AEROBIC EXERCISE TRAINING IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1

AEROBIC EXERCISE INDUCED FUNCTIONAL AND CELLULAR ADAPTATIONS IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1

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Lay Abstract

Myotonic dystrophy type 1 (DM1) is a genetic disorder that affects 1 in 8000 individuals. It primarily affects skeletal muscle, the heart and breathing resulting in a poor quality of life and a shortened lifespan in the persons diagnosed. There is currently no cure or treatment to help mitigate the symptoms of the disease. Aerobic exercise training is an easy and safe lifestyle intervention that has shown to improve fundamental symptoms of DM1 in mice. However, limited number of studies have examined the effects of aerobic training in patients diagnosed with DM1. Therefore, the purpose of this thesis was to examine the benefits of 12-weeks of cycling on function and strength of DM1 patients and to understand the molecular adaptations in skeletal muscle. Our observations demonstrate that cycling safely improved function, respiratory measures and increase muscle mass in a muscle wasting disease. Additionally, exercise induced changes in protein expression that are important for muscle health. Collectively, we demonstrate that exercise training is an efficacious and safe intervention that could help improve the health of patients with DM1.

Abstract

Myotonic dystrophy type 1 (DM1) is the most common adult muscular dystrophy affecting ~1/8000 people worldwide. DM1 is characterized by accelerated skeletal muscle weakness and wasting, myotonia and insulin resistance, ultimately causing impaired function and diminished quality of life. A trinucleotide (CTG) repeat expansion in the 3' region upstream of the *DMPK* gene results in dysregulation of several RNA binding proteins (RNABPs) important for muscle health such as MBNL and CUGBP1. Exercise was shown to ameliorate DM1 pathology in mice and to be safe for DM1 patients. This thesis aimed to investigate the muscular adaptations of 12-weeks of aerobic exercise in DM1 patients. Eleven DM1 patients (DM1, 42.6 ± 3 y) were recruited from the Neuromuscular and Neurometabolic clinic at McMaster University and age matched to healthy controls (CON, 42.5 ± 2 y). DM1 and CON performed incremental VO_{2peak} testing, muscle and spirometry functional tests and a skeletal muscle biopsy from the *Vastus lateralis*. After 12-weeks of training on a cycle ergometer (3x/wk @ ~65 %VO_{2peak}), DM1 patients completed post-testing. Exercise training significantly increased total lean mass (TLM) by ~ 1.6 kg ($p<0.05$) and fibre cross-sectional area by ~30 % in DM1 patients. Aerobic fitness was enhanced following training from 19.7 ± 1.5 mL/kg/min to 26.0 ± 2.1 mL/kg/min ($p<0.05$). Furthermore, training improved 6-min walk test, timed up & go, and 5X sit-to-stand scores ($p<0.05$). Mechanistically, exercise modestly altered expression of RNABPs, and augmented mitochondrial function and protein content. This is the first study to comprehensively investigate the effects of aerobic training on muscle health and function in DM1. Our data provides evidence that exercise training can augment fitness, functional capacity and muscle mass in DM1. Further understanding the influence of exercise on DM1 pathology could outline the efficacy of a simple life intervention and provide insight for future pharmacological discoveries for DM1.

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List of Abbreviations

AICAR – 5-aminoimidazole-4- carboxamide-1-β-D-ribofuranoside

ALS – Amyotrophic lateral sclerosis

AMPK – AMP activated protein kinase

ASO – Antisense oligonucleotides

BIN1 – Bridging integrator 1

CDK4 – Cyclin D3-dependent kinase 4

CDM – Congenital myotonic dystrophy type 1

CLCN1 – Skeletal muscle chloride voltage-gated channel 1

CoQ10 – Coenzyme Q10

CRISPR/Cas9 – Clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9

cTNT – Cardiac troponin T

CUGBP1 – CUG binding protein Elav-like family member 1

DM1 – Myotonic dystrophy type 1

DM2 – Myotonic dystrophy type 2

DMD – Duchenne muscular dystrophy

DMPK – Dystrophia myotonica protein kinase

DTNA – Dystrobrevin alpha

GSK3β – Glycogen synthase kinase 3 beta

IRα – Insulin receptor alpha subunit

IRβ – Insulin receptor beta subunit

IR-A – Insulin receptor alpha subunit isoform A

IR-B – Insulin receptor alpha subunit isoform B

MBNL1 – Muscleblind like protein 1

MBNL2 – Muscleblind like protein 2

mtDNA – Mitochondrial encoded DNA

NMD – Neuromuscular disorder

OMM – Outer mitochondrial membrane

OPA1 – Mitochondrial dynamin like GTPase OPA1

PCR – Polymerase chain reaction

PKC – Protein kinase C

PROMM – Proximal myotonic myopathy

RNABP – RNA binding protein

ROS – Reactive oxygen species

SIX5 – SIX homeobox 5

SMA – Spinal muscular atrophy

SOS1 – SOS Ras/Rac guanine nucleotide exchange factor 1

SRC – Proto-oncogene tyrosine protein kinase

UTR – Untranslated region

VO_{2peak} – Maximal cardiorespiratory fitness

ZNF9 – Zing finger 9

Review of the Literature

INTRODUCTION

Neuromuscular disorders (NMDs) are characterized by a defect in the lower motor neurons (including the anterior horn cells), neuromuscular junction or skeletal muscle (Mcdonald 2012). There are over 500 genetic mutations that can be directly linked to NMDs such as spinal muscular atrophy (SMA), Duchenne muscular dystrophy (DMD), myotonic dystrophy type 1 and 2 (DM1/DM2) (Mcdonald 2012). However, several NMDs do not have a genetic origin and are acquired such as amyotrophic lateral sclerosis (ALS), dermatomyositis and autoimmune myasthenia gravis. Symptoms associated with an NMD may be difficult to precisely isolate to a single anatomical region of the peripheral never, NMJ or muscle. Several NMDs present with multisystemic involvement often affecting skeletal muscle, the heart and the brain; however, several systemic defects are disease specific (Mcdonald 2012). Due to the heterogeneity of NMDs, age of onset can vary from infancy to late adulthood. Children with NMDs will often have delayed motor milestones, hypotonia, gait abnormalities or respiratory defects whereas adults will often complain of accelerated muscle weakness, poor balance, extreme fatigue and/or difficulties with speech and swallowing.

DM1 is the most common adult onset muscular dystrophy and second most common muscular dystrophy after DMD (Chau and Kalsotra 2015). It is an autosomal dominant disorder affecting ~1/600 people in parts of Quebec and ~1/8000 people worldwide. DM1 is caused by a microsatellite 'CTG' DNA repeat expansion in the 3' untranslated region (UTR) of the Dystrophia Myotonica Protein Kinase (*DMPK*) gene that is found on chromosome 19q13.3 (Brook et al. 1992; Mahadevan et al. 1992). Several theories are proposed as to how the trinucleotide repeat expansion results in the molecular pathophysiology of DM1. Through a toxic gain of function mechanism, accumulation of *DMPK* transcripts result in an imbalance of critical RNA binding proteins

(RNABPs) disrupting regular mRNA splicing processes (Davis et al. 1997; Taneja et al. 1995). Although RNABPs dysregulation is the primary hypothesis behind DM1 pathology, other theories focus on the loss of function of the DMPK protein and neighboring genes at the locus of the DM1 mutation (Klesert et al. 1997; Thornton et al. 1997). Clinical features commonly seen in patients with DM1 are multisystemic in nature and include; skeletal muscle loss and weakness, myotonia, hypersomnolence, cognitive impairment, premature cataracts, frontal balding, cardiac arrhythmias and respiratory difficulties (Thornton 2014). Ultimately, this results in impaired physical function, poor quality of life, and a significantly reduced lifespan.

There is no cure or specific treatment for DM1. Several strategies are being tested at the pre-clinical and clinical stages to directly target *DMPK,* some of which include antisense oligonucleotides (ASOs), small molecules and gene editing. Other strategies focus on mitigating disease progression and severity. Exercise training has been shown to elicit positive health and functional outcomes in several neuromuscular disorders. Recent evidence shows that resistance exercise can effectively reduce skeletal muscle loss in DM1 patients (M. P. Roussel, Hébert, and Duchesne 2020; M. P. Roussel et al. 2019), while aerobic exercise improves overall muscle function in DM1 murine models (Manta et al. 2019; Sharp, Cox, and Cooper 2019). However, there is contrasting evidence to whether exercise could improve muscle function in human DM1 patients and the underlying mechanism of how training could mitigate DM1 pathogenesis remains unknown.

This review will (1) summarize the genetic factors and primary hypothesis that influence DM1 severity and pathology, (2) discuss emerging therapies developed to treat DM1, and (3) outline the evidence behind exercise training as an effective therapy and the potential physiological pathways through which it could mitigate DM1 pathophysiology.

MYOTONIC DYSTROPHY: DM1 VS DM2

Myotonic dystrophy (also known as Steinert's disease) was initially described by Hans Steinert in 1909 with patients presenting with muscle weakness and myotonia during adulthood. Following its discovery, several clinical studies observed a dominant inheritance and wide multisystemic involvement including skeletal, cardiac and smooth muscle, as well as the eyes, brain, respiratory and gastrointestinal systems (Cho and Tapscott 2007). After the 1992 discovery of the 'CTG' repeat expansion in the *DMPK* locus, it became clear that a subpopulation of patients presented with the DM phenotype but lacked the 'CTG' mutation, or any mutation related to other myotonic disorders. Furthermore, these patients exhibited an early pattern of proximal limb muscle weakness; whereas, DM1 patients typically present with weakness of the distal muscles. Therefore, the terms DM2 or proximal myotonic myopathy (PROMM) were proposed for patients exhibiting these characteristics. It wasn't until 1998 that DM2 was linked to a 'CCTG' tetranucleotide repeat expansion in the intronic region of the Zinc Finger 9 (*ZNF9*) gene (Liquori et al. 2001).

As previously mentioned, the estimated prevalence of DM1 is ~1/8000 people, however, there are limited epidemiological studies on DM2. As a result, prevalence rates are not agreed upon but are estimated to be fewer than in DM1. Nucleotide repeat lengths vary largely in the general population, ranging anywhere from 5 to 37 repeats and 10 to 33 repeats for *DMPK* and *ZNF9* genes, respectively (Brook et al. 1992; Liquori et al. 2001). DM1 symptoms begin to manifest at a minimum 50 repeats, and some can reach upwards of 3000 'CTG' repeats in the most severe prognoses. As for DM2, majority of patients have more than 3,500 'CCTG' repeats. It is common in DM1 families to see subsequent generations with more severe symptoms and earlier onset as a result of rapid amplifications of 'CTG' microsatellite mutation, a phenomenon known as anticipation (Redman et al. 1993). This, in part, gives rise to different types of DM1 including

congenital, child-onset, and adult-onset DM1. In contrast to DM1, intergenerational increases in repeat size are not seen in DM2 (Day et al. 2003) and therefore onset of symptoms generally occurs generation to generation during teenage or adulthood years.

CLINICAL PROPERTIES OF DM1

DM1 presents with a wide spectrum of clinical characteristics varying from fetal death to mild late-onset, with disease severity positively correlated with the number of 'CTG' repeats. Those with 50-100 repeats exhibit mild symptoms and may go undiagnosed; whereas, greater than 1000 repeats are commonly seen in the incredibly severe, congenital form of DM1. However, the correlation is not particularly accurate for repeat lengths in between both extremes and therefore cannot be used to accurately predict future disease severity. The large variability in repeat lengths, unpredictable instability and intergenerational anticipation results in the different types of DM1 with varying clinical presentation.

Congenital DM1 (CDM) is the most severe phenotype and accounts for approximately 15% of DM1 patients. There is a strong bias for maternal inheritance over paternal, and the majority of congenital DM1 cases have over 1000 CTG repeats in the 3' UTR of the *DMPK* locus (Morales et al. 2015). Prenatally, a CDM fetus has reduced movement and delayed development. After birth, they present with low muscle tone and experience feeding or respiratory difficulties resulting in majority of cases requiring ventilatory support. Around 1 in 5 CDM cases result in neonatal death; whereas, the remaining patients develop into their childhood years commonly experiencing cognitive and learning disabilities, and delayed development of motor milestones (Moxley, Ciafaloni, and Guntrum 2015).

Childhood DM1 is the least common of all DM1 types, accounting for less than 10% of total DM1 cases. Unlike CDM, childhood DM1 is passed on either maternally or paternally. Symptoms usually manifest between 1 and 10 years of age and are largely psychiatric in nature (Thornton 2014). Children often experience cognitive impairments, excessive somnolence, mood disorders and deficits in attention span. Over time, patients may develop classical DM1 features in teenage and adulthood years.

Adult/Classical DM1 patients begin to experience symptoms during or after their second decade and account for majority of DM1 patients. Myotonia of the hands, tongue and jaw muscles are usually the initial features of classical DM1. Patients also experience accelerated weakness and wasting of limb muscles that presents in a distal to proximal pattern. Weakness in the plantar and dorsiflexors results in balance impairments and increased fall risk while weakness in the diaphragm and other muscles involved in breathing result in respiratory failure being the leading cause of death in adult onset DM1. Prolonged PR and QRS intervals are seen in approximately two-thirds of classical DM1, which causes heart block and is the second most common cause of death (Thijs et al. 1998). Other symptoms include premature cataracts, gastrointestinal defects, daytime sleepiness and increased risk of cancer (Moxley, Ciafaloni, and Guntrum 2015).

