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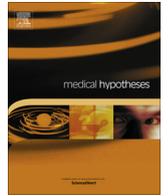
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# Revisiting the dystrophin-ATP connection: How half a century of research still implicates mitochondrial dysfunction in Duchenne Muscular Dystrophy aetiology



Cara A. Timpani<sup>a</sup>, Alan Hayes<sup>a,b,c</sup>, Emma Rybalka<sup>a,b,c,\*</sup>

<sup>a</sup> Centre for Chronic Disease, College of Health and Biomedicine, Victoria University, Melbourne, Australia

<sup>b</sup> Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Melbourne, Australia

<sup>c</sup> Australian Institute of Musculoskeletal Science, Western Health, Victoria, Australia

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## ABSTRACT

Duchenne Muscular Dystrophy (DMD) is a fatal neuromuscular disease that is characterised by dystrophin-deficiency and chronic Ca<sup>2+</sup>-induced skeletal muscle wasting, which currently has no cure. DMD was once considered predominantly as a metabolic disease due to the myriad of metabolic insufficiencies evident in the musculature, however this aspect of the disease has been extensively ignored since the discovery of dystrophin. The collective historical and contemporary literature documenting these metabolic nuances has culminated in a series of studies that importantly demonstrate that metabolic dysfunction exists independent of dystrophin expression and a mild disease phenotype can be expressed even in the complete absence of dystrophin expression. Targeting and supporting metabolic pathways with anaplerotic and other energy-enhancing supplements has also shown therapeutic value. We explore the hypothesis that DMD is characterised by a systemic mitochondrial impairment that is central to disease aetiology rather than a secondary pathophysiological consequence of dystrophin-deficiency.

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## Introduction

Duchenne Muscular Dystrophy (DMD) is the most prevalent muscular dystrophy afflicting ~1 in 3500–5000 live born males [1,2]. Regarded as a debilitating and fatal skeletal muscle disease, it is characterised by muscular weakness, exercise intolerance and progressive deterioration of skeletal muscle. Sufferers are generally confined to a wheelchair by 12 years of age with cardiorespiratory failure ultimately ensuing by the third decade of life [3,4]. 100 years after DMD was first described [5], the cause was identified as a gene mutation on the short arm of the X-chromosome [6]. The product of this mutation is the ablation of dystrophin, a 427 kDa rod-shaped [7] protein usually associated with the sarcolemma of muscle fibres via a complex of glycoproteins. The presence of dystrophin and associated glycoproteins provides integrity and rigidity to the fibre, however dystrophin-

deficiency and the secondary reduction of these glycoproteins [8] renders the fibres more susceptible to damage as they become structurally unstable and exceedingly porous to the extracellular environment. As a result, excessive calcium (Ca<sup>2+</sup>) influx, poor Ca<sup>2+</sup> handling, activation of proteases/lipases and mitochondrial Ca<sup>2+</sup> overload precede muscle degeneration. Over time, and as regeneration fails, fatty and connective tissue replacement culminates in non-functional muscle tissue.

In a bid to cure this progressive and fatal muscle wasting, the majority of research since 1987 has focused on genetically manipulating the disease by reintroducing the dystrophin gene (or a miniature version) back into the genome [9–11] and pharmacological intervention [12,13]. While some success has been observed with exon skipping and termination codon read-through trials (as reviewed in [14]), many complications of genetic therapy, including immunological reaction to delivery vectors, affordability and suitability [15] have been reported. As yet, there is no cure. Currently, corticosteroid treatment is used to delay muscular weakness and prolong function but has reported side effects including cardiomyopathy, weight gain, cataracts, hypertension, cushingoid features and osteoporosis [16].

\* Corresponding author at: Western Centre for Health, Research & Education, Sunshine Hospital, PO Box 294, Furlong Road, St Albans, Victoria 3021, Australia. Tel.: +61 3 8395 8226.

E-mail address: [emma.rybalka@vu.edu.au](mailto:emma.rybalka@vu.edu.au) (E. Rybalka).

Prior to the discovery of dystrophin (and by several research groups afterwards), DMD was considered to be a disease of metabolic origin, with a strong body of literature demonstrating deficiency of key metabolic systems and regulators, including the mitochondria. As mitochondria constitute the ubiquitous adenosine triphosphate (ATP)-producing machinery of the cell and consequently play a crucial role in signalling cell death, their dysfunction seemingly induces a myriad of physiological events that underscore, or at least exacerbate, dystrophinopathy. Deficits encompassing the cytosolic enzymes of glycolysis [17–21] and the purine nucleotide cycle (PNC) [22,23], and the mitochondrial enzymes of the Tricarboxylic Acid (TCA) cycle [21,24] and Electron Transport Chain (ETC) [25–27] have been consistently reported in DMD sufferers, female carriers and animal models of the disease. Severely reduced ATP content [28–32] is the downstream consequence of these deficits and has been observed in skeletal muscle from both DMD patients and animal models. Dysregulation of cellular energy homeostasis has a variety of consequences for muscle including (1) impaired contractile apparatus function leading to reduced strength, ambulatory capacity and exercise tolerance; (2) impaired intracellular  $\text{Ca}^{2+}$  buffering leading to loss of homeostasis and  $\text{Ca}^{2+}$ -induced degeneration; (3) reduced protein synthesis; and (4) reduced satellite cell activation, replication, migration and differentiation leading to a markedly decreased capacity for regeneration of damaged muscle fibres. The mitochondria are also explicitly involved in maintaining, and are strongly regulated by, cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]$ ). Heightened mitochondrial  $[\text{Ca}^{2+}]$  increases ATP synthesis until overload induces permeability transition, collapses the mitochondrial membrane potential and signals apoptotic cellular death pathways. Thus an important question that could be asked is: What comes first, the chicken ( $\text{Ca}^{2+}$ -induced pathophysiology) or the egg (mitochondrial dysfunction)? That impaired metabolism has been observed in *mdx* myoblasts independent of dystrophin-deficiency (dystrophin is not phenotypically expressed until myoblastic fusion into myotubes) [33], indeed suggests an intrinsic metabolic deficiency. Metabolic impairment is also evident in a variety of tissues and cells from DMD patients and animal models that express a different dystrophin isoform – these include liver [34,35], heart [36,37] and brain [38–42]. Collectively, the literature strongly suggests that DMD is characterised by a systemic metabolic impairment, which is central to the aetiology of the disease and not secondary to the pathophysiology as currently accepted.

In 1992, and following 30 years of clinical research, Bonsett and Rudman [43] published a timely article in *Medical Hypotheses* that offered compelling evidence to highlight that DMD is predominantly underscored by metabolic impairment at the mitochondrial level, and that this can be anaplerotically “corrected” using high dose adenylosuccinic acid (ASA) treatment. Since this publication, and despite mounting literature indicating the same perturbations in animal models of DMD, metabolic therapy – with the exception of dietary creatine monohydrate supplementation – is still not a mainstay of DMD treatment. We suggest that re-defining DMD as a metabolic myopathy and strategically treating it as such, could improve patient outcomes and quality of life.

## Hypothesis

Our hypothesis challenges the currently accepted pathophysiological paradigm describing DMD aetiology, which pinpoints dystrophin-deficiency-induced  $\text{Ca}^{2+}$  homeostasis de-regulation as the primary defect. We hypothesise that DMD is primarily a mitochondrial myopathy, in which the inability to generate sufficient quantities of ATP to fuel  $\text{Ca}^{2+}$  buffering from myofibres, induces the pathophysiological cascade of events leading to muscle wasting and fatty and connective tissue infiltration.

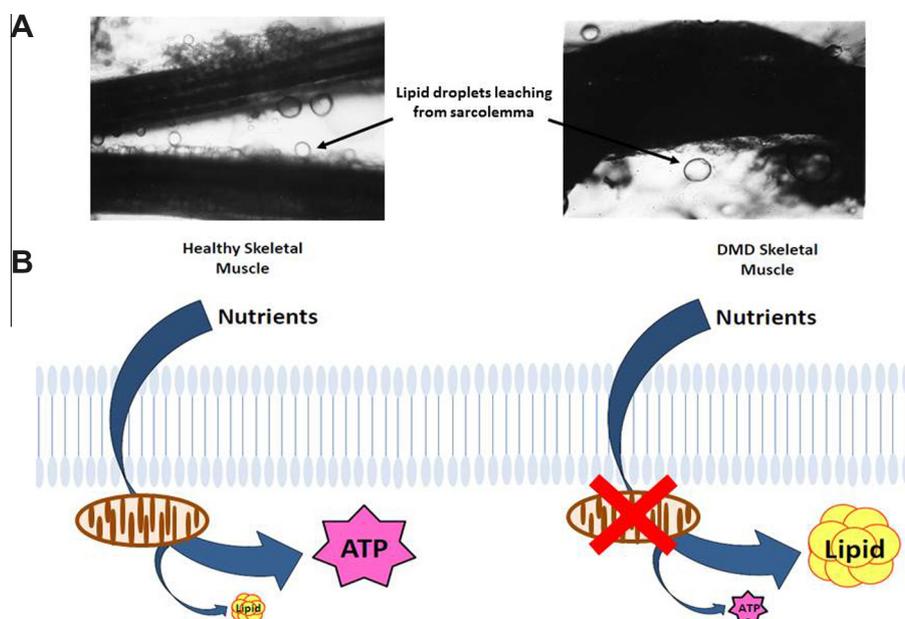
## Arguments to support the hypothesis

### *A plethora of metabolic deficiencies in dystrophin-deficient muscle*

The earliest literature of Meryon [44] and Duchenne [45] – who are renowned for reporting the first cases of DMD and the collective pathological manifestations of the disease, respectively – described the gross anatomical observations of DMD skeletal muscle fibres. A prominent feature of these fibres was intrafibrillar lipid accumulation. In whole fibre preparations, lipids are present extensively within the sarcoplasm and attached to the sarcolemma, and leach into the extracellular fluid from damaged fibres (Fig. 1A) [46,47]. This feature has also been reported in histological preparations using fat-specific stains [48,49]. Intracellular lipid droplets are a normal feature of healthy skeletal muscle, albeit in lesser abundance, in which they are located proximal to the sarcoplasmic reticulum and mitochondria to act as energy reservoirs [50]. As skeletal muscle has a high affinity for fatty acid oxidation as ATP demand increases, these reservoirs act as important regulators of cellular energy homeostasis during metabolic stress. The early work of Charles Bonsett’s laboratory on cultured human myocytes highlights an equivalent propensity for healthy and DMD cells to produce intracellular lipid droplets when supplemented with nutrient dense 20% foetal bovine serum (FBS) superfluous to cellular nutrient demand i.e. when nutrients are supplied and uptaken in excess of cellular requirements, intrafibrillar lipid accumulation is a natural consequence [46]. While reducing the FBS concentration induced concurrent reductions in lipid accumulation in the healthy myocytes until lipid accumulation was absent, DMD myocytes continued to produce lipid droplets irrespective of serum concentration [46] highlighting a reduced capacity for metabolism that culminates in enhanced production of lipids at the cellular level (Fig. 1B). [24]. Intramyofibrillar lipid accumulation is also characteristic of obese, type 2 diabetic patients and aged skeletal muscle [51–55] indicating comparable metabolic dysfunction amongst these disease states. In a subsequent study, the same group provided evidence indicating this phenomenon was due to isocitrate dehydrogenase (IDH) dysfunction/deficiency as the addition of isocitrate to DMD cells induced significant lipid formation [24].

In dystrophin-deficient skeletal muscle from human DMD patients and animal models, however, metabolic dysfunction is not limited to IDH, but is widespread across multiple metabolic pathways [56] and culminates in resting ATP levels that are at least 50% of healthy control levels [28–32]. In intensely exercised, healthy skeletal muscle, physiological fatigue mechanisms ensure that ATP demand does not exceed production capacity – a ~40% drop in resting ATP levels appears to be the critical maintenance threshold such to trigger these mechanisms and reduce demand on the metabolic system (as reviewed in [57]). Thus, compared to healthy skeletal muscle, resting dystrophin-deficient muscle consistently maintains sub-threshold ATP levels which are likely incompatible with long-term cell survival. Taken in context of Bonsett’s research, it is not that dystrophin-deficient muscle has a lesser requirement for ATP synthesis, in fact the exact opposite is true. Intrafibrillar lipid production and accumulation even in the presence of minute nutrient provision seems ostensibly linked to an intrinsic metabolic defect that limits the conversion of nutrients into cellular energy (ATP).

