PUBLICATION CERTIFICATE

Sr. No.	Name of the publication	Name of the journal	Publication status
1.	Mini-JAG1 peptide treatment enables patient-derived immortalized myoblast cultures to resist calcimycin-induced mortality	CIBTech Journal of Zoology	Published
2.	Is the fundamental pathology in Duchenne's Muscular Dystrophy caused by a failure of glycogenolysis-glycolysis in costameres	Journal of Genetics	Published
3.	Jagged1 overexpression increased mitochondrial reactive oxygen species in DMD knockout zebrafish and patient-derived myoblast cultures	Indian Journal of Biochemistry and Biophysics	Under review
4.	The Jagged1 overexpression rescues muscle function in a week-old dystrophic zebrafish larvae	BMC Musculoskeletal disorders	Under review

Journal of Genetics (2023)102:13
https://doi.org/10.1007/s12041-022-01410-w

REVIEW ARTICLE

Is the fundamental pathology in Duchenne's muscular dystrophy caused by a failure of glycogenolysis–glycolysis in costameres?

VISHAKHA NESARI[1], SURESH BALAKRISHNAN[1] and UPENDRA NONGTHOMBA[2]*

[1]*Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390 002, India*
[2]*Indian Institute of Science, Bengaluru 560 012, India*
*For correspondence. E-mail: upendra@iisc.ac.in, upendra.nongthomba@gmail.com.

Received 28 June 2022; revised 16 September 2022; accepted 27 October 2022

Abstract. Duchenne muscular dystrophy (DMD) is the most common form of progressive childhood muscular dystrophy associated with weakness of limbs, loss of ambulation, heart weakness and early death. The mutations causing either loss-of-expression or function of the full-length protein dystrophin (Dp427) from the *DMD* gene are responsible for the disease pathology. Dp427 forms a part of the large dystroglycan complex, called DAPC, in the sarcolemma, and its absence derails muscle contraction. Muscle biopsies from DMD patients show an overactivation of excitation-contraction-coupling (ECC) activable calcium incursion, sarcolemmal ROS production, NHE1 activation, IL6 secretion, etc. The signalling pathways, like Akt/PBK, STAT3, p38MAPK, and ERK1/2, are also hyperactive in DMD. These pathways are responsible for post-mitotic trophic growth and metabolic adaptation, in response to exercise in healthy muscles, but cause atrophy and cell death in dystrophic muscles. We hypothesize that the metabolic background of repressed glycolysis in DMD, as opposed to excess glycolysis seen in cancers or healthy contracting muscles, changes the outcome of these 'growth pathways'. The reduced glycolysis has been considered a secondary outcome of the cytoskeletal disruptions seen in DMD. Given the cytoskeleton-crosslinking ability of the glycolytic enzymes, we hypothesize that the failure of glycogenolytic and glycolytic enzymes to congregate is the primary pathology, which then affects the subsarcolemmal cytoskeletal organization in costameres and initiates the pathophysiology associated with DMD, giving rise to the tissue-specific differences in disease progression between muscle, heart and brain. The lacunae in the regulation of the key components of the hypothesized metabolome, and the limitations of this theory are deliberated. The considerations for developing future therapies based on known pathological processes are also discussed.

Keywords. Duchenne muscular dystrophy; muscle; metabolism; glycolysis; therapeutic interventions.

The *DMD* locus, the protein complex of dystrophin and the disease aetiology

Duchenne muscular dystrophy (DMD) is a severe, childhood-onset, progressive muscle-degenerative disease. Clinical symptoms typically include progressive muscle weakness apparent by around 3–5 years of age, pseudo-hypertrophy of calf muscles, Growers' sign, joint contractures, and loss of ambulation by 10–12 years of age. Patients surviving beyond 20 years often develop heart weakness, kyphoscoliosis, and breathing difficulties due to the diminishing capacity of the respective muscles. Some patients suffer from nonprogressive cognitive delay or disability. Lung failure was the primary cause of death before ventilators became available; now, it is majorly attributed to heart failure (Wittlieb-Weber *et al*. 2020). A whole range of severity and disease progression is seen in patients with several genetic aberrations in the causal gene *Dystrophin* (*DMD/DYS)* (Bladen *et al*. 2015; Juan-Mateu *et al*. 2015).

The human *DMD* locus on chromosome Xp2.1 forms one of the largest genes, with 79 exons spanning 2.4 Mbp, coding for a ∼14-kb mRNA (Koenig *et al*. 1987), which produces the protein dystrophin (Hoffman *et al*. 1987). The *DMD* gene transcribes several minor isoforms of dystrophin, abbreviated as Dp40, Dp71, Dp116, and Dp260, from internal promoters, but the absence of only the functional full-length isoform Dp427 is associated with DMD. The full-length isoform is expressed in skeletal muscle, heart, and brain from three different promoters. The two separate promoters produce Dp427b and Dp427p in brain regions, while

Published online: 09 January 2023

the Dp427m isoform from the third promoter is expressed in skeletal and cardiac muscles (reviewed by Blake *et al*. 2002). Mutations generating partially functional Dp427m lead to milder and late-onset Becker's muscular dystrophy (BMD). This locus is also responsible for X-linked dilated cardiomyopathy (XLDCM) without skeletal muscle phenotype. There could be several plausible reasons for the lack of muscle pathology in XLDCM (reviewed by Nakamura 2015), but the most accepted view is that the skeletal muscles alone upregulate the brain-specific isoforms of Dp427, and are hence spared (Muntoni *et al*. 1995).

The Dp427m protein is associated with the large membrane-associated dystroglycan complex (DGC), forming a larger complex called dystrophin associated protein complex (DAPC). The DAPC consists of roughly 28–30 proteins, connecting the extracellular matrix (ECM) to the subsarcolemmal cytoskeleton called 'costameres' (Peter *et al*. 2011) for lateral force transmission during muscle contraction (Ramaswamy *et al*. 2011). The costameric cytoskeleton is composed of several intermediate filaments like desmin, vinculin, dystrophin, nonsarcomeric F-actin, actinins, and microtubules. The DAPC with integrins forms a major hub by binding the costameric structures from the interior and laminins (also called merosin) in the ECM to perform structural, scaffolding, enzymatic and signalling functions, several of which are disturbed in DMD (Ervasti 2007). The mechanical fragility during muscle contraction is still considered primary pathology, which consequently leads to membrane lesions and ion incursion, oxidative stress, aberrant signalling, apoptosis or necrosis, inflammation, fibrosis and ultimately loss of functional muscles.

The molecular details behind most of these pathological pathways have been discerned with the help of the *mdx*-mouse model of DMD. The therapeutic strategies that ameliorated the *mdx* pathology have consistently failed during clinical trials. Newer approaches, such as gene therapies, are starting to show promise in clinical trials, but are not applicable or accessible to all patients. In addition, the immune response towards vectors or transgene continues to be a cause of concern.

The disease escape by Jagged1 overexpression in a severe golden retriever model of DMD (GRMD) shows that dystrophin or upregulation of its embryonic paralogue utrophin need not be invoked to rescue this disease (Vieira *et al*. 2015). This demands a look at the pathological processes involved into more defined cause-effect relations, which could then direct the therapeutic approaches independent of dystrophin, hence useful for all DMD patients. This review discusses the more accepted hypotheses and their contribution to understanding disease pathology. Also, the possibility of a lack of glycogenolysis–glycolysis derived energy in costameres as the primary initiator of pathology and considerations for therapy development based on the collectivity of pathological processes described in DMD.

The mechanical fragility and membrane damage hypothesis

Classically, the DAPC and integrins bound to trimeric laminins in the ECM, and several cytoskeletal elements in the subsarcolemmal region, provide mechanical support or stability during muscle contraction. The absence of dystrophin causing increased membrane lesion, injury, and death has been proved beyond doubt by several early studies. Thus, the prevalent hypothesis is that the protein dystrophin provides a passive scaffold or shock absorber to the subsarcolemmal cytoskeleton during muscle contraction, which is compromised in DMD (Le *et al*. 2018). The direct mechanical stiffness measurements with the help of atomic force microscopy on *mdx* myotubes found contrasting results (Puttini *et al*. 2009; Canato *et al*. 2010). While recent human *in vitro* muscle culture studies showed early fatigue in dystrophic conditions (Uchimura *et al*. 2021). It is now known that in the subsarcolemmal regions, the actin and microtubule filaments are actively reformed to maintain force and structure during muscle contraction. This goes against the idea of maintaining passive tension by binding of dystrophin to the cytoskeleton.

Yet, the idea of DAPC, integrin-mediating mechanical forces, has some foundation. Sarcospan, a small protein of these complexes, increases binding between DAPC and integrin. Human and mouse sarcospan overexpression rescued pathology in *mdx* (Gibbs *et al*. 2016) and improved cardiac phenotype (Parvatiyar *et al*. 2019). The sarcospan-mediated utrophin-DGC-integrinα7β1 amelioration of DMD requires Akt/PKB activation (Marshall *et al*. 2012); however, utrophin (Utr) can rescue *mdx* even in the absence of sarcospan (Gibbs *et al*. 2021) indicating that these mechanisms are much more complicated. However, the fact that the never-resting cardiac muscles, suspended in the chest cavity, develop the pathology about a decade after the skeletal muscles do not support this mechanical fragility hypothesis. Despite the downregulation of DAPC components in DMD (Ervasti *et al*. 1990), later studies found only a small fraction of DAPCs contain dystrophin or Utrophin, and complex formation is not affected in its absence (Johnson *et al*. 2013). While overexpression of dystroglycan or sarcoglycan did not rescue *mdx* pathology, double knockout increased the severity of disease in *mdx* (Zhu *et al*. 2001; Hoyte *et al*. 2004; Li *et al*. 2009), indicating that intersecting but distinct pathological mechanisms in DMD than other forms of MDs.

As a part of costameres, integrin can possibly compensate for many of the DAPC functions. The protective functions of integrins against overload exercise are well-known (reviewed by Boppart and Mahmassani 2019). The overexpression of β1D alone or α7β1 integrin (major isoforms of muscle integrins) is able to amend the pathology in *mdx* (Burkin *et al*. 2001; Liu *et al*. 2012). Compensatory integrin

upregulation is seen in animal models and patient biopsy samples (Hodges *et al*. 1997). The gold-standard therapy, treatment with steroids, has also been shown to work via integrin activation (Wuebbles *et al*. 2013) and has consistently delayed several aspects of the disease progression in patients, despite its side effects (Schram *et al*. 2013; Matthews *et al*. 2016; Marden *et al*. 2020).

Although the membrane damage hypothesis was based on patient biopsy findings (Mokri and Engel 1975), later studies could not support this (reviewed in detail by Hutter 1992), as the properties of lipids contribute toward the fluidity of membranes more than that of proteins. The mutations in several DAPC components have been associated with other muscular dystrophies; the absence of desmin, vinculin or merosin, which causes a more severe form of congenital muscular dystrophy (MDC1A), does not cause membrane damage (reviewed by Ervasti 2007). Many of these proteins are other members of costameres capable of regulating F-actin in subsarcolemmal regions where dystrophin functions.

In normal muscles, it is known that membrane lesions routinely appear during exercise but get repaired either in a Ca^{2+} and ATP-dependent manner (reviewed by Barthélémy *et al*. 2018) or mediated by dysferlin. In DMD, however, dysferlin-mediated membrane repair is upregulated (Vila *et al*. 2017; Capitanio *et al*. 2020). This suggests that, given the high levels of Ca^{2+} and dysferlin, it must perhaps be the shortage of ATP itself which is the limiting factor for membrane repair. Hence, low energy production during contraction is more likely to result in increased membrane lesions in DMD.

The dynamics of costameric cytoskeleton organization require energy

Microtubules (MT) and nonsarcomeric F-actin form an active cytoskeleton in the costameres. The extensive microtubule (MT) networks of muscles are in perpendicular orientation to sarcomeres; hence, are important for horizontal force transmission and synchronization of the Z-lines. Studies have demonstrated that when this microtubule network is disrupted, there is an increase in cellular ROS and calcium levels (Kerr *et al*. 2015; Coleman *et al*. 2021). Since dystrophin is an MT-binding protein, its absence is hypothesized to directly result in MT-mediated initiation of pathology (Rando *et al*. 2002) via increased ROS and calcium (Prosser *et al*. 2013; Kerr *et al*. 2015; Coleman *et al*. 2021). But this cause-effect correlation is confounded by the observation that increased ROS itself can mediate MT disruptions (Loehr *et al*. 2018).

During muscle contraction, MT dynamics increase. In contracting *mdx* muscles, the initial rate of MT polymerization is high but is not maintained, forming short and dense MT networks (Oddoux *et al*. 2019). This MT shortening cannot be linked to the lack of dystrophin, which inhibits the polymerization of MT (Belanto *et al*. 2014). On the other hand, the high MT density in dystrophic muscles is attributed to low depolymerization due to the detyrosination of tubulin (Kerr *et al*. 2015). This detyrosination is considered to be the consequence of the stable (polymerized) state of microtubules rather than the cause (Sanyal *et al*. 2021). Interestingly, the detyrosinated tubulins cannot repolymerize, and retyrosination of tubulin by the tubulin tyrosine ligase (TTL) requires ATP and L-tyrosine. The repolymerization of monomeric actin and tubulins also requires the exchange of bound ADP and GDP for ATP and GTP, respectively. This phenomenon emphasizes that there must perhaps be an underlying ATP crisis in the case of DMD.

A couple of findings vindicate this argument. First, the fact that in *mdx,* utrophin or micro-dystrophin, both lacking the MT binding domains, rescued the pathology without restoring microtubule organization (Belanto *et al*. 2016).

Second, the phenomenon of increased free monomeric tubulin binding to voltage-dependent anion channel (VDAC) and reduced ADP stimulation of mitochondria does not hold true for the pathology in DMD (Ramos *et al*. 2020). Together, these point against the microtubule theory of pathology initiation. Hence, we hypothesize that low energy and amino acid levels affect the dynamics of the costameric cytoskeleton resulting in disorganization.

The glycolysis-derived energy for calcium reabsorption during ECC

Membrane depolarization, in response to neuronal stimulation, causes the ingress of extracellular Ca^{2+}, and the activation of ryanodine responsive (Ryr) calcium release channels, in the sarcoplasmic reticulum (SR), leading to the amplification of excitation contraction coupling (ECC). The sudden ingress of calcium allows myosin and actin to come in contact, thus, allowing contraction. These calcium spikes are crucial for muscle activity. Hence, several types of calcium channels and pumps function coordinately. In DMD, several types of calcium channels are aberrant, resulting in pathology mediated by persistent levels of calcium and calcium-dependent proteases (reviewed by Mareedu *et al*. 2021). Yet, therapeutic targeting of these has consistently failed, pointing to defects in calcium reabsorption (reviewed by Duan *et al*. 2021).

