123]. Indirect linkage to the extracellular matrix component laminin [46] is provided through associations via the cysteine-rich domain with the dystroglycan sub-complex [45, 126] of dystrophin-associated proteins (Figure 1). The main function of dystroglycans, sarcoglycans and associated surface glycoproteins is stabilizing the sarcolemmal membrane during the mechanical rigors of muscle contraction [15, 25, 91, 101]. In muscular dystrophy, almost all dystrophin-associated proteins are greatly reduced in their relative density [26, 90, 91]. The COOH-terminus of dystrophin mediates binding of adapter and signaling proteins, which in turn mediates associations with membrane-bound ion channels [96, 98]. Primary genetic abnormalities in dystrophin, and dystrophin-

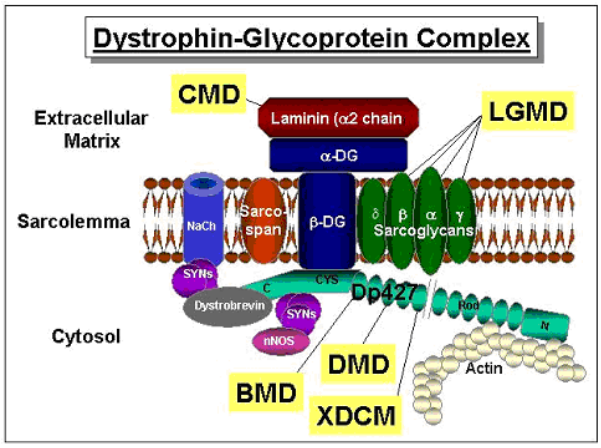


*Figure 1. Composition of the Dystrophin-Glycoprotein Complex and its involvement in muscular dystrophies. The Dp427 isoform of the membrane cytoskeletal protein dystrophin forms an indirect link between laminin of the extracellular matrix and the sub-sarcolemmal actin cytoskeleton. Binding occurs through a motif proximal to the COOH-terminus of Dp427 to the β-subunit of the dystroglycan (DG) complex, which in turn is tightly coupled to the highly glycosylated merosin binding-protein α-dystroglycan (α-DG). Two actin-binding domains in the N-terminal domain and rod domain distal to the N-terminus link the dystrophin-glycoprotein complex to the actin cytoskeleton. Within the muscle surface membrane, dystrophin is associated with a second set of proteins termed the sarcoglycans (SG) and sarcospan. It is believed that the primary role of the dystrophin-glycoprotein complex is to maintain muscle membrane integrity during excitation-contraction-relaxation cycles. The COOH-terminus of dystrophin binds to the dystrophin-like protein dystrobrevin. Both dystrophin and dystrobrevin contain syntrophin binding motifs, and through PDZ motif interactions are believed to bind proteins such as sodium ion channels (NaCh) and neuronal nitric oxide synthase (nNOS). Primary genetic abnormalities in dystrophin, sarcoglycans or laminin lead to various neuromuscular disorders such as Duchenne muscular dystrophy (DMD), Becker's muscular dystrophy (BMD), X-linked dilated cardiomyopathy (XDCM), congenital muscular dystrophy (CMD) and limb-girdle muscular dystrophy (LGMD).*

associated elements, such as sarcoglycans or laminin, lead to various neuromuscular disorders [15, 25] such as Duchenne muscular dystrophy (DMD), Becker's muscular dystrophy (BMD), X-linked dilated cardiomyopathy (XDCM), congenital muscular dystrophy (CMD) and limb-girdle muscular dystrophy (LGMD) (Figure 1).

With the discovery of at least eight promoter regions spanning the length of the entire dystrophin gene sequence, several isoforms of dystrophin have been identified [2, 25]. Three full-length dystrophin isoforms have been characterized, termed brain (Dp427-B), muscle (Dp427-M) and purkinje cell (Dp427-P) dystrophin. Alternate splicing of internal promoters results in the truncated isoforms of 260 kDa, 140 kDa, 116 kDa, and 71 kDa. Alternate splicing at the 3' end of dystrophin mRNA results in the generation of different isoforms of full-length dystrophin [36], as well as the shorter Dp71 isoform, the latter modifications resulting in the transposition of Dp71 from the subsarcolemmal region to the cytoplasm [43]. A chromosome 6-encoded autosomal homologue of dystrophin, termed dystrophin-related protein or utrophin, contains high sequence similarity to dystrophin [14, 65]. A 395 kDa submembranous protein, utrophin is predominantly located at the myotendinous and neuromuscular junctions, where it functions to anchor nicotinic acetylcholine receptors through interactions with a surface glycoprotein complex [75, 92]. Full-length utrophin has also been found in brain [57]. The cell biological role played by different dystrophin isoforms in the central nervous system [27] and how dystrophin mutations trigger mental retardation in a subpopulation of Duchenne patients [76] is poorly understood. Several splice variants of utrophin also exist, a 116 kDa and a 71 kDa C-terminal transcript, as well as a 62 kDa N-terminal transcript found in glioma cells [87, 128].