A plethora of isolated deficits in the cellular energy system have been reported in dystrophin-deficient skeletal muscle from human patients and animal models, which would both individually and collectively contribute to this failure of energy homeostasis (summarised in Table 1). Due to the strong and multifaceted allosteric regulation of metabolism by associated up- and down-stream products and reactants, one broken link in the metabolic chain would induce deleterious consequences at multiple levels



**Fig. 1.** Accumulation of intramyofibrillar lipids is a feature of dystrophin-deficient skeletal muscle and a hypothetical consequence of mitochondrial dysfunction. Lipid droplets are evident in the sarcoplasm, the sarcolemma and leaching into the extracellular fluid of isolated dystrophin deficient myofibres (Photographs courtesy of Bonsett [182]; reproduced with the permission of C.C. Thomas Publisher Ltd). In a hypothetical model to explain this phenomenon (B), nutrients are typically oxidised by the mitochondria to synthesise ATP in healthy skeletal muscle (left) with minimal directed to intracellular lipid production. In dystrophic muscle (right), the capacity to utilise nutrients for ATP synthesis is significantly impaired which coincides with an increased propensity to produce intracellular lipid (adapted from Bonsett, unpublished). We hypothesise that this feature is a consequence of mitochondrial dysfunction that is independent of the absence of dystrophin protein.

**Table 1**  
Summary of the metabolic deficits in the metabolic pathways of dystrophic skeletal muscle.

	Defect description	DMD model	References
Macronutrient uptake and availability	Normal glucose uptake but ↓ glucose content ↓ GLUT4 mRNA and protein expression in aged diaphragm ↓ Gluconeogenic precursors (alanine and glutamine) ↑ Fructose content	Human DMD and <i>mdx</i> mouse <i>mdx</i> mouse Human DMD Human DMD	[183–185] [75] [183,185,186] [187]
Glycolysis	↓ Glucose-6-phosphate ↓ Phosphofructokinase activity, ↓ sensitivity to allosteric regulation ↓ Aldolase ↓ Pyruvate kinase activity ↓ Lactate dehydrogenase ↓ Lactate production and acidification	Human DMD Human DMD and <i>mdx</i> mouse  Human DMD Human DMD Human DMD Human DMD and <i>mdx</i> mouse	[19] [18–20,78,79]  [17,19,188] [20,189] [18,19,103] [31,183,190]
Glycogen storage and utilisation	↑ Glycogen content ↓ Phosphorylase activity ↓ Phosphoglucomutase activity	Human DMD and <i>mdx</i> mouse Human DMD and <i>mdx</i> mouse Human DMD and <i>mdx</i> mouse	[121,124,191] [17–20,99,101,190–195] [20,102]
Fat oxidation	↓ Palmitate oxidation  ↓ Palmitoylcarnitine and malate oxidation ↓ Total carnitine ↓ Fatty acid transport into mitochondria	Human DMD patients and carriers Human DMD Human DMD Human DMD	[95,196,197]  [95,183,198–202] [95,183,199–202] [202]
Creatine phosphagen system	↓ PCr concentration  ↓ Cr concentration ↓ TCr ↓ PCr/Pi ↓ PCr/ATP ↑ Urinary Cr excretion (due to ↓ turnover)	Human DMD and <i>mdx</i> mouse  Human DMD and <i>mdx</i> mouse Human DMD and <i>mdx</i> mouse Human DMD Human DMD Human DMD	[28,84,85,87,183,203–205]  [28,183,184,205,206] [28,207,208] [85,203,209] [85,117,203] [210]
Purine nucleotide cycle	↓ IMP concentration ↓ Adenylate kinase content and activity ↑ Uric acid excretion	Human DMD <i>mdx</i> mouse Human DMD	[86] [23] [86,211,212]

spanning the entire metabolic system – thus pinpointing the precise defect becomes difficult. Indeed, the only established physical link between the dystrophin protein and the metabolic pathways is via neuronal nitric oxide synthase (nNOS). Dystrophin-deficiency

results in the secondary loss of nNOS [61]. In skeletal muscle, nNOS generates NO which is a key intracellular signalling molecule with strong metabolic regulatory capacity that has effects on contraction, blood flow, glucose uptake and metabolism [62,63]. In healthy

muscles, nNOS localises to the subsarcolemma bound to the dystrophin protein complex (DPC), and more specifically, the syntrophins. The absence of dystrophin disrupts the formation of the DPC [8] and affects nNOS localisation [58,59]. As nNOS exists unbound in the cytosol of *mdx* mouse (genetically homologous murine model of DMD) skeletal muscle and subsequently becomes a substrate for the calpain proteases, a 25-fold decrease in nNOS activity [59–61] and content is observed [62–65]. In the skeletal muscle of DMD patients, nNOS is absent in the pellet fraction of biopsy samples (confirmed by both enzyme assay and Western blot) [58,59]. Additionally, nNOS mRNA in both human DMD [66] and *mdx* [59,67] skeletal muscle is reduced, and as a consequence, endogenous NO production is significantly decreased [68–70]. nNOS-generated NO appears to play a key role in facilitating glucose uptake by stimulating glucose transporter 4 (GLUT4) translocation at rest [71] and during contraction [72]. Despite the reduction of nNOS in *mdx* skeletal muscle, basal (resting) glucose uptake has been shown to be equivalent to control muscle [21,73,74] with GLUT4 expression also normal in young animals [75,76]. However, GLUT4 expression (and its mRNA) decreases in the diaphragm of older *mdx* mice [75], which is important clinically as the diaphragm is the only *mdx* muscle to undergo progressive degenerative wasting throughout the lifespan as per the human disease [77]. Decreased mRNA expression in the older *mdx* mice suggests that disease progression may affect protein expression of GLUT4 and therefore the ability to bring glucose into muscle fibres sufficient to maintain energy production. nNOS also exerts strong regulatory capacity over the key rate-limiting glycolytic enzyme, phosphofructokinase (PFK) in a non-NO-mediated manner [78]. In both DMD and *mdx* muscle samples, reduced PFK activity is observed [18–20,78,79] and this seems ostensibly linked to the significant down-regulation of both glycogen and glucose metabolism in dystrophin-deficient muscle (summarised in Table 1) [56,80]. In healthy muscle, PFK co-localises at the sarcolemma alongside nNOS and is inhibited by high concentrations of ATP and activated by ADP and other by-products of ATP hydrolysis (as reviewed in [81]). As ATP concentration is diminished in dystrophic muscle, PFK activity should, logically, be increased to promote ATP synthesis and energy balance. However, altered allosteric regulation of PFK has been observed in *mdx* muscle [78] suggesting that PFK fails to respond appropriately to normal stimuli. Despite the soluble and cytoskeleton-bound PFK enzymes being distributed normally, the sensitivity of PFK to its allosteric regulators is reduced [79]. This indicates a functional change in PFK properties and/or its modulation, which significantly reduces its activity and likely contributes to the overall metabolic deficit in dystrophic muscle. Reintroduction of nNOS into *mdx* skeletal muscle has shown some benefit in improving glucose and glycogen metabolism (in addition to reducing membrane degradation and muscle inflammation) [78,82], such to improve exercise tolerance which was attributed to the positive allosteric effect nNOS exhibited on PFK [78].

While the secondary loss of nNOS abundance and function goes toward accounting for the widespread depression of glycolytic and glycogenolytic function observed in dystrophin-deficient muscle, its effect on downstream oxidative ATP production is unclear and yet to be characterised. While glycolytic perturbations would plausibly induce secondary reductions in mitochondrial metabolism as a direct result of reduced pyruvate flux through the system, the subsequent activation of stress-responsive metabotropic transcription factors should theoretically activate fat oxidation and stimulate mitochondrial biogenesis to restore ATP production as a compensatory mechanism. This would alleviate pressure on the creatine phosphagen and purine nucleotide salvage pathways. However, as summarized in Table 1, this is not the case in dystrophin-deficient muscle, and as such,

all signs seemingly point toward the mitochondria as the key site of metabolic anomaly.

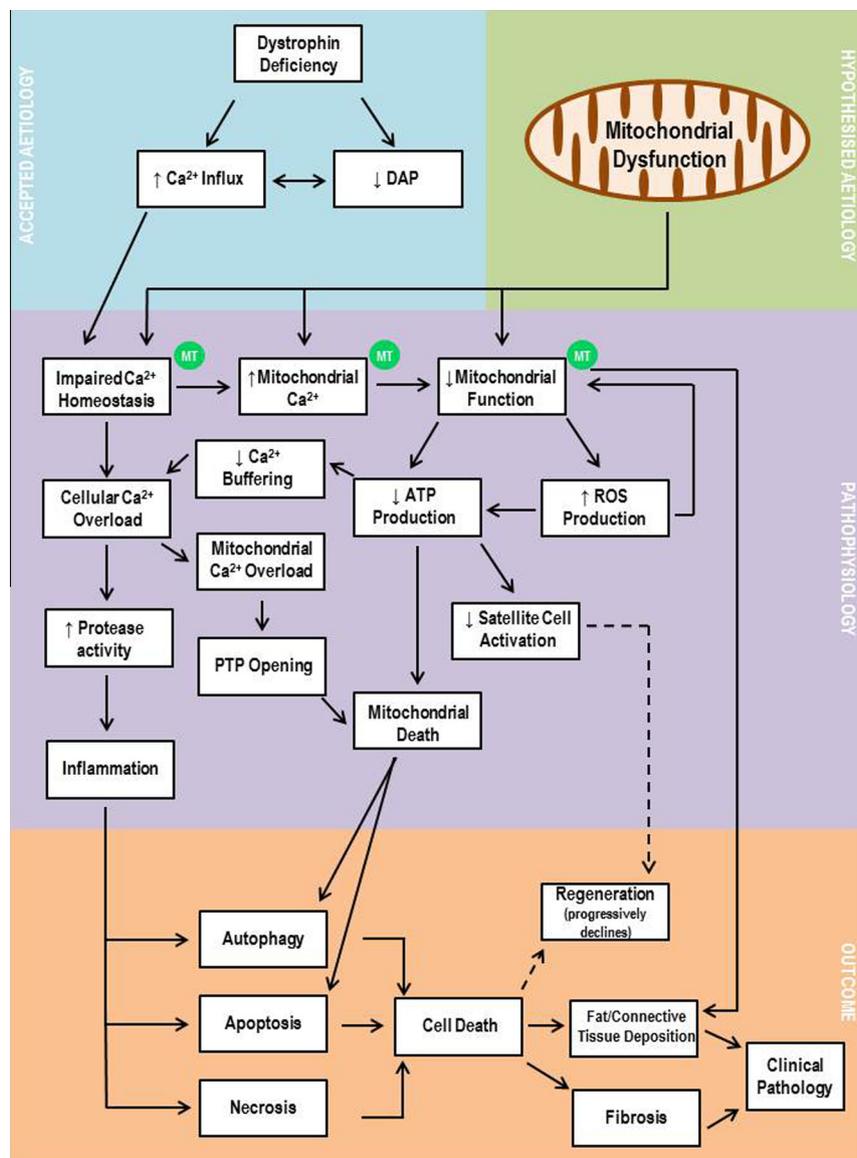
#### *All signs point to the mitochondria*

The fundamental role of the mitochondria is ATP synthesis, thus they are the major cellular regulators of energy homeostasis. More recently mitochondria have emerged as playing an important role in the regulation of initiating apoptotic cell death. Mitochondria are adept at sensing and responding to intracellular changes in energy balance to maintain homeostasis, but once the metabolic insult exceeds regulatory capacity, mitochondrial dysfunction ensues. Prolonged mitochondrial stress can initiate apoptosis when dissipation of the mitochondrial membrane potential, release of cytochrome c and/or caspases and opening of the mitochondrial transition pore occurs (as reviewed in [83]).

*Functional abnormalities in dystrophic mitochondria.* Mitochondrial dysfunction in dystrophic skeletal muscle is well documented and a key contributor to the reductions (up to 50%) in resting ATP content [27–32,79,84–89], with decreased ATP content in the brain of DMD patients also evident [39]. Impaired handling of substrates including pyruvate [21,25–27,33,90–95], malate [25–27,91–93] and glutamate [26,27,94] (with glutamate content increased in *mdx* diaphragm [96]) have been consistently reported to produce lower oxidation rates compared to healthy controls, even in combination with other substrates. Addition of succinate, on the other hand, has been shown to either restore [21,25,97,98] or at least partially restore oxidation rates to control levels [26,27,91,92]. This is a widely reported feature of dystrophin-deficient muscle metabolism and as published by us recently, indicates that the metabolic deficit may be located at complex I of the ETC [27]. Alternatively, as it appears that some enzymes of the TCA function abnormally – including succinic CoA synthetase, aconitase, malate dehydrogenase and IDH [99–103] – which would result in decreased production of reducing equivalents at the TCA level, the ability of succinate to restore oxidative phosphorylation may lie in its ability to bypass a defective TCA system and stimulate complex II of the ETC directly.