VARIABILITY IN DM1: 'CTG' REPEAT INSTABILITY AND GENETIC MODIFIERS

Despite a modest correlation between repeat size and disease severity, predicting future disease progression still remains difficult. The 'CTG' repetitive sequence is highly unstable and contributes to the variable nature of the disease. Instability of 'CTG' repeat sequence results in constant generation of new alleles with varying lengths that are bias towards expansion (Redman et al. 1993; De Temmerman et al. 2004). Thus, offspring of affected parents are more likely to

have larger mutations, earlier onset of the disease, and to be phenotypically more severe. Furthermore, intergenerational anticipation is influenced by the sex and repeat size of the transmitting parent. Paternal transmission coincides with expansions that result in a more severe phenotype of classical DM1 and have a greater frequency of repeat contraction (~10 %) (Ashizawa et al. 1994). However, affected mothers with as low as 80 to 250 CTG repeats are still at high risk of CDM transmission (Morales et al. 2015). Furthermore, only 3 % of cases through maternal transmission demonstrate contraction within the repeat tract (Ashizawa et al. 1994). Studies examining repeat lengths show expansions at the level of the spermatozoa and immature oocytes of DM1 patients suggesting instability during DNA repair and/or during germline cell division (G. Jansen et al. 1994; De Temmerman et al. 2004). Further analysis of the 'CTG' expansions in spermatogonia of DM1 mouse models elucidates that repeat amplification occurs prior to the meiosis process (Savouret et al. 2004). Aside from intergenerational anticipation, 'CTG' repeats also show a pattern of somatic instability where the 'CTG' sequence expands at a different rate in different tissues throughout a person's life (Thornton, Johnson, and Moxley 1994). Interestingly, 'CTG' repeats exhibits a greater degree of instability in non-dividing cells than dividing cells. For example, repeats in muscle, heart and brain are 3-25X larger than repeats measured in hematopoietic cells (Masayuki Nakamori et al. 2013). Therefore, 'CTG' repeats of blood DNA are a better indication of the inherited mutation size.

Several different factors have been suggested to influence the stability of the DM1 mutation. A small portion of DM1 patients (4-9 %) present with an interruption in their 'CTG' expanded allele. CGG, CCG, CTC and CAG interruptions all have a stabilizing effect on the repeat tract further influencing disease onset and progression (Tomé et al. 2018; Santoro et al. 2017). Most frequently CCG and CGG interruptions have been shown to reduce anticipation and develop

milder symptoms with a later onset (Braida et al. 2010). However, many aspects remain unknown such as the number of interruptions needed for a meaningful improvement in phenotype, the location of interruptions and the types. Epigenetic modifiers have also shown correlations with repeat stability and disease severity. Specifically, abnormal methylation levels around the *DMPK* locus has been implicated in influencing CDM transmission and DM1 symptom severity (Barbé et al. 2017; Légaré et al. 2019). A study by Barbé and colleagues measured methylation levels in blood samples of several DM1 patients. Interestingly, 95 % of maternally transmitted CDM patients showed increased methylation upstream of *DMPK* while only 9% of adult DM1 cases displayed little to no methylation despite a great overlap in 'CTG' repeat number (Barbé et al. 2017). Furthermore, no upstream methylation was found in paternally transmitted DM1 cases. Collectively, this suggests that several factors other than 'CTG' repeat length contribute to 'CTG' repeat instability and disease severity.

PATHOGENESIS OF DM1

Initial hypothesis: role of a novel kinase (DMPK) and adjacent proteins

Early focus was on the newly discovered DMPK protein, where the DM1 mutation lies, as the primary cause of pathogenesis. DMPK functions as a serine/threonine kinase and is ubiquitously expressed in different tissue types (Dunne, Walch, and Epstein 1994; Timchenko et al. 1995). However, its most highly expressed in cardiac and skeletal muscle in healthy individuals making it a prime candidate for elucidating DM1 pathology (Whiting et al. 1995; Lam et al. 2000). Immunoblotting studies in DM1 patients show a lower expression of DMPK in the majority of cases; however, some patients displayed normal or elevated levels of DMPK protein (Hofmann-Radvanyi and Junien 1994). Transgenic mice with homozygous deletions for *Dmpk* were then

studied to further clarify the role of DMPK in DM1. DMPK null mice developed only a late onset mild myopathy that failed to mimic the severe phenotype seen in patients (Gert Jansen et al. 1996; Reddy et al. 1996). Conversely, mice with heterozygous and homozygous mutations of *Dmpk* acquire cardiac conduction defects that are commonly seen in DM1 patients. This suggests that DMPK deficiency is directly or indirectly involved in cardiac defects in DM1 patients but has little to no involvement in muscle impairments.

The 'CTG' repetitive sequence has been shown to alter chromatin structure around the *DMPK* locus (Otten and Tapscott 1995) making it plausible that expression of neighbouring genes could be compromised. In fact, SIX homeobox 5 (*SIX5*) is located downstream of the 3'UTR of *DMPK* and its promoter region in very close proximity to the DM1 mutation. *In vivo* studies further confirmed this hypothesis showing reduced SIX5 protein expression in DM1 fibroblasts and myoblasts (Klesert et al. 1997; Thornton et al. 1997). Furthermore, SIX5 deficient mice developed pre-mature cataracts recapitulating one of the common symptoms seen in DM1 patients (Klesert et al. 2000; Sarkar et al. 2000). However, SIX5 haploinsufficiency failed to explain other hallmark symptoms such as myotonia, muscle wasting and weakness.

RNA Toxicity

Collectively, the strong similarities between DM1 and DM2 symptoms, despite having different mutations, and failure for DMPK and SIX5 deficiency to account for major features of DM1, are supportive evidence for a more generalized 'CUG' RNA toxicity theory. The resultant *DMPK* transcripts aggregate to form stable, double-stranded hairpin loops that cannot be exported into the cytoplasm but are trapped within the myonuclei. These foci disturb the expression and localization of several RNABPs ultimately resulting in a loss of function or toxic gain of function mechanism. Notably, the muscleblind like protein family (MBNL1-3) are a group of proteins

known to regulate splicing of numerous pre-mature mRNA and play a role in RNA stability. MBNL1 and MBNL2 is widely expressed in different tissues such as skeletal muscle, cardiac muscle, and the brain while MBNL3 is predominantly seen in the placenta. In DM1, MBNLs are sequestered by the 'CUG' foci within the myonuclei resulting in a loss of functional MBNL protein in the cytoplasm and ultimately impaired splicing. In fact, a transgenic knockout of MBNL1 accounts for the majority of mis-splicing events in DM1 (Du et al. 2010), and recapitulated muscle and eye impairments seen in DM1 (Kanadia, Johnstone, et al. 2003). CUG binding protein Elav-like family member 1 (CUGBP1) is another RNABP that displays aberrant function in DM1. Contrary to the name and MBNL, CUGBP1 is not sequestered within the 'CUG' RNA repeats but through hyperphosphorylation of protein kinase-C (PKC) and hyperactivation of glycogen synthase kinase 3 beta (GSK3β), CUGBP1 activity and expression are augmented in DM1 pathology. In contrast to MBNL, CUGBP1 function to promote embryonic isoforms of several genes. Furthermore, overexpression of CUGBP1 in mice further mimics missplicing events and myopathy seen in MBNL1 knockouts and DM1 patients. Interestingly, antagonistic MBNL and CUGBP1 regulation of exon splicing is dependent on binding motifs. Interaction of MBNL and CUGBP1 to the downstream intron results in exon inclusion while binding to the upstream intron results in skipping (Du et al. 2010; Kalsotra et al. 2008). Collectively, alterations in MBNL and CUGBP1 result in increased fetal isoforms of many genes that are unable to meet the functional demands of mature tissue.

Hundreds of missplicing events have been reported in DM1 patients (Masayuki Nakamori et al. 2013; E. T. Wang et al. 2019; Ho et al. 2004); however, this review will focus on genes that have been implicated in the canonical symptoms in DM1. It is important to note that mutation size, in blood or muscle, does not correlate with splicing defects further reiterating the key role of

RNABP dysregulation (Masayuki Nakamori et al. 2013). Myotonia is the inability of a muscle to relax following maximal stimulation and is commonly seen in finger flexors and jaw muscles and is attributed to a lack of functional skeletal muscle chloride voltage-gated channel 1 (CLCN1) protein resulting in poor chloride ions conductance at the level of the sarcolemma. Fetal isoform of *CLCN1* contains exon 7a (intron 2) and its inclusion/exclusion is regulated by both MBNL1 and CUGBP1. Therefore, majority of *CLCN1* transcripts in adult DM1 patients are of the fetal isoform and are dysfunctional due to a premature stop codon. Mice overexpressing CUGBP1 or a deficiency in MBNL1 display abnormal splicing of *Clcn1* and increased myotonic discharges; however, appropriate muscle relaxation is restored with overexpression of MBNL1 in a mouse model of DM1.

Rapid skeletal muscle weakness associated with wasting is one of the most common and initial symptoms than DM1 patients report which has been strongly correlated with aberrant bridging integrator 1 (*BIN1*) splicing. In healthy muscle, correct splicing of *BIN1* is necessary for assembly of transverse tubules (T-tubules) which are crucial for excitation-contraction coupling. DM1 muscle exhibits lower inclusion of exon 11 of *BIN1* impairing T-tubule formation contributing to muscle weakness. Other alternative splicing events have also been discovered and are highly correlated with skeletal muscle weakness, in addition to *BIN1*. Abnormal splicing of dystrobrevin alpha (*DTNA*; exons 11A and 12) and SOS Ras/Rac guanine nucleotide exchange factor 1 (*SOS1*; exon 25) disturb muscle growth pathways further exacerbating the muscle weakness and wasting phenotype (M. Nakamori et al. 2008; Rojas et al. 1999). Insulin resistance is another commonly seen feature in DM1 patients which is primarily regulated by insulin receptors (*IR*). *IR* is made up of two alpha subunits (*IR-*α), which has two isoforms IR-A (- exon 11) and IR-B (+ exon 11), and two beta subunits (*IR-*β) (Moller et al. 1989). In line with the adult

to fetal isoform switch, insulin resistance can be linked to increased abundance of the lower signaling IR-A. Despite similar levels of IR protein expression between DM1 and healthy muscle, DM1 display abnormal glucose clearance and diminished insulin responsiveness which can only be explained by an increased IR-A to IR-B ratio (Savkur, Philips, and Cooper 2001).

Secondary Mitochondrial Myopathy

Considering the strong similarities in characteristics of DM1 and aging such as muscle weakness, cataracts and insulin sensitivity, it is likely that mitochondrial dysfunction could be a contributing factor exacerbating the DM1 phenotype. In fact, several studies have discovered signs of mitochondrial abnormalities such as the presence of ragged-red fibres (Ono et al. 1986; Isashiki et al. 1989), cytochrome *c* oxidase deficient fibres (Isashiki et al. 1989; Sahashi et al. 1992), and reduced activity of key mitochondrial enzymes (Isashiki et al. 1989). Furthermore, deletions in mitochondrial DNA (mtDNA) have been detected in DM patients using polymerase chain reaction (PCR) technique (Sahashi et al. 1992), but not with southern blotting (Vita et al. 1993). It is important to take into consideration that these studies were performed prior to the discovery of the DM1 mutation or genetic testing as a routine clinical practice for diagnosis, and therefore the sample population may also include DM2 patients or patients with other myotonic disorders. Others proposed that mtDNA may play a role in disease severity given the exclusive maternal transmission of CDM; however, this was later found not to be the case (Poulton et al. 1995).

In vivo studies attempted to underpin the role of DMPK protein in mitochondrial function and ROS regulation. The majority (>80%) of DMPK protein was found to be localized at the outer mitochondrial membrane (OMM) with the remaining DMPK protein being expressed evenly in the cytosol and nucleus (Pantic et al. 2013). Moreover, DMPK protein improved buffering of ROS by forming a multimeric complex with hexokinase II and tyrosine protein kinase (SRC) at the

OMM (Pantic et al. 2013). Therefore, DMPK insufficiency could, in part, explain the higher levels of markers of lipid peroxidation, reactive oxygen species (ROS) and blunted abundance of antioxidants seen in DM1 patient samples (Ihara et al. 1995; Siciliano et al. 2001; Toscano et al. 2005; Kumar et al. 2014). Furthermore, plasma coenzyme Q10 (CoQ10) concentration was significantly lower in DM1 patients, as compared to healthy individuals (Ihara et al. 1995; Siciliano et al. 2001). CoQ10 concentration was negatively correlated with 'CTG' repeat length signifying a repeat depending influence in CoQ10 deficiency (Siciliano et al. 2001).

García-Puga and colleagues recently assessed mitochondrial health in patient derived fibroblasts (García-puga et al. 2020). They showed that DM1 fibroblast cells had low ATP production, abnormalities in mitochondrial fusion and fission, impaired metabolism and increase ROS production (García-puga et al. 2020). Interestingly, abnormal splicing of exon 4b of the mitochondrial fusion related protein OPA1 is present in DM1 muscle and shows a moderate correlation with muscle impairment (Masayuki Nakamori et al. 2013). These results further emphasize the interplay between DM1 pathology and mitochondrial dysfunction. Given the current literature, there is a need for further investigations of the link between mitochondrial oxygen consumption, dynamics and ROS regulation in DM1 associated muscle dysfunction.

CURRENT TREATMENTS AND POTENTIAL THERAPY

There is no current treatment that can profoundly alter DM1 progression. As a result, standard care is primarily based on mitigating symptoms. Regular testing of forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) are important for detecting respiratory impairments. Furthermore, electrocardiograms are routine for the early diagnosis of heart blocks. Screening for cataracts, type 2 diabetes, and endocrinopathies (especially hypogonadism) are usually done yearly. Some patients are prescribed mexiletine for the anti-myotonia effects and

others with hypersomnolence take modafinil (MacDonald, Hill, and Tarnopolsky 2002). With further disease progression and muscle loss, patients may need ankle-foot orthotics or other gait assistive devices in order to reduce fall risk, injury and improve mobility.