## Abnormal Calcium Handling and Muscular Dystrophy

Although the primary defect in DMD is the loss of the membrane cytoskeletal protein dystrophin resulting from specific mutations in the human DMD gene [2], the secondary molecular mechanisms leading ultimately to muscle degeneration have yet to be elucidated. Abnormal $Ca^{2+}$ homeostasis has been implicated in rendering skeletal muscle cells more susceptible to necrosis, as outlined in Figure 2. Studies on the intracellular $Ca^{2+}$ concentration in dystrophic muscle has revealed conflicting results as to whether or not $Ca^{2+}$ is present at higher concentrations in the cytosol of resting dystrophic skeletal muscle. Initial studies on the intracellular $Ca^{2+}$ concentration of DMD skeletal muscle biopsies revealed a large increase in the intracellular $Ca^{2+}$ concentration [10, 51]. Staining of muscle biopsies from DMD patients using the $Ca^{2+}$ deposit-sensitive histochemical stain alizarin red and the $Ca^{2+}$-sensitive von Kossa method and glyoxyl-bis-(2-hydroxyanil) showed an increase in positive-reacting cells in DMD skeletal muscle [10, 23, 88]. An elevation in cytosolic $Ca^{2+}$ levels using similar methodology was also demonstrated in mdx skeletal muscle [42].

Initial studies using fura-2, a fluorescent $Ca^{2+}$ indicator whose emission spectra alters upon $Ca^{2+}$ chelation, have revealed elevated levels of $Ca^{2+}$ in dystrophic muscle [121, 125]. Mongini et al. [83] demonstrated markedly increased $Ca^{2+}$ levels both in resting and in stimulated cultured skeletal muscle cells from DMD patients. This

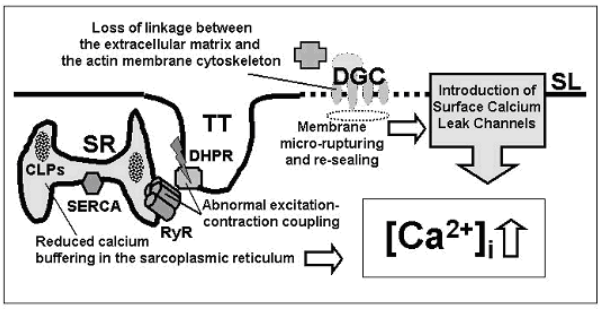


*Figure 2: Abnormal calcium handling in dystrophic skeletal muscle. In normal muscle, sarcolemma (SL) membrane depolarization induces a conformational change in the voltage-sensor of the transverse tubules (TT), the dihydropyridine receptor (DHPR). Direct physical interactions between the II-III loop domain of the $\alpha_1$-DHPR and a cytoplasmic domain of the $Ca^{2+}$-release channel, the ryanodine receptor (RyR), initiate the fast release of $Ca^{2+}$-ions from the luminal stores of the sarcoplasmic reticulum (SR). Transient elevation in cystosolic $Ca^{2+}$-levels cause, via the troponin-tropomyosin complex, actomyosin-mediated fibre contraction. The rapid re-uptake of $Ca^{2+}$-ions is facilitated by the SERCA type $Ca^{2+}$-pumps of the longitudinal tubules and terminal cisternae. Luminal $Ca^{2+}$-ions are mostly sequestered by the high-capacity, medium affinity $Ca^{2+}$-binding protein calsequestrin and its high-molecular-mass isoforms, the calsequestrin-like proteins (CLPs). In muscular dystrophy, excitation-contraction coupling and overall $Ca^{2+}$-handling is impaired. Reduction in the dystrophin-glycoprotein complex (DGC) results in the loss of the linkage between the extracellular matrix and the actin membrane cytoskeleton. Consequently, surface membrane micro-rupturing and re-sealing in dystrophic fibres introduces $Ca^{2+}$-leak channels into the muscle periphery triggering a pathophysiological $Ca^{2+}$-concentration in the subsarcolemmal cytosol. In addition, reduced $Ca^{2+}$-buffering within the SR lumen intensifies this abnormlity in ion handling. Elevated $Ca^{2+}$-levels are believed to cause increased proteolytic degradation of muscle proteins thereby rendering fibres more susceptible to necrosis.*