Various enzymes of the TCA cycle (in addition to complex V of the ETC) are regulated by increases in intramitochondrial  $[Ca^{2+}]$ . Pyruvate dehydrogenase (PDH) (indirectly activated by  $Ca^{2+}$ -activated phosphatase),  $\alpha$ -ketoglutarate dehydrogenase and IDH (at higher concentrations) are all allosterically activated as mitochondrial matrix  $[Ca^{2+}]$  rises (as reviewed in [104]). This normally results in the anaplerotic expansion of TCA-generated reducing equivalents and a greater chemiosmotic drive for, and faster speed of, ATP production at complex V. These enzymes should theoretically be stimulated in dystrophic muscle (as free intracellular  $Ca^{2+}$  is considerably higher at rest and during contraction [105–109]) to increase  $Ca^{2+}$  buffering and remove the pathological stimulus. However, normal stimulation of these enzymes by increased  $[Ca^{2+}]$  appears to be absent in dystrophic muscle as evidenced by decreased IDH activity [100]. If IDH fails to activate in response to the extremely high  $[Ca^{2+}]$  observed in DMD, it may be that other  $Ca^{2+}$ -sensitive enzymes are not responding appropriately either. The consequence of this is insufficient ATP production and  $Ca^{2+}$  buffering capacity leading to amplification of the pathological stimulus (i.e.  $[Ca^{2+}]$ ). As it has been recently demonstrated that *mdx* mitochondria hypersensitively respond to a  $Ca^{2+}$  load to prematurely open the permeability transition pore (channel that initiates mitochondrial death) [110], we suggest that the inability of dystrophic mitochondria to respond to an overwhelming  $Ca^{2+}$  stimulus by ramping up ATP production, favours premature induction of pro-apoptotic pathways such that cell death is the only viable outcome (Fig. 2).

Reduced oxidation rates of the substrates that channel through the TCA cycle appears to culminate at the ETC. In saponin-skinned



**Fig. 2.** Challenging the accepted paradigm of DMD aetiology: the potential for metabolic therapy to anaplerotically “correct” dystrophin-deficiency-mediated pathology. In the accepted aetiology of DMD (blue box), the pathophysiology (purple box) and clinical outcomes (orange box) are the result of dystrophin- and DAP-complex-deficiency-mediated  $\text{Ca}^{2+}$  influx and homeostatic deregulation. Mitochondrial dysfunction is a secondary consequence of cellular  $\text{Ca}^{2+}$  overload. In our hypothesised aetiology of DMD (green box), inherent mitochondrial dysfunction is the precursor to dystrophic pathophysiology and not a secondary consequence of dystrophin-deficiency as currently accepted. Inherent mitochondrial dysfunction would limit the ATP production required to buffer  $\text{Ca}^{2+}$  from myofibres and organelles and maintain regenerative capacity, thus driving the clinical phenotype. The application of metabolic therapy (MT) (green circles) would target the impaired  $\text{Ca}^{2+}$  homeostasis, increased mitochondrial  $\text{Ca}^{2+}$  load and mitochondrial dysfunction to effectively buffer the  $\text{Ca}^{2+}$  influx induced by dystrophin/DAP-deficiency and prevent the subsequent pathophysiology. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

*mdx* skeletal muscle fibres, the maximal rate of respiration, as stimulated by the addition of ADP, was nearly 50% lower regardless of the substrate used [26]. Similarly, isolated dystrophic mitochondria function at ~60% of maximal respiration control rates [26,88], while a biopsy from a DMD patient revealed similar respiratory deficits [26]. Additionally, reduced ADP-stimulated [91–93,97,111,112] and basal respiration has been reported [33,111–114], with further reductions observed as the disease progresses [94]. The ability of mitochondria to aptly respond to the increased  $[\text{Ca}^{2+}]$  and requirements of dystrophic muscle appears to be further impaired as the spare respiratory reserve, which indicates the ability for the ETC to increase ATP production in response to metabolic challenge, is reduced by ~60% [111]. Such a deficit can be accounted for by reduced activities of the ETC enzymes. In *mdx* fibres of the quadriceps, the activities of rotenone-sensitive

NADH:cytochrome *c* reductase, succinate:cytochrome *c* reductase and cytochrome *c* oxidase were found to be 50% of that in normal fibres [26], with a 20–35% reduction in the activities of complexes I, II and IV in *mdx* fibres of the tibialis anterior. Moreover, in both the fast-twitch extensor digitorum longus and slow-twitch soleus of the *mdx* mouse, NADH activity is reduced [115], with *mdx* myoblasts expressing a decreased complex III and V content [33]. There is also a significant decrease in the expression of genes encoding the subunits of complexes I, II, III and IV in DMD muscle [99]. Ultimately, the maximal ATP synthesis rate is reduced by up to 75% in mitochondria isolated from dystrophic skeletal muscle [27,88]. Similar respiratory dysfunction is observed in the brain of the *mdx* mouse. Decreased activity of complexes I and IV is observed throughout various sections of the brain [42] indicating that despite not being strongly involved in the pathological progression

of the disease, the brain still manifests similar metabolic deficits as per the skeletal musculature.

In addition to the content and activity of isolated complexes of the mitochondrial respiratory chain, functional measures of mitochondrial performance are challenged in dystrophin-deficient skeletal muscle. Dystrophic mitochondria exhibit reduced respiratory control, ADP/oxygen (O) and P/O ratios [25,88,90,92,94,97,98,113,116], all of which indicate that dystrophic mitochondria are not as tightly coupled as healthy mitochondria, thus reducing the phosphorylation potential [117] as evidenced by the 40% reduction in ATP produced per O<sub>2</sub> molecule consumed [88]. Uncoupling refers to any process that impacts upon the P/O ratio and subsequently depletes the potential energy. This includes loss of protons due to inefficient proton pumping by the ETC complexes, leak of electrons from the respiratory chain and activity of uncoupling proteins – all of which dissipate the mitochondrial membrane potential. While uncoupling is thought to provide protective effects as it can buffer reactive oxygen species (ROS) produced by electron leak from the respiratory chain, prolonged uncoupling can lead to severe mitochondrial impairment and death [118].

The NAD/NADH ratio is an important regulator of metabolism [119]. NAD is a cofactor at multiple sites of the TCA cycle and in glycolysis, where it is reduced to NADH and oxidised at complexes I, III and IV of the ETC. This generates the mitochondrial membrane potential which is the driving force for ATP production. Therefore, maintaining the NAD/NADH ratio is imperative, albeit seemingly difficult in dystrophic muscle due to the decreased total intramitochondrial NAD pool [91]. Moreover, as the NADH produced at the glycolytic level is dependent upon the malate-aspartate and glycerol-3-phosphate shuttles to enter the mitochondria, and these rely on glutamate oxidation (which is notably decreased in dystrophic muscle [26,27,94]), glycolysis-generated NADH may be largely prevented from contributing to respiration. Together, this indicates that the NAD/NADH ratio is unable to suitably modulate metabolic function due to other confounding factors that impair the maintenance of the NAD and NADH pool at the mitochondrial level.

*Structural abnormalities in dystrophic mitochondria.* Proper mitochondrial structure and locale is also important to function and is compromised in dystrophin-deficient states. Mitochondria exist in two distinct pools – located beneath the sarcolemma (subsarcolemmal) and at the I band and intermyofibrillar space of the contractile apparatus (intermyofibrillar) [120]. Subsarcolemmal mitochondria account for 10–15% of the mitochondrial pool and supply ATP for Ca<sup>2+</sup> handling, ion transport, membrane function and the peripheral nuclei, while also assisting with glucose homeostasis and lipid utilisation [120]. In contrast, intermyofibrillar mitochondria constitute up to 90% of the mitochondrial pool and provide ATP for contraction. Intermyofibrillar mitochondria differ from subsarcolemmal mitochondria in that they maintain a higher respiratory rate via increased mitochondrial enzyme activity [120]. Despite their differences, both pools of mitochondria share a networking system that allows them to translocate to areas of increased metabolic demand. Thus, mitochondria are extremely responsive to changes in isolated regions of the intracellular environment.

In *mdx* skeletal muscle, a decrease in mitochondrial mass has been reported [114,115]. This is partnered with a decrease in the density of subsarcolemmal mitochondria and the accrual of intermyofibrillar mitochondria around necrotic and regenerating fibres with no change in overall mitochondrial number [88]. This suggests that either the subsarcolemmal mitochondria are translocating to support the intermyofibrillar mitochondrial pool or require the presence of dystrophin for scaffolding to remain at their proper location. Decreased density would be detrimental as

the subsarcolemmal mitochondria play a role in Ca<sup>2+</sup> handling and lipid metabolism, which may partially explain the inability to appropriately handle the stress applied by Ca<sup>2+</sup> and the deficits observed in  $\beta$ -oxidation. Moreover, in human DMD biopsies, an increased population of dense and dilated mitochondria have been observed [121–123] along with changes in cristae shape and density [49]. Swollen mitochondria are also evident in *mdx* mouse skeletal muscle [110,124,125] along with morphologically abnormal cristae structure [110]. While morphological changes of the mitochondria are generally characteristic of fibres undergoing degeneration and necrosis, it appears this swollen morphology may exist outside of an environment conducive to swelling [27]. Isolated *mdx* mitochondria bathed in a Ca<sup>2+</sup>-free environment are more swollen than mitochondria isolated from healthy animals [27]. While this could be a residual effect of an extreme pre-isolation *in vivo* Ca<sup>2+</sup> environment, it may also be an inherent feature of the disease as alterations in mitochondrial architecture, morphology and localisation are apparent in female DMD carriers that express dystrophin and do not manifest dystrophinopathy [126]. If so, this inherent swollen morphology would affect mitochondrial functionality, as changes in cristae shape have recently been shown to alter ETC supercomplex assembly [127] which would deleteriously impact upon their function. Together, this decrease in mitochondrial mass and inherent swollen morphology strongly suggest that metabolic impairments in DMD are an inherent feature of the genotype, and this is exacerbated – but not caused by – the persistent elevation of Ca<sup>2+</sup>.

#### *Dystrophic muscle does not respond normally to master energy signals*

In healthy skeletal muscle, ATP depletion induced by metabolic, nutritional and/or environmental stressors (including intense exercise and hypoglycaemia) stimulates ATP-producing pathways to restore energy homeostasis [128]. One important regulator of this switch from ATP-consuming (anabolic) to ATP-producing (catabolic) pathways is adenosine monophosphate-activated protein kinase (AMPK), a major sensor of cellular energy status. Induced by rises in the AMP/ATP ratio, AMPK stimulates glucose uptake, glycolysis, fatty acid oxidation [128,129] and various TCA cycle and ETC enzymes [130], while also modulating expression of a suite of genes – including PGC-1 $\alpha$  – that increase mitochondrial biogenesis. Thus, AMPK activation favours the oxidative fibre phenotype, which is highly beneficial for dystrophic muscle as this fibre type is less affected by the disease [131] and therefore may offer protection from damage. In addition, AMPK appears to play a significant role in muscle remodelling as it stimulates autophagy. Autophagy is a catabolic pathway that breaks down cellular components when they are in excess or damaged, or, to provide fuel sources in times of metabolic challenge [132]. Therefore, AMPK is a positive stimulator of metabolism, controlling the supply of fuel to various metabolic pathways and initiating remodelling to improve muscle structure and function. Considering that AMPK positively modulates metabolism, stimulates targeted remodelling of muscle to improve oxidative capacity and is activated by ATP depletion, AMPK activation should, theoretically, be enhanced in dystrophic skeletal muscle. Indeed, Pauly et al. [110] demonstrate a higher basal AMPK activation in *mdx* diaphragm, highlighting that metabolic stress-induced signalling pathways are appropriately activated in dystrophic muscle. When the AMPK-activator metabolite 5'-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside (AICAR) was given to *mdx* myotubes *in vitro* and to 6 week old *mdx* mice via daily intraperitoneal injection, AMPK activation was further enhanced compared to untreated conditions. In the *mdx* mice in particular, AICAR treatment had several beneficial effects including increased activation of autophagic signalling proteins, maximal force production and time to permeability transition pore opening in response to Ca<sup>2+</sup> challenge

[110]. Most noteworthy however, was that despite AICAR inducing a ~50% increase in activated AMPK, only minimal increases were observed in acetyl CoA carboxylase phosphorylation (the downstream target of AMPK activity) and this failed to induce any changes in oxidative metabolism including activity of citrate synthase, cytochrome oxidase and mitochondrial O<sub>2</sub> consumption. This data importantly highlights that while dystrophin-deficient muscle can ably detect metabolic stress, the downstream response of the metabolic systems to AMPK phosphorylation fails to improve ATP synthesis to abate this stress.