The Ca^{2+}-ATPases (SERCA) in the SR reabsorb Ca^{2+} by expending ATP. The SERCA-1 overexpression rescued the more severe DMD/Utr double knockout mice (Mázala *et al*. 2015). SERCA activity can be modulated in two ways. First, available ATP. In fact, creatine supplementation, by increasing the phosphocreatine energy stores, improved the Ca^{2+} handling via Ca^{2+}/ATPase activity, in *mdx* (Pulido *et al*. 1998). The creatine-phosphocreatine cycles ATP only for a few seconds, and thereafter, glycolysis-derived ATP is used for SERCA and other ion pumps during the early stage of contraction. Second, the

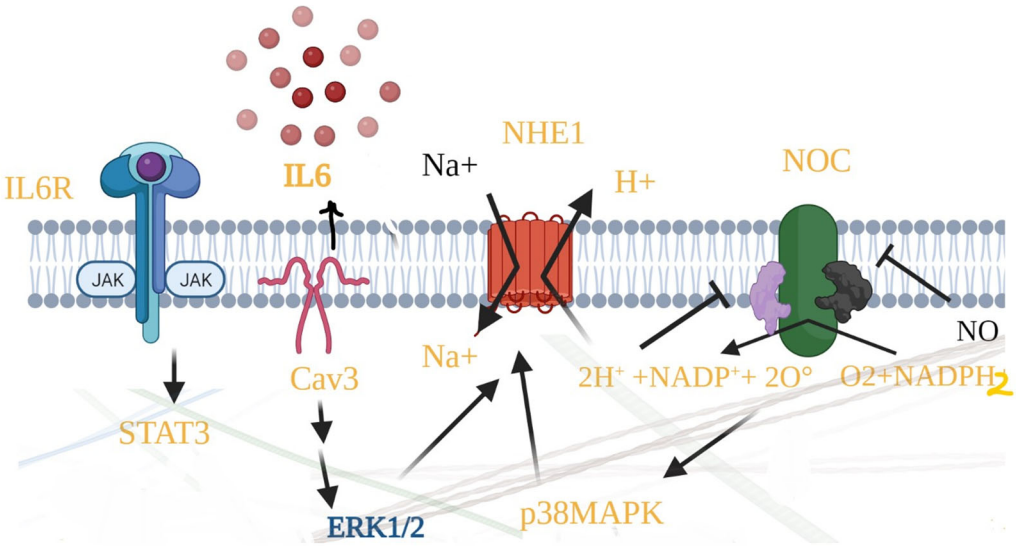

Figure 1. ECC or mechanical stretch activable processes involved in the pathology of DMD.

muscle-specific Acyl or acetyl phosphatase (ACYP2) can remove phosphate from SERCA to regulate calcium. Interestingly, ACYP2 can increase the glycolytic volume by removing product inhibition of enzymes of the pathway (Stefani *et al*. 1997), and the TCA volume by cleaving acetyl phosphate (Xu *et al*. 2018). However, in DMD, ACYP2 has been reported to have low activity (Landi *et al*. 1986), but its implications remain unstudied. This finding further corroborates the underlying energy deficiency in DMD.

The higher NADPH levels lead to elevated ROS burst and NHE1 activation

The primary sources of ROS in dystrophic muscles are considered to be mitochondria (Hughes *et al*. 2019), xanthine oxidase (XO) (Lindsay *et al*. 2018) and NADPH oxidase complex (NOC). First, calcium persistence is considered to cause mitochondrial ROS generation (Rybalka *et al*. 2014), which in turn is attributed to the low activity of SERCA in DMD (previous section). Second, the possible reasons for XO upregulation in DMD could be due to high purine breakdown. This can also increase uric acid, as seen in patients. However, clinical trials with XO inhibitors were discontinued due to a lack of benefits.

Third, there are five different *Nox* genes that encode the catalytic subunit of the NOC, of which Nox2 and Nox4 are active in muscles. Nox4 is constitutive and ER-associated, while Nox2, present in the cell membrane complex, is activated by mechanical stretch during exercise (Xia *et al*. 2003), and converts NADPH2 and O_2 to $NADP^+$, $2\,\overset{\circ}{O}$, and $2H^+$. The ROS augments membrane depolarization during ECC and force production (reviewed by Roy *et al*. 2017), and is necessary for adaptation to exercise (reviewed by Powers *et al*. 2010). The NOC can auto-inactivate as local acidic pH from its own H^+ generation can reduce its activity significantly (Brennan-Minnella *et al*. 2015). To continue its activity, contracting muscles expulse H^+ ions in exchange for Na^+ through a channel called NHE (NHE1 is predominant in the heart muscles). In fact, this Na^+ in turn can further increase Ca^{2+} ingress by Na^+/Ca^{2+} exchangers. This sequence of mechanical stretch- NOC–NHE1 activation should be reversed at the end of the ECC. The NHE1 inactivation would increase local pH and inactivate NOC.

The Nox2 containing NOC is responsible for the pathogenic levels of ROS generated in the muscles of *mdx* mice (Whitehead *et al*. 2010). In DMD, one of the reasons for the higher oxidative burst from NOC could be high NADPH levels (Gupte *et al*. 2006) generated by the increased pentose phosphate pathway (PPP) metabolism. Another possibility is the lack of an inactivating mechanism at the end of the contraction. NHE1 is hyperactive, contributing to DMD pathology in skeletal and cardiac muscles (Bkaily and Jacques 2017). Although NHE1 hyperactivity has been attributed to ATP receptor P2A in *mdx* (Iwata *et al*. 2007), its other regulators like p38 MAPK, ERK1/2, calcineurin, PTPN11, etc. are anomalous in DMD, as discussed in following sections. NHE1 inhibitors, as therapy for DMD, are under clinical trials (Previtali *et al*. 2020) as they potentially target skeletal muscle and heart pathology simultaneously. It is important to note that the calcium ingress, membrane ROS production, and NHE activation are all part of the normal ECC; hence therapies targeting these may affect normal muscle function and should be considered carefully.

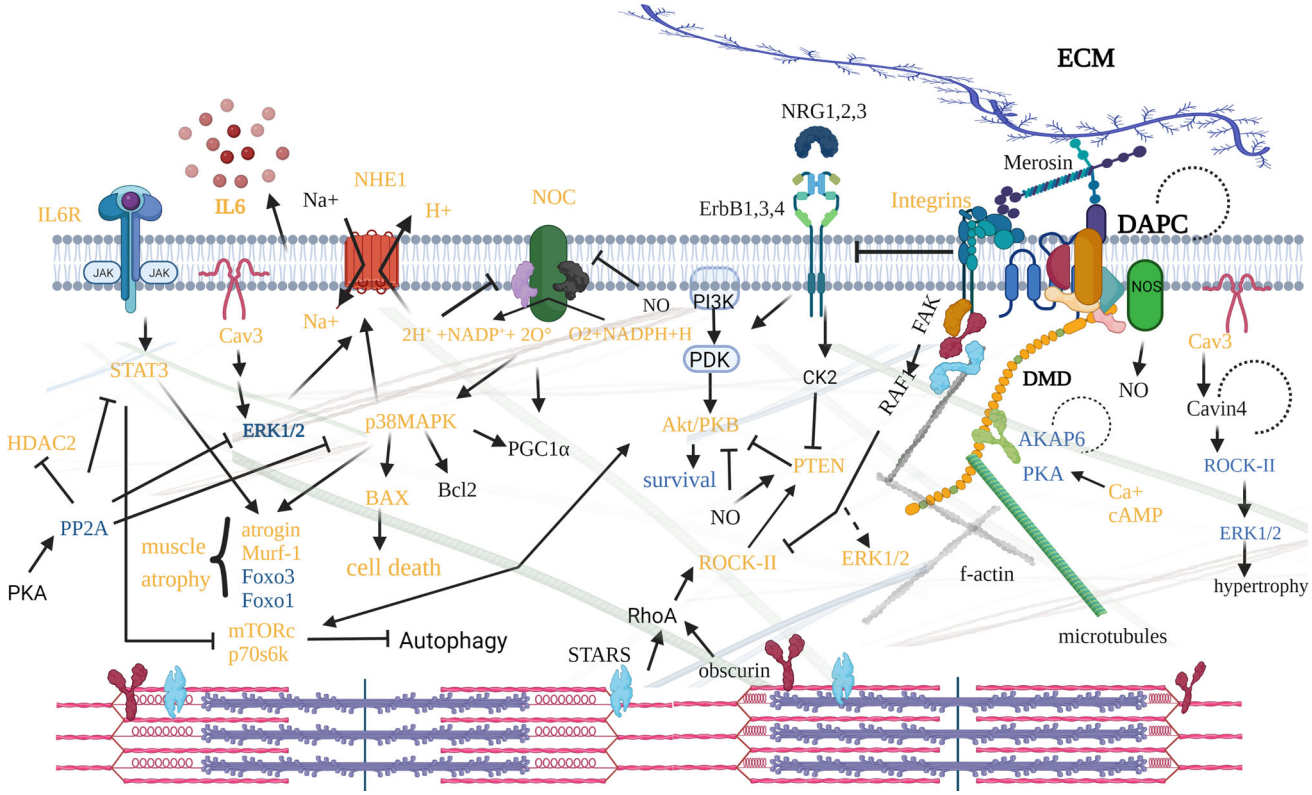

Figure 2. The pathology-associated pathways in detail. Yellow represents hyperactive pathways, and blue represents hypoactive pathways. Black represents either unaffected or undetermined activity. The dotted semicircle represents the mislocalization of proteins.

The hyperactive 'growth pathways' elicit pathology in DMD against the backdrop of altered metabolite fluxes

Muscle contraction and growth are intrinsically linked. The interesting feature of DMD is activity-induced muscle atrophy via hyperactivity of the very signalling pathways that stimulate growth in response to exercise in normal muscles (figure 2). Some of these pathways include p38 MAPK, ERK1/2, and AKT signalling. The activation of the p38MAPK pathway during exercise occurs via several mechanisms (Combes *et al*. 2015), as it is required for metabolic adaptation (reviewed by Bengal *et al*. 2020) by stabilizing and activating PGC1-α (Fan *et al*. 2004). In cardiac muscles, p38MAPK activity protects against stress-mediated telomere shortening (Ludlow *et al*. 2017). However, in DMD, the reasons behind why P38MAPK activates Bax-mediated apoptosis (Wissing *et al*. 2014), instead of PGC1-α remain unclear. The presence, in DMD patients, of other activators of PGC1-α, like ROS (Nalbandian *et al*. 2019), calcium-calmodulin (Handschin *et al*. 2003) suggests that PGC1-α activity might, in fact, be high, although it has not reported. Moreover, in DMD, PGC1-α could be behind upregulation of its target uncoupling protein 2 (UCP2) (Markham *et al*. 2017), which is thought to shunt the mitochondrial electron potential towards heat production instead of ATP.

The ERK1/2, via a broad range of effectors, stimulates Akt/PKB-independent muscle growth (Hodson *et al*. 2019) in response to exercise (reviewed by Long *et al*. 2004). The ERK1/2 activation occurs in two different microenvironments in skeletal muscles; (i) membrane-associated caveolin-3-cavin4-mediated (similar to heart, discussed in caveolae section), and (ii) sarcomere-associated proteins, like obscurin and STARS, which activate the ERK1/2 via ROCK in a Rho/Rac dependent manner (Ford-Speelman *et al*. 2009). Protein levels of obscurin are upregulated, while the STARS, RhoA and SRF are downregulated in DMD patients (Capitanio *et al*. 2020). Downstream of ERK1/2, lies in different effectors, such as Janus Kinase (JNK) (Long *et al*. 2004), the CREB binding protein (CBP), and Sphingosine kinase. However, the details of its contribution to dystrophic pathology remain elusive. On the other hand, the overexpression of CPB (a part of histone acetylase complex CBP/p300) rescued the *Sapje* zebrafish model of DMD (Bajanca and Vandel 2017). Sphingosine Kinase (S1PK) generates sphingosine-1-phosphate (S1P) (Pitson *et al*. 2003). Increasing S1P levels, improved regeneration (Ieronimakis *et al*. 2013), in a STAT3-dependent manner (Loh *et al*. 2012), to rescue the *mdx* pathology, when its degradation enzyme, SP lyase (SPL), was chemically inhibited. This suggests that in dystrophic muscles, ERK1/2 is unable to either activate its targets, such as S1PK or CBP, or, despite their activation, these effectors are nonfunctional.

Interestingly, it has been shown that nutritional and acetylation status can have an impact on PGC1-α and S1PK activity (Dominy *et al*. 2010; Yu *et al*. 2012), determining their pro-growth function. Thus, the lack of glycolysis-derived acetyl and other metabolites, in DMD, can subvert the expected outcomes of these growth and survival pathways.

Akt/PKB and PTEN regulating muscle mass in DMD-an indirect consequence of low glycolysis?

The Akt/PKB is a well-known pathway for cell survival and growth in various tissues, including skeletal muscles, by stimulating protein synthesis and inhibiting proteasomal degradation (reviewed by Schiaffino and Mammucari 2011). As an autophagy inhibitor, Akt/PKB is not activated in response to muscle contraction, but rather responds to nutritional cues, like insulin (Deldicque *et al*. 2008). One of its inactivating phosphatases, PTEN, gets activated by sarcomere-associated RhoA during normal muscle contraction. However, there is controversy because, despite the hyperactivity of PTEN (Feron *et al*. 2009), in DMD, the Akt/PKB remains highly active (Peter and Crosbie 2006). Other factors, such as regenerating fibres (Peter and Crosbie 2006), or upregulated integrin signalling (Boppart *et al*. 2011) could contribute to Akt/PKB hyperactivity in this case.

Besides stimulating protein synthesis, Akt/PKB-mTORc signalling promotes muscle growth by inhibiting autophagy. Although beneficial in normal muscles, this same pathway, in DMD, contributes to pathology, also by reducing the efficiency of autophagy (De Palma *et al*. 2012; Yazid and Hung-Chih 2021). While chemical inhibition of PTEN rescued the dystrophic phenotype in animal models (Alexander *et al*. 2014; Vieira *et al*. 2017; Yue *et al*. 2021), Akt/PKB activation by IGF1 treatment failed to improve muscle function during clinical trials despite improving muscle mass in DMD patients (Rutter *et al*. 2020). This reiterates that although nutritional cues or ECC-activable signalling are intact in DMD, their outcomes are altered, which could be due to the unique metabolic background of dystrophic muscles.

The muscle protein synthesis and autophagic renewal connected to energy starvation

Autophagy removes dysfunctional organelles and recycles material for the proper functioning of cells and tissues. The details of the autophagic pathway in skeletal muscles have been reviewed extensively (Xia *et al*. 2021), where AMPK and mTOR emerge as major positive and negative regulators, respectively. The trimeric AMPK, which has several subunit isoforms responding to different cues, acts as a major energy sensor in several tissues (Kjøbsted *et al*. 2018) to mitigate energy scarcity by upshifting catabolism. When activated by increasing AMP/ATP, and ADP/ATP ratios in contracting muscles, AMPK stimulates glucose uptake (Cokorinos *et al*. 2017), glycogenolysis, and proteolysis by removing the mTOR-mediated block on autophagy (reviewed by Garcia and Shaw 2017); inhibits FA synthesis and stimulates FAO (Wu *et al*. 2017).