was confirmed by studies with mdx fibres, demonstrating increases in cytosolic $Ca^{2+}$ levels in dystrophin-deficient myotubes [6]. However, this finding was not universally confirmed. Several other groups reported no difference in the resting values of intracellular $Ca^{2+}$ concentrations in dystrophic muscle fibers using fura-2 analogs [41, 63, 97]. Possibly differences in experimental methodology in the use of fura-2 could have resulted in some contradicting findings [4, 47, 104]. Other $Ca^{2+}$ indicators have been used to determine whether or not an increase in intracellular $Ca^{2+}$ levels exists in dystrophic skeletal muscle. Using the fluorescent indicator indo-1, resting levels of intracellular $Ca^{2+}$ concentrations in isolated skeletal muscle fibers were demonstrated to be similar in mdx mice compared to normal [22], as well as in DMD myotubes [99]. Depolarization of these skeletal muscle fibers showed similar changes in intracellular $Ca^{2+}$ levels indicating that these muscle fibers are capable of handling changes in $Ca^{2+}$ levels in response to membrane depolarization.

A critical evaluation of previous experimental procedures by Hopf et al. [47] confirmed the original result of Turner et al. [119-121], demonstrating increased cytosolic $Ca^{2+}$ concentrations in dystrophic mouse myotubes. This was also shown by the analysis of the characteristics of calcium-activated $K^+$ channels [73]. Using cell-attached and inside-out patch-clamp techniques, a three-fold increase in intracellular $Ca^{2+}$ concentrations, in particular at the subsarcolemmal region, was demonstrated in mdx muscle fibers compared to controls [73]. It thus appears that the intracellular $Ca^{2+}$ concentration in dystrophic skeletal muscle is not uniformly elevated within the cytosol but this phenomena is restricted to the subsarcolemma [3, 37, 38, 119]. Interestingly, transfection of full-length dystrophin is capable of lowering the elevation in intracellular $Ca^{2+}$ [33, 70], suggesting a direct linkage between surface membrane destabilization in dystrophin-deficient muscle and abnormal $Ca^{2+}$ handling.

## Membrane Abnormalities in Muscular Dystrophy

The predominant muscle fibres to be affected in DMD are the fast-twitch glycolytic type IIB muscle fibers [79, 124]. It is speculated that the burst-like stimulation pattern of these fibres overwhelms the integrity of the dystrophin-deficient surface membrane, making them more susceptible to membrane micro-rupturing and $Ca^{2+}$ influx. Smaller caliber fibres, such as extraocular and toe muscles, appear not to be as severely affected as bulk type IIB fibers of limb and torso muscles [34, 55]. In concurrence with this phenomena, the sarcolemmal water channel aquaporin-4, localized to the plasma membrane by interactions with the dystrophin-glycoprotein complex [1] has been demonstrated to have a higher deficiency in mdx mouse type IIB muscle fibres than other muscle fibre types [40]. During the mechanical events of muscle contraction, force pressure may lead to the formation of transient micro-ruptures in the sarcolemma. In normal muscle, plasma membrane tears or wounds have been demonstrated to increase several-fold during eccentric exercise [71]. Re-sealing of these wounds has been demonstrated to be a $Ca^{2+}$-dependent process, requiring the influx of extracellular $Ca^{2+}$-ions [30, 32], resulting in locally occurring vesicle exocytosis events [8, 9, 111, 117]. These vesicles, by adding plasma membrane, seal areas of micro-disruption of the sarcolemma. Dystrophic muscle membranes, on the other hand, undergo tearing more often than normal cell membranes, the frequency of which markedly increases during exercise [20]. These membrane ruptures, reported in both necrotic and non-necrotic muscle fibers, allow for increased passage of muscle-

specific proteins such as creatine kinase, pyruvate kinase, myoglobin and parvalbumin, as well as intracellular enzymes from the cytoplasm [29, 52, 56, 89, 102].