The plasticity of the skeletal musculature in response to isolated and chronic exposure to metabolic stress is afforded via the induction of a slow-type oxidative phenotype. In a study that compared global gene expression responses between skeletal muscle from metabolically-challenged endurance-trained individuals who had been previously sedentary, and DMD patients, ~90 genes were shown to be modulated identically [56]. This data highlights that strong metabolic challenge is a feature of dystrophinopathy and that it invokes similar responses as per chronic endurance exercise. However, while the expression of genes regulating oxidative phosphorylation was increased following endurance training as expected, they were differentially down regulated in muscle from DMD patients [56]. These included genes of carbohydrate, glycogen and mitochondrial metabolism [56]. Thus, while DMD muscle adapts on a genetic level to metabolic stress as per endurance trained athletes, this stress seems not to induce the regular adaptations at the mitochondrial level to enhance the ATP production capacity of the skeletal musculature. It has been well established in the literature that inducing type I oxidative fibre type transformations pharmacologically and genetically affords therapeutic value to dystrophin-deficient skeletal muscle by reducing the rate of disease progression [133–136], as type II fibres are preferentially affected [131,137], which can be promoted by the activation of AMPK [115,132,138–140] and its downstream targets [114,141–143]. However, it has recently been suggested that the beneficial effects of a slow type I phenotype is functionally related to enhanced utrophin A expression – in dystrophin/utrophin double knock-out mice AICAR administration afforded no benefit – in comparison, therapeutic benefit was observed in *mdx* mice [140]. Thus whether AMPK activation can suitably induce benefits at the mitochondrial level to buffer metabolic demand remains unclear.

Another important role of AMPK is regulation of the targeted removal of dysfunctional organelles/structures via autophagy. It has been observed that *mdx* diaphragm is laden with dysfunctional mitochondria characterised by morphological abnormalities and an increased propensity to open the permeability transition pore [110]. The removal of dysfunctional mitochondria is strongly regulated in healthy skeletal muscle [144], albeit background mitophagic activity is typically low due to the relatively low ratio of unhealthy/healthy mitochondria. However, in dystrophic skeletal muscle, there is reduced propensity for sufficient and/or functional mitophagy leading to the accumulation of defective mitochondria, particularly in the subsarcolemmal pool [145]. Ineffective autophagic signalling induction has been demonstrated in *mdx* [145] and human DMD [110,145] skeletal muscle with AMPK activation seemingly central to the problem. Both AICAR- [110] and low protein diet-induced AMPK activation demonstrably increases the activity of pro-autophagic pathways and ameliorates the dystrophic condition [110], indicating that improving the clearance of dysfunctional mitochondria is beneficial. Thus while Pauly et al. [110] have demonstrated enhanced endogenous AMPK signalling in dystrophin-deficient skeletal muscle, it appears insufficient to match the extent of mitochondrial pathology evident in DMD without therapeutic support.

#### *Revisiting the dystrophin-ATP connection: is a mitochondrial disease at the heart of DMD?*

Several hypothetical review and original research papers have both historically and more recently proposed that the lack of dystrophin protein may not be the primary cause of the progressive and fatal degeneration observed in DMD, but rather a co-morbidity [33,43,146,147]. We have described a plethora of mitochondrial defects (in addition to many others of substrate feeder pathways that are allosterically regulated by the functional capacity of the mitochondria) that are also commonly observed in mitochondrial diseases and in senescence. Indeed, DMD shares common metabolic and mitopathological features with various mitochondrial diseases and with aged skeletal muscle, including often comparable symptomology. In addition a more recent study has shown that mitochondrial dysfunction exists in “pre” dystrophin-deficient myoblasts prior to the “typical” cascade of events that are commonly believed to cause the progressive muscle degeneration and wasting evident in DMD [33]. Collectively, this literature importantly suggests a mitochondrial aetiology of DMD.

Because skeletal muscle accounts for ~40–50% of body weight and ~30% of oxygen consumption at rest, it is an important regulator of overall metabolism. As such, mitochondrial deficits manifest vastly in the skeletal musculature and myopathy is thus characteristic of many mitochondrial diseases. Mitochondrial disease can arise from mutations in the maternally inherited mitochondrial DNA (mtDNA), and less commonly in the nuclear DNA. mtDNA resides in the matrix and encodes for the hydrogen pumping regions of the respiratory chain complexes, highlighting its integral role in the regulation of metabolism [148]. However due to its proximity to the respiratory chain, mtDNA is extremely vulnerable to mutation, most commonly by ROS produced by the respiratory complexes [149,150]. Initially, this has minimal effect on mitochondrial function, until the number of mutant mtDNA outnumbers wild-type mtDNA. As mutant mtDNA accumulates, the bioenergetical capacity of the cell diminishes. Various diseases result from mtDNA mutations and manifest themselves as multi-systemic diseases. These mitochondrial diseases share common features with DMD including mental impairment, skeletal muscle weakness, cardiomyopathy and multisystem metabolic dysfunction [148,151]. Reduced activities of complex I, III, IV and V of the ETC, increased ROS production and decreased ATP synthesis are common nuances of mitochondrial diseases and DMD [151]. The fact that dystrophin is encoded and expressed normally in these diseases, but that they share clinical features with dystrophinopathy indicates the potential for a common disease origin that is not linked to dystrophin-deficiency.

As the majority of ETC complexes (excluding complex II) are partially encoded by mtDNA and reports exist that describe mitochondrial dysfunction in DMD carriers that express dystrophin normally, maternal mtDNA inheritance would be a likely theoretical origin of such a mitochondrial mutation. Female carriers of the dystrophin gene mutation on one of their X chromosomes commonly express normal levels of dystrophin (albeit sporadic dystrophin-deficient fibres have been reported [152]). As such, they do not manifest DMD. However, despite lacking phenotypic pathology, deficits in mitochondrial responses to exercise have been reported. Carriers are unable to perform muscle work at the same level as controls and their P<sub>i</sub>/PCr ratio is higher for corresponding work levels [153,154]. This supports an inability of the mitochondria to sufficiently replenish the Cr/PCr system during activity. Post-exercise recovery of the PCr/inorganic phosphate (P<sub>i</sub>) ratio is also much slower in carriers [153], demonstrating that mitochondrial insufficiency is also apparent at rest. Additionally,

sharp increases in serum CK activity are observed following exercise in carriers but are absent in healthy exercised individuals [155,156]. Notably, the co-occurrence of a mtDNA mutation in a family with extensive history of DMD has also been observed [157], which adds further credence to the ideation of mtDNA mutation underscoring DMD pathology.

If not inherited, another likely origin of mtDNA mutation is via the rapidly progressive accumulation of ROS-induced mutations that are not too dissimilar to those that underscore senescence as described in the popular *mtDNA accumulation theory of aging* (reviewed in [158]). Aging muscle shares many symptomatic characteristics of dystrophic muscle including fatigability, muscular weakness and atrophy, and mitochondrial dysfunction. In aging muscle, it appears that accumulation of mutant mtDNA leads to mitochondria characterised by decreased oxidative capacity, increased oxidative stress and decreased ATP synthesis [148,159] which impairs muscular function and viability. Of note, a characteristic feature of senescent mitochondria is a reduction in spare respiratory capacity [160] which renders mitochondria unable to adapt to increased energy demand, thus promoting fatigue, exercise intolerance and progressive muscle wasting (sarcopenia) which are all clinical features of DMD. Indeed, both aged and dystrophic muscles display deregulation of the same genes involved in metabolism [161] which highlights once more the possibility of mtDNA mutation involvement in DMD.

Perhaps one of the more compelling pieces of evidence that mitochondrial dysfunction is an inherent feature of DMD is a recent finding by Onopiuk and colleagues [33]. Using myoblasts from control and *mdx* mice, it was observed that *mdx* myoblasts exhibit changes to several mitochondrial functional parameters including decreased basal oxygen consumption, increased mitochondrial membrane potential and ROS production (~70% higher) and decreased complex III and V content [33]. Remarkably, these metabolic changes are observed at a time when dystrophin is yet to be expressed in myoblasts [162]. In both control and *mdx* myoblasts, dystrophin expression was negligible, despite an mRNA transcript evident in control myoblasts [33]. Myoblasts express a different metabolic phenotype to myotubes including a greater dependence on glycolysis [62]. Lactate production via glycolysis pacifies ~60% of energy demand (due to its conversion to pyruvate via LDH) [62] and *mdx* myoblasts demonstrably produce more lactate [33] indicating heavy reliance on glycolytic flux. This appears to be pertinent to *mdx* myoblasts as the basal rate of respiration following the addition of glucose and pyruvate was depressed compared to controls [33], suggesting that further oxidation of intermediates in the mitochondria is impaired. The authors concluded that the metabolic dysfunction in *mdx* myoblasts is independent of dystrophin-deficiency as deficits were observed in *mdx* myoblasts prior to the time of dystrophin expression.

There are now also several case studies in the literature documenting either dual mtDNA and nuclear dystrophin gene mutations in family pedigrees [157] or dystrophin gene abnormalities with pseudometabolic presentation but dystrophin protein expression [163,164]. A case study by Wong and colleagues [157] describes the presentation of an adolescent male with a strong family history of DMD but who does not express the genotype himself, with complicated seizure disorder, congenital heart disease and developmental delay. Suspected mitochondrial respiratory chain disorder was confirmed in which low levels of heteroplasmic A3243G mutation was detected in the mtDNA. The diagnosis of MELAS disorder was made following respiratory enzyme analysis that revealed significantly elevated complex IV activity without gross mitochondrial cytopathy (albeit some mitochondria displayed altered cristae structure and were morphometrically abnormal). The patient carried low mutant loads in all tissues analysed – 6%, 8%, 12%, 17% and 9% for blood, hair follicle, buccal

mucosa, skeletal muscle and skin fibroblast cultures, respectively. The mutation appeared to have occurred *de novo* as it was not detected in the maternal mtDNA from blood, hair follicle or buccal mucosa cells, albeit this could not be confirmed given the relatively low mutant load found in the patient. Similar cases of mtDNA mutation in the background of other, more severe nuclear gene mutations (such as cystic fibrosis and spinal muscular atrophy) have been reported by the same group [165,166] highlighting the propensity for dual mitochondrial and nuclear gene mutations – perhaps as a result of modifier gene induction – that are difficult to diagnose due to broad and often competing symptomologies.

The pseudometabolic presentation of DMD due to missense mutations in the dystrophin gene has also been documented. Romero and colleagues [163] report three male adolescents presenting with exercise-induced myalgia, muscle stiffness, and myoglobinuria following strenuous exercise – all symptoms of metabolic diseases including glycogen storage disorder, fatty acid oxidation disorder and mitochondrial cytopathy. All patients were found to have a hemizygous T-to-C mutation in exon 15 of the DMD gene resulting in an amino acid substitution of leucine to proline at codon 575. Immunohistochemical staining of dystrophin and other proteins of the DPC was normal as was western blot analysis for dystrophin quantity and size. A further two reports of the same missense mutation inducing recurrent rhabdomyolysis has been reported [167]. These case studies highlight symptoms characteristic of metabolic disease that are seemingly induced by dystrophin gene point mutations but which are not phenotypically associated with dystrophin protein expression abnormalities.