In the past two decades, several innovative technologies have shown promising evidence in pre-clinical models of DM1 with a select number currently being tested in clinical trials. Various therapeutic strategies have been designed to target several steps of DM1 pathogenesis including; 'CTG' DNA mutations, *DMPK* transcripts, MBNL1-CUG repeat interaction or downstream pathways. Arguably one of the most advanced strategies is gene editing through Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 technology. Promising results in myoblasts derived from DM1 patients and DM1 mouse models were seen with the use of complementary RNA sequence that binds to either the 5' or 3' end of the mutant tract. 'CTG' repeat mutation was completely excised without affecting the expression of surrounding genes (van Agtmaal et al. 2017). More importantly, nuclear RNA foci were significantly reduced and DM1 associated spliceopathy was improved (van Agtmaal et al. 2017). Others have been able to replicate these benefits and improve splicing in different cell lines using other protocols (Batra et al. 2017; Provenzano et al. 2017). Although CRISP/Cas9 approaches showed favourable outcomes, concerns over target specificity, delivery and distribution to affected tissue with adequate concentrations are some of the drawbacks that limit its progress into clinical trials. Another effective avenue is the inhibition or suppression of the transcription of *DMPK* through the use of small molecules. Pentamidine, an FDA approved drug for treating pneumonia, has been repurposed due to its ability reduced the number of RNA foci and correct mis-splicing patterns of *IR* and cardiac troponin T (*cTNT*) in cell culture and DM1 mouse models (Warf et al. 2009). The mechanism of action has been attributed to the direct binding of Pentamidine to 'CTG' repeats

suppressing the transcription of the mutant transcripts in a dose dependent manner and liberating MBNL1 (Coonrod et al. 2013). Likewise, actinomycin D, an anticancer drug, binds to GC rich regions at the *DMPK* locus to suppress transcription and improve splicing in pre-clinical models of DM1 (Siboni et al. 2015).

It still remains unclear whether transcriptional silencing of *DMPK* via small molecules will translate to clinical populations. Hence, others have focused on manipulating the RNA 'CUG' structure. Antisense oligonucleotides (ASOs) have recently been approved for DMD and SMA and therefore, have proven efficacy in NMDs. For DM1, ASO's have been synthesized to target binding sites on 'CUG' repeats in order to degrade the foci and reduced MBNL sequestration. An ASO made up of complementary CAG repeats was able to significantly reduce the amount of *DMPK* mRNA while improving aberrant splicing in a DM1 murine model (Mulders et al. 2009). Alternatively, other ASOs designed to target binding sites outside the 'CUG' expansion showed tremendous improvements in DM1 mice by reducing 'CUG' RNA, liberating MBNL1 and improving splicing (Wheeler et al. 2012). Furthermore, its ability to reduce myotonia and improve myopathy displayed in DM1 resulted in its use in phase 1 and 2 clinical trials. Unfortunately, the use of ASO poses similar challenges to CRISP/Cas9 technology resulting in termination of the clinical trial due to insufficient concentrations of the ASO in patient biopsies. However, oral administration of erythromycin, a commonly prescribed antibiotic, displaced MBNL1 and inhibited its binding to CUG expansions in DM1 mice (Masayuki Nakamori et al. 2016). In turn, several splicing events were corrected and myotonia was improved. Erythromycin is currently being tested in a phase II clinical trial in Japan.

Lastly, small molecules have been synthesized to manipulate downstream pathways that are mis-regulated as a result of the DM1 mutation. Namely, GSK3β, PKC and AMP activated

protein kinase (AMPK) have all been targeted in order to alter their expression and activity pattern. As previously mentioned, hyperactivation of GSK3β in DM1 muscle resulted in the pathological overexpression and increased activation of CUGBP1 through the inhibition of cyclin D3-dependent kinase 4 (CDK4) (K. Jones et al. 2012). Furthermore, GSK3β inhibition with lithium treatment corrected CDK4 expression, improved muscle weakness and ameliorated myotonia in DM1 mice. Therefore, GSK3β inhibitors have garnered attention for their therapeutic potential of correcting CUGBP1 activity. In fact, assessment of the drug, Tideglusib, recently completed phase 1 and 2 clinical trial as a GSK3β inhibitor in DM1 patients (clinicaltrials.gov: NCT02858908). Newly published results from the phase 2 single blind clinical trial highlights that 12-week of Tideglusib (400 mg or 1000 mg) administration was safely tolerated with no serious adverse events in 16 patients with the congenital and childhood form of DM1 (Horrigan et al. 2020). Furthermore, significant improvements were seen in cognitive and neuromuscular symptoms. These results support the safety and efficacy of the kinase inhibitor which led to an ongoing phase 3 trial (clinicaltrials.gov: NCT03692312). The use of PKC inhibitors in DM1 mice resulted in the preservation of skeletal muscle and have further been proposed as a therapeutic avenue to correct dysregulation of CUGBP1 activity (G. Wang et al. 2009); however, no clinical trials are currently being investigated with PKC inhibition.

Finally, dysregulated AMPK signaling has been reported in the skeletal muscle of DM1 mice (Brockhoff et al. 2017). Specifically, a mouse model of DM1 and patient myoblasts both had blunted activation of AMPK (Ravel-chapuis et al. 2018; Brockhoff et al. 2017). Pharmacological AMPK activation via administration of either 5-aminoimidazole-4- carboxamide-1-β-D-ribofuranoside (AICAR) or metformin, a well-known type 2 diabetes treatment, significantly improved myotonia, reduced the number of CUG positive myonuclei, corrected splicing events of

several genes and increased total CLCN1 protein levels in DM1 mice (Ravel-chapuis et al. 2018; Brockhoff et al. 2017). In 40 patients with DM1, 52-weeks of metformin treatment significantly improved mobility compared to placebo but had no effect on alternative splicing events, myotonia or muscle strength (Bassez et al. 2018). A phase 3 clinical trial is currently underway that will investigate the efficacy of 24 months of metformin treatment in 194 DM1 patients (clinicaltrialregister.eu: 2018-000692-32).

EXERCISE TRAINING IN DM1

Despite remarkable advances in therapeutics designed to combat DM1, there still remains a critical need for an immediate intervention to mitigate disease progression and as adjunctive therapy for future interventions (including gene therapy). Exercise training has proven beneficial physiological and cellular adaptations in NMDs such as ALS, DMD and SMA (M. Jansen et al. 2013; Clawson et al. 2018; Madsen et al. 2015; Ng, Mikhail, and Ljubicic 2019). A recent review by Roussel and colleagues (M. Roussel et al. 2019) summarized the current evidence of exercise training in patients with DM1. Overall, physical activity appears to be safe and well tolerated by DM1 patients. However, evidence supporting the efficacy of training on canonical symptoms and molecular biology of DM1 is unclear and limited. Early studies by Lindeman et al (Lindeman et al. 1995, 1999) demonstrated that resistance exercise training in myotonic dystrophy (DM – unspecified) patients had no adverse effects on the integrity of skeletal muscle as measured by circulating myoglobin levels. The randomized control trial consisted of progressive lower limb home-based resistance training exercises 3 times per week for 12 weeks. No change was observed in overall strength or function between exercised and sedentary DM1 patients; however, non-significant increases in muscular endurance were observed in most patients. Later, Tollbäck (Tollbäck et al. 1999) and colleagues examined the benefits of isolated unilateral knee extensions

in six DM patients through a within-subject study design. Following 12 weeks of progressive knee extensions, the exercised limb experienced a ~33 % increase in strength as compared to the non-exercised limb. No changes were observed in cross-sectional area of knee extensor muscles as measured by magnetic resonance imaging, but five out of six patients showed an increase in the cross-sectional area of type I muscle fibres (Tollbäck et al. 1999). Histopathology markers such as centrally located myofibres and fibre size variable did not differ between the two limbs further echoing the safety of exercise training in DM patients.

More recently, Roussel et al (M. P. Roussel, Hébert, and Duchesne 2020) comprehensively assessed the effect of resistance training on strength, function, mobility and muscle fibre size in 11 DM1 patients. Following 12 weeks of lower limb supervised resistance training, DM1 patients showed a statistically significant increase in 1 repetition max values of knee extension, leg press, hip abduction and squat strength. Furthermore, significant improvements in function and mobility were seen following the exercise intervention as measured by 30s sit to stand test and 10-meter walking speed, respectively, but no changes in fibre size were observed. In summary, resistance exercise training is a safely tolerated intervention with majority of evidence suggesting improved muscular strength but limited evidence to support increases in muscle size. Contrasting evidence are likely due to different exercise protocols, methods of measurements and the low sample size.

Aerobic exercise training studies in DM1 literature are scant and it is difficult to be conclusive regarding the effects. To date, only two studies have examined the use of aerobic exercise training as a potential therapy for DM1. Wright et al (Wright et al. 1996) investigated the benefits of walking in five DM patients. The exercise intervention included 12 weeks of walking 3-4 times per week at 50 to 60 % of max heart rate. Overall, walking improved submaximal heart rate and systolic blood pressure while not significantly increasing cardiorespiratory fitness

(VO_{2peak}) or maximal power output. Several years later, Ørngreen et al (Ørngreen, Olsen, and Vissing 2005) examined the effect of 12 weeks of cycle ergometry in 12 DM1 patients. Cycling intensity was set at 65 % at VO_{2peak} for ~ 35 min and a total of 50 training session were performed within the 12 weeks. Three patients had low adherence to the exercise program (< 65%) and were excluded from the final analysis. Most importantly, the cycling intervention was safely tolerated as measured by creatine kinase levels before and after the intervention. Cycling greatly improved cardiorespiratory fitness and maximal power output by 14 % and 11 %, respectively. Furthermore, ATPase staining of muscle biopsies showed an increase in fibre cross-sectional area of both oxidative type I fibres and glycolytic type IIA fibres. Overall, aerobic exercise training elicited improvements in cardiorespiratory fitness and some evidence suggesting increases in fibre size. Future trials further assessing aerobic training adaptations on function, strength and basic DM1 biology are needed to definitively conclude the potential therapeutic benefits in DM1 patients.

Brady et al (Brady, MacNeil, and Tarnopolsky 2014) aimed to define the long-term benefits of physical activity in DM1 patients. A retrospective analysis of 63 DM1 patients showed that habitually active patients scored significantly higher grip strength, knee extension and elbow flexion strength as compared to age- and CTG repeat matched sedentary DM1 patients. Physical activity was defined as participating in structured exercise more than two times per week; however, the authors did not differentiate between resistance or aerobic based exercise. Nevertheless, this study emphasizes the importance of exercise for maintenance of muscular strength in a large cohort of DM1 patients. Preferential atrophy and weakness in the hands and wrists are commonly seen in DM1 patients and may significantly limit independence during activities of daily living with disease progression. Therefore, Aldehag and colleagues (Aldehag, Jonsson, and Ansved 2005; Aldehag et al. 2013) employed a 12 week hand and wrist resistance based program as a

rehabilitation intervention. Indeed, training significantly improved forces produced by wrist and finger extensors and flexors, and improved fine motor skills but not influence myotonia. Multidisciplinary exercise interventions have also been studied for their efficacy in DM patients. Moon et al investigated the benefits of full body home-based resistance training in combination with quinine sulfate, anti-myotonia drug, supplementation in seven DM patients (Moon et al. 1996). Following the 6-month intervention, strength of the upper and lower limb significantly increased as well as significant improvements in muscle relaxation time were recorded. Others examined the efficacy of a 60-minute exercise program consisting of flexibility, strength, balance and aerobic components for 14 weeks in randomized trial of 35 DM1 patients (Kierkegaard et al. 2011). The multi-component rehabilitation program showed no evidence of positive or harmful effects when compared to non-exercised DM1 controls.

The molecular adaptations of both resistance and aerobic exercise in healthy individuals are impressively researched and will not be included in this review. However, recent efforts of exercise training studies in pre-clinical models of DM1 aimed to elucidate training induced molecular signaling pathways in DM1 muscle. Manta et al recently demonstrated that voluntary wheel running in DM1 rodents significantly improved the muscular phenotype, reduced myotonic discharge and reduced the frequency of atrophic muscle fibres (Manta et al. 2019). Furthermore, wheel running in DM1 mice reduced the number of CUG positive myonuclei thereby liberating MBNL1 and improving splicing abnormalities in skeletal muscle (Manta et al. 2019; Ravel-chapuis et al. 2018). Recently after, Sharp et al showed that 10-weeks of progressive treadmill running in DM1 mice increased skeletal muscle weight and oxidative capacity while further replicating improvements in splicing and reduction in CUG expansions (Sharp, Cox, and Cooper 2019). Collectively, this data suggested an AMPK mediated mechanism of adaptation by which

the DM1 phenotype can be mitigated (Dial et al. 2018; Ng, Manta, and Ljubicic 2018). In fact, similar adaptations were seen in DM1 mice with pharmacological activation of AMPK as previously mentioned above (Brockhoff et al. 2017; Ravel-chapuis et al. 2018).

THESIS OBJECTIVES AND HYPOTHESIS

Recent evidence in murine models of DM1 have highlighted the efficacy of aerobic exercise in DM1 biology while early training studies in patients demonstrate the safety of several exercise modalities. However, to date only one study has investigated the effects of moderate intensity aerobic training and failed to measure clinically relevant measures of function, strength and respirometry. DM1 patient studies further lack molecular and mitochondrial investigations in response to aerobic training. Addressing these limitations will further provide support for the therapeutic potential of exercising training in DM1 patients. Therefore, the purpose of this study is three fold: 1) Investigate exercise induced changes in functional, strength and other clinically relevant measures, 2) Explore mitochondrial function and content in DM1 patients relative to healthy controls and adaptations in response to exercise training, and 3) Outlining alterations in RNABP expression in DM1 patients following exercise training. We hypothesize that exercise training will elicit clinical, molecular and mitochondrial benefits in DM1 patients.

REFERENCES

Agtmaal, Ellen L. van, Laurène M. André, Marieke Willemse, Sarah A. Cumming, Ingeborg D.G. van Kessel, Walther J.A.A. van den Broek, Geneviève Gourdon, et al. 2017. "CRISPR/Cas9-Induced (CTG·CAG)n Repeat Instability in the Myotonic Dystrophy Type 1 Locus: Implications for Therapeutic Genome Editing." *Molecular Therapy* 25 (1): 24–43. https://doi.org/10.1016/j.ymthe.2016.10.014.

Akinbiola, O O, S A Adeniran, and O Ogunlade. 2019. "Effects of an 8 Week Continuous Exercise Training on the Electrocardiogram and Physiological Parameters of Institutional Security Personnel in Nigeria." *Asian J. Biol. Sci.* 12 (2): 342–48. https://doi.org/10.3923/ajbs.2019.342.348.

Aldehag, Anna Sandin, Hans Jonsson, and Tor Ansved. 2005. "Effects of a Hand Training Programme in Five Patients with Myotonic Dystrophy Type 1." *Occupational Therapy International* 12 (1): 14–27. https://doi.org/10.1002/oti.12.