In the mdx mouse model of X-linked muscular dystrophy, mechanical stress induced by exercise has been shown to result in an increase in the number of membrane wounds compared to normal muscle [13, 105, 122]. Chemical markers such as procion yellow [12, 68, 9], procion red [80], evans blue [74, 112] and peroxidase [81], as well as extracellular fluid markers such as albumin [23] and radioactively labeled ion species [68] have been demonstrated to accumulate in dystrophic skeletal muscle cells more readily than in normal muscle cells. Dyes accumulate to a larger degree in dystrophic fibers, whereby the frequency of dye-positive dystrophic cells increases with exercise. These sarcolemmal defects can be detected in muscle fibres that show no other structural abnormality and are believed to be indicative of early muscle fibre trauma [81, 112]. Therefore, the higher fragility of the plasma membrane in muscular dystrophy can be directly correlated with the loss of dystrophin. On the other hand, the pressure required for the rupture of cell membranes of normal and dystrophic fibers using patch-clamp assays does not differ [38, 39, 50]. A difference in the stress, strain, or energy required to rupture isolated muscles could not be determined [62]. However, the stiffness of the subsarcolemmal cytoskeleton is several-fold decreased in mdx myotubes [93].

## Elevated Intracellular Calcium in Response to Mechanical Stress

A higher and persistent increase in the concentration of intracellular $Ca^{2+}$ has been demonstrated in skeletal muscle after sustained periods of mechanical stress. Jockusch and co-workers [77, 78] have challenged myotubes from mdx mice by hyper-osmosis, a method of mimicking mechanical stress on the membrane cytoskeletal complex. They could clearly show a lower stress resistance in DMD [78] and mdx myotubes [77] compared to normal controls. The mean frequency of $Ca^{2+}$ deposits observed in DMD skeletal muscle fibers increased several-fold in hyper-contracted fibers [13]. Although DMD myotubes do not spontaneously contract in culture, induction of contraction may be achieved by co-culturing with rat spinal chord explants [50]. When co-cultered myotubes were induced to spontaneously contract, intracellular levels of $Ca^{2+}$ increased dramatically [50]. Similar results were achieved with mdx myotubes [48, 119]. Levels of intracellular $Ca^{2+}$ returned to normal levels when cells were chronically treated with tetrodotoxin to inhibit the spontaneous contractions [48, 50]. Initially, it was postulated that increased membrane tearing seen in mdx mouse muscle leads to muscle necrosis by infiltration of $Ca^{2+}$ ions through micro-ruptures, eventually overwhelming cell repair mechanisms [23, 86, 103]. However, an increase in the quantity of membrane tears would evoke a rise in the intracellular concentration of $Na^{+}$ ions, driven along a steep sarcolemmal concentration gradient. This has been found not to be the case, with both normal levels and influx rates of $Na^{+}$ ions detected in mdx mouse muscle [120]. Therefore, elevated intracellular $Ca^{2+}$ seen at the subsarcolemmal compartment cannot be due to infiltration of substantial levels of $Ca^{2+}$ through transient membrane micro-ruptures.

## Calcium-Dependent Proteolysis and Muscular Dystrophy

Despite the controversy about the extent and exact micro-domain of pathophysiological $Ca^{2+}$ influx into dystrophic fibres, it is now relatively well established that the intracellular $Ca^{2+}$ concentration is increased close to the dystrophin-deficient regions of the sarcolemma [4]. As outlined above, exercise-induced sarcolemmal micro-ruptures are several-fold increased in dystrophic fibers, ultimately raising cytosolic $Ca^{2+}$ levels [13, 49]. The marked elevation of $Ca^{2+}$ in dystrophic muscle may contribute to activation of $Ca^{2+}$-dependent proteases, such as skeletal muscle-specific calpains [4, 86, 109, 110, 119]. Calpains have been implicated in the proteolysis of vesicle-bound $Ca^{2+}$ leak channels, constitutively activating these channels [3, 4, 69]. Using an artificial fluorogenic calpain substrate Boc-Leu-Met-CMAC, the rate of proteolysis in resting mdx myotubes was determined to be markedly elevated, dependent on the activity of plasma membrane $Ca^{2+}$ leak channels [3]. Nagy and Samaha [86] previously determined increased calpain II activity in dystrophic fibers, which could be inhibited by protease inhibitors and the $Ca^{2+}$-chelators EDTA and EGTA. Spencer and Tidball [109] found an increase in levels of calpain in mdx muscle, with a redistribution of calpain from the Z-discs to the cytosol. An increase in both m- and mu-calpains was shown in necrotic and regenerating fibers in mdx mouse muscle, as a result of post-translational regulation [110].