It seems, however, that in dystrophic muscles, AMPK-based energy-sensing mechanisms fail despite high levels of its stimulators, like ADP, liver kinase B1 (LKB1), and calcium/calmodulin-dependent protein kinase kinase (CaMKK). As discussed in the previous section, the reduced efficiency of autophagy has largely been attributed to Akt/PBK-mTOR in DMD. Independent of Akt/PKB, phospholipase D (PLD) can also activate mTOR via its product phosphatidic acid (O'Neil *et al*. 2009). Because PLD is mechanically stimulated by contraction (Vandenburgh *et al*. 1993), its higher activity in DMD (Touboul *et al*. 2005; Dabaj *et al*. 2021) could contribute to the pathology.

Stimulation of autophagy by Simvastatin (Verhaart *et al*. 2021; Mucha *et al*. 2021) or knocking out its negative regulator TRAF6 (Hindi *et al*. 2014) failed to ameliorate the *mdx* pathology. Treatment with aminoimidazole-4-carboxamide ribonucleotide (AICAR), a known activator of AMPK, ameliorated pathology in *mdx* (Ljubicic *et al*. 2014; Bueno Júnior *et al*. 2012; Pauly *et al*. 2012) in a utrophin-dependent manner (Al-Rewashdy *et al*. 2015), although utrophin upregulation alone, as therapy, has not been successful yet (Wilkinson *et al*. 2020). It seems that the AMPK-based catabolic shift is only transiently required during contraction as long-term AMPK activation attenuated the adaptive metabolic response (Ljubicic *et al*. 2012). However, AICAR treatment stimulates IL6 expression from human myotubes (Weigert *et al*. 2007), which is considered pathological in DMD, and hence, it may not serve as a promising drug candidate for human patients. Another activator of AMPK, Metformin, has shown nonstatistical, but clinically relevant improvement in patients, when supplemented with L-citrulline amino acid (Hafner *et al*. 2019). Thus, when viewed in the context of energy starvation, stimulation of autophagy allows proteins to be used for energy production, but results in loss of muscle mass, and its inhibition preserves muscle structure, but reduces the energy needed for muscle function; this is the tight-rope toed by DMD.

The mystery of dysfunctional phosphatases in DMD

As discussed previously, muscle contraction results in activation of the calcium cascade and CaM Kinases, ERK1/2, p38MAPKs, etc. To counterbalance the activities of these kinases, several phosphatases also get activated. Although PP2A impinges on and inhibits several of the hyperactive signalling pathways described in DMD pathology (review by Eichhorn *et al*. 2009), it has not yet been considered a target for treating DMD. For example, compound FTY720,

an activator of PP2A (Saddoughi *et al*. 2013), has not been evaluated in DMD models. FTY720 has ameliorated LGMD2C in mouse models (Heydemann 2017), though the rescue is attributed to S1PK receptor (S1PKR) activation and not that of PP2A. PP2A can oppose cardiac hypertrophy via HDAC2 inhibition (Yoon *et al*. 2018), making it a promising therapeutic candidate.

The calcium–calmodulin-dependent phosphatase (calcineurin) PP2B activity is low despite high calcium in DMD. Its overexpression or activation rescued the *mdx* phenotype (Stupka *et al*. 2006; Delacroix *et al*. 2018). The calcineurin rescue is considered to be via activation of a transcription programme required for the transition of fast to slow oxidative type muscles, which are preserved longer in DMD. But, in DMD, the antagonist of calcineurin-mediated muscle-type transition, myospryn/AKAP6, is already low, as it depends on dystrophin for its localization and activity (Kielbasa *et al*. 2011). AKAP6 mislocalization also deregulates PKA activity (Reynolds *et al*. 2008) in the subsarcolemmal compartment, which, among its many targets, also regulates PP2A and PP1.

During exercise, PKA is also activated by cAMP generated by adenyl cyclases. The cAMP and cGMP act as second messengers to nitric oxide signalling, which is considered to be low in DMD. Hence, increasing cAMP/cGMP by inhibiting cAMP/cGMP degrading phosphodiesterase (PDE) seemed like a promising two-fold therapy. However, two such PDE inhibitors, sildenafil and tadalafil, failed in clinical trials, exacerbating patient conditions (Timpani *et al*. 2020).

Protein phosphatase-1 (PP1) localizes in different compartments in skeletal muscle. The glycogen-bound PP1 regulates intermediary metabolism via PKG, where its activity depends on insulin and adrenalin signalling (reviewed by Aggen *et al*. 2000). PP1 is upregulated in a GSK3β-dependent manner in *mdx* (Villa-Moruzzi *et al*. 1996), despite the low intermediary metabolism in dystrophic muscles (discussed in later sections). The GSK3β (and GSK3α), identified for regulating glycogen synthase (GS) activity, does not respond to muscle contraction (Wojtaszewski *et al*. 2001), and can inhibit PGC1-α, and calcineurin signalling. GSK3β has a reciprocal inhibitory relation with insulin/IGF1-stimulated Akt/PKB (reviewed by Mirzoev *et al*. 2021). Thus, inhibiting GSK3β can improve several aspects of pathological processes, including reducing glucose levels by glycogen synthesis, but cannot stimulate glycogenolysis–glycolysis in response to exercise.

The caveolae, caveolin-3 and IL6-STAT3 dysbalance in DMD

There is an increase in membrane caveolae and associated protein caveolin-3 (Cav3) in DMD patient muscles (Repetto *et al*. 1999; van Westering *et al*. 2020). The Cav3 upregulation could either be an adaptative response to differentiation and mechanical defects seen in dystrophic muscles (Pradhan and Prószyński 2020) or a direct consequence of p38MAPK hyperactivity (Galbiati *et al*. 1999). The Cav3 and its associated cavins 1, 2, 3, and 4 localize to membranes of various cells, while Cav4 is enriched in heart and skeletal muscles. Cav4/MRUC activates Rho/ROCK pathway during cardiac dysfunction and activates the ERK1/2 pathway in caveolae to drive cardiac hypertrophy (Ogata *et al*. 2008, 2014). Recent findings suggest that the association of Cav4 with the membrane is disrupted in the *mdx* heart, which cannot be rescued with mini dystrophin (Wang *et al*. 2021).

IL6 released from exercising muscles (Pedersen and Febbraio 2008) activates STAT3 transiently, as opposed to constitutively, in DMD. IL6 is considered pathological as its inhibition rescued DMD/Utrn DKO mice (Wada *et al*. 2017). IL6 also upregulates neuregulin (NRG/HRG) cleavage-mediated ligand activation (Juretić *et al*. 2017), which correlates with exercise-induced activation of its ErbB (1,2,3,4) receptors (Lebrasseur *et al*. 2003). The study also found that IL6-NRG-ErbB stimulates utrophin transcription in *mdx* via the ERK pathway (Juretić *et al*. 2017). IL6 is also considered an energy sensor (Kistner *et al*. 2022) and can stimulate glucose and fatty acid oxidation in contracting muscles (Al-Khalili *et al*. 2006; Trinh *et al*. 2021), possibly via AMPK (Carey *et al*. 2006). Thus, the underlying metabolic deficiency could be driving the IL6 secretion in DMD.

Chronic activation of STAT3 can lead to muscle atrophy via Murf-1 and atrogin1 (Zhang *et al*. 2013). However, in dystrophic conditions, Murf-1, atrogin-1, CHIP or NEDD4 are not responsible for the observed pathological proteasomal degradation (Assereto *et al*. 2016). Studies on DMD/Utrn DKO mice have shown that STAT3 is required for the repair and regeneration via the muscle stem cells (Zhu *et al*. 2001), and thus it may not be a promising therapy candidate. The mitochondrial localization of STAT3 depends on p300-mediated acetylation (Xu *et al*. 2016), which is considered as an important modulator of energy metabolism in various tissues, including the heart (Meier *et al*. 2017). Although muscle-specific knockout of STAT3 does not hamper energy production and exercise capacity (Dent *et al*. 2019) in nondystrophic mice, it could be a contributing factor in DMD, because of the disturbed metabolic landscape, which needs further investigation.

The histone deacetylases inhibition mitigates low acetyl levels to improve patient histopathology

The emerging details of all classes of HDACs on skeletal muscles and exercise have been reviewed (Tian *et al*. 2020). Many of the HDACs are involved in myogenesis, as expected. The activity of only HDAC2, out of HDAC1, 2, 3, and 8, in DMD patient samples, was found to be high (Colussi *et al*. 2008). Though reduced NO levels could contribute to higher HDAC2 activity in DMD (Colussi *et al*. 2008), increasing NO signalling as a strategy for BMD and

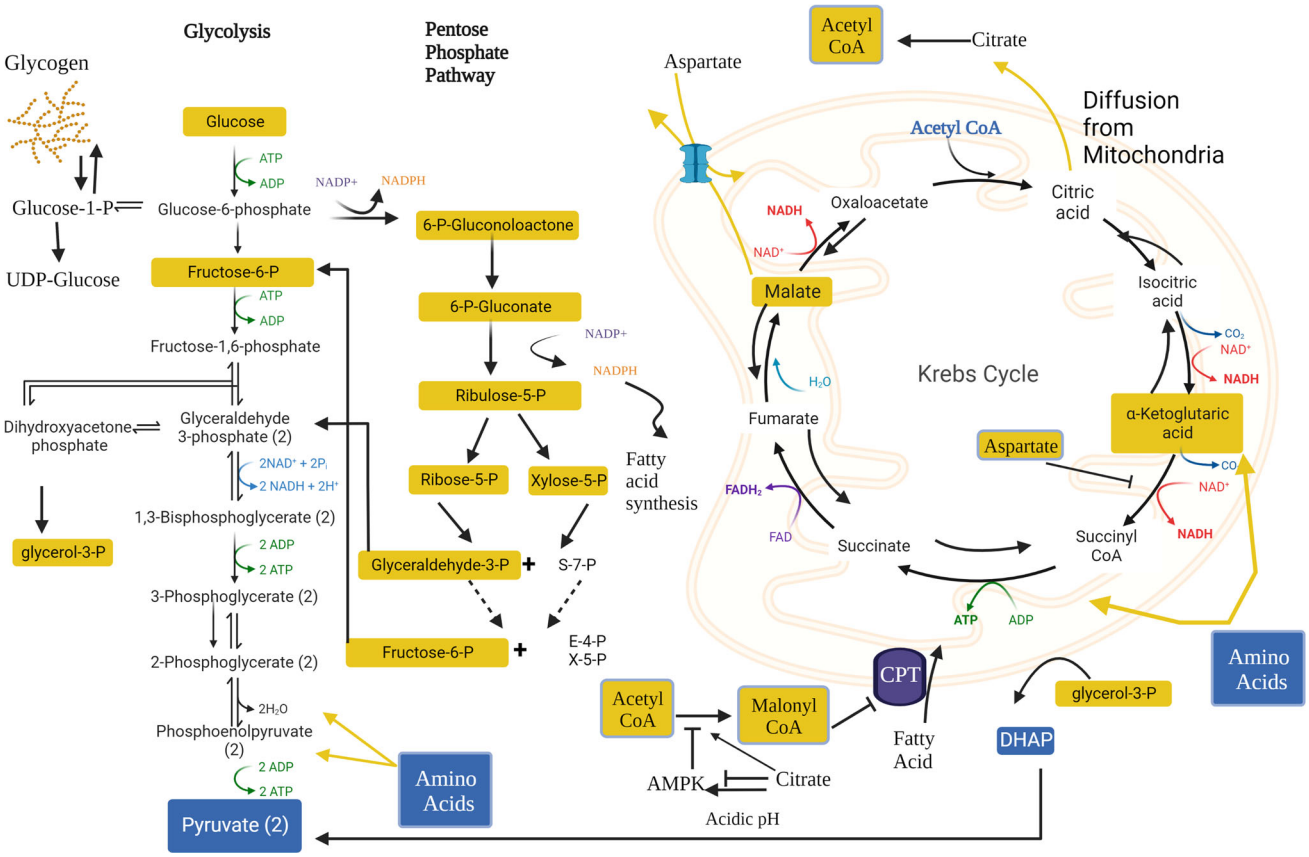

Figure 3. Glucose metabolism pathways. Yellow represents higher metabolite levels; blue represents lower metabolite levels.

DMD therapy failed. The knockout of HDAC1-2 in nondystrophic mice resulted in a blockage of autophagy in muscles, whereas a high-fat diet removed the block, improving muscle function (Moresi *et al*. 2012). In a recent study (Li *et al*. 2021), HDAC2 overexpression protected muscles against cigarette smoke-induced atrophy and senescence. Further, the HDAC class1 and class2 inhibitors reduced SERCA activity in zebrafish muscles (Simmonds and Seebacher 2017). The SERCA activity is required for calcium reabsorption in muscles, and its lower activity contributes to higher calcium-mediated pathology in DMD (Schneider *et al*. 2013; Voit *et al*. 2017; Mareedu *et al*. 2021). Thus, HDAC2 inhibitors as a therapy for DMD should be considered carefully.

The exercise-mediated nuclear export of HDAC4 and HDAC5 in an AMPK and CaMKII-dependent manner has been reported in healthy individuals (McGee *et al*. 2009) but has not been described in dystrophic conditions yet. Whether the activities of HDAC 4, 5, 7, and 9 are altered in DMD patients is not known. The pan HDAC inhibitor 'Givinostat' improved muscle pathology in DMD patients, for which clinical evaluation for functional aspects is ongoing (Bettica *et al*. 2016). HDAC inhibition may mitigate the low acetyl levels, by influencing the acetylation span of proteins, despite limiting acetyl availability. This could be the reason behind the positive outcomes of the HDAC inhibitor treatment on patient muscle biopsies (NCT02851797) though functional improvements are yet to be seen.

The stimulation of another class of deacetylases, NAD^+ dependent sirtuins or SIRTs is also being developed as DMD therapy. Most of the evidence of SIRT involvement has been indirect, via rescue in the *mdx* model by resveratrol (reviewed by Kuno and Horio 2016). Though SIRT1, in DMD patients, was shown to be upregulated at transcript levels, its protein level in plasma remained unchanged (Rossi *et al*. 2021). The activity of sirtuins has not yet been reported in DMD patient muscles. Unlike the metabolic enzymes, sirtuins convert NAD^+ to NAM with their activity; thus, activating sirtuins could lead to a reduction of NAD levels, which are already low in DMD. However, efforts to enrich NAD^+ in muscles by supplementation with precursors or inhibition of ADP-ribosyl cyclases (that use NAD^+) did not improve the *mdx* pathology (Frederick *et al*. 2020). During glycolysis or mitochondrial TCA, only the redox state of NAD is interchanged without changing the total concentrations; thus, NAD concentration, by itself, cannot improve energy levels if glycolysis is repressed.