Aldehag, Anna Sandin, Hans Jonsson, Jan Lindblad, Anders Kottorp, Tor Ansved, and Marie Kierkegaard. 2013. "Effects of Hand-Training in Persons with Myotonic Dystrophy Type 1-a Randomised Controlled Cross-over Pilot Study." *Disability and Rehabilitation* 35 (21): 1798–1807. https://doi.org/10.3109/09638288.2012.754952.

Angane, Esha Y, and Amit A Navare. 2016. "Effects of Aerobic Exercise on Pulmonary Function Tests in Healthy Adults." *International Journal of Research in Medical Sciences* 4 (6): 2059–63.

Ashizawa, Tetsuo, M. Anvret, M. Baiget, J. M. Barceló, H. Brunner, A. M. Cobo, B. Dallapiccola, et al. 1994. "Characteristics of Intergenerational Contractions of the CTG Repeat in Myotonic Dystrophy." *American Journal of Human Genetics* 54 (3): 414–23.

Barbé, Lise, Stella Lanni, Arturo López-Castel, Silvie Franck, Claudia Spits, Kathelijn Keymolen, Sara Seneca, et al. 2017. "CpG Methylation, a Parent-of-Origin Effect for Maternal-Biased Transmission of Congenital Myotonic Dystrophy." *American Journal of Human Genetics* 100 (3): 488–505. https://doi.org/10.1016/j.ajhg.2017.01.033.

Bassez, Guillaume, Etienne Audureau, Jean-yves Hogrel, Sandrine Baghdoyan, Hamza Bhugaloo, Philippe Le Corvoisier, and Marc Peschanski. 2018. "Improved Mobility with Metformin in Patients with Myotonic Dystrophy Type 1 : A Randomized Controlled Trial." *Brain* 141 (10): 2855–65. https://doi.org/10.1093/brain/awy231.

Batra, Ranjan, David A. Nelles, Elaine Pirie, Steven M. Blue, Ryan J. Marina, Harrison Wang, Isaac A. Chaim, et al. 2017. "Elimination of Toxic Microsatellite Repeat Expansion RNA by RNA-Targeting Cas9." *Cell* 170 (5): 899-912.e10. https://doi.org/10.1016/j.cell.2017.07.010.

Brady, Lauren I., Lauren G. MacNeil, and Mark A. Tarnopolsky. 2014. "Impact of Habitual Exercise on the Strength of Individuals with Myotonic Dystrophy Type 1." *American Journal of Physical Medicine & Rehabilitation / Association of Academic Physiatrists* 93 (9): 739–50. https://doi.org/10.1097/PHM.0000000000000088.

Braida, Claudia, Rhoda K.A. Stefanatos, Berit Adam, Navdeep Mahajan, Hubert J.M. Smeets, Florence Niel, Cyril Goizet, et al. 2010. "Variant CCG and GGC Repeats within the CTG Expansion Dramatically Modify Mutational Dynamics and Likely Contribute toward Unusual Symptoms in Some Myotonic Dystrophy Type 1 Patients." *Human Molecular Genetics* 19 (8): 1399–1412. https://doi.org/10.1093/hmg/ddq015.

Brockhoff, Marielle, Nathalie Rion, Kathrin Chojnowska, Tatiana Wiktorowicz, Christopher

Eickhorst, Beat Erne, Stephan Frank, et al. 2017. "Targeting Deregulated AMPK / MTORC1 Pathways Improves Muscle Function in Myotonic Dystrophy Type I." *Journal of Clinical Investigation* 127 (2): 549–63.

Brook, J D, M E McCurrach, H G Harley, A J Buckler, D Church, H Aburatani, K Hunter, V P Stanton, J P Thirion, and T Hudson. 1992. "Molecular Basis of Myotonic Dystrophy: Expansion of a Trinucleotide (CTG) Repeat at the 3' End of a Transcript Encoding a Protein Kinase Family Member." *Cell* 68 (4): 799–808.

Chau, Anthony, and Auinash Kalsotra. 2015. "Developmental Insights into the Pathology of and Therapeutic Strategies for DM1: Back to the Basics." *Developmental Dynamics* 244 (3): 377–90. https://doi.org/10.1002/DVDY.24240.

Chen, Zhi-Ping, Terry J Stephens, Sid Murthy, Benedict J Canny, Mark Hargreaves, Lee A Witters, Bruce E Kemp, Glenn K Mcconell, and Glenn Mcconell. 2003. "Effect of Exercise Intensity on Skeletal Muscle AMPK Signaling in Humans." *Diabetes* 52 (9): 2205–12. http://diabetes.diabetesjournals.org.libaccess.lib.mcmaster.ca/content/diabetes/52/9/2205.full.pdf.

Cho, Diane H., and Stephen J. Tapscott. 2007. "Myotonic Dystrophy: Emerging Mechanisms for DM1 and DM2." *Biochimica et Biophysica Acta - Molecular Basis of Disease* 1772 (2): 195–204. https://doi.org/10.1016/j.bbadis.2006.05.013.

Clawson, Lora L., Merit Cudkowicz, Lisa Krivickas, Benjamin R. Brooks, Mohammed Sanjak, Peggy Allred, Nazem Atassi, et al. 2018. "A Randomized Controlled Trial of Resistance and Endurance Exercise in Amyotrophic Lateral Sclerosis." *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration* 19 (3–4): 250–58. https://doi.org/10.1080/21678421.2017.1404108.

Cogliati, Sara, Christian Frezza, Maria Eugenia Soriano, Tatiana Varanita, Ruben Quintana-Cabrera, Mauro Corrado, Sara Cipolat, et al. 2013. "Mitochondrial Cristae Shape Determines Respiratory Chain Supercomplexes Assembly and Respiratory Efficiency." *Cell* 155 (1): 160–71. https://doi.org/10.1016/j.cell.2013.08.032.

Coonrod, Leslie A., Masayuki Nakamori, Wenli Wang, Samuel Carrell, Cameron L. Hilton, Micah J. Bodner, Ruth B. Siboni, et al. 2013. "Reducing Levels of Toxic RNA with Small Molecules." *ACS Chemical Biology* 8 (11): 2528–37. https://doi.org/10.1021/cb400431f.

Davis, Brigid M, Mila E Mccurrach, Krishan L Taneja, Robert H Singer, and David E Housman. 1997. "Expansion of a CUG Trinucleotide Repeat in the 3 ′ Untranslated Region of Myotonic Dystrophy Protein Kinase Transcripts Results in Nuclear Retention of Transcripts." *Proceedings of the National Academy of Sciences* 94 (14): 7388–93.

Day, J. W., K. Ricker, J. F. Jacobsen, L. J. Rasmussen, K. A. Dick, W. Kress, C. Schneider, et al. 2003. "Myotonic Dystrophy Type 2: Molecular, Diagnostic and Clinical Spectrum." *Neurology* 60 (4): 657–64. https://doi.org/10.1212/01.WNL.0000054481.84978.F9.

Dial, Athan G, Sean Y Ng, Alexander Manta, and Vladimir Ljubicic. 2018. "The Role of AMPK in Neuromuscular Biology and Disease." *Trends in Endocrinology & Metabolism* 29 (5): 300–312. https://doi.org/10.1016/j.tem.2018.02.010.

Du, Hongqing, Melissa S. Cline, Robert J. Osborne, Daniel L. Tuttle, Tyson A. Clark, John Paul Donohue, Megan P. Hall, et al. 2010. "Aberrant Alternative Splicing and Extracellular Matrix Gene Expression in Mouse Models of Myotonic Dystrophy." *Nature Structural and Molecular Biology* 17 (2): 187–93. https://doi.org/10.1038/nsmb.1720.

Dunne, Patrick W, E Timothy Walch, and Henry F Epstein. 1994. "Phosphorylation Reactions of Recombinant Human Myotonic Dystrophy Protein Kinase and Their Inhibition."

Biochemistry 33 (35): 10809–14. https://doi.org/10.1021/bi00201a031.
Enright, Paul L. 2003. "The Six-Minute Walk Test." *Respiratory Care* 48 (8): 783–85.
García-puga, Mikel, Ander Saenz-antoñanzas, Roberto Fernández-torrón, Adolfo Lopez De, and Ander Matheu. 2020. "Myotonic Dystrophy Type 1 Cells Display Impaired Metabolism and Mitochondrial Dysfunction That Are Reversed by Metformin." *Aging* 12 (7): 6260–75.
Gramegna, Laura Ludovica, Maria Pia Giannoccaro, David Neil Manners, Claudia Testa, Stefano Zanigni, Stefania Evangelisti, Claudio Bianchini, et al. 2018. "Mitochondrial Dysfunction in Myotonic Dystrophy Type 1." *Neuromuscular Disorders* 28 (2): 144–49. https://doi.org/10.1016/j.nmd.2017.10.007.
Ha, Andrew H, Mark A Tarnopolsky, T Graham Bergstra, Girish M Nair, Atif Al-qubbany, and Jeff S Healey. 2012. "Predictors of Atrio-Ventricular Conduction Disease , Long-Term Outcomes in Patients with Myotonic Dystrophy Types I and II" 35 (October): 1262–69. https://doi.org/10.1111/j.1540-8159.2012.03351.x.
Harley, Helen G, J David Brook, Shelley A Rundle, Stephen Crow, William Reardon, Alan J Buckler, Peter S Harper, David E Housman, and Duncan J Shaw. 1992. "Expansion of an Unstable DNA Region and Phenotypic Variation in Myotonic Dystrophy" 355 (November 1991): 1991–92.
Hawkins, A. M., C. L. Hawkins, K. Abdul Razak, T. K. Khoo, K. Tran, and R. V. Jackson. 2019. "Respiratory Dysfunction in Myotonic Dystrophy Type 1: A Systematic Review." *Neuromuscular Disorders* 29 (3): 198–212. https://doi.org/10.1016/j.nmd.2018.12.002.
Ho, Thai H, Nicolas Charlet-b, G Michael, Gopal Singh, S Maurice, and Thomas A Cooper. 2004. "Muscleblind Proteins Regulate Alternative Splicing" 23 (15): 3103–12. https://doi.org/10.1038/sj.emboj.7600300.
Hofmann-Radvanyi, Helen, and Claudine Junien. 1994. "MYOTONIC DYSTROPHY: OVER-EXPRESSION OR/AND UNDER-EXPRESSION? A CRITICAL REVIEW ON A CONTROVERSIAL POINT." *Neuromuscular Disorders* 3 (5): 497–501.
Horrigan, Joseph, Tiago Bernardino Gomes, Mike Snape, Nikoletta Nikolenko, Alison McMorn, Stuart Evans, Alex Yaroshinsky, Oscar Della Pasqua, Sean Oosterholt, and Hanns Lochmüller. 2020. "A Phase 2 Study of AMO-02 (Tideglusib) in Congenital and Childhood Onset Myotonic Dystrophy Type 1 (DM1)." *Pediatric Neurology*. https://doi.org/10.1016/j.eplepsyres.2019.106192.
Huston, Tim P., James C. Puffer, and WM. MacMillan Rodney. 1985. "The Athletic Heart Syndrome." *The New England Journal of Medicine* 313 (1): 24–32.
Hyatt, Hayden, Rafael Deminice, Toshinori Yoshihara, and Scott K. Powers. 2019. "Mitochondrial Dysfunction Induces Muscle Atrophy during Prolonged Inactivity: A Review of the Causes and Effects." *Archives of Biochemistry and Biophysics* 662 (November 2018): 49–60. https://doi.org/10.1016/j.abb.2018.11.005.
Ihara, Yuetsu, Akitane Mori, Reiko Namba, and Keigo Nobukuni. 1995. "Free Radicals , Lipid Peroxides and Antioxidants in Blood of Patients with Myotonic Dystrophy." *Neurology* 242 (4): 119–22.
Isashiki, Y, E Kawabata, N Ohba, I Higuchi, M Nakagawa, M Osame, E Kawabata, et al. 1989. "Mitochondrial Abnormalities in Extraocular Muscles in Myotonic Dystrophy." *Neuro-Opthalmology* 9 (2): 115–22. https://doi.org/10.3109/01658108909007466.
Jansen, G., P. Willems, M. Coerwinkel, W. Nillesen, H. Smeets, L. Vits, C. Howeler, H. Brunner, and B. Wieringa. 1994. "Gonosomal Mosaicism in Myotonic Dystrophy Patients: Involvement of Mitotic Events in (CTG)(n) Repeat Variation and Selection against Extreme