The increase in $Ca^{2+}$-induced proteolysis may permanently alter the activity of vesicle-bound $Ca^{2+}$ leak channels rendering the channels constitutively active [4]. Exocytosis of $Ca^{2+}$ leak channel-containing membrane vesicles reseals exercise-induced membrane lesions. This inserts these constitutively active channels into the membrane, resulting in persistent $Ca^{2+}$ influx at localized sites. The ion movement gives rise to a circle of events, $Ca^{2+}$ causing increased proteolysis, proteolysis causing increased influx of $Ca^{2+}$ [4]. Accumulation of $Ca^{2+}$ can be demonstrated to occur mostly in the sarcolemmal area, in particular at regions that underlie plasma membrane defects [73]. Studies by Carlson [16, 18] have also determined an abnormal leakage of $Ca^{2+}$ into the cytoplasm of mdx and DMD myotubes. However, the leakiness of the membrane is attributed to abnormalities in the nicotinic acetylcholine receptor population. Using cell-attached patches, instability of nicotinic acetylcholine receptor function was determined. An increase in small-conductance events was noted, attributed to $Ca^{2+}$-leak activity.

This alternative idea, the nicotinic acetylcholine receptor aggregation hypothesis, is based on the fact that agrin and dystrophin/utrophin-glycoprotein complexes show interactions [11]. This theory suggests that the absence of dystrophin disrupts the integrity of the membrane cytoskeleton, thereby disturbing the proper aggregation of ion channels and neurotransmitter receptors [17].

## Sarcoplasmic Reticulum Calcium Cycling and Muscular Dystrophy

As illustrated in Figure 2, excitation-contraction coupling and $Ca^{2+}$-handling is impaired in muscular dystrophy. In normal fibres, the proper cycling of $Ca^{2+}$ is a prerequisite for optimum excitation-contraction coupling and muscle relaxation. $Ca^{2+}$-induced contraction of skeletal muscle is induced by the release of $Ca^{2+}$ from the luminal stores of the sarcoplasmic reticulum through the ryanodine receptor $Ca^{2+}$-release channel complex. Transient opening of the RyR1 isoform of the $Ca^{2+}$-release channel is triggered by direct physical interactions with the voltage-sensing dihydropyridine receptor of the junctional transverse tubules [85]. Relaxation-inducing re-uptake of cytosolic $Ca^{2+}$ is mediated by the sarcoplasmic reticulum $Ca^{2+}$-ATPases of the longitudinal tubules and terminal cisternae. Direct measurement of $Ca^{2+}$ levels in the dystrophic sarcoplasmic reticulum by Robert et al. [100] revealed a higher steady state level at rest and a larger drop after depolarization in mdx myotubes. Although earlier studies have shown impaired maximum velocity of $Ca^{2+}$-uptake [54], the total $Ca^{2+}$-ATPase activity does not seem to be impaired in dystrophic skeletal muscle fibres [28].

Within the lumen of the sarcoplasmic reticulum, a large proportion of $Ca^{2+}$ is maintained bound to the high capacity $Ca^{2+}$-binding protein calsequestrin [72, 127]. While the expression of the ryanodine receptor, the dihydropyridine receptor and calsequestrin is not affected in muscular dystrophy, a drastic decline in calsequestrin-like proteins of 150 to 220 kDa was observed in dystrophic microsomes using one-dimensional immunoblotting, two-dimensional immunoblotting with isoelectric focusing, diagonal two-dimensional blotting technique and immuno precipitation [28]. In analogy to these pathobiochemical findings, the overall $Ca^{2+}$-binding capacity was also found to be significantly reduced in the sarcoplasmic reticulum of mdx microsomes [28]. The reduction in $Ca^{2+}$-binding proteins might be directly involved in triggering impaired $Ca^{2+}$-sequestration within the lumen of the sarcoplasmic reticulum (Figure 3). Hence, disturbed subsarcolemmal $Ca^{2+}$-levels appear to influence $Ca^{2+}$-cycling resulting in distinct changes in the expression profile of a subset of $Ca^{2+}$-handling proteins. This secondary abnormality might be an important factor in the progressive functional decline of dystrophic muscle fibres [28]. The sarcoplasmic retculum $Ca^{2+}$-release channel is directly influenced by interactions with luminal calsequestrin aggregates. Thus the major $Ca^{2+}$ reservoir component is also