The theory of glycolysis as secondary pathology in DMD

Since the first description of the disease, its aspects of metabolic and energy deficiency have been studied in detail (reviewed by Timpani *et al*. 2015). The main features of dystrophic muscles are low glycogenolysis, glycolysis, fatty acid oxidation (FAO), excess amino acid catabolism, fatty acid synthesis, and PPP (figure 3). The TCA cycle is blocked at the α-ketoglutarate stage, lowering ETC-OXPHOS and ATP synthesis. The levels of metabolites are correspondingly high glucose, fructose-6-P, α-ketoglutarate, phosphatidic acid, sphingolipids and ADP, and low acetyl, pyruvate, branched amino acids, glutamine/glutamate and ATP (Sharma *et al*. 2003; Srivastava *et al*. 2018, 2020; Dabaj *et al*. 2021). Despite the pervasive glycolytic deficiency observed, because octanoate (whose catabolism is independent of glycolysis) failed to rescue the mitochondrial defects (Chinet *et al*. 1994), the mitochondrial deficiency was considered as an independent and primary defect. This does not correlate with the fact that primary mitochondrial deficiency invariably upregulates glycolysis, as seen in mitochondrial dystrophies (Arnold *et al*. 1985). The common mitochondrial pathology should also affect more oxidative tissues (brain and heart) earlier than less oxidative, glycolytic-type muscle, which contrasts with what is seen in DMD.

The early study compared glycogen and glucose metabolism between neurogenic muscle diseases and progressive muscular dystrophies, like DMD, LGMD, myotonic dystrophy, FSHD, and found that early-stage repression of these processes is common to all muscular dystrophies, and, hence, should be considered as a secondary pathology instead (Di Mauro *et al*. 1967). However, there are several key differences in terms of disease onset, abnormalities of ECC, slow versus fast muscle type affected, or defects of muscle formation, that set these diseases apart from DMD, where repression of glucose metabolism could be a secondary pathology or an adaptive response. For example, in the case of myotonic dystrophy, there is prolonged contraction (inability to return to resting state), where repression of glycogenolysis–glycolysis could be adaptive.

The detailed discussion about several types of muscular dystrophies with respect to glucose metabolism is beyond the scope of this review. The distinguishing aspects of DMD are normal resting ATP/ADP ratio and normal ECC (Mancinelli *et al*. 1989), which makes the failure of glycogenolysis–glycolysis more pertinent to the pathological mechanism. If dystrophic muscles are mechanically inefficient at contraction, there should be compensatory metabolic upregulation even during rest, which is not seen in DMD. The increased proteolysis and amino acid catabolism for energy production also oppose the notion that glycolysis is actively or adaptively repressed in DMD. The high α-ketoglutarate level inhibits ATP synthase (Chin *et al*. 2014), and increases fibrosis (Ge *et al*. 2018). Yet, an α-ketoglutarate supplement was able to rescue the *mdx* pathology (Cai *et al*. 2018). This suggests that blocking the TCA cycle at α-ketoglutarate, is a mitochondrial adaptive response in the face of the limited availability of glucose-derived pyruvate, so as to limit protein degradation. As discussed in the previous sections, the mechanical force stabilization theory does not explain the small percentage of dystrophin-containing DGC or the slower disease manifestation in the heart.

The reduced glycogenolysis–glycolysis converges on shift to PPP and altered fat and amino acid metabolism from myoblast stages

An elegant radiolabelling study (Ellis 1980) showed that, in DMD, the glucose from glycolysis is shunted to glycerol-3-phosphate (G3P), rather than to pyruvate, and PPP-derived pyruvate is used for mitochondrial oxidation, which increases NADPH generation. The G3P and NADPH contribute to high FA synthesis and fat accumulation. The calcium and thyroid-responsive GPD2 enzyme usually reconverts G3P to DHAP, and, ultimately, to pyruvate. The G3P accumulation in DMD suggests that GPD2 activity is reduced (Liu *et al*. 2018).

The calcium spike during ECC also stimulates the activity of mitochondrial pyruvate dehydrogenase activity to increase pyruvate oxidation. Both the resting-induced (Liang 1986; Chinet *et al*. 1994; Kuznetsov *et al*. 1998; Rybalka *et al*. 2014) and the exercise-induced (Faist *et al*. 2001) mitochondrial O_2 consumption has been reported to be low in DMD. This may be either due to the reduced efficiency of OXPHOS or the lower content of ETC proteins in the mitochondria. ADP-phosphorylation per molecule of O_2 consumed, represented as the P/O ratio, remains similar between the control, *mdx* and in patients, indicating that the efficiency of OXPHOS is unchanged among these contexts (Olson *et al*. 1968). On the other hand, the low mitochondrial ETC content in both *mdx* and patient biopsy (Kuznetsov *et al*. 1998), and lower Co-enzyme A (CoA) levels in *mdx* (Jato-Rodriguez *et al.* 1975) could explain the reduced capacity of the dystrophic mitochondria to utilize not just pyruvate but fatty acids too.

The mechanisms of protein transport into mitochondria are only now emerging. For example, one such protein, Mortalin/mtHsp70 (also known as glucose response protein (GRP75), is low in *mdx* but high in patients (Thakur *et al*. 2018; Capitano *et al.* 2020). GRP75 gets activated by low glucose levels; hence, increased levels of GRP75 could be a compensatory response to its lower activity. However, further investigation is needed to establish the role of GRP75 and other proteins in the mechanisms of protein transport into dystrophic mitochondria.

The FAO is regulated at FA transport into mitochondria via carnitine palmitoyl transferase (CPT). A blockage of the α-ketoglutarate in the TCA cycle causes citrate diffusion

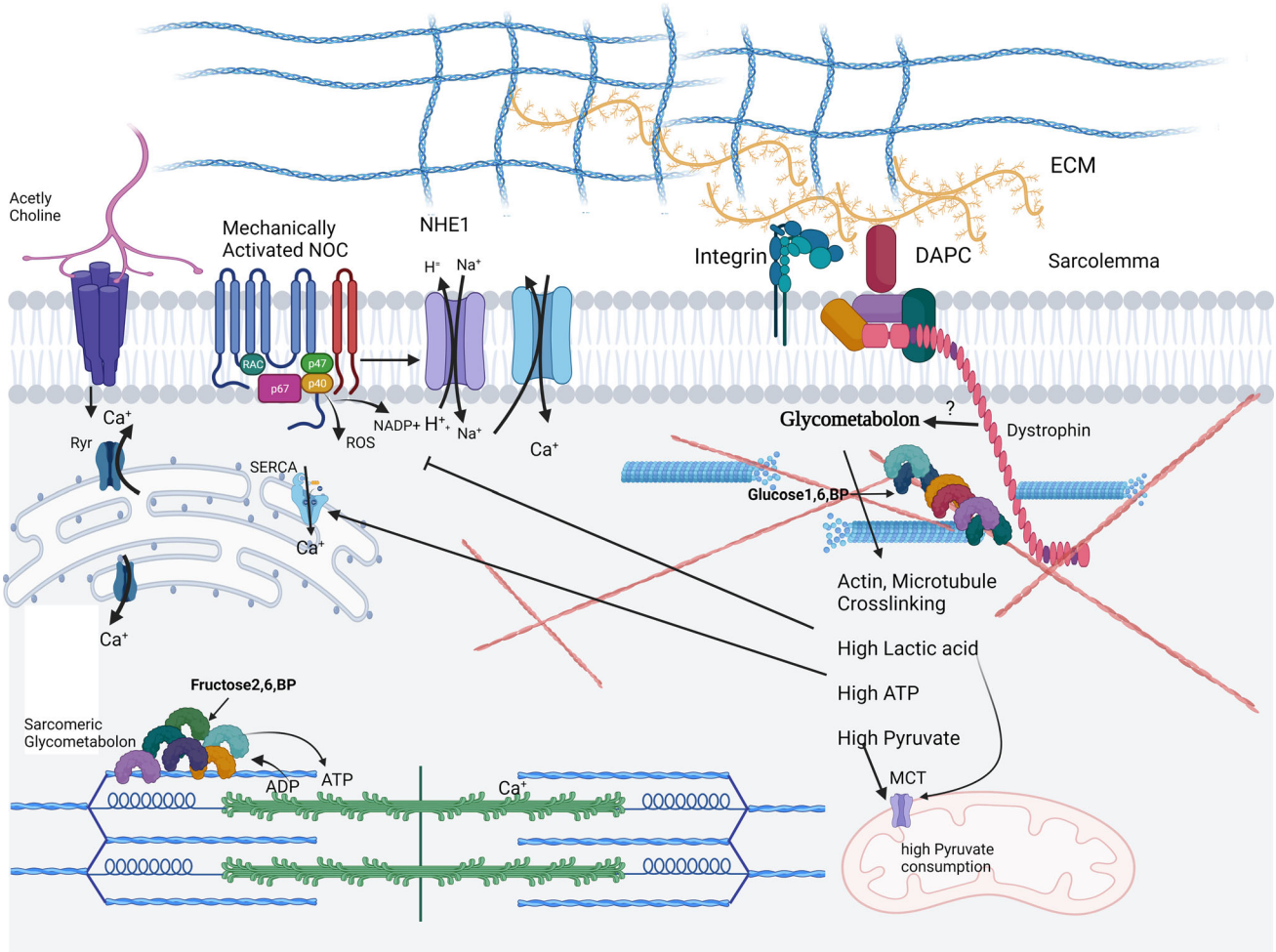

Figure 4. Theory of pathology initiation as a result of reduced costameric glycometabolic action.

from the mitochondria into the cytoplasm, as all the TCA reactions can occur reversibly except α-ketoglutarate dehydrogenase and citrate synthase (Chinopoulos 2013). Citrate diffused into the cytoplasm can activate acetyl CoA carboxylase (ACC), leading to the production of malonyl CoA that inhibits CPT, thus reducing FAO (Martin and Vagelos 1962). The lactic acid accumulation accompanies a reduction in pH, which changes the effects of citrate on ACC and AMPK, the negative regulator of ACC, thus allowing FAO to be upregulated in contracting muscles. Lactic acid, which has many essential physiological functions (reviewed by Sun *et al*. 2017) is significantly reduced in DMD and BMD patients (Ellis 1980), which might be relevant to pathology.

The lactic acid secreted by contracting muscles also brings metabolic and transcriptional changes to myoblasts (Zhang *et al*. 2019), possibly contributing to the poor regeneration seen in DMD. Myoblast proliferation and myotube formation require switching up of both glucose and fatty acid metabolism (Fulco *et al*. 2008; Ryall *et al*. 2015; Abreu, 2018; Nalbandian *et al*. 2020). However, the expression of phosphofructokinase-2 (PFK2), a key regulatory enzyme of glycolysis, begins only towards the end of differentiation (Vandoolaeghe *et al*. 1999). This suggests that an alternate regulation to activate glycogenolysis–glycolysis must exist during the myoblast stages, which is affected in DMD myoblasts (Timpani *et al*. 2020). So, glucose-1,6-bisphosphate, another key regulator of glycogenolysis and glycolysis, which also inhibits the PPP (reviewed by Beitner 1984, 1993) in contracting muscles, might be responsible for activating glycogenolysis–glycolysis in myoblasts too. Hence, in DMD, lack of this alternative arm of glycolysis from myoblast stages could wire the metabolism towards excess PPP, and decreased glycolysis, which together induce FA synthesis (as described in the previous section) on the one hand, and affect the ability of the dystrophic muscle to oxidize FA on the other. This results, in DMD, an increased dependence on amino acid catabolism for energy production, which in turn, reduces amino acids available for and stimulates protein synthesis (Blomstrand *et al*. 2006; Rennie *et al*. 2006), thus, shifting the balance towards atrophy.

Alternate arm of glycolysis that additionally stimulates glycogenolysis

Glycogen is utilized in various ways in different tissues. It is very important for the proper functioning of skeletal muscles, supports extra work in the heart (reviewed by Depre *et al*. 1999), has shown to be localized in the area of higher neuronal activity in the brain (Vaishnavi *et al*. 2010), and to promote myoblast survival under stress (Lytridou *et al*. 2020). The glycogen synthesis mostly depends on nutritional cues and responds less to a contraction in skeletal muscles (Wojtaszewski *et al*. 2001; reviewed by Mirzoev *et al*. 2021). But glycogenolysis needs to be tightly linked with muscle contraction factors other than AMP, ADP, Pi and Ca^{2+} (reviewed by Katz 2022). The glucose-1-phosphate generated by glycogenolysis can inhibit muscle glycogen phosphorylase, which is less sensitive to inhibition by glucose-6-phosphate. The enzyme phosphoglucomutase (PGM) interconverts glucose-1-phosphate and glucose-6-phosphate depending on their ratios, and, hence, the glycogenolysis rate depends on G6P channelling into glycolysis. The PFK2 product fructose-2,6-bisphosphate can only activate the glycolytic enzymes already assembled onto stable sarcomeric A and Z bands but not the free cytoplasmic enzymes. The subsarcolemmal cytoskeleton during muscle contraction is unstable and dynamically reconstructed, requiring free cytoplasmic enzymes of glycolysis to get activated and assemble into congregates by crosslinking cytoskeletal filaments. The glucose-1,6-bisphosphate activates several free cytoplasmic enzymes, like PGM, GAPDH, PEP, PFK1 and pyruvate kinase, to meet the energy needs of costameric dynamics and ion channels (reviewed by Beitner 1984, 1993). The dependence on glycogenolysis, the contribution of tissue-specific PFK2 isozyme to glycolytic regulation, and the manifold increase in glycolytic flux between the resting and the active state decides the vulnerability to G1, 6BP deficiency, which fits the disease progression seen in muscles, heart and brain for DMD.

The plausible involvement of dystrophin and lacunae in glycolytic congregation theory

There exists an inverse relationship between glucose and fatty acids. The excess glucose/glycogen can stimulate fatty acid synthesis. The excess FA, in turn, stimulates cGMP synthesis, which can inhibit G1,6BP synthesis, repressing glycogenolysis–glycolysis (Bassols *et al*. 1991). The dystrophic myoblasts synthesize FA at a higher rate than the control, even under low serum conditions, which can give rise to defects of glucose metabolism (Bonsett *et al*. 1979). This can also partly explain the disease exacerbation caused by the PDE inhibitors, which increase cGMP levels.