Expansion in Sperm." *American Journal of Human Genetics* 54 (4): 575–85.
Jansen, Gert, Patricia J T A Groenen, Dietmar Bachner, Paul H K Jap, Frank Oerlemans, Walther Van Den Broek, Barbel Gohlsch, et al. 1996. "Abnormal Myotonic Dystrophy Protein Kinase Levels Produce Only Mild Myopathy in Mice." *Nature Genetics* 13 (3): 316–24. https://doi.org/1061-4036 (Print).
Jansen, Merel, Nens Van Alfen, Alexander C.H. Geurts, and Imelda J.M. De Groot. 2013. "Assisted Bicycle Training Delays Functional Deterioration in Boys with Duchenne Muscular Dystrophy: The Randomized Controlled Trial 'No Use Is Disuse.'" *Neurorehabilitation and Neural Repair* 27 (9): 816–27. https://doi.org/10.1177/1545968313496326.
Jones, Karlie, Christina Wei, Polina Iakova, Enrico Bugiardini, Christiane Schneider-Gold, Giovanni Meola, James Woodgett, James Killian, Nikolai A. Timchenko, and Lubov T. Timchenko. 2012. "GSK3β Mediates Muscle Pathology in Myotonic Dystrophy." *Journal of Clinical Investigation* 122 (12): 4461–72. https://doi.org/10.1172/JCI64081.
Jones, Paul W, Kai M Beeh, Kenneth R Chapman, Marc Decramer, Donald A Mahler, and Jadwiga A Wedzicha. 2014. "Minimal Clinically Important Differences in Pharmacological Trials." *Am J Respir Crit Care Med.* 189 (3): 250–55. https://doi.org/10.1164/rccm.201310-1863PP.
Kalsotra, Auinash, Xinshu Xiao, Amanda J. Ward, John C. Castle, Jason M. Johnson, Christopher B. Burge, and Thomas A. Cooper. 2008. "A Postnatal Switch of CELF and MBNL Proteins Reprograms Alternative Splicing in the Developing Heart." *Proceedings of the National Academy of Sciences of the United States of America* 105 (51): 20333–38. https://doi.org/10.1073/pnas.0809045105.
Kanadia, Rahul N., Carl R. Urbinati, Valerie J. Crusselle, Defang Luo, Young Jae Lee, Jeffrey K. Harrison, S. Paul Oh, and Maurice S. Swanson. 2003. "Developmental Expression of Mouse Muscleblind Genes Mbnl1, Mbnl2 and Mbnl3." *Gene Expression Patterns* 3 (4): 459–62. https://doi.org/10.1016/S1567-133X(03)00064-4.
Kanadia, Rahul N, Karen A Johnstone, Ami Mankodi, Codrin Lungu, Charles A. Thornton, Douglas Esson, Adrian M Timmers, William W Hauswirth, and Maurice S. Swanson. 2003. "A Muscleblind Knockout Modet for Myotonic Dystrophy." *Science* 302 (5): 1978–80.
Kierkegaard, Marie, Karin Harms-Ringdahl, Lars Edström, Lotta Widén Holmqvist, and Anna Tollbäck. 2011. "Feasibility and Effects of a Physical Exercise Programme in Adults with Myotonic Dystrophy Type 1: A Randomized Controlled Pilot Study." *Journal of Rehabilitation Medicine* 43 (8): 695–702. https://doi.org/10.2340/16501977-0833.
Klesert, Todd R, Diane H Cho, John I Clark, James Maylie, John Adelman, Lauren Snider, Eric C. Yuen, Philippe Soriano, and Stephen J Tapscott. 2000. "Mice Deficient in Six5 Develop Cataracts : Implications for Myotonic Dystrophy." *Nature Genetics* 25 (1): 105–9.
Klesert, Todd R, Anne D Otten, Thomas D Bird, and Stephen J. Tapscott. 1997. "Trinucleotide Repeat Expansion at the Myotonic Dystrophy Locus Reduces Expression of DMAHP." *Nature Genetics* 16 (5): 1–5.
Kumar, A., V. Kumar, S. K. Singh, S. Muthuswamy, and S. Agarwal. 2014. "Imbalanced Oxidant and Antioxidant Ratio in Myotonic Dystrophy Type 1." *Free Radical Research* 48 (4): 503–10. https://doi.org/10.3109/10715762.2014.887847.
Kuyumcu-Martinez, N Muge, Guey-shin Wang, and Thomas A Cooper. 2007. "Increased Steady-State Levels of CUGBP1 in Myotonic Dystrophy 1 Are Due to PKC-Mediated Hyperphosphorylation." *Molecular Cell* 28 (1): 68–78.

https://doi.org/10.1016/j.molcel.2007.07.027.
Lam, L T, Y C N Pham, Nguyen Man, and G E Morris. 2000. "Characterization of a Monoclonal Antibody Panel Shows That the Myotonic Dystrophy Protein Kinase , DMPK , Is Expressed Almost Exclusively in Muscle and Heart." *Human Molecular Genetics* 9 (14): 2167–74.
Légaré, Cécilia, Gayle Overend, Simon Pierre Guay, Darren G. Monckton, Jean Mathieu, Cynthia Gagnon, and Luigi Bouchard. 2019. "DMPK Gene DNA Methylation Levels Are Associated with Muscular and Respiratory Profiles in DM1." *Neurology: Genetics* 5 (3). https://doi.org/10.1212/NXG.0000000000000338.
Lindeman, Eline, Pieter Leffers, Frank Spaans, Jan Drukker, Jos Reulen, Maria Kerckhoffs, and Albere Köke. 1995. "Strength Training in Patients with Myotonic Dystrophy and Hereditary Motor and Sensory Neuropathy: A Randomized Clinical Trial." *Archives of Physical Medicine and Rehabilitation* 76 (7): 612–20. https://doi.org/10.1016/S0003-9993(95)80629-6.
Lindeman, Eline, Frank Spaans, Jos Reulen, Pieter Leffers, and Jan Drukker. 1999. "Progressive Resistance Training in Neuromuscular Patients. Effects on Force and Surface EMG." *Journal of Electromyography and Kinesiology* 9 (6): 379–84. https://doi.org/10.1016/S1050-6411(99)00003-6.
Liquori, C. L., K. Ricker, M. L. Moseley, J. F. Jacobsen, W. Kress, S. L. Naylor, J. W. Day, and L. P.W. Ranum. 2001. "Myotonic Dystrophy Type 2 Caused by a CCTG Expansion in Intron I of ZNF9." *Science* 293 (5531): 864–67. https://doi.org/10.1126/science.1062125.
MacDonald, J. R., J. D. Hill, and Mark A. Tarnopolsky. 2002. "Modafinil Reduces Excessive Somnolence and Enhances Mood in Patients with Myotonic Dystrophy." *Neurology* 59 (12): 1876–80. https://doi.org/10.1212/01.WNL.0000037481.08283.51.
Madsen, Karen Lindhardt, Regitze Sølling Hansen, Nicolai Preisler, Frank Thøgersen, Martin Peter Berthelsen, and John Vissing. 2015. "Training Improves Oxidative Capacity, but Not Function, in Spinal Muscular Atrophy Type III." *Muscle & Nerve* 52 (2): 240–44. https://doi.org/10.1002/mus.24527.
Mahadevan, Mani, Catherine Tsilfidis, Luc Sabourin, Gary Shutler, Gert Jansen, Catherine Neville, Monica Narang, et al. 1992. "Myotonic Dystrophy Mutation : An Unstable CTG Repeat in the 3 ′ Untranslated Region of the Gene." *Science* 255 (5049): 1253–55.
Malandish, Abbas, Bakhtyar Tartibian, Zeinab Sheikhlou, Roghaiyeh Afsargharehbagh, and Mohammad Rahmati. 2020. "The Effects of Short-Term Moderate Intensity Aerobic Exercise and Long-Term Detraining on Electrocardiogram Indices and Cardiac Biomarkers in Postmenopausal Women." *Journal of Electrocardiology* 60: 15–22. https://doi.org/10.1016/j.jelectrocard.2020.03.004.
Mankodi, Ami, Carl R Urbinati, Qiu-ping Yuan, Richard T Moxley, Valeria Sansone, Matt Krym, Donald Henderson, Martin Schalling, Maurice S Swanson, and Charles A Thornton. 2001. "Muscleblind Localizes to Nuclear Foci of Aberrant RNA in Myotonic Dystrophy Types 1 and 2." *Human Molecular Genetics* 10 (19): 2165–70.
Manta, Alexander, Derek W Stouth, Donald Xhuti, Leon Chi, Irena A Rebalka, Jayne M Kalmar, Thomas J Hawke, and Vladimir Ljubicic. 2019. "Chronic Exercise Mitigates Disease Mechanisms and Improves Muscle Function in Myotonic Dystrophy Type 1 Mice." *Journal of Physiology* 597 (5): 1361–81. https://doi.org/10.1113/JP277123.
Mcdonald, Craig M. 2012. "Clinical Approach to the Diagnostic Evaluation of Hereditary and Acquired Neuromuscular Diseases." *Physical Medicine and Rehabilitation Clinics of N Am* 23 (3): 495–563. https://doi.org/10.1016/j.pmr.2012.06.011.

Miller, Jill W, Carl R Urbinati, Patana Teng-umnuay, Myrna G Stenberg, Barry J Byrne, Charles A Thornton, and Maurice S Swanson. 2000. "Recruitment of Human Muscleblind Proteins to (CUG) n Expansions Associated with Myotonic Dystrophy." *EMBO J.* 19 (17): 4439–48.

Moller, David E., Atsushi Yokota, Jose F. Caro, and Jeffrey S. Flier. 1989. "Tissue-Specific Expression of Two Alternatively Spliced Insulin Receptor Mrnas in Man." *Molecular Endocrinology* 3 (8): 1263–69. https://doi.org/10.1210/mend-3-8-1263.

Moon, Jae Ho, Young Moo Na, Seong Woong Kang, and Han Soo Lee. 1996. "The Changes in Muscle Strength and Relaxation Time after a Comprehensive Rehabilitation Program for Patients with Myotonic Dystrohy." *Yonsei Medical Journal* 37 (4): 237–42.

Morales, Fernando, Melissa Vásquez, Patricia Cuenca, Domingo Campos, Carolina Santamaría, Gerardo Del Valle, Roberto Brian, Mauricio Sittenfeld, and Darren G. Monckton. 2015. "Parental Age Effects, but No Evidence for an Intrauterine Effect in the Transmission of Myotonic Dystrophy Type 1." *European Journal of Human Genetics* 23 (5): 646–53. https://doi.org/10.1038/ejhg.2014.138.

Moxley, Richard T, Emma Ciafaloni, and Debra Guntrum. 2015. "Myotonic Dystrophy." In *Neuromuscular Disorders of Infancy, Childhood, and Adolescence 2e*, Second Edi, 697–718. Elsevier Inc. https://doi.org/10.1016/B978-0-12-417044-5.00037-8.

Mulders, Susan A M, Walther J A A Van Den Broek, Thurman M Wheeler, Huib J E Croes, Derick G Wansink, and Charles A Thornton. 2009. "Triplet-Repeat Oligonucleotide-Mediated Reversal of RNA Toxicity in Myotonic Dystrophy." *Proceedings of the National Academy of Sciences* 106 (33): 13915–20.

Nakamori, M., T. Kimura, T. Kubota, T. Matsumura, H. Sumi, H. Fujimura, M. P. Takahashi, and S. Sakoda. 2008. "Aberrantly Spliced Alpha-Dystrobrevin Alters Alpha-Syntrophin Binding in Myotonic Dystrophy Type 1." *Neurology* 70 (9): 677–85. https://doi.org/10.1212/01.wnl.0000302174.08951.cf.

Nakamori, Masayuki, Krzysztof Sobczak, Araya Puwanant, Katy Eichinger, Shree Pandya, Jeannne Dekdebrun, R Heatwole, et al. 2013. "Splicing Biomarkers of Disease Severity in Myotonic Dystrophy." *Annals of Neurology* 74 (6): 862–72. https://doi.org/10.1002/ana.23992.Splicing.

Nakamori, Masayuki, Katarzyna Taylor, Hideki Mochizuki, Krzysztof Sobczak, and Masanori P. Takahashi. 2016. "Oral Administration of Erythromycin Decreases RNA Toxicity in Myotonic Dystrophy." *Annals of Clinical and Translational Neurology* 3 (1): 42–54. https://doi.org/10.1002/acn3.271.

Nederveen, Joshua P., George Ibrahim, Stephen A. Fortino, Tim Snijders, Dinesh Kumbhare, and Gianni Parise. 2020. "Variability in Skeletal Muscle Fibre Characteristics during Repeated Muscle Biopsy Sampling in Human Vastus Lateralis." *Applied Physiology, Nutrition and Metabolism* 45 (4): 368–75. https://doi.org/10.1139/apnm-2019-0263.

Ng, Sean Y, Alexander Manta, and Vladimir Ljubicic. 2018. "Exercise Biology of Neuromuscular Disorders." *Applied Physiology, Nutrition, and Metabolism* 43 (11): 1194–1206. https://doi.org/10.1139/apnm-2018-0229.

Ng, Sean Y, Andrew Mikhail, and Vladimir Ljubicic. 2019. "Mechanisms of Exercise-Induced Survival Motor Neuron Expression in the Skeletal Muscle of Spinal Muscular Atrophy-like Mice." *Journal of Physiology* 587 (18): 4757–78. https://doi.org/10.1113/JP278454.

Ono, Seiitsu, Hiroshi Kurisaki, Kiyoharu Inouye, and Toru Mannen. 1986. "'Ragged-Red' Fibres in Myotonic Dystrophy." *Journal of the Neurological Sciences* 74 (2–3): 247–55.