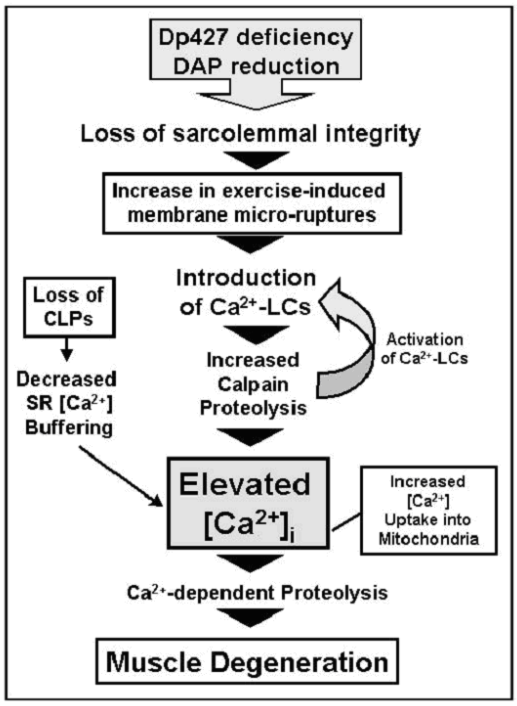


*Figure 3. Flow chart of the Calcium Hypothesis of Muscular Dystrophy. The mutation-induced deficiency in the Dp427 isoform of the membrane cytoskeletal protein dystrophin results in the drastic reduction of dystrophin-associated proteins (DAP). The loss of the linkage between the extracellular matrix component laminin and the actin membrane cytoskeleton, normally provided by the dystrophin-glycoprotein complex, impairs the structural integrity of the muscle plasmalemma. Consequently, dystrophin-deficient skeletal muscle membranes succumb to exercise-induced membrane ruptures more frequently than those of normal fibres. Transient micro-ruptures allow for the insertion of proteolytically-activated $Ca^{2+}$-leak channels into the sarcolemma around the area of disrupture during the natural processes of cell membrane resealing, thereby triggering a localized sub-sarcolemmal accumulation of $Ca^{2+}$-ions. The raised $Ca^{2+}$-levels allow for activation of $Ca^{2+}$-dependent proteases, causing further channel activation and general protein degradation. In addition, the reduced expression of several luminal calsequestrin-like proteins (CLPs) and a general decrease in the ability of the sarcoplasmic reticulum (SR) to properly sequester $Ca^{2+}$-ions allows for abnormal $Ca^{2+}$-buffering. This might be a contributing factor to the raised cytosolic $Ca^{2+}$-levels. In contrast, mitochondria in close proximity to ryanodine receptor $Ca^{2+}$-release channel complexes at the membrane face of the SR, probably buffer a certain degree of elevated $Ca^{2+}$-levels. Abnormalities in mitochondrial function, however, are proposed to lead to the production of pro-apoptotic factors, resulting ultimately in muscle degeneration.*

an endogenous regulator within the lumen of the SR [115]. $Ca^{2+}$-release mechanisms appear to be dependent on the phosphorylation state of calsequestrin aggreagtes. Taken together with findings of Takagi et al. [116], demonstrating increased leakiness of the SR, and De Luca et al. [31] suggesting alteration in E-C coupling in dystrophic mdx mice, loss of CLPs from the SR may influence the open probability of the RyR, causing leakage of $Ca^{2+}$ from the SR into the cytosol, and may thus alter overall $Ca^{2+}$-handling (Figure 3).