In myoblast and differentiated dystrophic muscles, excess fatty acid levels could be due to higher import or higher production. However, elevated FA import has not been reported in DMD, and the normal resting ATP/ADP ratio suggests basal FAO is unaffected. Hence, it is more likely that the glycogenolysis defects are stimulating excess fatty acid synthesis in DMD. The proteomics profiling of DMD and BMD patients also support our hypothesis, as the differentially regulated pathways between BMD and DMD strongly correlate with their respective severity of glucose metabolism defects (Capitanio *et al*. 2020).

The study also found reduced hexosamine synthesis and related metabolites in the differential proteomes, which depend on glucose-1-phosphate generated by either glycogen phosphorylase or PGM, both of which are known to be low in DMD. The defects in hexosamine and other glycosyl moiety synthesis or transfer to proteins of DGC also give rise to specific MDs, which vary in their nature from congenital to late onset. However, the disease onset and progression in such diseases are markedly different from those in the DMD case, suggesting that the hexosamine pathway is affected secondarily in DMD.

The major caveat with our hypothesis is the lack of direct evidence of the active congregation of glycogenolytic and glycolytic enzymes in stimulated muscles. There is no direct interaction between dystrophin and the key regulatory glycolytic enzymes, except for the catalytically deficient PGM5/aciculin (Wakayama *et al*. 2000), which can interact with other PGM enzymes. The dependence of ACYP2 activation on dystrophin-mediated processes during muscle contraction could contribute to reduced glycolytic volume in sarcomeric regions, primarily stimulated by the PFK2 product. But the regulation of ACYP2 during muscle contraction remains understudied to refute or support such a hypothesis. The relationship between free glycolytic enzymes binding to monomeric versus polymerized cytoskeleton is complex. The binding to the cytoskeleton also affects binding to other glycolytic enzymes, further adding to the intricacy of the congregate formation model (Menard *et al*. 2014). Yet, it remains a fact that during ECC, the volume of glycogenolysis–glycolysis increases a thousand folds despite high ROS and calcium, and continually reforming subsarcolemmal cytoskeleton. This is because other energy stores (like PCr and adenylate kinase) are too short-lived, and the activation of oxygen-dependent mitochondria is 100 times slower than the rate of glycolysis.

We hypothesize that dystrophin acts as a modulator of glycogenolytic–glycolytic stimulation during ECC in differentiated muscles, in addition to its canonical DGC-related functions. Its similar function could also exist during the asymmetric division of satellite cells (Dumont *et al*. 2015; Chang *et al*. 2016), which might predispose the resulting myoblast to higher PPP and lower glycolysis. Further studies are needed to unveil the regulation of key enzymes to generate a well-informed model, details of which could also be useful in heart and neuronal diseases with glycogenolytic defects.

It is possible that dystrophin lies under both positive and negative regulation, to control the glycolytic switch. Given that in cancers, glycolysis is classically upregulated, suggesting that the dystrophin could be targeted in tumourigenesis. Dystrophin-mediated positive and negative regulation of glycometabolism is possibly associated with its dual (oncogenic and tumour suppressor) role in various cancers (reviewed by Jones *et al*. 2021).

The summary considerations for future DMD therapies

Muscles, irrespective of many complexities, work on a simple paradigm—one ATP for single myosin head flex; hence, energy and function are inseparable. The glycolysis needs a quick upshift during contraction, and the accumulated metabolites are shunted to protein synthesis and repair. The failure of IGF-1 treatment to improve muscle function, despite the consequently increased muscle mass, points to the same. The hyperactivity of pathways like p38MAPK, ERK1/2, Akt/PKB etc. are similar to the scenario in cancer, yet the lack of excess glycolysis-derived metabolites changes the outcome from tumour growth in cancer to atrophy in DMD. Despite these 'growth pathways' driving pathology in differentiated dystrophic muscles, their inhibition could affect not just muscle regeneration but nontarget tissues as well, considering that DMD patients are in the 'growth phase' of their life. Similarly, sarcolemma-associated ROS production, NHE1 activation, and calcium ingress are all part of excitation–contraction coupling; hence, their inhibition can affect muscle function, even if there is a preservation of the structure. Thus, there is a need to understand further how metabolites modulate the 'dystrophic pathways' for therapies to restore muscle function.

Many adaptive responses, like integrin, dysferlin, filamin C (Capitanio *et al*. 2020; Han *et al*. 2021), α-ketoglutarate, etc., are already active in DMD, and further activated by steroid therapy (Sanson *et al*. 2020; Herbelet *et al*. 2020). Hence, creating therapies to override steroid use will require metabolic rescue (Timpani *et al*. 2020).

While chemical approaches have been struggling to increase slow oxidative type muscles that persevere longer in DMD, certain nutritional approaches have ameliorated the *Sgca*$^{-/-}$ model of dystrophy, with a transition to slow type muscles and additional mitigation of several of the pathological processes which are observed in DMD as well (Saclier *et al*. 2020). Thus, it might be worthwhile to adopt similar approaches for DMD.

Since its first description, DMD has been considered a metabolic disease, yet therapies targeting metabolism are very few (NCT04184882). Improving mitochondrial energy production significantly increased exon skipping efficiency (Ran *et al*. 2021), suggesting that metabolic targeting can, in fact, aid or complement advanced gene therapies. It should be considered that stimulating mitochondrial OXPHOS without stimulating glycolysis/FAO can only burden amino acid catabolism for fuel supply. Moreover, mitochondrial protein transport also remains an unexplored area for DMD.

Trifluoperazine, an FDA-approved drug for schizophrenia, is shown to increase glucose-1,6-bisphosphate availability by inhibiting its breakdown in rat skeletal muscles (Frucht *et al*. 1984). Improving glycogenolysis–glycolysis in the myoblast stage might improve autologous cell transplantation efforts. The molecular mechanisms of cytoskeleton-based glycometabolic regulation need further investigation as it can be productive for some muscular dystrophies, with secondary loss of glycogenolysis–glycolysis capacity. The improvement of resting metabolism can reduce energy deficit, thus delaying disease progression, but the cure will need contraction-activable glycolysis. It will be a challenging yet worthwhile attempt to develop a therapy for DMD.

Acknowledgements

We thank all researchers working on DMD and apologize to those whose work could not be cited due to space limitations or ignorance. A special thanks to Dr Isha Desai, Dr Saroj Jawkar and Amartya Mukherjee for endless discussion around the hypothesis and manuscript editing. Dr Somit Dutta, for his help with the figures. We also would like to thank the reviewer/s for their critical comments and suggestions. This review is part of a project funded by DST (SR/WOS-A/LS-273/2017) to VN and DST-SERB grant to UN (EMR/2016/004563).

Authors' contribution

VN, SB and UN conceived the idea, and VN wrote the initial draft with inputs from UN. All authors have read and agreed to the manuscript submitted.

References

Abreu P. 2018 Bioenergetics mechanisms regulating muscle stem cell self-renewal commitment and function. *Biomed. Pharmacother.* **103**, 463–472.

Aggen J. B., Nairn A. C. and Chamberlin R. 2000 Regulation of protein phosphatase-1. *Chem. Biol.* **7**, R13–R23.

Alexander M. S., Casar J. C., Motohashi N., Vieira N. M., Eisenberg I., Marshall J. L. *et al*. 2014 MicroRNA-486-dependent modulation of DOCK3/PTEN/AKT signaling pathways improves muscular dystrophy–associated symptoms. *J. Clin. Invest.* **124**, 2651–2667.

Al-Khalili L., Bouzakri K., Glund S., Lönnqvist F., Koistinen H. A. and Krook A. 2006 Signaling specificity of interleukin-6 action on glucose and lipid metabolism in skeletal muscle. *Mol. Endocrinol.* (Baltimore) **20**, 3364–3375.

Al-Rewashdy H., Ljubicic V., Lin W., Renaud J.-M. and Jasmin B. J. 2015 Utrophin A is essential in mediating the functional adaptations of mdx mouse muscle following chronic AMPK activation. *Hum. Mol. Genet.* **24**, 1243–1255.

Arnold D. L., Taylor D. J. and Radda G. K. 1985 Investigation of human mitochondrial myopathies by phosphorous magnetic resonance spectroscopy. *Ann. Neurol.* **18**, 189–196.

Assereto S., Piccirillo R., Baratto S., Scudieri P., Fiorillo C., Massacesi M. *et al*. 2016 The ubiquitin ligase tripartite-motif-protein 32 is induced in Duchenne muscular dystrophy. *Lab. Invest.* **96**, 862–871.

Bajanca F. and Vandel L. 2017 Epigenetic regulators modulate muscle damage in Duchenne muscular dystrophy model. *PLoS Curr* **9**, ecurrents, https://doi.org/10.1371/currents.md.f1e2379fa632f8135577333dd92ca83b.

Barthélémy F., Defour A., Lévy N., Krahn M. and Bartoli M. 2018 Muscle cells fix breaches by orchestrating a membrane repair ballet. *J. Neuromuscul. Dis.* **5**, 21–28.

Bassols A., Andrés V., Ballarín M., Mahy N., Carreras J. and Cusso R. 1991 Identification of guanine and adenine nucleotides as activators of glucose-1,6-bisphosphatase activity from rat skeletal muscle. *Arch. Biochem. Biophys.* **291**, 121–125.

Beitner R. 1984 Control of levels of glucose 1,6-bisphosphate. *Int. J. Biochem.* **16**, 579–585.

Beitner R. 1993 Control of glycolytic enzymes through binding to cell structures and by glucose-1,6-bisphosphate under different conditions. The role of Ca^{2+} and calmodulin. *Int. J. Biochem.* **25**, 297–305.

Belanto J. J., Mader T. L., Eckhoff M. D., Strandjord D. M., Banks G. B., Gardner M. K. *et al*. 2014 Microtubule binding distinguishes dystrophin from Utrophin. *Proc. Natl. Acad. Sci. USA* **111**, 5723–5728.

Belanto J. J., Olthoff J. T., Mader T. L., Chamberlain C. M., Nelson D. M., McCourt P. M. *et al*. 2016 Independent variability of microtubule perturbations associated with dystrophinopathy. *Hum. Mol. Genet.* **25**, 4951–4961.

Bengal E., Aviram S. and Hayek T. 2020 p38 MAPK in Glucose metabolism of skeletal muscle: beneficial or harmful? *Int. J. Mol. Sci.*, https://doi.org/10.3390/ijms21186480.

Bettica P., Petrini S., D'Oria V., D'Amico A., Catteruccia M., Pane M. *et al*. 2016 Histological effects of givinostat in boys with Duchenne muscular dystrophy. *Neuromuscul. Disord.* **26**, 643–649.

Bkaily G. and Jacques D. 2017 Na+-H+ exchanger and proton channel in heart failure associated with Becker and Duchenne muscular dystrophies. *Can. J. Physiol. Pharmacol.* **95**, 1213–1223.

Bladen C. L., Salgado D., Monges S., Foncuberta M. E., Kekou K., Kosma K. *et al*. 2015 The TREAT-NMD DMD global database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. *Hum. Mutat.* **36**, 395–402.

Blake D. J., Weir A., Newey S. E. and Davies K. E. 2002 Function and genetics of Dystrophin and Dystrophin-related proteins in muscle. *Physiol. Rev.* **82**, 291–329.

Blomstrand E., Eliasson J., Karlsson H. K. and Köhnke R. 2006 Branched-chain amino acids activate key enzymes in protein synthesis after physical exercise. *J. Nutr.* **136**, 269S-273S.

Bonsett C. A., Rudman A. and Elliott A. Y. 1979 Intracellular lipid in pseudohypertrophic muscular dystrophy tissue culture. *J. Ind. State Med. Assoc.* **72**, 184–187.

Boppart M. D. and Mahmassani Z. S. 2019 Integrin signaling: linking mechanical stimulation to skeletal muscle hypertrophy. *Am. J. Physiol. Cell Physiol.* **317**, C629–C641.

Boppart M. D., Burkin D. J. and Kaufman S. J. 2011 Activation of AKT signaling promotes cell growth and survival in α7β1 integrin-mediated alleviation of muscular dystrophy. *Biochim. Biophys. Acta.* **1812**, 439–446.

Brennan-Minnella A. M., Won S. J. and Swanson R. A. 2015 NADPH oxidase-2: linking glucose, acidosis, and excitotoxicity in stroke. *Antioxid. Redox Signal.* **22**, 161–174.

Bueno Júnior C. R., Pantaleão L. C., Voltarelli V. A., Bozi L. H. M., Brum P. C. and Zatz M. 2012 Combined effect of AMPK/PPAR agonists and exercise training in *mdx* mice functional performance. *PLoS ONE* **7**, e45699.

Burkin D. J., Wallace G. Q., Nicol K. J., Kaufman D. J. and Kaufman S. J. 2001 Enhanced expression of the α7β1 integrin reduces muscular dystrophy and restores viability in dystrophic mice. *J. Cell Biol.* **152**, 1207–1218.

Cai X., Yuan Y., Liao Z., Xing K., Zhu C., Xu Y. *et al*. 2018 α-Ketoglutarate prevents skeletal muscle protein degradation and muscle atrophy through PHD3/ADRB2 pathway. *FASEB J.* **32**, 488–499.

Canato M., Dal Maschio M., Sbrana F., Raiteri R., Reggiani C., Vassanelli S. *et al*. 2010 Mechanical and electrophysiological properties of the sarcolemma of muscle fibers in two murine models of muscle Dystrophy: *Col6a1*$^{-/-}$ and *Mdx*. *J. Biotechnol. Biomed.* **2010**, 981945.

Capitanio D., Moriggi M., Torretta E., Barbacini P., De Palma S., Viganò A. *et al*. 2020 Comparative proteomic analyses of Duchenne muscular dystrophy and Becker muscular dystrophy muscles: changes contributing to preserve muscle function in Becker muscular dystrophy patients. *J. Cachexia Sarcopenia Muscle.* **11**, 547–563.

Carey A. L., Steinberg G. R., Macaulay S. L., Thomas W. G., Holmes A. G., Ramm G. *et al*. 2006 Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes.* **55**, 2688–2697.

Chang N. C., Chevalier F. P. and Rudnicki M. A. 2016 Satellite cells in muscular dystrophy – lost in polarity. *Trends Mol. Med.* **22**, 479–496.

Chin R. M., Fu X., Pai M. Y., Vergnes L., Hwang H., Deng G. *et al*. 2014 The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. *Nature* **510**, 397–401.

Chinet A. E., Even P. C. and Decrouy A. 1994 Dystrophin-dependent efficiency of metabolic pathways in mouse skeletal muscles. *Experientia* **50**, 602–605.

Chinopoulos C. 2013 Which way does the citric acid cycle turn during hypoxia? The critical role of α-ketoglutarate dehydrogenase complex. *J. Neurosci. Res.* **91**, 1030–1043.

Cokorinos E. C., Delmore J., Reyes A. R., Albuquerque B., Kjøbsted R., Jørgensen N. O. *et al*. 2017 Activation of skeletal muscle AMPK promotes glucose disposal and glucose lowering in non-human primates and mice. *Cell Metab.* **25**, 1147–1159.