https://doi.org/10.1016/0022-510X(86)90110-3.
Ørngreen, Mette C., David B. Olsen, and John Vissing. 2005. "Aerobic Training in Patients with Myotonic Dystrophy Type 1." *Annals of Neurology* 57 (5): 754–57. https://doi.org/10.1002/ana.20460.
Otten, A.D., and S.J. Tapscott. 1995. "Triplet Repeat Expansion in Myotonic Dystrophy Alters the Adjacent Chromatin Structure." *Proceedings of the National Academy of Sciences* 92 (12): 5465–69.
Ozaki, H, J P Loenneke, R S Thiebaud, and T Abe. 2015. "Cycle Training Induces Muscle Hypertrophy and Strength Gain : Strategies and Mechanisms (Review)." *Acta Physiol Hung.* 102 (1): 1–22. https://doi.org/10.1556/APhysiol.102.2015.1.1.
Pantic, B, E Trevisan, A Citta, M P Rigobello, O Marin, P Bernardi, S Salvatori, and A Rasola. 2013. "Myotonic Dystrophy Protein Kinase (DMPK) Prevents ROS-Induced Cell Death by Assembling a Hexokinase II-Src Complex on the Mitochondrial Surface." *Cell Death and Disease* 4 (10): e858. https://doi.org/10.1038/cddis.2013.385.
Pelkonen, Margit, Irma-leena Notkola, Timo Lakka, Hannu O Tukiainen, Paula Kivinen, and Aulikki Nissinen. 2003. "Delaying Decline in Pulmonary Function with Physical Activity." *Am J Respir Crit Care Med.* 168 (4): 494–99. https://doi.org/10.1164/rccm.200208-954OC.
Philips, A V, L T Timchenko, and T A Cooper. 1998. "Disruption of Splicing Regulated by a CUG-Binding Protein in Myotonic Dystrophy." *Science (New York, N.Y.)* 280 (5364): 737–41.
Pisani, Valerio, Maria Beatrice Panico, Chiara Terracciano, Emanuela Bonifazi, Giovanni Meola, Giuseppe Novelli, Giorgio Bernardi, Corrado Angelini, and Roberto Massa. 2008. "Preferential Central Nucleation of Type 2 Myofibers Is an Invariable Feature of Myotonic Dystrophy Type 2." *Muscle and Nerve* 38 (5): 1405–11. https://doi.org/10.1002/mus.21122.
Podsiadlo, Diane, and Sandra Richardson. 1991. "The Timed "Up & Go": A Test of Basic Functional Mobility for Frail Elderlv Persons." *Journal of the American Geriatrics Society*, 142–48.
Poulton, J., H. G. Harley, J. Dasmahapatra, G. K. Brown, C. G. Potter, and B. Sykes. 1995. "Mitochondrial DNA Does Not Appear to Influence the Congenital Onset Type of Myotonic Dystrophy." *Journal of Medical Genetics* 32 (9): 732–35. https://doi.org/10.1136/jmg.32.9.732.
Provenzano, Claudia, Marisa Cappella, Rea Valaperta, Rosanna Cardani, Giovanni Meola, Fabio Martelli, Beatrice Cardinali, and Germana Falcone. 2017. "CRISPR/Cas9-Mediated Deletion of CTG Expansions Recovers Normal Phenotype in Myogenic Cells Derived from Myotonic Dystrophy 1 Patients." *Molecular Therapy - Nucleic Acids* 9 (December): 337–48. https://doi.org/10.1016/j.omtn.2017.10.006.
Ravel-chapuis, Aymeric, Ali Al-rewashdy, Guy Bélanger, and Bernard J Jasmin. 2018. "Pharmacological and Physiological Activation of AMPK Improves the Spliceopathy in DM1 Mouse Muscles." *Human Molecular Genetics* 27 (19): 3361–76. https://doi.org/10.1093/hmg/ddy245.
Reddy, Sita, Daniel B J Smith, Mark M Rich, John M Leferovich, Patricia Reilly, Brigid M Davis, Khoa Tran, et al. 1996. "Mice Lacking the Myotonic Dystrophy Protein Kinase Develop a Late Onset Progressive Myopathy Sita." *Nature Genetics* 13 (3): 325–35.
Redman, Joy B., Raymond G. Fenwick, Ying Hui Fu, Antonio Pizzuti, and C. Thomas Caskey. 1993. "Relationship Between Parental Trinucleotide GCT Repeat Length and Severity of Myotonic Dystrophy in Offspring." *JAMA: The Journal of the American Medical*

Association 269 (15): 1960–65. https://doi.org/10.1001/jama.1993.03500150072029.
Rojas, Jose M., Marianne Subleski, Juan J.R. Coque, Carmen Guerrero, Rosana Saez, Bao Qun Li, Eva Lopez, et al. 1999. "Isoform-Specific Insertion near the Grb2-Binding Domain Modulates the Intrinsic Guanine Nucleotide Exchange Activity of HSos1." *Oncogene* 18 (9): 1651–61. https://doi.org/10.1038/sj.onc.1202483.
Roussel, Marie-pier, Marika Morin, Cynthia Gagnon, and Elise Duchesne. 2019. "What Is Known about the Effects of Exercise or Training to Reduce Skeletal Muscle Impairments of Patients with Myotonic Dystrophy Type 1 ? A Scoping Review." *BMC Musculoskelet Disord* 20 (1): 101.
Roussel, Marie Pier, Luc J. Hébert, and Elise Duchesne. 2020. "Strength-Training Effectively Alleviates Skeletal Muscle Impairments in Myotonic Dystrophy Type 1." *Neuromuscular Disorders* 30 (4): 283–93. https://doi.org/10.1016/j.nmd.2020.02.015.
Roussel, Marie Pier, Marika Morin, Mélina Girardin, Anne Marie Fortin, Mario Leone, and Jean Mathieu. 2019. "Training Program - Induced Skeletal Muscle Adaptations in Two Men with Myotonic Dystrophy Type 1." *BMC Research Notes*, 1–6. https://doi.org/10.1186/s13104-019-4554-z.
Sahashi, Ko, Masashi Tanaka, Michinari Tashiro, Kinji Ohno Tohru Ibi, Akira Takahashi, and Takayuki Ozawa. 1992. "Increased Mitochondrial DNA Deletions in the Skeleal Muscle of Myotonic Dystrophy." *Gerontology* 38: 18–29.
Salvatori, Sergio, M. Fanin, C. P. Trevisan, S. Furlan, S. Reddy, J. I. Nagy, and C. Angelini. 2005. "Decreased Expression of DMPK: Correlation with CTG Repeat Expansion and Fibre Type Composition in Myotonic Dystrophy Type 1." *Neurological Sciences* 26 (4): 235–42. https://doi.org/10.1007/s10072-005-0466-x.
Santoro, M., M. Masciullo, G. Silvestri, G. Novelli, and A. Botta. 2017. "Myotonic Dystrophy Type 1: Role of CCG, CTC and CGG Interruptions within DMPK Alleles in the Pathogenesis and Molecular Diagnosis." *Clinical Genetics* 92 (4): 355–64. https://doi.org/10.1111/cge.12954.
Sarkar, Partha S, Binoy Appukuttan, Jennifer Han, Yoshihiro Ito, Cuiwei Ai, Wenli Tsai, Yang Chai, J Timothy Stout, and Sita Reddy. 2000. "Heterozygous Loss of Six5 in Mice Is Sufficient to Cause Ocular Cataracts." *Nature Genetics* 25 (1): 110–14.
Savkur, R S, A V Philips, and T A Cooper. 2001. "Aberrant Regulation of Insulin Receptor Alternative Splicing Is Associated with Insulin Resistance in Myotonic Dystrophy." *Nature Genetics* 29 (1): 40–47. https://doi.org/10.1038/ng704.
Savouret, Cédric, Corinne Garcia-Cordier, Jérôme Megret, Hein te Riele, Claudine Junien, and Geneviève Gourdon. 2004. "MSH2-Dependent Germinal CTG Repeat Expansions Are Produced Continuously in Spermatogonia from DM1 Transgenic Mice." *Molecular and Cellular Biology* 24 (2): 629–37. https://doi.org/10.1128/mcb.24.2.629-637.2004.
Sharp, Lydia, Diana C. Cox, and Thomas A. Cooper. 2019. "Endurance Exercise Leads to Beneficial Molecular and Physiological Effects in a Mouse Model of Myotonic Dystrophy Type 1." *Muscle and Nerve* 60 (6): 779–89. https://doi.org/10.1002/mus.26709.
Siboni, Ruth B., Masayuki Nakamori, Stacey D. Wagner, Adam J. Struck, Leslie A. Coonrod, Shanee A. Harriott, Daniel M. Cass, Matthew K. Tanner, and J. Andrew Berglund. 2015. "Actinomycin D Specifically Reduces Expanded CUG Repeat RNA in Myotonic Dystrophy Models." *Cell Reports* 13 (11): 2386–94. https://doi.org/10.1016/j.celrep.2015.11.028.
Siciliano, G., M. Mancuso, D. Tedeschi, M. L. Manca, M. R. Renna, V. Lombardi, A. Rocchi, F. Martelli, and L. Murri. 2001. "Coenzyme Q10, Exercise Lactate and CTG Trinucleotide

Expansion in Myotonic Dystrophy." *Brain Research Bulletin* 56 (3–4): 405–10. https://doi.org/10.1016/S0361-9230(01)00653-0.

Solbakken, Gro, Bård Bjørnarå, Eva Kirkhus, Bac Nguyen, Gunnar Hansen, Jan C. Frich, and Kristin Ørstavik. 2019. "MRI of Trunk Muscles and Motor and Respiratory Function in Patients with Myotonic Dystrophy Type 1." *BMC Neurology* 19 (1): 1–11. https://doi.org/10.1186/s12883-019-1357-8.

Steinberg, Gregory R. 2019. "AMP-Activated Protein Kinase : The Current Landscape for Drug Development." *Nature Reviews Drug Discovery* 18 (7): 527–51. https://doi.org/10.1038/s41573-019-0019-2.

Talan, D. A., R. A. Bauernfeind, W. W. Ashley, C. Kanakis, and K. M. Rosen. 1982. "Twenty-Four Hour Continuous ECG Recordings in Long-Distance Runners." *Chest* 82 (1): 19–24. https://doi.org/10.1378/chest.82.1.19.

Taneja, Krishan L, Mila McCurrach, Martin Schalling, David Housman, and Robert H Singer. 1995. "Foci of Trinucleotide Repeat Transcripts in Nuclei of Myotonic Dystrophy Cells and Tissues" 128 (6): 995–1002.

Tarnopolsky, Mark A., Erin Pearce, Katelyn Smith, and Boleslaw Lach. 2011. "Suction-Modified Bergström Muscle Biopsy Technique: Experience with 13,500 Procedures." *Muscle and Nerve* 43 (5): 717–25. https://doi.org/10.1002/mus.21945.

Temmerman, Nele De, Karen Sermon, Sara Seneca, Martine De Rycke, Pierre Hilven, Willy Lissens, André Van Steirteghem, and Inge Liebaers. 2004. "Intergenerational Instability of the Expanded CTG Repeat in the DMPK Gene: Studies in Human Gametes and Preimplantation Embryos." *American Journal of Human Genetics* 75 (2): 325–29. https://doi.org/10.1086/422762.

Thijs, C, J F Mirandolle, H B Anten, H J M Smeets, K E Chandler, and J P M Geraedts. 1998. "Age and Causes of Death in Adult-Onset Myotonic Dystrophy," 1557–63.

Thornton, Charles A. 2014. "Myotonic Dystrophy." *Neurologic Clinics* 32 (3): 705–19. https://doi.org/10.1016/j.ncl.2014.04.011.

Thornton, Charles A., Keith Johnson, and Richard T. Moxley. 1994. "Myotonic Dystrophy Patients Have Larger CTG Expansions in Skeletal Muscle than in Leukocytes." *Annals of Neurology* 35 (1): 104–7. https://doi.org/10.1002/ana.410350116.

Thornton, Charles A, James P Wym, Zachary Simmons, Carolyn Mcclain, and Richard T Moxley III. 1997. "Expansion of the Myotonic Dystrophy CTG Repeat Reduces Expression of the Flanking DMAHP Gene." *Nature Genetics* 16 (4): 407–9.

Timchenko, L, W Nastainczykt, T Schneidert, B Patel, F Hofmannt, and C T Caskey. 1995. "Full-Length Myotonin Protein Kinase (72 KDa) Displays Serine Kinase Activity." *Proceedings of the National Academy of Sciences* 92 (12): 5366–70.

Tollbäck, Anna, Stefan Eriksson, Anna Wredenberg, Göran Jenner, Roberto Vargas, Kristian Borg, and Tor Ansved. 1999. "Effects of High Resistance Training in Patients with Myotonic Dystrophy." *Scandinavian Journal of Rehabilitation Medicine* 31 (1): 9–16. https://doi.org/10.1080/003655099444678.

Tomé, Stéphanie, Elodie Dandelot, Céline Dogan, Alexis Bertrand, David Geneviève, Yann Péréon, Marie Simon, et al. 2018. "Unusual Association of a Unique CAG Interruption in 5′ of DM1 CTG Repeats with Intergenerational Contractions and Low Somatic Mosaicism." *Human Mutation* 39 (7): 970–82. https://doi.org/10.1002/humu.23531.

Toscano, A, S Messina, G M. Campo, R Di Leo, O Musumeci, C Rodolico, M Aguennouz, G Annesi, C Messina, and G Vita. 2005. "Oxidative Stress in Myotonic Dystrophy Type 1."

Free Radical Research 39 (7): 771–76.

Vihola, A., G. Bassez, G. Meola, S. Zhang, H. Haapasalo, A. Paetau, E. Mancinelli, et al. 2003. "Histopathological Differences of Myotonic Dystrophy Type 1 (DM1) and PROMM/DM2." *Neurology* 60 (11): 1854–57. https://doi.org/10.1212/01.WNL.0000065898.61358.09.

Vita, G, A Toscano, A Prelle, A Bordoni, N Checcarelli, N Bresolin, A Baradello, and C Messina. 1993. "Muscle Mitochondria Investigation in Myotonic Dystrophy Abstract." *Eur Neurol* 33: 423–27.

Wang, Eric T., Daniel Treacy, Katy Eichinger, Adam Struck, Joseph Estabrook, Hailey Olafson, Thomas T. Wang, et al. 2019. "Transcriptome Alterations in Myotonic Dystrophy Skeletal Muscle and Heart." *Human Molecular Genetics* 28 (8): 1312–21. https://doi.org/10.1093/hmg/ddy432.

Wang, Eric T, Amanda J Ward, Jennifer M Cherone, Jimena Giudice, Thomas T Wang, Daniel J Treacy, Nicole J Lambert, et al. 2015. "Antagonistic Regulation of MRNA Expression and Splicing by CELF and MBNL Proteins." *Genome Res.* 25 (6): 858–71. https://doi.org/10.1101/gr.184390.114.Freely.

Wang, Guey-shin, Xander H T Wehrens, A Thomas, Guey-shin Wang, Muge N Kuyumcu-martinez, Satyam Sarma, and Nitin Mathur. 2009. "PKC Inhibition Ameliorates the Cardiac Phenotype in a Mouse Model of Myotonic Dystrophy Type 1 Find the Latest Version : PKC Inhibition Ameliorates the Cardiac Phenotype in a Mouse Model of Myotonic Dystrophy Type 1." *Journal of Clinical Investigation* 119 (12): 3797–3806. https://doi.org/10.1172/JCI37976.for.

Warf, M. Bryan, Masayuki Nakamori, Catherine M. Matthys, Charles A. Thornton, and J. Andrew Berglund. 2009. "Pentamidine Reverses the Splicing Defects Associated with Myotonic Dystrophy." *Proceedings of the National Academy of Sciences of the United States of America* 106 (44): 18551–56. https://doi.org/10.1073/pnas.0903234106.

Wei, Christina, Lauren Stock, Leila Valanejad, Zachary A. Zalewski, Rebekah Karns, Jack Puymirat, David Nelson, et al. 2018. "Correction of GSK3β at Young Age Prevents Muscle Pathology in Mice with Myotonic Dystrophy Type 1." *FASEB Journal* 32 (4): 2073–85. https://doi.org/10.1096/fj.201700700R.