Most recently, several studies by the same group have documented the clinical history of canine models of muscular dystrophy [168–170] and dystrophin-deficient human DMD patients [171] that express a mild disease phenotype and in some instances, a normal lifespan, despite the absence of dystrophin. Zucconi et al. [168] and Zatz et al. [169] describe the clinical history of a golden retriever muscular dystrophy dog and its offspring, who display absent dystrophin production, unremarkable utrophin regulation, hallmark histopathological features of skeletal musculature and extreme elevations in serum CK levels as per phenotypically normal severely-affected dogs, but are seemingly able to buffer this to maintain muscle mass, ambulation and a normal life span. A similar canine colony has been reported in the Labrador retriever muscular dystrophy model [170] which also displays the absence of dystrophin – albeit the precise mutation on the dystrophin gene was not elucidated in this study – and are asymptomatic. This protection seems related to the maintenance of strong regenerative potential throughout the lifespan, albeit the precise mechanism through which muscle function is maintained despite the absence of dystrophin requires further investigation and characterisation. Finally, in human DMD patients, Zatz et al. [171] have reported half-brothers with comparable, minimal (near absent) levels of dystrophin expression, elevated serum CK levels and pathological histological parameters, but who express widely variable phenotypic progression of DMD. While one brother has progressed through a normal disease course with onset of symptoms at 3 years, diagnosis at 7 years and loss of ambulation at 9 years, the older brother shows mild signs of muscle weakness and physical dysfunction with mild calf hypertrophy, but maintains normal ambulatory capacity at 16 years of age. The same paper describes a third case of an unrelated male 16 year old adolescent who displayed normal phenotypic DMD at age 7 years when diagnosis was made, but whom now displays only mild weakness and calf hypertrophy and is fully ambulatory. Other isolated case studies exist documenting the complete absence of dystrophin expression but a mild DMD phenotype [172,173]. While modifier gene regulation likely accounts for such phenotypic differences, it is possible that in these rare cases of DMD, mitochondrial dysfunction is

spared or anomalies are corrected such that ATP demand is met to overcome the pathophysiological insult induced by dystrophin-deficiency – indeed, metabolism and cellular redox status are highly regulated by transcriptional modification and the induction of nuclear factors. At the very least, these cases highlight that the loss of dystrophin expression is not the sole contributor to the pathological deterioration of skeletal muscle in DMD, and while indeed promoting sarcolemmal leakiness and significant damage, dystrophin-deficiency can be effectively buffered by adaptive mechanisms in some instances.

#### Treating DMD as a metabolic disease

Bonsett & Rudman's seminal study illustrating the critical role of ATP depletion in dystrophinopathy and the vast potential for anaplerotic correction [43] was to be one of several papers documenting the beneficial effects of targeted metabolic therapy on the pathophysiological and clinical course of DMD. ASA is a product/reactant of the purine nucleotide cycle that has the dual function of producing fumarate to stimulate the TCA cycle and ADP resynthesis via purine nucleotide salvage pathway reactions, to ultimately increase mitochondrial ATP production and reduce the loss of purines from the muscle (into the blood stream) as xanthine and hypoxanthine. Comprising a 10 year clinical trial, ASA treatment induced vast improvements in Cr retention within, and histological features and the regenerative capacity of, dystrophic muscle, which was accompanied by improved energy levels, stamina and strength [43]. In cultured DMD cells, the addition of ASA was effective at removing the overwhelming presence of intracellular lipid droplets [24], thus apparently rectifying the metabolic dysfunction either at the allosteric or metabotropic transcriptional level. Notably, cessation of ASA therapy diminished the positive benefits observed during treatment of DMD patients [43], highlighting that ongoing support of the mitochondria is pivotal to mitigating disease progression. Similar clinically beneficial effects have been observed for allopurinol [30,86,174–177] – which inhibits xanthine oxidase activity via its active metabolite oxypurinol, and therefore seems to reduce the flux of xanthine from skeletal muscle during metabolic stress – and Cr [178–181] – which increases the total PCr pool and therefore the phosphorylation potential of skeletal muscle. Notably for allopurinol, the age at which supplementation begins is crucial for induction of beneficial effects, and as with ASA, clinical improvements diminish upon cessation of therapy [176]. These studies importantly highlight the potential for the clinical use of metabolic therapies and the necessity for further investigation into the ways in which such therapies can be enhanced to improve the phenotypic progression of DMD and the quality of life of patients.

#### Conclusions

Although the collective literature over the past 50 years has carefully documented the plethora of metabolic abnormalities consistently observed in DMD patients, genetic carriers and genotypically identical animal models of the disease, the significance of this data has been largely ignored. As a cure for DMD remains currently elusive, every effort must be made to consider all possibilities for improved characterisation and treatment of the disease. We hypothesise an aetiological nuance at the mitochondrial level that manifests in multiple deficiencies of various metabolic pathways to culminate in severe ATP insufficiency and clinical manifestation of the disease. Of course, it cannot be denied that changes induced by dystrophin-deficiency, including disruption of the DPC and failed  $\text{Ca}^{2+}$  homeostasis, play a role in the severe and progressive muscle wasting characteristic of DMD. However, if mitochondrial

defects do underlie DMD aetiology, then re-defining DMD as a metabolic myopathy and strategically targeting research funding, and treating it clinically as such, could improve patient outcomes and enhance quality of life.

#### Conflict of interest

No conflict of interest, financial or otherwise is declared by the authors.

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#### References

- [1] Emery A. Population frequencies of inherited neuromuscular diseases – a world survey. *Neuromuscul Disord* 1991;1:19.
- [2] P.A. Romitti, Y. Zhu, S. Puzhankara, et al., Prevalence of Duchenne and Becker muscular dystrophies in the United States, *pediatrics*, 2015.
- [3] Brooke M, Griggs R, Mendell J, Fenichel G, Shumate J. The natural history of Duchenne muscular dystrophy: a caveat for therapeutic trials. *Trans Am Neurol Assoc* 1981;106:195.
- [4] Brooke M, Fenichel G, Griggs R, et al. Duchenne muscular dystrophy patterns of clinical progression and effects of supportive therapy. *Neurology* 1989;39:475–475.
- [5] Duchenne GB. De la paralysie musculaire pseudo-hypertrophique ou paralysie myo-sclérotique. P. Asselin; 1868.
- [6] Hoffman EP, Brown RH, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 1987;51:919–28.
- [7] Koenig M, Monaco AP, Kunkel LM. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell* 1988;53:219–28.
- [8] Ohlendieck K, Campbell KP. Dystrophin-associated proteins are greatly reduced in skeletal muscle from mdx mice. *J Cell Biol* 1991;115:1685–94.
- [9] Wells DJ, Wells KE, Asante EA, et al. Expression of human full-length and minidystrophin in transgenic mdx mice: implications for gene therapy of Duchenne muscular dystrophy. *Hum Mol Genet* 1995;4:1245–50.
- [10] Clemens P, Kochanek S, Sunada Y, et al. In vivo muscle gene transfer of full-length dystrophin with an adenoviral vector that lacks all viral genes. *Gene Ther* 1996;3:965–72.
- [11] Sakamoto M, Yuasa K, Yoshimura M, et al. Micro-dystrophin cDNA ameliorates dystrophic phenotypes when introduced into mdx mice as a transgene. *Biochem Biophys Res Commun* 2002;293:1265–72.
- [12] Willmann R, Possekel S, Dubach-Powell J, Meier T, Ruegg MA. Mammalian animal models for Duchenne muscular dystrophy. *Neuromuscul Disord* 2009;19:241–9.
- [13] Matsumura CY, Perteille A, Albuquerque TC, Santo Neto H, Marques MJ. Diltiazem and verapamil protect dystrophin-deficient muscle fibers of MDX mice from degeneration: a potential role in calcium buffering and sarcolemmal stability. *Muscle Nerve* 2009;39:167–76.
- [14] Fairclough RJ, Wood MJ, Davies KE. Therapy for Duchenne muscular dystrophy: renewed optimism from genetic approaches. *Nat Rev Genet* 2013;14:373–8.
- [15] Cossu G, Sampaolesi M. New therapies for Duchenne muscular dystrophy: challenges, prospects and clinical trials. *Trends Mol Med* 2007;13:520–6.
- [16] Moxley R, Ashwal S, Pandya S, et al. Practice parameter: corticosteroid treatment of Duchenne dystrophy report of the quality standards subcommittee of the American academy of neurology and the practice committee of the child neurology society. *Neurology* 2005;64:13–20.
- [17] Dreyfus J-C, Schapira Georges, Schapira Fanny. Biochemical study of muscle in progressive muscular dystrophy. *J Clin Invest* 1954;33:794–7.
- [18] Di Mauro S, Angelini Corrado, Catani Claudia. Enzymes of the glycogen cycle and glycolysis in various human neuromuscular disorders. *J Neurol Neurosurg Psychiatry* 1967;30:411–5.
- [19] Hess J. Phosphorylase activity and glycogen, glucose-6-phosphate, and lactic acid content of human skeletal muscle in various myopathies. *J Lab Clin Med* 1965;66:452–63.
- [20] Chi MMY, Hintz CS, McKee D, et al. Effect of Duchenne muscular dystrophy on enzymes of energy metabolism in individual muscle fibers. *Metabolism* 1987;36:761–7.
- [21] Chinnet A, Even P, Decrouy A. Dystrophin-dependent efficiency of metabolic pathways in mouse skeletal muscles. *Cell Mol Life Sci* 1994;50:602–5.
- [22] van Bennekom C, Oerlemans FT, Kulakowski S, De Bruyn CH. Enzymes of purine metabolism in muscle specimens from patients with Duchenne-type muscular dystrophy. *Adv Exp Med Biol* 1984;165:447–50.