## Mitochondrial Calcium, Apoptosis and Muscular Dystrophy

Mitochondria play a central role in muscle bioenergetics and are proposed to be directly involved in muscle degeneration in dystrophic fibres [61, 66, 100]. Interestingly, an alternate transcript of the major brain isoform of dystrophin, Dp71, has been detected in mitochondria. This isoform, which lacks exon 78 of brain Dp71, was found to be upregulated in dystrophin-deficient tissue [19], although its biological function in mitochondria remains elusive. The release of higher $Ca^{2+}$ rates from the SR in dystrophic cells is believed to be buffered effectively by mitochondria proximal to the ryanodine receptor, suppressing abnormally elevated $Ca^{2+}$ levels within the cytosol [100]. Recent evidence has linked abnormal $Ca^{2+}$ concentrations within the SR with activation of apoptotic processes. Pinton et al., [95] suggest that overexpression of Bcl-2, the well established anti-apoptotic protein, results in a reduction of steady-state SR $Ca^{2+}$ levels, affecting $Ca^{2+}$ signaling to mitochondria, and reducing stimulation-induced $Ca^{2+}$ peaks in the mitochondrial matrix. However, over-expression of SERCA $Ca^{2+}$ pump units and a higher $Ca^{2+}$ accumulation within the SR, may accelerate spontaneous cell death by ICE protease-induced apoptosis [67]. Several candidates have been identified as possible contributors to apoptosis following derangements in $Ca^{2+}$ homeostasis. These include the opening of the permeability transition pore and the ensuing release of pro-apoptotic factors like cytochrome C, apoptosis-inducing factor, procaspases and ATP synthesis [24, 60]. Mitochondrial accumulation of $Ca^{2+}$ stimulates synthesis of ATP, and increasing levels of ATP in the cytosol are an prerequisite for energy metabolism during apoptosis [53]. $IP_3$-mediated $Ca^{2+}$ spikes cause opening of the permeability transition pore, invoking release of cytochrome C, potently initiating apoptosis by the activation of caspases and apoptosis-inducing factor [113, 114]. This suggests the existence of a causal link between the level of $Ca^{2+}$ storage and the activation-inhibition pattern of apoptosis [60].

Early changes in mitochondrial functions have been demonstrated in muscle of mdx mice with a reduction of the activity of the respiratory chain [61], as well as similar findings in human DMD biopsies [66]. Several groups have reported the presence of apoptotic fibers in both mdx mice and in DMD patients before muscular necrosis becomes evident [107, 118]. Spencer et al. [107] demonstrated by the use of a double mutant mdx mouse lacking both dystrophin and perforin, cytotoxic T-lymphocytes-mediated apoptosis, with perforin playing a role in the initiation of myonuclear apoptosis in mdx mouse skeletal muscle. The expression of several caspases and Granzyme B in DMD skeletal muscle have been established, as well as an increase of apoptotic myonuclei, bax, and bcl-2-positive myofibers [108, 109]. Positive correlation was detected between apoptotic nuclei and bax expression in skeletal muscle apoptosis in DMD [106]. Elevations in the levels of calmitine, a calcium-specific mitochondrial protein has been found in mdx mouse fast-twitch muscles, with a correlation between the quantity of calmitine present and the extent of $Ca^{2+}$ uptake into mitochondria [66].

## Conclusions

Based on the above described pathophysiological role of abnormal $Ca^{2+}$ handling in dystrophin-deficient muscle fibres, potential sites of pharmacological interventions present themselves at various $Ca^{2+}$ regulatory processes including the sarcolemma, sarcoplasmic retriculum and mitochondria. Thus, besides modern therapeutic approaches for the treatment of DMD, such as gene therapy or myoblast transfer therapy, traditional pharmacological treatment might represent a promising option. Potential pharmacological strategies include immuno-suppressive therapy, the drug-induced up-regulation of compensatory molecules such as an extra-junctional utrophin-glycoprotein complex, as well as the targeted introduction of protease inhibitors [106] such as carnitine-linked leupeptin [7] in order to inactivate $Ca^{2+}$-dependent calpain activity. In addition, studies by DeLuca et al. [31] on disturbed excitation-contraction coupling in dystrophic muscle fibres suggest a potential therapeutic role of taurine. Thus, the elucidation of impaired $Ca^{2+}$ fluxes, cycling and buffering in muscular dystrophy should lead to the design of novel treatment strategies for these devastating genetic muscle disorders in the not too distant future.

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