Wheeler, Thurman M., Andrew J. Leger, Sanjay K. Pandey, A. Robert Mac Leod, Thurman M. Wheeler, Seng H. Cheng, Bruce M. Wentworth, C. Frank Bennett, and Charles A. Thornton. 2012. "Targeting Nuclear RNA for in Vivo Correction of Myotonic Dystrophy." *Nature* 488 (7409): 111–17. https://doi.org/10.1038/nature11362.

Wheeler, Thurman M, John D Lueck, Maurice S Swanson, Robert T Dirksen, and Charles A Thornton. 2007. "Correction of ClC-1 Splicing Eliminates Chloride Channelopathy and Myotonia in Mouse Models of Myotonic Dystrophy" 117 (12). https://doi.org/10.1172/JCI33355.3952.

Whiting, Elisabeth J., James D. Waring, Katsuyuki Tamai, Martin J. Somerville, Maxwell Hincke, William A. Staines, Joh-e Ikeda, and Robert G Korneluk. 1995. "Characterization of Myotonic Dystrophy Kinase (DMK) Protein in Human and Rodent Muscle and Central Nervous Tissue." *Human Molecular Genetics* 4 (6): 1063–72.

Whitney, Susan L., Diane M. Wrisley, Gregory F. Marchetti, Michael A. Gee, Mark S. Redfern, and Joseph M. Furman. 2005. "Clinical Measurement of Sit-to-Stand Performance in People with Balance Disorders: Validity of Data for the Five-Times-Sit-to-Stand Test." *Physical Therapy* 85 (10): 1034–45. https://doi.org/10.1093/ptj/85.10.1034.

Wright, Nancy C., David D. Kilmer, Megan A. McCrory, Susan G. Aitkens, Bryan J. Holcomb,

and Edmund M. Bernauer. 1996. “Aerobic Walking in Slowly Progressive Neuromuscular Disease: Effect of a 12-Week Program.” *Archives of Physical Medicine and Rehabilitation* 77 (1): 64–69. https://doi.org/10.1016/S0003-9993(96)90222-1.

Zehender, Manfred, Thomas Meinertz, Joseph Keul, and Hansjörg Just. 1990. “ECG Variants and Cardiac Arrhythmias in Athletes: Clinical Relevance and Prognostic Importance.” *American Heart Journal* 119 (6): 1378–91. https://doi.org/10.1016/S0002-8703(05)80189-9.

Aerobic exercise induced functional and cellular adaptations in patients with myotonic dystrophy type 1

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ABSTRACT

Myotonic dystrophy type 1 (DM1) is a neuromuscular disorder that primarily affects adults. It affects approximately 1 in 8000 persons. DM1 patients often present with accelerated skeletal muscle weakness and wasting, myotonia and insulin resistance, that results in poor quality of life, limited functionality and reduced lifespan. A microsatellite (CTG) repeat expansion in the non-coding region of the dystrophia myotonica protein kinase (*DMPK*) gene results in abnormal expression and localization of several RNA binding proteins (RNABPs) important for muscle health such as MBNL and CUGBP1. Aerobic exercise in DM1 mice ameliorated canonical symptoms of DM1 pathology in mice. Additionally, exercise training in DM1 patients is safe and well tolerated. This study aimed to investigate the muscular adaptations of 12-weeks of aerobic exercise in eleven DM1 patients (DM1, 42.6 ± 3 y) recruited from the Neuromuscular and Neurometabolic clinic at McMaster University. Age and sex matched healthy controls (CON, 42.5 ± 2 y) were recruited for reference values. Both groups performed baseline testing including incremental VO_{2peak} testing, muscle and spirometry functional tests and a skeletal muscle biopsy from the *Vastus lateralis*. Only DM1 patients underwent 12-weeks of training on a cycle ergometer (3x/wk @ ~65 %VO_{2peak}) and completed follow-up testing. Cycle ergometry increased total lean mass (TLM) by ~ 1.6 kg ($p<0.05$) and fibre cross-sectional area by ~30 % in DM1 patients. Cardiorespiratory fitness improved following training from 19.7 ± 1.5 mL/kg/min to 26.0 ± 2.1 mL/kg/min ($p<0.05$). Furthermore, functional capacity was increased following training as measured by 6-min walk test, timed up & go, and 5X sit-to-stand scores ($p<0.05$). Mechanistically, exercise increased MBNL2 protein expression, and enhanced mitochondrial function and protein content. This is the first study to examine the effects of cycle ergometry training on functional, molecular and mitochondrial adaptation in DM1 patients. Our data support the safety of exercise

training in DM1 patients and provide new evidence for improved fitness, functional capacity and muscle mass in DM1. Further understanding the influence of exercise on DM1 pathology could outline the efficacy of a simple life intervention and provide insight for future pharmacological discoveries for DM1.

INTRODUCTION

Myotonic dystrophy type 1 (DM1) is the most commonly diagnosed muscular dystrophy amongst adults, and the second most common of all muscular dystrophies after Duchenne muscular dystrophy (DMD) (Chau and Kalsotra 2015). Disease inheritance is autosomal dominant and has been linked to a microsatellite trinucleotide 'CTG' repeat expansion in the 3' untranslated region (3'UTR) of the dystrophia myotonica protein kinase (*DMPK*) gene on chromosome 19q (Brook et al. 1992; Mahadevan et al. 1992). Healthy individuals possess up to 37 repeat expansions, while DM1 symptoms begin to manifest with 50-3000 repetitions of the 'CTG' mutation (Brook et al. 1992). DM1 is a progressive neuromuscular disorder (NMD) characterized by skeletal muscle wasting, weakness and myotonia primarily in distal muscles of the extremities (Thornton 2014). Furthermore, the DM1 phenotype presents with multisystemic involvement including insulin resistance, respiratory dysfunction, cardiac conduction defects, premature cataracts, cognitive impairment and gastrointestinal issues (Thornton 2014). The disease severity is moderately correlated to 'CTG' repeat size; where, individuals with greater repeat amplification present with a more severe phenotype (Harley et al. 1992; Redman et al. 1993).

The DM1 microsatellite repeat mutation results in nuclear accumulation of *DMPK* transcripts forming aggregates of double-stranded hairpin loops or foci (Taneja et al. 1995; Davis et al. 1997). 'CUG' nuclear foci trigger a dysregulation in the expression and localization of several RNA-binding proteins (RNABP) critical for correct splicing. Notably, the muscle-blind like protein family (MBNL), specifically isoforms MBNL1 and MBNL2, are widely expressed in various tissues and are critical during several steps in mRNA metabolism. MBNL1 and 2 possess a high affinity for 'CUG' sequence resulting in nuclear sequestration within the RNA foci and reduced functional MBNL in the cytoplasm (Mankodi et al. 2001; Miller et al. 2000). In contrast,

CUGBP Elav-like family member 1 protein (CUGBP1 or CELF1) functions antagonistically to MBNL promoting the splicing of fetal isoforms and is hyperactivated in individuals diagnosed with DM1 (Philips, Timchenko, and Cooper 1998; Kuyumcu-Martinez, Wang, and Cooper 2007). Collectively, *DMPK* toxic gain of function disrupts MBNL and CUGBP1 expression patterns resulting in the mis-splicing of several pre-mature mRNA transcripts (E. T. Wang et al. 2015), such as adult isoforms of insulin receptor (*IR*) and chloride voltage-gated channel (*CLCN1*). In DM1, there is a greater exclusion of exon 11 in *IR* and inclusion of exon 7a in *CLCN1* mRNA resulting in the canonical symptoms of DM1 like insulin resistance and myotonia, respectively (Wheeler et al. 2007; Masayuki Nakamori et al. 2013; Savkur, Philips, and Cooper 2001).

AMP-activated protein kinase (AMPK) is a key regulator of energy homeostasis primarily during energy deprivation (Steinberg 2019). Furthermore, its critical involvement in neuromuscular plasticity has garnered attention as a promising therapeutic target against NMDs such as DMD, spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) (Dial et al. 2018). Pharmacological activation of AMPK in murine models of DM1 reduced MBNL1 sequestration and improved splicing defects (Ravel-chapuis et al. 2018). In patients with DM1, 52 weeks of metformin supplementation , a well-known AMPK activator, significantly improved mobility in contrast to placebo administration (Bassez et al. 2018). Additionally, DM1 fibroblasts display reduced oxygen consumption rates and other indications of mitochondrial dysfunction that were ameliorated with metformin treatment (García-puga et al. 2020). Aerobic exercise training is a safe and potent physiological activator of AMPK (Chen et al. 2003). Exercise has been shown to induce physiological and molecular benefits in NMDs (Madsen et al. 2015; Ng, Mikhail, and Ljubicic 2019; Clawson et al. 2018; Brady, MacNeil, and Tarnopolsky 2014; Ng, Manta, and Ljubicic 2018; M. Roussel et al. 2019; M. Jansen et al. 2013). Recently, aerobic training in a

murine model of DM1 corrected spliceopathy, liberated MBNL1, and significantly reduced relaxation time (Manta et al. 2019; Sharp, Cox, and Cooper 2019; Ravel-chapuis et al. 2018). Previous studies suggested that exercise training has no effect on functional capacity in DM1 patients; however, this was likely due to low/insufficient exercise intensities (Wright et al. 1996; Kierkegaard et al. 2011). Conversely, other studies report improvements in aerobic capacity and muscle fibre size but did not implement measures to assess functional and other physiological adaptations (Ørngreen, Olsen, and Vissing 2005). Overall, DM1 patient trials have shown conflicting results and the benefits are largely unknown, especially at the cellular/mitochondrial level. Thus, the purpose of the current clinical trial was to comprehensively assess the adaptations to 12 weeks of aerobic cycling on functional, strength and cellular outcomes in patients with myotonic dystrophy type 1.

METHODS

Ethical approval. This trial was approved by the Hamilton Integrated Research Ethics Board (# 7091) and complied with the guidelines set out in the Canadian Tri-Council policy statement on ethical conduct for research involving humans. All participants were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. This study was registered on clinicaltrials.gov under study #: NCT04187482.

Participants. All DM1 patients at the Neuromuscular and Neurometabolic Clinic at McMaster University Medical Center (MUMC) were considered for this trial. A detailed schematic of the recruitment process can be found in Figure 1. Briefly, 33 out of 314 patients satisfied the age (18-60 y) and CTG (100-1000 repeats) repeat criteria and resided within or around the Greater Hamilton Area. A total of 13 DM1 patients were interested and recruited to participate in this

clinical trial (41.5 ± 3 y). Patients 10 and 11 dropped out after week 2 of the trial for personal reasons unrelated to the study. Therefore, a total of 11 participants (5M and 6F; 42.6 ± 3 y; DM1) completed the trial and were included in the final analysis. Leukocyte 'CTG' repeat length was measured for all patients at time of diagnosis and were not measured during the course of this trial. Eleven age and sex matched (5M and 6F; 42.5 ± 2 y; CON) healthy controls were recruited from the Hamilton community for reference values. All study participants performed < 2 h of structured aerobic activity per week and were asked to refrain from performing any exercise during the course of the study. Other exclusion criteria included smoking, obesity (BMI > 34.9 kg/m^2), diabetes (type 1 or 2), cardiovascular or respiratory disorders (other than a mild restrictive ventilatory defect), other genetic disorders, active musculoskeletal injuries or any other health complications that would preclude them from exercise training.

Study design. All participants completed 3 baseline visits to assess pre-exercise measures. Visit 1 included a brief health questionnaire, anthropometric measures, body composition, electrocardiogram (ECG) and maximal aerobic fitness test (VO_{2peak}). After 48 h, participants completed Visit 2 which consisted of functional, strength and spirometry testing. Finally, participants were asked to refrain from exercise for a minimum of 48 h and report to our laboratory fasted for Visit 3 to collect a blood sample and skeletal muscle biopsy from the *vastus lateralis*. Only the DM1 group completed a 12-week aerobic exercise intervention and follow up testing. Follow up testing was done on the week immediately after the last exercise sessions and consisted of the same 3 visits completed at baseline.