- [23] Ge Y, Molloy MP, Chamberlain JS, Andrews PC. Proteomic analysis of mdx skeletal muscle: great reduction of adenylate kinase 1 expression and enzymatic activity. *Proteomics* 2003;3:1895–903.
- [24] Bonsett C, Rudman A. Duchenne's muscular dystrophy: a tissue culture perspective. *Indiana Med J Indiana State Med Assoc* 1984;77:446.
- [25] Glesby MJ, Rosenmann E, Nylén EG, Wrogemann K, Serum CK. Calcium, magnesium, and oxidative phosphorylation in mdx mouse muscular dystrophy. *Muscle Nerve* 1988;11:852–6.
- [26] Kuznetsov AV, Winkler K, Wiedemann F, von Bossanyi P, Dietzmann K, Kunz WS. Impaired mitochondrial oxidative phosphorylation in skeletal muscle of the dystrophin-deficient mdx mouse. *Mol Cell Biochem* 1998;183:87–96.
- [27] Rybalka E, Timpani CA, Cooke MB, Williams AD, Hayes A. Defects in mitochondrial ATP synthesis in dystrophin-deficient Mdx skeletal muscles may be caused by complex I insufficiency. *PLoS ONE* 2014;9:e115763.
- [28] Ronzoni E, Wald S, Berg L, Ramsey R. Distribution of high energy phosphate in normal and dystrophic muscle. *Neurology* 1958;8:359–68.
- [29] Vignos Jr P, Warner J. Glycogen, creatine and high energy phosphate in human muscle disease. *J Lab Clin Med* 1963;62:579.
- [30] Tamari H, Ohtani Y, Higashi A, Miyoshino S, Matsuda I. Xanthine oxidase inhibitor in Duchenne muscular dystrophy. *Brain Dev* 1982;4:137–43.
- [31] Cole M, Rafael J, Taylor D, Lodi R, Davies K, Styles P. A quantitative study of bioenergetics in skeletal muscle lacking utrophin and dystrophin. *Neuromuscul Disord* 2002;12:247–57.
- [32] Shuttlewood R, Griffiths J. The purine nucleotide profile in mouse, chicken and human dystrophic muscle: an abnormal ratio of inosine plus adenine nucleotides to guanine nucleotides. *Clin Sci* 1982;62:113–5.
- [33] Onopiuk M, Brutkowski W, Wierzbicka K, et al. Mutation in dystrophin-encoding gene affects energy metabolism in mouse myoblasts. *Biochem Biophys Res Commun* 2009;386:463–6.
- [34] Howland JL, Challberg MD. Altered respiration and proton permeability in liver mitochondria from genetically dystrophic mice. *Biochem Biophys Res Commun* 1973;50:574–80.
- [35] Katyare SS, Challberg MD, Howland JL. Energy coupling in liver mitochondria from dystrophic mice: differential sensitivity of oxidative phosphorylation and  $\text{Ca}^{2+}$  uptake to  $\text{K}^+$ . *Metabolism* 1978;27:761–9.
- [36] Zhang W, Ten Hove M, Schneider JE, et al. Abnormal cardiac morphology, function and energy metabolism in the dystrophic mdx mouse: an MRI and MRS study. *J Mol Cell Cardiol* 2008;45:754–60.
- [37] Lewis C, Jockusch H, Ohlendeck K. Proteomic profiling of the dystrophin-deficient MDX heart reveals drastically altered levels of key metabolic and contractile proteins. *J Biomed Biotechnol* 2010;2010.
- [38] Bresolin N, Castelli E, Comi G, et al. Cognitive impairment in Duchenne muscular dystrophy. *Neuromuscul Disord* 1994;4:359–69.
- [39] Tracey I, Scott RB, Thompson CH, et al. Brain abnormalities in Duchenne muscular dystrophy: phosphorus-31 magnetic resonance spectroscopy and neuropsychological study. *Lancet* 1995;345:1260–4.
- [40] Tracey I, Dunn J, Parkes H, Radda G. An in vivo and in vitro  $^1\text{H}$ -magnetic resonance spectroscopy study of mdx mouse brain: abnormal development or neural necrosis? *J Neurol Sci* 1996;141:13–8.
- [41] Tracey I, Dunn JF, Radda GK. Brain metabolism is abnormal in the mdx model of Duchenne muscular dystrophy. *Brain* 1996;119:1039–44.
- [42] Tuon L, Comim CM, Fraga DB, et al. Mitochondrial respiratory chain and creatine kinase activities in mdx mouse brain. *Muscle Nerve* 2010;41:257–60.
- [43] Bonsett C, Rudman A. The dystrophin connection—ATP? *Med Hypotheses* 1992;38:139–54.
- [44] Meryon E. On granular and fatty degeneration of the voluntary muscles. *Med Chir Trans* 1852;35:73.
- [45] Duchenne G-B. De l'électrisation localisée et de son application à la pathologie. Baillière; 1861.
- [46] Bonsett C, Rudman A, Elliott AY. Intracellular lipid in pseudohypertrophic muscular dystrophy tissue culture. *J Indiana State Med Assoc* 1979;72:184–7.
- [47] Bonsett C, Rudman A. 'Oil globules' in Duchenne muscular dystrophy—History, demonstration, and metabolic significance. *Med Hypotheses* 1994;43:327–38.
- [48] Harriman D, Reed R. The incidence of lipid droplets in human skeletal muscle in neuromuscular disorders: a histochemical, electron-microscopic and freeze-etch study. *J Pathol* 1972;106:1–24.
- [49] Pearce G. Electron microscopy in the study of muscular dystrophy. *Ann N Y Acad Sci* 1966;138:138–50.
- [50] Watt MJ, Hoy AJ. Lipid metabolism in skeletal muscle: generation of adaptive and maladaptive intracellular signals for cellular function. *Am J Physiol Endocrinol Metab* 2012;302:E1315–28.
- [51] He J, Watkins S, Kelley DE. Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. *Diabetes* 2001;50:817–23.
- [52] Goodpaster BH, Theriault R, Watkins SC, Kelley DE. Intramuscular lipid content is increased in obesity and decreased by weight loss. *Metabolism* 2000;49:467–72.
- [53] Sinha R, Dufour S, Petersen KF, et al. Assessment of skeletal muscle triglyceride content by  $^1\text{H}$  nuclear magnetic resonance spectroscopy in lean and obese adolescents relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes* 2002;51:1022–7.
- [54] Hilton TN, Tuttle LJ, Bohnert KL, Mueller MJ, Sinacore DR. Excessive adipose tissue infiltration in skeletal muscle in individuals with obesity, diabetes mellitus, and peripheral neuropathy: association with performance and function. *Phys Ther* 2008;88:1336–44.
- [55] Delmonico MJ, Harris TB, Visser M, et al. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr* 2009;90:1579–85.
- [56] Timmons JA, Larsson O, Jansson E, et al. Human muscle gene expression responses to endurance training provide a novel perspective on Duchenne muscular dystrophy. *FASEB J* 2005;19:750–60.
- [57] Allen DG, Lamb G, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 2008;88:287–332.
- [58] Brennan JE, Chao DS, Xia H, Aldape K, Bredt DS. Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. *Cell* 1995;82:743–52.
- [59] Chang W-J, Iannaccone ST, Lau KS, et al. Neuronal nitric oxide synthase and dystrophin-deficient muscular dystrophy. *Proc Natl Acad Sci* 1996;93:9142–7.
- [60] Kameya S, Miyagoe Y, Nonaka I, et al. A1-syntrophin gene disruption results in the absence of neuronal-type nitric-oxide synthase at the sarcolemma but does not induce muscle degeneration. *J Biol Chem* 1999;274:2193–200.
- [61] Li D, Yue Y, Lai Y, Hakim CH, Duan D. Nitrosative stress elicited by nNOS $\mu$  delocalization inhibits muscle force in dystrophin-null mice. *J Pathol* 2011;223:88–98.
- [62] Leary SC, Battersby BJ, Hansford RG, Moyes CD. Interactions between bioenergetics and mitochondrial biogenesis. *Biochim Biophys Acta (BBA) Bioenerg* 1998;1365:522–30.
- [63] Thomas GD, Sander M, Lau KS, Huang PL, Stull JT, Victor RG. Impaired metabolic modulation of  $\alpha$ -adrenergic vasoconstriction in dystrophin-deficient skeletal muscle. *Proc Natl Acad Sci* 1998;95:15090–5.
- [64] Vaghy PL, Fang J, Wu W, Vaghy LP. Increased caveolin-3 levels in mdx mouse muscles. *FEBS Lett* 1998;431:125–7.
- [65] Judge LM, Haraguchin M, Chamberlain JS. Dissecting the signaling and mechanical functions of the dystrophin-glycoprotein complex. *J Cell Sci* 2006;119:1537–46.
- [66] Arning L, Jagiello P, Schara U, et al. Transcriptional profiles from patients with dystrophinopathies and limb girdle muscular dystrophies as determined by qRT-PCR. *J Neurol* 2004;251:72–8.
- [67] Crosbie RH, Straub V, Yun H-Y, et al. Mdx muscle pathology is independent of nNOS perturbation. *Hum Mol Genet* 1998;7:823–9.
- [68] Gücüyener K, Ergenekon E, Erbas D, Pinarli G, Serdaroğlu A. The serum nitric oxide levels in patients with Duchenne muscular dystrophy. *Brain Dev* 2000;22:181–3.
- [69] Kasai T, Abeyama K, Hashiguchi T, Fukunaga H, Osame M, Maruyama K. Decreased total nitric oxide production in patients with Duchenne muscular dystrophy. *J Biomed Sci* 2004;11:534–7.
- [70] Barton ER, Morris L, Kawana M, Bish LT, Toursel T. Systemic administration of L-arginine benefits mdx skeletal muscle function. *Muscle Nerve* 2005;32:751–60.
- [71] Balon TW, Nadler JL. Evidence that nitric oxide increases glucose transport in skeletal muscle. *J Appl Physiol* 1997;82:359–63.
- [72] Bradley SJ, Kingwell BA, McConnell GK. Nitric oxide synthase inhibition reduces leg glucose uptake but not blood flow during dynamic exercise in humans. *Diabetes* 1999;48:1815–21.
- [73] MacLennan PA, McArdle A, Edwards R. Acute effects of phorbol esters on the protein-synthetic rate and carbohydrate metabolism of normal and mdx mouse muscles. *Biochem J* 1991;275:477.
- [74] Even P, Decrouy A, Chinot A. Defective regulation of energy metabolism in mdx-mouse skeletal muscles. *Biochem J* 1994;304:649.
- [75] Olichon-Berthe C, Gautier N, Van Obberghen E, Le Marchand-Brustel Y. Expression of the glucose transporter GLUT4 in the muscular dystrophic mdx mouse. *Biochem J* 1993;291:257.
- [76] Raith M, Valencia RG, Fischer I, et al. Linking cytoarchitecture to metabolism: sarcolemma-associated plectin affects glucose uptake by destabilizing microtubule networks in mdx myofibers. *Skeletal Muscle* 2013;3:14.
- [77] Stedman HH, Sweeney HL, Shrager JB, et al. The mdx mouse diaphragm reproduces the degenerative changes of Duchenne muscular dystrophy. *Nature* 1991;352:536–9.
- [78] Wehling-Henricks M, Oltmann M, Rinaldi C, Myung KH, Tidball JG. Loss of positive allosteric interactions between neuronal nitric oxide synthase and phosphofructokinase contributes to defects in glycolysis and increased fatigability in muscular dystrophy. *Hum Mol Genet* 2009;18:3439–51.
- [79] Lilling G, Beitner R. Altered allosteric properties of cytoskeleton-bound phosphofructokinase in muscle from mice with X chromosome-linked muscular dystrophy (mdx). *Biochem Med Metab Biol* 1991;45:319–25.
- [80] Kotelnikova E, Shkrob MA, Pyatnitskiy MA, Ferlini A, Daraselia N. Novel approach to meta-analysis of microarray datasets reveals muscle remodeling-related drug targets and biomarkers in Duchenne muscular dystrophy. *PLoS Comput Biol* 2012;8:e1002365.
- [81] Wegener G, Krause U. Different modes of activating phosphofructokinase, a key regulatory enzyme of glycolysis, in working vertebrate muscle. *Biochem Soc Trans* 2002;30:264–9.
- [82] Wehling M, Spencer MJ, Tidball JG. A nitric oxide synthase transgene ameliorates muscular dystrophy in mdx mice. *J Cell Biol* 2001;155:123–32.
- [83] Galluzzi L, Kepp O, Kroemer G. Mitochondria: master regulators of danger signalling. *Nat Rev Mol Cell Biol* 2012;13:780–8.
- [84] Davis B, Nagy B, Samaha FJ. Duchenne muscular dystrophy: adenosine triphosphate and creatine phosphate content in muscle. *Neurology* 1981;31:916–9.