Coleman A. K., Joca H. C., Shi G., Lederer W. J. and Ward C. W. 2021 Tubulin acetylation increases cytoskeletal stiffness to regulate mechanotransduction in striated muscle. *J. Gen. Physiol.* **153**, e202012743.

Colussi C., Mozzetta C., Gurtner A., Illi B., Rosati J., Straino S. *et al*. 2008 HDAC2 blockade by nitric oxide and histone deacetylase inhibitors reveals a common target in Duchenne muscular dystrophy treatment. *Proc. Natl. Acad. Sci. USA* **105**, 19183–19187.

Combes A., Dekerle J., Webborn N., Watt P., Bougault V. and Daussin F. N. 2015 Exercise-induced metabolic fluctuations influence AMPK, p38-MAPK and CaMKII phosphorylation in human skeletal muscle. *Physiol. Rep.* **3**, e12462.

Dabaj I., Ferey J., Marguet F., Gilard V., Basset C., Bahri Y. *et al*. 2021 Muscle metabolic remodelling patterns in Duchenne muscular dystrophy revealed by ultra-high-resolution mass spectrometry imaging. *Sci. Rep.* **11**, 1906.

De Palma C., Morisi F., Cheli S., Pambianco S., Cappello V., Vezzoli M. *et al*. 2012 Autophagy as a new therapeutic target in Duchenne muscular dystrophy. *Cell Death Dis.* **3**, e418.

Delacroix C., Hyzewicz J., Lemaitre M., Friguet B., Li Z., Klein A. *et al*. 2018 Improvement of dystrophic muscle fragility by short-term voluntary exercise through activation of Calcineurin pathway in mdx mice. *Am. J. Pathol.* **188**, 2662–2673.

Deldicque L., Atherton P., Patel R., Theisen D., Nielens H., Rennie M. J. *et al*. 2008 Decrease in Akt/PKB signalling in human

skeletal muscle by resistance exercise. *Eur. J. Appl. Physiol.* **104**, 57–65.
Dent J. R., Hetrick B., Tahvilian S., Sathe A., Greyslak K., LaBarge S. A. *et al*. 2019 Skeletal muscle mitochondrial function and exercise capacity are not impaired in mice with knockout of STAT3. *J. Appl. Physiol.* **127**, 1117–1127.
Depre C., Vanoverschelde J.-L.J. and Taegtmeyer H. 1999 Glucose for the heart. *Circulation* **99**, 578–588.
Di Mauro S., Angelini C. and Catani C. 1967 Enzymes of the glycogen cycle and glycolysis in various human neuromuscular disorders. *J. Neurol. Neurosurg. Psychiat.* **30**, 411–415.
Dominy J. E. Jr., Lee Y., Gerhart-Hines Z. and Puigserver P. 2010 Nutrient-dependent regulation of PGC-1alpha's acetylation state and metabolic function through the enzymatic activities of Sirt1/GCN5. *Biochim. Biophys. Acta.* **1804**, 1676–1683.
Duan D., Goemans N., Takeda S., Mercuri E. and Aartsma-Rus A. 2021 Duchenne muscular dystrophy. *Nat. Rev. Dis. Primers.* **7**, 13.
Dumont N. A., Wang Y. X., Maltzahn J., Von Pasut A., Bentzinger C. F., Brun C. E. *et al*. 2015 Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. *Nat. Med.* **21**, 1455–1463.
Eichhorn P. J. A., Creyghton M. P. and Bernards R. 2009 Protein phosphatase 2A regulatory subunits and cancer. *Biochim. Biophys. Acta Rev. Cancer.* **1795**, 1–15.
Ellis D. A. 1980 Intermediary metabolism of muscle in Duchenne muscular dystrophy. *Br. Med. Bull.* **36**, 165–172.
Ervasti J. M. 2007 Dystrophin, its interactions with other proteins, and implications for muscular dystrophy. *Biochim. Biophys. Acta. Mol. Basis. Dis.* **1772**, 108–117.
Ervasti J. M., Ohlendieck K., Kahl S. D., Gaver M. G. and Campbell K. P. 1990 Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle. *Nature* **345**, 315–319.
Faist V., König J., Höger H. and Elmadfa I. 2001 Decreased mitochondrial oxygen consumption and antioxidant enzyme activities in skeletal muscle of dystrophic mice after low-intensity exercise. *Ann. Nutr. Metab.* **45**, 58–66.
Fan M., Rhee J., St-Pierre J., Handschin C., Puigserver P., Lin J. *et al*. 2004 Suppression of mitochondrial respiration through recruitment of p160 myb binding protein to PGC-1alpha: Modulation by p38 MAPK. *Genes Dev.* **18**, 278–289.
Feron M., Guevel L., Rouger K., Dubreil L., Arnaud M. C., Ledevin M. *et al*. 2009 PTEN contributes to profound PI3K/Akt signaling pathway deregulation in dystrophin-deficient dog muscle. *Am. J. Pathol.* **174**, 1459–1470.
Ford-Speelman D. L., Roche J. A., Bowman A. L. and Bloch R. J. 2009 The Rho-Guanine nucleotide exchange factor domain of Obscurin activates RhoA signaling in skeletal muscle. *Mol. Biol. Cell.* **20**, 3905–3917.
Frederick D. W., McDougal A. V., Semenas M., Vappiani J., Nuzzo A., Ulrich J. C. *et al*. 2020 Complementary NAD+ replacement strategies fail to functionally protect dystrophin-deficient muscle. *Skelet. Muscle.* **10**, 30.
Frucht H., Kaplansky M. and Beitner R. 1984 Increase in glucose 1,6-bisphosphate levels, activation of phosphofructokinase and phosphoglucomutase, and inhibition of glucose 1,6-bisphosphatase in muscle induced by trifluoperazine. *Biochem. Med.* **31**, 122–129.
Fulco M., Cen Y., Zhao P., Hoffman E. P., McBurney M. W., Sauve A. A. *et al*. 2008 Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Dev. Cell.* **14**, 661–673.
Galbiati F., Volonté D., Engelman J. A., Scherer P. E. and Lisanti M. P. 1999 Targeted down-regulation of Caveolin-3 is sufficient to inhibit myotube formation in differentiating C2C12 myoblasts: transient activation of p38 mitogen-activated protein kinase is required for induction of caveolin-3 expression and subsequent myotube formation. *J. Biol. Chem.* **274**, 30315–30321.
Garcia D. and Shaw R. J. 2017 AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance. *Mol. Cell.* **66**, 789–800.
Ge J., Cui H., Xie N., Banerjee S., Guo S., Dubey S. *et al*. 2018 Glutaminolysis promotes collagen translation and stability via a-ketoglutarate-mediated mTOR activation and proline hydroxylation. *Am. J. Respir. Cell Mol. Biol.* **58**, 378–390.
Gibbs E. M., Marshall J. L., Ma E., Nguyen T. M., Hong G., Lam J. S. *et al*. 2016 High levels of sarcospan are well tolerated and act as a sarcolemmal stabilizer to address skeletal muscle and pulmonary dysfunction in DMD. *Hum. Mol. Genet.* **25**, 5395–5406.
Gibbs E. M., McCourt J. L., Shin K. M., Hammond K. G., Marshall J. L. and Crosbie R. H. 2021 Loss of sarcospan exacerbates pathology in *mdx* mice, but does not affect utrophin amelioration of disease. *Hum. Mol. Genet.* **30**, 149–159.
Gupte S. A., Levine R. J., Gupte R. S., Young M. E., Lionetti V., Labinskyy V. *et al*. 2006 Glucose-6-phosphate dehydrogenase-derived NADPH fuels superoxide production in the failing heart. *J. Mol. Cell. Cardiol.* **41**, 340–349.
Hafner P., Bonati U., Klein A., Rubino D., Gocheva V., Schmidt S. *et al*. 2019 Effect of combination l-Citrulline and Metformin treatment on motor function in patients with Duchenne muscular dystrophy: A randomized clinical trial. *JAMA Netw. Open.* **2**, e1914171–e1914171.
Han S., Cui C., Zhao X., Zhang Y., Zhang Y., Zhao J. *et al*. 2021 Filamin C regulates skeletal muscle atrophy by stabilizing dishevelled-2 to inhibit autophagy and mitophagy. *Mol. Ther. Nucleic Acids* **27**, P147–P164.
Handschin C., Rhee J., Lin J., Tarr P. T. and Spiegelman B. M. 2003 An autoregulatory loop controls peroxisome proliferator-activated receptor γ coactivator 1α expression in muscle. *Proc. Natl. Acad. Sci. USA* **100**, 7111–7116.
Herbelet S., Rodenbach A., De Paepe B. and De Bleecker J. L. 2020 Anti-Inflammatory and general glucocorticoid physiology in skeletal muscles affected by Duchenne muscular dystrophy: exploration of steroid-sparing agents. *Int. J. Mol. Sci.* **21**, 4596.
Heydemann A. 2017 Severe murine limb-girdle muscular dystrophy type 2C pathology is diminished by FTY720 treatment. *Muscle Nerve* **56**, 486–494.
Hindi S. M., Sato S., Choi Y. and Kumar A. 2014 Distinct roles of TRAF6 at early and late stages of muscle pathology in the mdx model of Duchenne muscular dystrophy. *Hum. Mol. Genet.* **23**, 1492–1505.
Hodges B. L., Hayashi Y. K., Nonaka I., Wang W., Arahata K. and Kaufman S. J. 1997 Altered expression of the alpha7beta1 integrin in human and murine muscular dystrophies. *J. Cell Sci.* **110**, 2873–2881.
Hodson N., West D., Philp A., Burd N. A. and Moore D. R. 2019 Molecular regulation of human skeletal muscle protein synthesis in response to exercise and nutrients: a compass for overcoming age-related anabolic resistance. *Am. J. Physiol. Cell Physiol.* **317**, C1061–C1078.
Hoffman E. P., Brown R. H. and Kunkel L. M. 1987 Dystrophin: The protein product of the Duchenne muscular dystrophy locus. *Cell* **51**, 919–928.
Hoyte K., Jayasinha V., Xia B. and Martin P. T. 2004 Transgenic overexpression of Dystroglycan does not inhibit muscular dystrophy in *mdx* mice. *Am. J. Pathol.* **164**, 711–718.
Hughes M. C., Ramos S. V., Turnbull P. C., Rebalka I. A., Cao A., Monaco C. M. F. *et al*. 2019 Early myopathy in Duchenne muscular dystrophy is associated with elevated mitochondrial H_2O_2 emission during impaired oxidative phosphorylation. *J. Cachexia Sarcopenia Muscle* **10**, 643–661.

Hutter O. F. 1992 The membrane hypothesis of Duchenne muscular dystrophy: Quest for functional evidence. *J. Inherit. Metab. Dis.* **15**, 565–577.

Ieronimakis N., Pantoja M., Hays A. L., Dosey T. L., Qi J., Fischer K. A. *et al*. 2013 Increased sphingosine-1-phosphate improves muscle regeneration in acutely injured mdx mice. *Skelet. Muscle* **3**, 20.

Iwata Y., Katanosaka Y., Hisamitsu T. and Wakabayashi S. 2007 Enhanced Na+/H+ exchange activity contributes to the pathogenesis of muscular dystrophy via involvement of P2 receptors. *Am. J. Pathol.* **171**, 1576–1587.

Jato-Rodriguez J., Liang C. R., Lin C. H., Hudson A. J. and Strickland K. P. 1975 Comparison of the intermediary metabolism of fatty acids in denervated and dystrophic murine skeletal muscle. *J. Neurol. Neurosurg. Psychiatry* **38**, 1083–1089.

Johnson E. K., Li B., Yoon J. H., Flanigan K. M., Martin P. T. and Ervasti J. 2013 Identification of new dystroglycan complexes in skeletal muscle. *PLoS ONE* **8**, e73224.

Jones L., Naidoo M., Machado L. R. and Anthony K. 2021 The Duchenne muscular dystrophy gene and cancer. *Cell. Oncol.* **44**, 19–32.

Juan-Mateu J., Gonzalez-Quereda L., Rodriguez M. J., Baena M., Verdura E., Nascimento A. *et al*. 2015 DMD mutations in 576 dystrophinopathy families: A step forward in genotype-phenotype correlations. *PLoS One* **10**, e0135189.

Juretić N., Díaz J., Romero F., González G., Jaimovich E. and Riveros N. 2017 Interleukin-6 and neuregulin-1 as regulators of utrophin expression via the activation of NRG-1/ErbB signaling pathway in mdx cells. *Biochim. Biophys. Acta. Mol. Basis Dis.* **1863**, 770–780.

Katz A. 2022 A century of exercise physiology: key concepts in regulation of glycogen metabolism in skeletal muscle. *Eur. J. Appl. Physiol.* **122**, 1751–1772.

Kerr J. P., Robison P., Shi G., Bogush A. I., Kempema A. M., Hexum J. K. *et al*. 2015 Detyrosinated microtubules modulate mechanotransduction in heart and skeletal muscle. *Nat. Commun.* **6**, 8526.

Kielbasa O. M., Reynolds J. G., Wu C. L., Snyder C. M., Cho M. Y., Weiler H. *et al*. 2011 Myospryn is a calcineurin-interacting protein that negatively modulates slow-fiber-type transformation and skeletal muscle regeneration. *FASEB J.* **25**, 2276–2286.

Kistner T. M., Pedersen B. K. and Lieberman D. E. 2022 Interleukin 6 as an energy allocator in muscle tissue. *Nat. Metab.* **4**, 170–179.

Kjøbsted R., Hingst J. R., Fentz J., Foretz M., Sanz M. N., Pehmøller C. *et al*. 2018 AMPK in skeletal muscle function and metabolism. *FASEB J.* **32**, 1741–1777.

Koenig M., Hoffman E. P., Bertelson C. J., Monaco A. P., Feener C. and Kunkel L. M. 1987 Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. *Cell* **50**, 509–517.

Kuno A. and Horio Y. 2016 SIRT1: A novel target for the treatment of muscular dystrophies. *Oxid. Med. Cell. Longev.* **2016**, 6714686.

Kuznetsov A. V., Winkler K., Wiedemann F. R., Von Bossanyi P., Dietzmann K. and Kunz W. S. 1998 Impaired mitochondrial oxidative phosphorylation in skeletal muscle of the dystrophin-deficient mdx mouse. *Mol. Cell. Biochem.* **183**, 87–96.

Landi N., Nassi P., Liguri G., Bobbi S., Sbrilli C. and Marconi G. 1986 Sarcoplasmic reticulum Ca2+-ATPase and acylphosphatase activities in muscle biopsies from patients with Duchenne muscular dystrophy. *Clin. Chim. Acta.* **158**, 245–251.

Le S., Yu M., Hovan L., Zhao Z., Ervast J. and Yan J. 2018 Dystrophin as a molecular shock absorber. *ACS Nano* **12**, 12140–12148.