- [85] Newman RJ, Bore PJ, Chan L, et al. Nuclear magnetic resonance studies of forearm muscle in duchenne dystrophy. *Br Med J (Clin Res Ed)* 1982;284:1072–4.
- [86] Camiña F, Novo-Rodríguez MI, Rodríguez-Segade S, Castro-Gago M. Purine and carnitine metabolism in muscle of patients with Duchenne muscular dystrophy. *Clin Chim Acta* 1995;243:151–64.
- [87] Dunn J, Frostick S, Brown G, Radda G. Energy status of cells lacking dystrophin: an in vivo/in vitro study of mdx mouse skeletal muscle. *Biochim Biophys Acta (BBA) Mol Basis Dis* 1991;1096:115–20.
- [88] Percival JM, Siegel MP, Knowels G, Marcinek DJ. Defects in mitochondrial localization and ATP synthesis in the mdx mouse model of Duchenne muscular dystrophy are not alleviated by PDE5 inhibition. *Hum Mol Genet* 2013;22:153–67.
- [89] Bertorini TE, Palmieri GMA, Griffin J, et al. Chronic allopurinol and adenine therapy in Duchenne muscular dystrophy: effects on muscle function, nucleotide degradation, and muscle ATP and ADP content. *Neurology* 1985;35:61.
- [90] Liang RCR. Studies on mitochondria from dystrophic skeletal muscle of mice. *Biochem Med Metab Biol* 1986;36:172–8.
- [91] Martens M, Jankulovska L, Neymark M, Lee C. Impaired substrate utilization in mitochondria from strain 129 dystrophic mice. *Biochim Biophys Acta (BBA) Bioenerg* 1980;589:190–200.
- [92] Bhattacharya SK, Johnson PL, Thakar JH. Reversal of impaired oxidative phosphorylation and calcium overloading in the in vitro cardiac mitochondria of CHF-146 dystrophic hamsters with hereditary muscular dystrophy. *J Neurol Sci* 1993;120:180–6.
- [93] Faist V, König J, Höger H, Elmadaf I. Decreased mitochondrial oxygen consumption and antioxidant enzyme activities in skeletal muscle of dystrophic mice after low-intensity exercise. *Ann Nutr Metab* 2001;45:58–66.
- [94] Olson E, Vignos P, Woodlock J, Perry T. Oxidative phosphorylation of skeletal muscle in human muscular dystrophy. *J Lab Clin Med* 1968;71:231.
- [95] Shumate JB, Carroll JE, Brooke MH, Choksi RM. Palmitate oxidation in human muscle: comparison to CPT and carnitine. *Muscle Nerve* 1982;5:226–31.
- [96] Griffin J, Williams H, Sang E, Clarke K, Rae C, Nicholson J. Metabolic profiling of genetic disorders: a multitissue <sup>1</sup>H nuclear magnetic resonance spectroscopic and pattern recognition study into dystrophic tissue. *Anal Biochem* 2001;293:16–21.
- [97] Nylen EG, Wroegemann K. Mitochondrial calcium content and oxidative phosphorylation in heart and skeletal muscle of dystrophic mice. *Exp Neurol* 1983;80:69–80.
- [98] Ionășescu V, Luca N, Vuia O. Respiratory control and oxidative phosphorylation in the dystrophic muscle. *Acta Neurol Scand* 1967;43:564–72.
- [99] Chen YW, Zhao P, Borup R, Hoffman EP. Expression profiling in the muscular dystrophies: identification of novel aspects of molecular pathophysiology. *J Cell Biol* 2000;151:1321–36.
- [100] Dudley RW, Khairallah M, Mohammed S, Lands L, Des Rosiers C, Petrof BJ. Dynamic responses of the glutathione system to acute oxidative stress in dystrophic mouse (mdx) muscles. *Am J Physiol-Regul Integr Comp Physiol* 2006;291:R704–10.
- [101] Carberry S, Brinkmeier H, Zhang Y, Winkler CK, Ohlendieck K. Comparative proteomic profiling of soleus, extensor digitorum longus, flexor digitorum brevis and interosseus muscles from the mdx mouse model of Duchenne muscular dystrophy. *Int J Mol Med* 2013;32(3):544–56.
- [102] Matsumura CY, de Oliveira BM, Durbeek J, Marques MJ. Isobaric tagging-based quantification for proteomic analysis: a comparative study of spared and affected muscles from mdx mice at the early phase of dystrophy. *PLoS ONE* 2013;8:e65831.
- [103] Cao A, Macciotta A, Fiorelli G, Mannucci P, Ideo G. Chromatographic and electrophoretic pattern of lactate and malate dehydrogenase in normal human adult and foetal muscle and in muscle of patients affected by Duchenne muscular dystrophy. *Enzymol Biol Clin* 1965;7:156–66.
- [104] Gellerich FN, Gizatullina Z, Trumbeckaitė S, et al. The regulation of OXPHOS by extra mitochondrial calcium. *Biochim Biophys Acta (BBA) Bioenerg* 2010;1797:1018–27.
- [105] P.R. Turner, T. Westwood, C.M. Regen, R.A. Steinhardt, Increased protein degradation results from elevated free calcium levels found in muscle from mdx mice, 1988.
- [106] MAclennan PA, McArdle A, Edwards R. Effects of calcium on protein turnover of incubated muscles from mdx mice. *Am J Physiol Endocrinol Metab* 1991;260:594–8.
- [107] Kämper A, Rodemann H. Alterations of protein degradation and 2-D protein pattern in muscle cells of MDX and DMD origin. *Biochem Biophys Res Commun* 1992;189:1484–90.
- [108] Hopf F, Turner P, Denetclaw W, Reddy P, Steinhardt R. A critical evaluation of resting intracellular free calcium regulation in dystrophic mdx muscle. *Am J Physiol Cell Physiol* 1996;271:C1325–39.
- [109] Bakker A, Head S, Williams D, Stephenson D. Ca<sup>2+</sup> levels in myotubes grown from the skeletal muscle of dystrophic (mdx) and normal mice. *J Physiol* 1993;460:1–13.
- [110] Pauly M, Daussin F, Burelle Y, et al. AMPK activation stimulates autophagy and ameliorates muscular dystrophy in the mdx mouse diaphragm. *Am J Pathol* 2012;181:583–92.
- [111] Schuh RA, Jackson KC, Khairallah RJ, Ward CW, Spangenburg EE. Measuring mitochondrial respiration in intact single muscle fibers. *Am J Physiol Regul Integr Comp Physiol* 2012;302:R712–9.
- [112] Passaquini AC, Renard M, Kay L, et al. Creatine supplementation reduces skeletal muscle degeneration and enhances mitochondrial function in *mdx* mice. *Neuromuscul Disord* 2002;12:174–82.
- [113] Wroegemann K, Jacobson B, Blanchaer M. On the mechanism of a calcium-associated defect of oxidative phosphorylation in progressive muscular dystrophy. *Arch Biochem Biophys* 1973;159:267.
- [114] Godin R, Daussin F, Matecki S, Li T, Petrof BJ, Burelle Y. PGC1 $\alpha$  gene transfer restores mitochondrial biomass and improves mitochondrial calcium handling in post-necrotic mdx MOUSE skeletal muscle. *J Physiol* 2012;590(21):5487–502.
- [115] Jahnke VE, Van Der Meulen JH, Johnston HK, et al. Metabolic remodeling agents show beneficial effects in the dystrophin-deficient mdx mouse model. *Skeletal Muscle* 2012;2:16.
- [116] Jato-Rodríguez J, Hudson A, Strickland K. Activities of enzymes of the citric acid cycle and electron transport chain in the skeletal muscle of normal and dystrophic mice (strain 129). *Enzyme* 1972;13:286.
- [117] Kemp G, Taylor D, Dunn J, Frostick S, Radda G. Cellular energetics of dystrophic muscle. *J Neurol Sci* 1993;116:201–6.
- [118] Kadenbach B. Intrinsic and extrinsic uncoupling of oxidative phosphorylation. *Biochim Biophys Acta (BBA) Bioenerg* 2003;1604:77–94.
- [119] Stein LR, Imai S-I. The dynamic regulation of NAD metabolism in mitochondria. *Trends Endocrinol Metab* 2012;23:420–8.
- [120] Hood DA. Invited review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J Appl Physiol* 2001;90:1137–57.
- [121] Watkins SC, Cullen MJ. A qualitative and quantitative study of the ultrastructure of regenerating muscle fibres in Duchenne muscular dystrophy and polymyositis. *J Neurol Sci* 1987;82:181–92.
- [122] Mastaglia F, Papadimitriou J, Kakulas B. Regeneration of muscle in Duchenne muscular dystrophy: an electron microscope study. *J Neurol Sci* 1970;11:425–44.
- [123] Cullen M, Fulthorpe J. Stages in fibre breakdown in Duchenne muscular dystrophy: an electron-microscopic study. *J Neurol Sci* 1975;24:179–200.
- [124] Cullen M, Jaros E. Ultrastructure of the skeletal muscle in the X chromosome-linked dystrophic (mdx) mouse. *Acta Neuropathol* 1988;77:69–81.
- [125] Tidball JG, Albrecht DE, Lokensgard BE, Spencer MJ. Apoptosis precedes necrosis of dystrophin-deficient muscle. *J Cell Sci* 1995;108:2197–204.
- [126] Afifi AK, Bergman RA, Zellweger H. A possible role for electron microscopy in detection of carriers of Duchenne type muscular dystrophy. *J Neurol Neurosurg Psychiatry* 1973;36:643–50.
- [127] Cogliati S, Frezza C, Soriano Maria E, et al. Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell* 2013;155:160–71.
- [128] Viollet B, Andreelli F, Jorgensen SB, et al. Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. *Biochem Soc Trans* 2003;31:216–9.
- [129] Wang Y, Liang Y, Vanhoutte PM. SIRT1 and AMPK in regulating mammalian senescence: a critical review and a working model. *FEBS Lett* 2011;585:986–94.
- [130] Winder W, Holmes B, Rubink D, Jensen E, Chen M, Holloszy J. Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol* 2000;88:2219–26.
- [131] Webster C, Silberstein L, Hays AP, Blau HM. Fast muscle fibers are preferentially affected in Duchenne muscular dystrophy. *Cell* 1988;52:503–13.
- [132] Ljubicic V, Jasmin BJ. AMP-activated protein kinase at the nexus of therapeutic skeletal muscle plasticity in Duchenne muscular dystrophy. *Trends Mol Med* 2013;19(10):614–62.
- [133] Chakkalakal JV, Stocksley MA, Harrison M-A, et al. Expression of utrophin A mRNA correlates with the oxidative capacity of skeletal muscle fiber types and is regulated by calcineurin/NFAT signaling. *Proc Natl Acad Sci* 2003;100:7791–6.
- [134] Chakkalakal JV, Harrison M-A, Carbonetto S, Chin E, Michel RN, Jasmin BJ. Stimulation of calcineurin signaling attenuates the dystrophic pathology in mdx mice. *Hum Mol Genet* 2004;13:379–88.
- [135] Angus LM, Chakkalakal JV, Méjat A, et al. Calcineurin-NFAT signaling, together with GABP and peroxisome PGC-1 $\alpha$ , drives utrophin gene expression at the neuromuscular junction. *Am J Physiol Cell Physiol* 2005;289:C908–17.
- [136] Chakkalakal JV, Michel SA, Chin ER, Michel RN, Jasmin BJ. Targeted inhibition of Ca<sup>2+</sup>/calmodulin signaling exacerbates the dystrophic phenotype in mdx mouse muscle. *Hum Mol Genet* 2006;15:1423–35.
- [137] Karpatti G, Carpenter S, Prescott S. Small-caliber skeletal muscle fibers do not suffer necrosis in mdx mouse dystrophy. *Muscle Nerve* 1988;11:795–803.
- [138] Ljubicic V, Miura P, Burt M, et al. Chronic AMPK activation evokes the slow, oxidative myogenic program and triggers beneficial adaptations in mdx mouse skeletal muscle. *Hum Mol Genet* 2011;20(17):ddr265.
- [139] Ljubicic V, Khogali S, Renaud J-M, Jasmin BJ. Chronic AMPK stimulation attenuates adaptive signaling in dystrophic skeletal muscle. *Am J Physiol Cell Physiol* 2012;302:C110–21.
- [140] Al-Rewashdy H, Ljubicic V, Lin W, Renaud J-M, Jasmin BJ. Utrophin A is essential in mediating the functional adaptations of mdx mouse muscle following chronic AMPK activation. *Hum Mol Genet* 2014;24(5):1243–55.

- [141] Bueno Júnior CR, Pantaleão LC, Voltarelli VA, Bozi LHM, Brum PC, Zatz M. Combined effect of AMPK/PPAR agonists and exercise training in *mdx* mice functional performance. *PLoS ONE* 2012;7:45699.
- [142] Handschin C, Kobayashi YM, Chin S, Seale P, Campbell KP, Spiegelman BM. PGC-1 $\alpha$  regulates the neuromuscular junction program and ameliorates Duchenne muscular dystrophy. *Genes Dev* 2007;21:770–83.
- [143] Selsby JT, Morine KJ, Pendrak K, Barton ER, Sweeney HL. Rescue of dystrophic skeletal muscle by PGC-1 $\alpha$  involves a fast to slow fiber type shift in the *mdx* mouse. *PLoS ONE* 2012;7:e30063.
- [144] Sandri M. Autophagy in skeletal muscle. *FEBS Lett* 2010;584:1411–6.
- [145] De Palma C, Perrotta C, Pellegrino P, Clementi E, Cervia D. Skeletal muscle homeostasis in Duchenne muscular dystrophy: modulating autophagy as a promising therapeutic strategy. *Front Aging Neurosci* 2014;6.
- [146] Lucas-Heron B. Muscular degeneration in Duchenne's dystrophy may be caused by a mitochondrial defect. *Med Hypotheses* 1995;44:298–300.
- [147] Kelly-Wornden M, Thomas E. Mitochondrial dysfunction in duchenne muscular dystrophy. *Open J Endocr Metab Dis* 2014;2014.
- [148] Wallace DC. Mitochondrial diseases in man and mouse. *Science* 1999;283:1482–8.
- [149] Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci* 1997;94:514–9.
- [150] Lin P-H, Lee S-H, Su C-P, Wei Y-H. Oxidative damage to mitochondrial DNA in atrial muscle of patients with atrial fibrillation. *Free Radical Biol Med* 2003;35:1310–8.
- [151] DiMauro S, Davidzon G. Mitochondrial DNA and disease. *Ann Med* 2005;37:222–32.
- [152] Bonilla E, Schmidt B, Samitt CE, et al. Normal and dystrophin-deficient muscle fibers in carriers of the gene for Duchenne muscular dystrophy. *Am J Pathol* 1988;133:440.
- [153] Barbiroli B, Funicello R, Ferlini A, Montagna P, Zaniol P. Muscle energy metabolism in female DMD/BMD carriers: a <sup>31</sup>P-MR spectroscopy study. *Muscle Nerve* 1992;15:344–8.
- [154] Barbiroli B, Funicello R, Iotti S, Montagna P, Ferlini A, Zaniol P. <sup>31</sup>P-NMR spectroscopy of skeletal muscle in Becker dystrophy and DMD/BMD carriers: altered rate of phosphate transport. *J Neurol Sci* 1992;109:188–95.
- [155] Stephens J, Lewin E. Serum enzyme variations and histological abnormalities in the carrier state in Duchenne dystrophy. *J Neurol Neurosurg Psychiatry* 1965;28:104.
- [156] Thomson W. The biochemical identification of the carrier state in X-linked recessive (Duchenne) muscular dystrophy. *Clin Chim Acta Int J Clin Chem* 1969;26:207.
- [157] Wong LJC, Wladyka C, Mardach-Verdon R. A mitochondrial DNA mutation in a patient with an extensive family history of Duchenne muscular dystrophy. *Muscle Nerve* 2004;30:118–22.
- [158] Hiona A, Leeuwenburgh C. The role of mitochondrial DNA mutations in aging and sarcopenia: implications for the mitochondrial vicious cycle theory of aging. *Exp Gerontol* 2008;43:24–33.
- [159] Dirks AJ, Hofer T, Marzetti E, Pahor M, Leeuwenburgh C. Mitochondrial DNA mutations, energy metabolism and apoptosis in aging muscle. *Ageing Res Rev* 2006;5:179–95.
- [160] Desler C, Hansen TL, Frederiksen JB, Marcker ML, Singh KK, Juel Rasmussen L. Is there a link between mitochondrial reserve respiratory capacity and aging? *J Aging Res* 2012;2012.
- [161] Baron D, Magot A, Ramstein G, et al. Immune response and mitochondrial metabolism are commonly deregulated in DMD and aging skeletal muscle. *PLoS ONE* 2011;6:e26952.
- [162] Klamut H, Zubrzycka-Gaarn E, Bulman D, et al. Myogenic regulation of dystrophin gene expression. *Br Med Bull* 1989;45:681–702.
- [163] Romero NB, De Lonlay P, Lléns S, et al. Pseudo-metabolic presentation in a Duchenne muscular dystrophy symptomatic carrier with 'de novo' duplication of dystrophin gene. *Neuromuscul Disord* 2001;11:494–8.
- [164] Veerapandiyam A, Shashi V, Jiang YH, Gallentine WB, Schoch K, Smith EC. Pseudometabolic presentation of dystrophinopathy due to a missense mutation. *Muscle Nerve* 2010;42:975–9.
- [165] Lam C, Lau C, Williams J, Chan Y, Wong L. Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) triggered by valproate therapy. *Eur J Pediatr* 1997;156:562–4.
- [166] Wong LC, Perng C, Hsu C, et al. Compensatory amplification of mtDNA in a patient with a novel deletion/duplication and high mutant load. *J Med Genet* 2003;40. e125–e125.
- [167] Aartsma-Rus A, Van Deutekom JC, Fokkema IF, Van Ommen GJB, Den Dunnen JT. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve* 2006;34:135–44.
- [168] Zucconi E, Valadares MC, Vieira NM, et al. Ringo: discordance between the molecular and clinical manifestation in a golden retriever muscular dystrophy dog. *Neuromuscul Disord* 2010;20:64–70.
- [169] Zatz M, Vieira NM, Zucconi E, et al. A normal life without muscle dystrophin. *Neuromuscul Disord* 2015;25(5):371–4.
- [170] Vieira NM, Guo LT, Estrela E, Kunkel LM, Zatz M, Shelton GD. Muscular dystrophy in a family of Labrador retrievers with no muscle dystrophin and a mild phenotype. *Neuromuscul Disord* 2015;25(5):363–70.
- [171] Zatz M, Pavanello R, Lazar M, et al. Milder course in Duchenne patients with nonsense mutations and no muscle dystrophin. *Neuromuscul Disord* 2014;24:986–9.
- [172] Dubowitz V. Enigmatic conflict of clinical and molecular diagnosis in Duchenne/Becker muscular dystrophy. *Neuromuscul Disord* 2006;16:865–6.
- [173] M. Castro-Gago, Milder course in Duchenne patients with nonsense mutations and no, 2015.
- [174] Thomson WHS, Smith I. X-linked recessive (Duchenne) muscular dystrophy (DMD) and purine metabolism: effects of oral allopurinol and adenylate. *Metabolism* 1978;27:151–63.
- [175] Castro-Gago M, Lojo S, Novo I, Del Río R, Peña J, Rodríguez-Segade S. Effects of chronic allopurinol therapy on purine metabolism in Duchenne muscular dystrophy. *Biochem Biophys Res Commun* 1987;147:152–7.
- [176] Thomson W, Smith I. Allopurinol in Duchenne's muscular dystrophy. *N Engl J Med* 1978;299:101.
- [177] Kulakowski S, Renoirte P, de Bruyn C. Dynamometric and biochemical observations in Duchenne patients receiving allopurinol. *Neuropediatrics* 1981;12:92.
- [178] Felber S, Skladal D, Wyss M, Kremser C, Koller A, Sperl W. Oral creatine supplementation in Duchenne muscular dystrophy: a clinical and <sup>31</sup>P magnetic resonance spectroscopy study. *Neurol Res* 2000;22:145.
- [179] Tarnopolsky M, Martin J. Creatine monohydrate increases strength in patients with neuromuscular disease. *Neurology* 1999;52:854–854.
- [180] Tarnopolsky M, Mahoney D, Vajsar J, et al. Creatine monohydrate enhances strength and body composition in Duchenne muscular dystrophy. *Neurology* 2004;62:1771–7.
- [181] Banerjee B, Sharma U, Balasubramanian K, Kalaivani M, Kalra V, Jagannathan NR. Effect of creatine monohydrate in improving cellular energetics and muscle strength in ambulatory Duchenne muscular dystrophy patients: a randomized, placebo-controlled <sup>31</sup>P MRS study. *Magn Reson Imaging* 2010;28:698–707.
- [182] Bonsett CA. *Studies of Pseudohypertrophic Muscular Dystrophy*. Springfield, Ill: Thomas; 1969.
- [183] Sharma U, Atri S, Sharma M, Sarkar C, Jagannathan N. Skeletal muscle metabolism in Duchenne muscular dystrophy (DMD): An in-vitro proton NMR spectroscopy study. *Magn Reson Imaging* 2003;21:145–53.
- [184] Nishio H, Wada H, Matsuo T, Horikawa H, Takahashi K, Nakajima T, et al. Glucose, free fatty acid and ketone body metabolism in Duchenne muscular dystrophy. *Brain Dev* 1990;12:390–402.
- [185] Hankard RG, Hammond D, Haymond MW, Darmaun D. Oral glutamine slows down whole body protein breakdown in Duchenne muscular dystrophy. *Pediatr Res* 1998;43:222–6.
- [186] Hankard R. Duchenne muscular dystrophy: A model for studying the contribution of muscle to energy and protein metabolism. *Reprod Nutr Dev* 1998;38:181–6.
- [187] Ellis DS, Strickland JM. Differences in the metabolism of glucose between normal and dystrophic human muscle. *Biochem J* 1972;130:17–8.
- [188] Thomson WHS, Leyburn P, Walton JN. Serum enzyme activity in muscular dystrophy. *Brit Med J* 1960;1276–81.
- [189] Zatz M, Rapaport D, Vainzof M, Passos-Bueno MR, Bortolini ER, Pavanello RCM, et al. Serum creatine-kinase (CK) and pyruvate-kinase (PK) activities in Duchenne (DMD) as compared with Becker (BMD) muscular dystrophy. *J Neurol Sci* 1991;102:190–6.
- [190] Ellis D. Intermediary metabolism of muscle in Duchenne muscular dystrophy. *Brit Med Bull* 1980;36:165–72.
- [191] Stapleton DI, Lau X, Flores M, Trieu J, Gehrig SM, Chee A, et al. Dysfunctional muscle and liver glycogen metabolism in *mdx* dystrophic mice. *PLoS ONE* 2014;9:e91514.
- [192] Mastaglia F, Kakulas B. Regeneration in Duchenne muscular dystrophy: A histological and histochemical study. *Brain* 1969;92:809–18.
- [193] Engel A. Duchenne dystrophy. In: Engel A, Banker BQ, editors. *Myology*. McGraw-Hill: New York; 1986. p. 1185–240.
- [194] Ronzoni E, Berg L, Landau W. Enzyme studies in progressive muscular dystrophy. *Res. Publ. Ass. Nerv. Ment. Dis.* 1960;38:721–9.
- [195] Petell JK, Marshall NA, Leberz HG. Content and synthesis of several abundant glycolytic enzymes in skeletal muscles of normal and dystrophic mice. *Int J Biochem* 1984;16:61–7.
- [196] Lin CH, Hudson AJ, Strickland KP. Fatty acid oxidation by skeletal muscle mitochondria in Duchenne muscular dystrophy. *Life Sci* 1972;11:355–62.
- [197] Carroll JE, Norris BJ, Brooke MH. Defective [U-14 C] palmitic acid oxidation in Duchenne muscular dystrophy. *Neurology* 1985;35:96–7.
- [198] Scholte H, Luyt-Houwen I, Busch H, Jennekens F. Muscle mitochondria from patients with Duchenne muscular dystrophy have a normal beta oxidation, but an impaired oxidative phosphorylation. *Neurology* 1985;35:1396–7.
- [199] Borum PR, Broquist HP, Roelofs RI. Muscle carnitine levels in neuromuscular disease. *J Neurol Sci* 1977;34:279–86.
- [200] Carrier HN, Berthillier G. Carnitine levels in normal children and adults and in patients with diseased muscle. *Muscle Nerve* 1980;3:326–34.
- [201] Carroll JE, Villadiego A, Brooke MH. Increased long chain acyl CoA in Duchenne muscular dystrophy. *Neurology* 1983;33:1507–10.
- [202] Le Borgne F, Guyot S, Logerot M, Beney L, Gervais P, Demarquois J. Exploration of lipid metabolism in relation with plasma membrane properties of Duchenne muscular dystrophy cells: Influence of L-carnitine. *PLoS ONE* 2012;7:e49346.
- [203] Griffiths RD, Cady EB, Edwards RHT, Wilkie DR. Muscle energy metabolism in Duchenne dystrophy studied by <sup>31</sup>P-NMR: Controlled trials show no effect of allopurinol or ribose. *Muscle Nerve* 1985;8:760–7.

- [204] Pulido S, Passaquin A, Leijendekker W, Challet C, Wallimann T, Rüegg U. Creatine supplementation improves intracellular  $\text{Ca}^{2+}$  handling and survival in *mdx* skeletal muscle cells. *FEBS Lett* 1998;439:357–62.
- [205] Fitch CD, Rahmanian M. Creatine entry into skeletal muscle of normal and of dystrophic mice. In: *Proceedings of the society for experimental biology and medicine*. Society for experimental biology and medicine (New York, NY). Royal Society of Medicine; 1969. p. 236–9.
- [206] Martins-Bach AB, Bloise AC, Vainzof M, Rahnamaye Rabbani S. Metabolic profile of dystrophic *mdx* mouse muscles analyzed with in vitro magnetic resonance spectroscopy (MRS). *Magn Reson Imaging* 2012;30:1167–76.
- [207] Louis M, Raymackers JM, Debaix H, Lebacqz J, Francaux M. Effect of creatine supplementation on skeletal muscle of *mdx* mice. *Muscle Nerve* 2004;29:687–92.
- [208] Ionasescu R, Kaeding L, Feld R, Witte D, Cancilla P, Stern LZ. Alterations in creatine kinase in fresh muscle and cell cultures in Duchenne dystrophy. *Ann Neurol* 1981;9:394–9.
- [209] Younkin DP, Berman P, Sladky J, Chee C, Bank W, Chance B.  $^3\text{P}$  NMR studies in Duchenne muscular dystrophy. *Neurology* 1987;37:165.
- [210] Benedict JD, Kalinsky HJ, Scarrone LA, Wertheim AR, Stetten Jr D. The origin of urinary creatine in progressive muscular dystrophy. *J Clin Invest* 1955;34:141.
- [211] Bertorini TE, Palmieri GMA, Airozo D, Edwards NL, Fox IH. Increased adenine nucleotide turnover in Duchenne muscular dystrophy. *Pediatr Res* 1981;15:1478–82.
- [212] de Bruyn C, Kulakowski S, van Bennekom C, Renoirte P, Müller M. Purine metabolism in Duchenne muscular dystrophy. *Adv Exp Med Biol* 1980;122:177.