Lebrasseur N. K., Coté G. M., Miller T. A., Fielding R. A. and Sawyer D. B. 2003 Regulation of neuregulin/ErbB signaling by contractile activity in skeletal muscle. *Am. J. Physiol. Cell Physiol.* **284**, C1149–C1155.

Li C., Deng Z., Zheng G., Xie T., Wei X., Huo Z. *et al*. 2021 Histone deacetylase 2 suppresses skeletal muscle atrophy and senescence via NF-κB signaling pathway in cigarette smoke-induced mice with emphysema. *Int. J. Chron. Obstruct. Pulmon. Dis.* **16**, 1661–1675.

Li D., Long C., Yue Y. and Duan D. 2009 Sub-physiological sarcoglycan expression contributes to compensatory muscle protection in mdx mice. *Hum. Mol. Genet.* **18**, 1209–1220.

Liang R. C. R. 1986 Studies on mitochondria from dystrophic skeletal muscle of mice. *Biochem. Med. Metab. Biol.* **36**, 172–178.

Lindsay A., McCourt P. M., Karachunski P., Lowe D. A. and Ervasti J. M. 2018 Xanthine oxidase is hyper-active in Duchenne muscular dystrophy. *Free Radic. Biol. Med.* **129**, 364–371.

Liu J., Milner D. J., Boppart M. D., Ross R. S. and Kaufman S. J. 2012 β1D chain increases α7β1 integrin and laminin and protects against sarcolemmal damage in *mdx* mice. *Hum. Mol. Genet.* **21**, 1592–1603.

Liu X., Qu H., Zheng Y., Liao Q., Zhang L., Liao X. *et al*. 2018 Mitochondrial glycerol 3-phosphate dehydrogenase promotes skeletal muscle regeneration. *EMBO Mol. Med.* **10**, e9390.

Ljubicic V., Burt M., Lunde J. A. and Jasmin B. J. 2014 Resveratrol induces expression of the slow, oxidative phenotype in mdx mouse muscle together with enhanced activity of the SIRT1-PGC-1α axis. American journal of physiology. *Am. J. Physiol. Cell Physiol.* **307**, C66–C82.

Ljubicic V., Khogali S., Renaud J.-M. and Jasmin B. J. 2012 Chronic AMPK stimulation attenuates adaptive signaling in dystrophic skeletal muscle. *Am. J. Physiol. Cell Physio.* **302**, C110–C121.

Loehr J. A., Wang S., Cully T. R., Pal R., Larina I. V., Larin K. V. *et al*. 2018 NADPH oxidase mediates microtubule alterations and diaphragm dysfunction in dystrophic mice. *eLife* **7**, e31732.

Loh K. C., Leong W.-I., Carlson M. E., Oskouian B., Kumar A., Fyrst H. *et al*. 2012 Correction: Sphingosine-1-Phosphate enhances satellite cell activation in dystrophic muscles through a S1PR2/STAT3 signaling pathway. *PLoS ONE* **7**, 10.

Long C. Y., Widegren U. and Zierath J. R. 2004 Exercise-induced mitogen-activated protein kinase signalling in skeletal muscle. *Proc. Nutr. Soc.* **63**, 227–232.

Ludlow A. T., Gratidão L., Ludlow L. W., Spangenburg E. E. and Roth S. M. 2017 Acute exercise activates p38 MAPK and increases the expression of telomere-protective genes in cardiac muscle. *Exp. Physiol.* **102**, 397–410.

Lytridou A. A., Demetriadou A., Christou M., Potamiti L., Mastroyiannopoulos N. P., Kyriacou K. *et al*. 2020 Stbd1 promotes glycogen clustering during endoplasmic reticulum stress and supports survival of mouse myoblasts. *J. Cell Sci.* **133**, 244855.

Mancinelli E., Sardini A., D'Aumiller A., Meola G., Martucci G., Cossu G. *et al*. 1989 Properties of acetylcholine-receptor activation in human Duchenne muscular dystrophy myotubes. *Proc. Royal Soc. B.* **237**, 247–257.

Marden J. R., Freimark J., Yao Z., Signorovitch J., Tian C. and Wong B. L. 2020 Real-world outcomes of long-term prednisone and deflazacort use in patients with Duchenne muscular dystrophy: experience at a single, large care center. *J. Comp. Eff. Res.* **9**, 177–189.

Mareedu S., Million E. D., Duan D. and Babu G. J. 2021 Abnormal calcium handling in Duchenne muscular dystrophy: mechanisms and potential therapies. *Front. Physiol.* **12**, 647010.

Markham L. W., Brinkmeyer-Langford C. L., Soslow J. H., Gupte M., Sawyer D. B., Kornegay J. N. *et al*. 2017 GRMD cardiac and

skeletal muscle metabolism gene profiles are distinct. *BMC Med. Genom.* **10**, 14–16.
Marshall J. L., Holmberg J., Chou E., Ocampo A. C., Oh J., Lee J. *et al*. 2012 Sarcospan-dependent Akt activation is required for utrophin expression and muscle regeneration. *J. Cell Biol.* **197**, 1009–1027.
Martin D. B. and Vagelos P. R. 1962 Mechanism of tricarboxylic acid cycle regulation of fatty acid synthesis. *Biochem. Biophys. Res. Commun.* **7**, 101–106.
Matthews E., Brassington R., Kuntzer T., Jichi F. and Manzur A. Y. 2016 Corticosteroids for the treatment of Duchenne muscular dystrophy. *Cochrane Database Syst. Rev.* **2016**, CD003725.
Mázala D. A. G., Pratt S. J. P., Chen D., Molkentin J. D., Lovering R. M. and Chin E. R. 2015 SERCA1 overexpression minimizes skeletal muscle damage in dystrophic mouse models. *Am. J. Physiol. Cell Physiol.* **308**, C699–C709.
McGee S. L., Fairlie E., Garnham A. P. and Hargreaves M. 2009 Exercise-induced histone modifications in human skeletal muscle. *J. Physiol.* **587**, 5951–5958.
Meier J. A., Hyun M., Cantwell M., Raza A., Mertens C., Raje V. *et al*. 2017 Stress-induced dynamic regulation of mitochondrial STAT3 and its association with cyclophilin D reduce mitochondrial ROS production. *Sci. Signal.* **10**, 2aag2588.
Menard L., Maughan D. and Vigoreaux J. 2014 The structural and functional coordination of glycolytic enzymes in muscle: evidence of a metabolon? *Biology* **3**, 623–644.
Mirzoev T. M., Sharlo K. A. and Shenkman B. S. 2021 The role of gsk-3β in the regulation of protein turnover, myosin phenotype, and oxidative capacity in skeletal muscle under disuse conditions. *Int. J. Mol. Sci.* **22**, 5081.
Mokri B. and Engel A. G. 1975 Duchenne dystrophy: electron microscopic findings pointing to a basic or early abnormality in the plasma membrane of the muscle fiber. *Neurology* **25**, 1111–1120.
Moresi V., Carrer M., Grueter C. E., Rifki O. F., Shelton J. M., Richardson J. A. *et al*. 2012 Histone deacetylases 1 and 2 regulate autophagy flux and skeletal muscle homeostasis in mice. *Proc. Natl. Acad. Sci. USA* **109**, 1649–1654.
Mucha O., Podkalicka P., Kaziród K., Samborowska E., Dulak J. and Łoboda A. 2021 Simvastatin does not alleviate muscle pathology in a mouse model of Duchenne muscular dystrophy. *Skelet. Muscle.* **11**, 21.
Muntoni F., Wilson L., Marrosu G., Marrosu M. G., Cianchetti C., Mestroni L. *et al*. 1995 A mutation in the dystrophin gene selectively affecting dystrophin expression in the heart. *J. Clin. Invest.* **96**, 693–699.
Nakamura A. 2015 X-linked dilated cardiomyopathy: a cardiospecific phenotype of dystrophinopathy. *Pharmaceuticals* **8**, 303–320.
Nalbandian M., Radak Z. and Takeda M. 2019 N-acetyl-L-cysteine prevents lactate-mediated PGC1-alpha expression in C2C12 myotubes. *Biology* **8**, 8–15.
Nalbandian M., Radak Z. and Takeda M. 2020 Lactate metabolism and satellite cell fate. *Front. Physiol.* **11**, 610983.
Oddoux S., Randazzo D., Kenea A., Alonso B., Zaal K. J. M. and Ralston E. 2019 Misplaced Golgi elements produce randomly oriented Microtubules and aberrant cortical arrays of Microtubules in dystrophic skeletal muscle fibers. *Front. Cell Dev. Biol.* **7**, 176.
Ogata T., Naito D., Nakanishi N., Hayashi Y. K., Taniguchi T., Miyagawa K. *et al*. 2014 MURC/Cavin-4 facilitates recruitment of ERK to caveolae and concentric cardiac hypertrophy induced by α1-adrenergic receptors. *Proc. Natl. Acad. Sci. USA* **111**, 3811–3816.
Ogata T., Ueyama T., Isodono K., Tagawa M., Takehara N., Kawashima T. *et al*. 2008 MURC, a muscle-restricted coiled-coil protein that modulates the Rho/ROCK pathway, induces cardiac dysfunction and conduction disturbance. *Mol. Cell. Biol.* **28**, 3424–3436.
Olson E., Vignos P. J. Jr., Woodlook J. and Perry T. 1968 Oxidative phosphorylation of skeletal muscle in human muscular dystrophy. *J. Lab. Clin. Med.* **71**, 220–231.
O'Neil T. K., Duffy L. R., Frey J. W. and Hornberger T. A. 2009 The role of phosphoinositide 3-kinase and phosphatidic acid in the regulation of mammalian target of rapamycin following eccentric contractions. *J. Physiol.* **587**, 3691–3701.
Parvatiyar M. S., Brownstein A. J., Kanashiro-Takeuchi R. M., Collado J. R., Dieseldorff Jones K., M., Gopal J. *et al*. 2019 Stabilization of the cardiac sarcolemma by sarcospan rescues DMD-associated cardiomyopathy. *JCI Insight.* **5**, e123855.
Pauly M., Daussin F., Burelle Y., Li T., Godin R., Fauconnier J. *et al*. 2012 AMPK activation stimulates autophagy and ameliorates Muscular dystrophy in the mdx mouse diaphragm. *Am. J. Pathol.* **181**, 583–592.
Pedersen B. K. and Febbraio M. A. 2008 Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol. Rev.* **88**, 1379–1406.
Peter A. K. and Crosbie R. H. 2006 Hypertrophic response of Duchenne and Limb-girdle muscular dystrophies is associated with activation of Akt pathway. *Exp. Cell Res.* **312**, 2580–2591.
Peter A. K., Cheng H., Ross R. S., Knowlton K. U. and Chen J. 2011 The costamere bridges sarcomeres to the sarcolemma in striated muscle. *Prog. Pediatr. Cardiol.* **31**, 83–88.
Pitson S. M., Moretti P. A., Zebol J. R., Lynn H. E., Xia P., Vadas M. A. *et al*. 2003 Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. *EMBO J.* **22**, 5491–5500.
Powers S. K., Duarte J., Kavazis A. N. and Talbert E. E. 2010 Reactive oxygen species are signalling molecules for skeletal muscle adaptation. *Exp. Physiol.* **95**, 1–9.
Pradhan B. S. and Prószyński T. J. 2020 A role for Caveolin-3 in the pathogenesis of muscular dystrophies. *Int. J. Mol. Sci.* **21**, 8736.
Previtali S. C., Gidaro T., Díaz-Manera J., Zambon A., Carnesecchi S., Roux-Lombard P. *et al*. 2020 Rimeporide as a first- in-class NHE-1 inhibitor: Results of a phase Ib trial in young patients with Duchenne muscular dystrophy. *Pharmacol. Res.* **159**, 104999.
Prosser B. L., Khairallah R. J., Ziman A. P., Ward C. W. and Lederer W. J. 2013 X-ROS signaling in the heart and skeletal muscle: stretch-dependent local ROS regulates [Ca2+]i. *J. Mol. Cell. Cardiol.* **58**, 172–181.
Pulido S. M., Passaquin A. C., Leijendekker W. J., Challet C., Wallimann T. and Ruegg U. T. 1998 Creatine supplementation improves intracellular Ca^{2+} handling and survival in *mdx* skeletal muscle cells. *FEBS Lett.* **439**, 357–362.
Puttini S., Lekka M., Dorchies O. M., Saugy D., Incitti T., Ruegg U. T. *et al*. 2009 Gene-mediated restoration of normal myofiber elasticity in dystrophic muscles. *Mol. Ther.* **17**, 19–25.
Ramaswamy K. S., Palmer M. L., van der Meulen J. H., Renoux A., Kostrominova T. Y., Michele D. E. *et al*. 2011 Lateral transmission of force is impaired in skeletal muscles of dystrophic mice and very old rats. *J. Physiol.* **589**, 1195–1208.
Ramos S. V., Hughes M. C., Delfinis L. J., Bellissimo C. A. and Perry C. G. R. 2020 Mitochondrial bioenergetic dysfunction in the D2.mdx model of Duchenne muscular dystrophy is associated with microtubule disorganization in skeletal muscle. *PLoS ONE* **15**, e0237138.
Ran N., Lin C., Leng L., Han G., Geng M., Wu Y. *et al*. 2021 MOTS-c promotes phosphorodiamidate morpholino oligomer uptake and efficacy in dystrophic mice. *EMBO Mol. Med.* **13**, e12993.
Rando T. A. 2002 Oxidative stress and the pathogenesis of muscular dystrophies. *Am. J. Phys. Med. Rehabil.* **81**, S175–S186.

Rennie M. J., Bohé J., Smith K., Wackerhage H. and Greenhaff P. 2006 Branched-chain amino acids as fuels and anabolic signals in human muscle. *J. Nutr.* **136**, 264S-S268.

Repetto S., Bado M., Broda P., Lucania G., Masetti E., Sotgia F. *et al*. 1999 Increased number of Caveolae and Caveolin-3 overexpression in Duchenne muscular dystrophy. *Biochem. Biophys. Res. Commun.* **261**, 547–550.

Reynolds J. G., McCalmon S. A., Donaghey J. A. and Naya F. J. 2008 Deregulated protein kinase A signaling and Myospryn expression in muscular dystrophy. *J. Biol. Chem.* **283**, 8070–8074.

Rossi R., Falzarano M. S., Osman H., Armaroli A., Scotton C., Mantuano P. *et al*. 2021 Circadian genes as exploratory biomarkers in DMD: results from both the mdx mouse model and patients. *Front. Physiol.* **12**, 1031.

Roy J., Galano J.-M., Durand T., Le Guennec J.-Y. and Chung-Yung Lee J. 2017 Physiological role of reactive oxygen species as promoters of natural defenses. *FASEB J.* **31**, 3729–3745.

Rutter M. M., Wong B. L., Collins J. J., Sawnani H., Taylor M. D., Horn P. S. *et al*. 2020 Recombinant human insulin-like growth factor-1 therapy for 6 months improves growth but not motor function in boys with Duchenne muscular dystrophy. *Muscle Nerve* **61**, 623–631.

Ryall J. G., Dell'Orso S., Derfoul A., Juan A., Zare H., Feng X. *et al*. 2015 The NAD(+)-dependent SIRT1 deacetylase translates a metabolic switch into regulatory epigenetics in skeletal muscle stem cells. *Cell Stem Cell.* **16**, 171–183.

Rybalka E., Timpani C. A., Cooke M. B., Williams A. D. and Hayes A. 2014 Defects in mitochondrial ATP synthesis in dystrophin-deficient mdx skeletal muscles may be caused by complex I insufficiency. *PLoS ONE* **9**, e115763.

Saclier M., Bonfanti C., Antonini S., Angelini G., Mura G., Zanaglio F. *et al*. 2020 Nutritional intervention with cyanidin hinders the progression of muscular dystrophy. *Cell Death Dis.* **11**, 127.

Saddoughi S. A., Gencer S., Peterson Y. K., Ward K. E., Mukhopadhyay A., Oaks J. *et al*. 2013 Sphingosine analogue drug FTY720 targets I2PP2A/SET and mediates lung tumour suppression via activation of PP2A-RIPK1-dependent necroptosis. *EMBO Mol. Med.* **5**, 105–121.

Sanson M., Vu Hong A., Massourides E., Bourg N., Suel L., Amor F. *et al*. 2020 miR-379 links glucocorticoid treatment with mitochondrial response in Duchenne muscular dystrophy. *Sci Rep* **10**, 9139.

Sanyal C., Pietsch N., Ramirez Rios S., Peris L., Carrier L. and Moutin M.-J. 2021 The detyrosination/re-tyrosination cycle of tubulin and its role and dysfunction in neurons and cardiomyocytes. *Semin. Cell Dev. Biol.*, https://doi.org/10.1016/j.semcdb.2021.12.006.

Schiaffino S. and Mammucari C. 2011 Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. *Skelet. Muscle* **1**, 4.

Schneider J. S., Shanmugam M., Gonzalez J. P., Lopez H., Gordan R., Fraidenraich D. *et al*. 2013 Increased sarcolipin expression and decreased sarco(endo)plasmic reticulum Ca^{2+} uptake in skeletal muscles of mouse models of Duchenne muscular dystrophy. *J. Muscle Res. Cell Motil.* **34**, 349–356.

Schram G., Fournier A., Leduc H., Dahdah N., Therien J., Vanasse M. *et al*. 2013 All-cause mortality and cardiovascular outcomes with prophylactic steroid therapy in Duchenne muscular dystrophy. *J. Am. Coll. Cardiol.* **61**, 948–954.

Sharma U., Atri S., Sharma M. C., Sarkar C. and Jagannathan N. R. 2003 Skeletal muscle metabolism in Duchenne muscular dystrophy (DMD): an in-vitro proton NMR spectroscopy study. *Magn. Reson. Imaging* **21**, 145–153.

Simmonds A. I. M. and Seebacher F. 2017 Histone deacetylase activity modulates exercise-induced skeletal muscle plasticity in zebrafish (Danio rerio). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **313**, R35–R43.

Srivastava N. K., Yadav R., Mukherjee S. and Sinha N. 2018 Perturbation of muscle metabolism in patients with muscular dystrophy in early or acute phase of disease: In vitro, high resolution NMR spectroscopy based analysis. *Clin. Chim. Acta.* **478**, 171–181.

Srivastava N. K., Mukherjee S. and Mishra V. N. 2020 Metabolic disturbance in patients with muscular dystrophy and reflection of altered enzyme activity in dystrophic muscle: one critical view. *J. Biomed. Res. Environ. Sci.* **1**, 393–403.

Stefani M., Taddei N. and Ramponi G. 1997 Insights into acylphosphatase structure and catalytic mechanism. *Cell. Mol. Life Sci.* **53**, 141–151.

Stupka N., Plant D. R., Schertzer J. D., Emerson T. M., Bassel-Duby R., Olson E. N. *et al*. 2006 Activated calcineurin ameliorates contraction-induced injury to skeletal muscles of mdx dystrophic mice. *J. Physiol.* **575**, 645–656.

Sun S., Li H., Chen J. and Qian Q. 2017 Lactic acid: no longer an inert and end-product of glycolysis. *Physiology* **32**, 453–463.

Thakur S. S., Swiderski K., Ryall J. G. and Lynch G. S. 2018 Therapeutic potential of heat shock protein induction for muscular dystrophy and other muscle wasting conditions. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **373**, 20160528.

Tian H., Liu S., Ren J., Lee J. K. W., Wang R. and Chen P. 2020 Role of histone deacetylases in skeletal muscle physiology and systemic energy homeostasis: implications for metabolic diseases and therapy. *Front. Physiol.* **11**, 949.

Timpani C. A., Hayes A. and Rybalka E. 2015 Revisiting the dystrophin-ATP connection: How half a century of research still implicates mitochondrial dysfunction in Duchenne Muscular Dystrophy aetiology. *Med. Hypotheses* **85**, 1021–1033.

Timpani C. A., Mamchaoui K., Butler-Browne G. and Rybalka E. 2020 Nitric oxide (NO) and Duchenne muscular dystrophy: NO way to go? *Antioxidants* **9**, 1268.

Touboul D., Brunelle A., Halgand F., De La Porte S. and Laprévote O. 2005 Lipid imaging by gold cluster time-of-flight secondary ion mass spectrometry: application to Duchenne muscular dystrophy. *J. Lipid Res.* **46**, 1388–1395.

Trinh B., Peletier M., Simonsen C., Plomgaard P., Karstoft K., Klarlund P. B. *et al*. 2021 Blocking endogenous IL-6 impairs mobilization of free fatty acids during rest and exercise in lean and obese men. *Cell Rep. Med.* **2**, 100396.

Uchimura T., Asano T., Nakata T., Hotta A. and Sakurai H. 2021 A muscle fatigue-like contractile decline was recapitulated using skeletal myotubes from Duchenne muscular dystrophy patient-derived iPSCs. *Cell Rep. Med.* **2**, 100298.

Vaishnavi S. N., Vlassenko A. G., Rundle M. M., Snyder A. Z., Mintun M. A. and Raichle M. E. 2010 Regional aerobic glycolysis in the human brain. *Proc. Natl. Acad. Sci. USA* **107**, 17757–17762.

van Westering T., Johansson H. J., Hanson B., Coenen-Stass A., Lomonosova Y., Tanihata J. *et al*. 2020 Mutation-independent proteomic signatures of pathological progression in murine models of Duchenne muscular dystrophy. *Mol. Cell Proteom.* **19**, 2047–2068.

Vandenburgh H. H., Shansky J., Karlisch P. and Solerssi R. L. 1993 Mechanical stimulation of skeletal muscle generates lipid-related second messengers by phospholipase activation. *J. Cell. Physiol.* **155**, 63–71.

Vandoolaeghe P., Gueuning M.-A. and Rousseau G. G. 1999 M-type 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase is the product of a late muscle differentiation gene. *Biochem. Biophys. Res. Commun.* **259**, 250–254.

Verhaart I., Cappellari O., Tanganyika-de Winter C., L., Plomp J. J., Nnorom S., Wells K. E. *et al*. 2021 Simvastatin treatment does

not ameliorate muscle pathophysiology in a mouse model for Duchenne muscular dystrophy. *J. Neuromuscul. Dis.* **8**, 845–863.
Vieira N. M., Elvers I., Alexander M. S., Moreira Y. B., Eran A., Gomes J. P. *et al*. 2015 Jagged 1 rescues the Duchenne muscular dystrophy phenotype. *Cell* **163**, 1204–1213.
Vieira N. M., Spinazzola J. M., Alexander M. S., Moreira Y. B., Kawahara G., Gibbs D. E. *et al*. 2017 Repression of phosphatidylinositol transfer protein α ameliorates the pathology of Duchenne muscular dystrophy. *Proc. Natl. Acad. Sci. USA* **114**, 6080–6085.
Vila M. C., Rayavarapu S., Hogarth M. W., Van der Meulen J. H., Horn A., Defour A. *et al*. 2017 Mitochondria mediate cell membrane repair and contribute to Duchenne muscular dystrophy. *Cell Death Differ.* **24**, 330–342.
Villa-Moruzzi E., Puntoni F. and Marin O. 1996 Activation of protein phosphatase-1 isoforms and glycogen synthase kinase-3β in muscle from mdx mice. *Int. J. Biochem.* **28**, 13–22.
Voit A., Patel V., Pachon R., Shah V., Bakhutma M., Kohlbrenner E. *et al*. 2017 Reducing sarcolipin expression mitigates Duchenne muscular dystrophy and associated cardiomyopathy in mice. *Nat. Commun.* **8**, 1068.
Wada E., Tanihata J., Iwamura A., Takeda S., Hayashi Y. K. and Matsuda R. 2017 Treatment with the anti-IL-6 receptor antibody attenuates muscular dystrophy via promoting skeletal muscle regeneration in dystrophin-/utrophin-deficient mice. *Skelet. Muscle* **7**, 23.
Wakayama Y., Inoue M., Kojima H., Murahashi M., Shibuya S., Yamashita S. *et al*. 2000 Aciculin and its relation to dystrophin: immunocytochemical studies in human normal and Duchenne dystrophy quadriceps muscles. *Acta Neuropathol.* **99**, 654–662.
Wang H., Marrosu E., Brayson D., Wasala N. B., Johnson E. K., Scott C. S. *et al*. 2021 Proteomic analysis identifies key differences in the cardiac interactomes of dystrophin and micro-dystrophin. *Hum. Mol. Genet.* **30**, 1321–1336.
Weigert C., Düfer M., Simon P., Debre E., Runge H., Brodbeck K. *et al*. 2007 Upregulation of IL-6 mRNA by IL-6 in skeletal muscle cells: role of IL-6 mRNA stabilization and Ca^{2+}-dependent mechanisms. *Am. J. Physiol. Cell Physiol.* **293**, C1139–C1147.
Whitehead N. P., Yeung E. W., Froehner S. C. and Allen D. G. 2010 Skeletal muscle NADPH oxidase is increased and triggers stretch-induced damage in the mdx mouse. *PLoS One* **5**, e15354.
Wilkinson I. V. L., Perkins K. J., Dugdale H., Moir L., Vuorinen A., Chatzopoulou M. *et al*. 2020 Chemical proteomics and phenotypic profiling identifies the Aryl hydrocarbon receptor as a molecular target of the Utrophin modulator Ezutromid. *Angew. Chem. Int. Ed. Engl.* **59**, 2420–2428.
Wissing E. R., Boyer J. G., Kwong J. Q., Sargent M. A., Karch J., McNally E. M. *et al*. 2014 P38α MAPK underlies muscular dystrophy and myofiber death through a Bax-dependent mechanism. *Hum. Mol. Genet.* **23**, 5452–5463.
Wittlieb-Weber C. A., Knecht K. R., Villa C. R., Cunningham C., Conway J., Bock M. J. *et al*. 2020 Risk factors for cardiac and non-cardiac causes of death in males with Duchenne muscular dystrophy. *Pediatr. Cardiol.* **41**, 764–771.
Wojtaszewski J. F. P., Nielsen P., Kiens B. and Richter E. A. 2001 Regulation of glycogen synthase Kinase-3 in human skeletal muscle: effects of food intake and bicycle exercise. *Diabetes* **50**, 265–269.
Wu W., Feng J., Jiang D., Zhou X., Jiang Q., Cai M. *et al*. 2017 AMPK regulates lipid accumulation in skeletal muscle cells through FTO-dependent demethylation of N6-methyladenosine. *Sci. Rep.* **7**, 41606.
Wuebbles R. D., Sarathy A., Kornegay J. N. and Burkin D. J. 2013 Levels of α7 integrin and laminin-α2 are increased following prednisone treatment in the mouse and GRMD dog models of Duchenne muscular dystrophy. *Dis. Model Mech.* **6**, 1175–1184.
Xia Q., Huang X., Huang J., Zheng Y., March M. E., Li J. *et al*. 2021 The role of autophagy in skeletal muscle diseases. *Front. Physiol.* **12**, 291.
Xia R., Webb J. A., Gnall L. L., Cutler K. and Abramson J. J. 2003 Skeletal muscle sarcoplasmic reticulum contains a NADH-dependent oxidase that generates superoxide. *Am. J. Physiol. Cell Physiol.* **285**, C215–C221.
Xu W. J., Wen H., Kim H. S., Ko Y. J., Dong S. M., Park I. S. *et al*. 2018 Observation of acetyl phosphate formation in mammalian mitochondria using real-time in-organelle NMR metabolomics. *Proc. Natl. Acad. Sci. USA* **115**, 4152–4157.
Xu Y. S., Liang J. J., Wang Y., Zhao X. J., Xu L., Xu Y. *et al*. 2016 STAT3 undergoes acetylation-dependent mitochondrial translocation to regulate pyruvate Metabolism. *Sci. Rep.* **6**, 39517.
Yazid M. D. and Hung-Chih C. 2021 Perturbation of PI3K/Akt signaling affected autophagy modulation in dystrophin-deficient myoblasts. *Cell Commun. Signal.* **19**, 105.
Yoon S., Kook T. and Min H. K. 2018 PP2A negatively regulates the hypertrophic response by dephosphorylating HDAC2 S394 in the heart. *Exp. Mol. Med.* **50**, 1–14.
Yu H., Shao Y., Gao L., Zhang L., Guo K., Wu C. *et al*. 2012 Acetylation of sphingosine kinase 1 regulates cell growth and cell-cycle progression. *Biochem. Biophys. Res. Commun.* **417**, 1242–1247.
Yue F., Song C., Huang D., Narayanan N., Qiu J., Jia Z. *et al*. 2021 PTEN inhibition ameliorates muscle degeneration and improves muscle function in a mouse model of Duchenne muscular dystrophy. *Mol. Ther.* **29**, 132–148.
Zhang D., Tang Z., Huang H., Zhou G., Cui C., Weng Y. *et al*. 2019 Metabolic regulation of gene expression by histone lactylation. *Nature* **574**, 575–580.
Zhang L., Pan J., Dong Y., Tweardy D. J., Dong Y., Garibotto G. *et al*. 2013 Stat3 activation links a C/EBPδ to myostatin pathway to stimulate loss of muscle mass. *Cell Metab.* **18**, 368–379.
Zhu X., Hadhazy M., Groh M. E., Wheeler M. T., Wollmann R. and McNally E. M. 2001 Overexpression of γ-sarcoglycan induces severe muscular dystrophy: implications for the regulation of sarcoglycan assembly. *J. Biol. Chem.* **276**, 21785–21790.

Corresponding editor: Durgadas P. Kasbekar