Training intervention. Exercise protocol consisted of three training sessions per week for a 12-week period on an electronically braked cycle ergometer (Lode, Groningen, Netherlands). Each training session began with a 3-min warm up at 25 watts (W) followed by 30-min at 65% of max

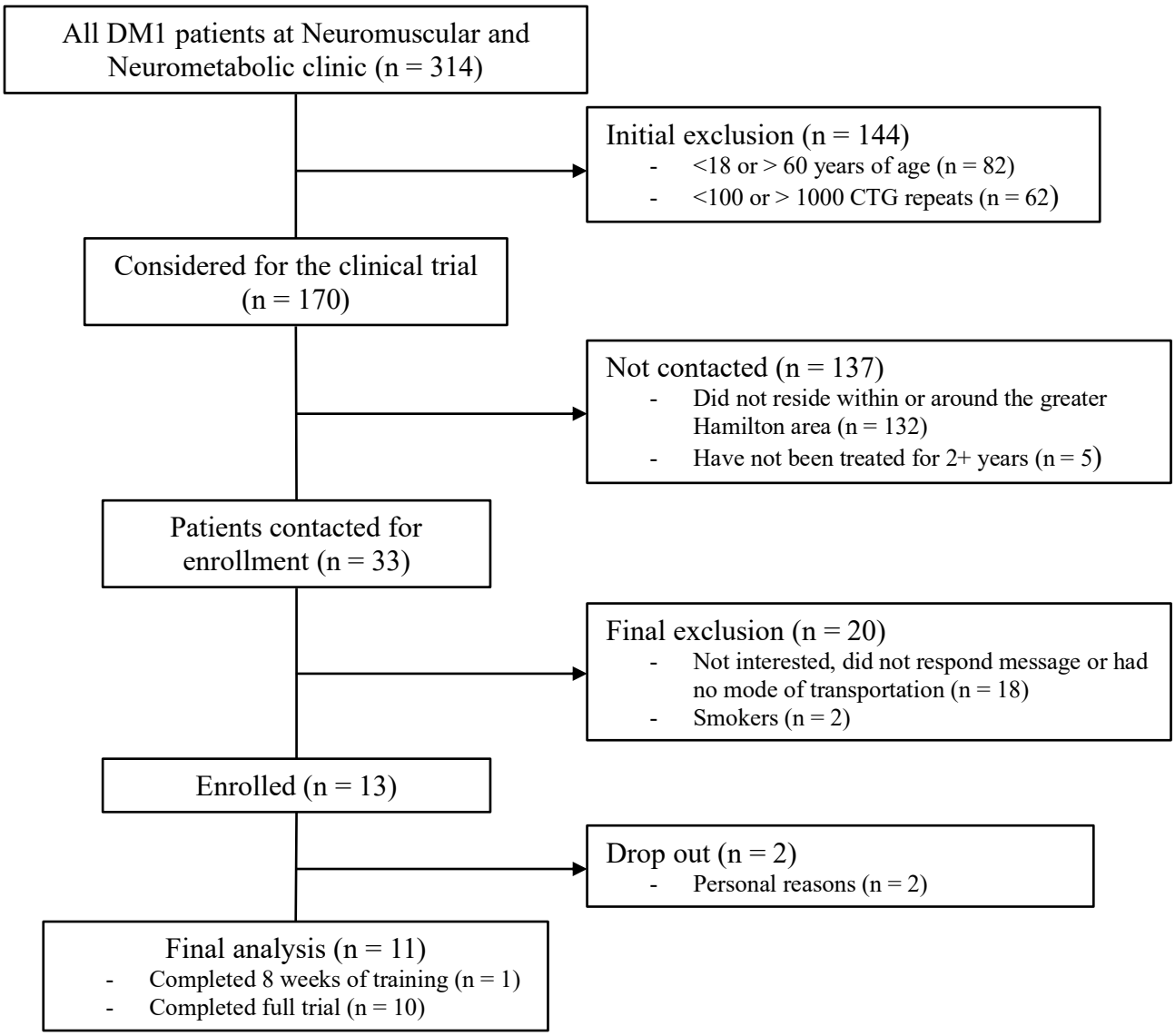

Figure 1. Flowchart of patient population and study enrollment

workload determined during VO_{2peak} test (W_{peak}) and ended with a 2-min cool down at 25 W. Training intensity progressively increased to 35-min at 75%W_{peak} by week 12. All training sessions were completed in our laboratory and supervised by a trained professional.

Spirometry and ECG. Participants performed a series of three strong inhalations, followed by a strong exhalation in a seated position to measure forced vital capacity (FVC) and forced expiratory volume (FEV1) using Sentrysuite software (Vyaire Medical, Mettawa, IL, USA). FVC and FEV1 values are expressed as absolute and as a percentage of predicted. DM1 patients underwent a standard 12-lead ECG (GE Healthcare, Milwaukee, WI) before and after performing VO_{2peak} test to assess for arrythmias. An electrophysiology cardiologist was consulted if any patients had a PR interval > 225 ms or QRS complex > 125 ms prior to performing any strenuous exercise. At baseline, one patient had a prolonged PR interval, two patients had prolonged QRS complexes and one patient had prolonged PR and QRS. Following the exercise intervention, the same patient had a prolonged PR interval, only one patient still had a prolonged QRS complex and the same patient had a prolonged PR and QRS.

Maximal aerobic fitness test ($VO_{2\ peak}$). Peak oxygen uptake was measured using a metabolic cart with a gas collection system (Moxus Modular Metabolic System, AEI Technology, Pittsburgh, PA, USA) during a double-leg incremental cycle ergometer test (Lode). The test began with a 2-minute warm up at 30W, after which the power was progressively increased by 15W for DM1 participants and 30W for healthy control participants every minute until participants reached volitional exhaustion or a cadence below 50 rpm. Heart rate was continuously monitored throughout the test using a heart rate monitor (Polar A3, Lake Success, NY, USA). VO_{2peak} was defined as the highest oxygen consumption recorded over a 15 s period and maximal workload was the highest power output reached during the test. Following test completion, participants

completed a 3-min cool-down period pedaling against zero resistance.

Body composition. Dual-Energy X-ray Absorptiometry (DEXA; GE Lunar Prodigy, Madison, WI, USA) scan was used to measure fat mass, total lean mass (TLM) and body composition before and after exercise intervention.

Functional testing. In the present study, a total of three tests were used to assess functional capacity: 6-min walk test, 5 time sit-to-stand and timed up and go test. The 6-minute walk test (6MWT) score is an indicator of muscular endurance (Enright 2003). Participants were given standardized instructions and test was done in a flat, 15 m hallway. Five times sit-to-stand test (5X STS) was used as a measure of functional strength of the lower limbs and timed up and go (TUG) test was implemented to assess functional mobility and agility. Both tests were completed as previously described (Whitney et al. 2005; Podsiadlo and Richardson 1991).

Strength testing. Participants completed isometric unilateral maximal knee extension on a dynamometer (System 3, Biodex Medical Systems, Shirley, NY, USA) at a 90° angle on the right leg. Contractions were performed in triplicate for 5 s with 30 s rest in between and max torque (N•m) achieved was recorded. A hand dynamometer was used to assess maximal grip strength (Jamar, Sammons, Bolingbrook, IL, USA) and lateral/key pinch grip (B&L Engineering, Santa Ana, CA, USA) for both the right and left hand. Participants were instructed to maximally contract for 3 s with 30 s rest between each trial. Measurements were done in triplicates on each hand and highest force achieved was used.

Muscle biopsy. A resting muscle biopsy was obtained from the mid-section of the *vastus lateralis* during the last visit of baseline and follow up testing. The biopsy leg was randomized between participants, but Pre and Post biopsies were taken from the same leg with >1.5 cm between incisions and the proximal/distal sequence was randomized. Participants were asked to refrain

from all food/drinks (except water) and exercise for 12 and 48 h, respectively. Biopsy samples were obtained using a suction-modified Bergström needle under local anaesthesia, as previously described (Tarnopolsky et al. 2011). Approximately 20 mg of muscle was embedded in optimal cutting temperature (OCT) medium for histological and immunofluorescence experiments, and ~10 mg was immersed in pre-chilled solution for mitochondrial respiration analysis. The remaining samples were immediately frozen in liquid nitrogen and stored in a -80° freezer until further analyses.

Protein extraction. Approximately 20 mg of frozen muscle was powdered using a cell crusher (Cellcrusher, Cork, Ireland) then placed in RIPA buffer (1:20, Thermo Fisher Scientific, Burlington, ON, Canada) with Halt protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Samples were then sonicated (Qsonica L.L.C, Newtown, CT, USA) on ice at 75 % power and incubated at 4°C on a rotating mixer for 30 min. Finally, supernatant was collected after homogenates were centrifuged at 12,000 g for 10 min. Protein concentration in each sample was determined using a standard bicinchoninic protein assay (BCA; Thermo Fisher Scientific) and all samples were diluted with 4x loading buffer and ultra-pure water to a final concentration of 2 μg/μL.

Western blotting. Samples (20-30 μg) were separated on a 4-20% Criterion TGX precast protein gel (Bio-Rad Laboratories, Mississauga, ON, Canada) for 10 min at 70 V then for 30 min at 240 V. Afterwards, proteins were transferred onto nitrocellulose transfer pack (Bio-Rad Laboratories) using a Trans-Blot Turbo transfer system (Bio-Rad Laboratories). Subsequently, membranes were stained with Ponceau S solution (Sigma-Aldrich, Oakville, ON, Canada), imaged and placed in blocking solution (5% BSA in 1X TBST) for 1 hour. Membranes were then incubated overnight at 4°C with primary antibodies listed in Table S1. Blots were then washed in 1X TBST for 3 x 5

min and incubated in the appropriate secondary antibody for 1 h at room temperature. Next, membranes were washed in 1X TBST buffer for 3 x 5 min again prior to applying luminol based enhanced chemiluminescence (ECL) reagent (Bio-Rad Laboratories) for visualization. Finally, proteins were imaged using ChemiDoc MP Imaging System (Bio-Rad Laboratories) and quantified using Image Lab software (Bio-Rad Laboratories). All bands were normalized to their respective Ponceau prior to analysis.

Histochemical staining. OCT embedded samples were sectioned into 10 μm cross-sections on a cryostat (Leica Biosystems, Concord, ON, Canada) and stained for haematoxylin and eosin. Slides were then dehydrated with successive washes of 70, 95, and 100% ethanol, and further dried with xylene then mounted with Permount (Thermo Fisher Scientific). Images were captured using Nikon 90i eclipse upright microscope and Nikon elements software (Nikon Instruments, Melville, NY, USA). Centrally nucleated myofibres were analyzed using ImageJ 2.0 software and were defined as a muscle fibre with a minimum of 1 myonuclei not in contact with the periphery. An average 252 ± 14 myofibres were analyzed per sample.

Immunoflorescence staining. Samples were sectioned into 10 μm thick and mounted on glass slides. Briefly, slides were rinsed in 1X PBS then blocked with 10% goat serum in 1 % BSA in 1X PBS and incubated overnight at 4 °C in primary antibodies (Table S1) for myosin heavy chain (MHC) I, MHC IIA and MHC IIX (Developmental Studies Hybridoma Bank, Iowa City, IA, USA; DSHB). Slides were once again rinsed in 1X PBS and the appropriate secondary antibodies were applied (Alexa Fluor 488, 555 or 647, Thermo Fisher Scientific) prior to mounting with fluorescence mounting medium (Dako, Burlington, ON, Canada). Muscle cross-sections were imaged with CoolSNAP HQ2 fluorescence camera (Nikon Instruments) at 20X magnification.

Fibre cross-sectional area analysis. Fibre type analysis and cross-sectional area (CSA) were done using Nikon NIS Elements AR 4.40 software (Nikon Instruments). The entire muscle sample was analyzed for fibre type distribution analysis and approximately 60 % of fibres were used to obtain accurate measures for CSA (Nederveen et al. 2020). An average of 83 ± 6, 92 ± 4 and 38 ± 5 fibres were circled for type I, type IIA and type IIX, respectively.

Preparation of permeabilized muscle fibres. Immediately after biopsy collection, ~10 mg piece of muscle was immersed in pre-chilled BIOPS solution (50 mM MES Hydrate, 7.23 mM MES K_2EGTA, 2.77mM CaK_2EGTA, 20 mM imidazole, 0.5 mM dithiothreitol, 20 mM taurine, 5.77 mM ATP, 15 mM PCr, and 6.56 mM $MgCl_2$•6 H_2O; pH 7.1). Under a light microscope, samples were stripped of connective tissue, blood and fat using fine-tip forceps. Muscle fibres were then separated and divided into two roughly even bundles. Each bundle was washed on a rotating mixer for 30 min at 4°C in BIOPS solution that was treated with 40 μg/mL saponin. Lastly, muscle bundles were washed in MiR05 (0.5 mM EGTA, 10 KH_2PO_4, 3 mM $MgCl_2$•6 H_2O, 60 mM K-lactobionate, 20 mM Hepes, 20 mM taurine, 110 mM sucrose and 1 g/L BSA; pH 7.1) for 15 min at 4°C prior to analysis.

Mitochondrial respiration. Mitochondrial respiration experiments were performed in 2 mL of MIR05 at 37°C using Oroboros Oxygraph-2k (Oroboros Instruments, Innsburck, Austria). MiR05 contained 5 μM blebbistatin, to avoid spontaneous contraction of muscle fibres, and 20 mM creatine during all experiments. Each assay began with oxygenating each chamber to an O_2 concentration of ~350 μM. After steady state was reached, respiration was stimulated with an ADP titration (25 μM, 100 μM, 500 μM, 1000 μM, 2000 μM, 4000 μM, 8000 μM) in the presence of pyruvate (5 mM) and malate (2 mM). Glutamate (5 mM) was then added to measure complex I maximal respiration. Subsequently, succinate (20 mM) and rotenone (0.5 μM) were added to

assess complex I+II and complex II maximal respiration, respectively. To ensure mitochondrial membrane integrity, cytochrome *c* (10 μM) was added to confirm <10 % change in respiration (CON: -1.0 ± 0.9%, DM1-PRE: -0.4 ± 0.7 % and DM1-POST: -0.1 ± 1.2%). Two samples from control participants were of poor quality and therefore were not included in the final analysis. All experiments were performed in duplicates and averaged for each participant. Data was expressed relative to wet weight of each bundle.

Blood analysis. Heparinized blood was collected during visit 3 of baseline and follow up testing in a fasted state. Samples were immediately centrifugated at 4°C for 15 min at 2000 g. Plasma was then collected and sent to the CORE facility at McMaster University Medical Center (CLIA certified laboratory) for further analysis of fasting blood glucose (GLUF), creatine kinase (CK), creatinine, bilirubin, alanine transaminase (ALT) and gamma-glutamyl transpeptidase (GTT) levels.

Statistical analysis. Purpose of this trial is to primarily investigate exercise-induce clinical and physiological benefits in DM1 patients. Therefore, a paired Student's t-test was implemented to determine exercise changes in DM1 patients. An ordinary one-way ANOVA was employed to determine significance between control participants and DM1 patients Pre and Post exercise training intervention. All statistical analysis was completed on GraphPad Prism software version 6 (GraphPad Software, La Jolla, CA, USA). All data is expressed as mean ± SEM. Statistical significance was accepted at $P < 0.05$.

RESULTS

Participant characteristics. Individual patient characteristics can be seen in Table 1 along with CON averages. By design, DM1 and CON groups did not statistically differ in age (42.6 ± 3 y and

42.5 ± 2 y, respectively), weight (68.1 ± 4.8 kg and 69.7 ± 2.7 kg, respectively), height (1.68 ± 0.03 m and 1.72 ± 0.03 m, respectively) or body mass index (BMI; 23.9 ± 1.3 kg/m^2 and 23.6 ± 0.6 kg/m^2, respectively).

Exercise adherence and safety. Adherence was measured by how many supervised training sessions each participant attended. Patient 5 dropped out of the trial after 8 weeks of training due to unrelated personal reasons and completed 18/24 exercise sessions (75%). However, patient 5 completed follow up testing (excluding VO_{2peak} test) and was therefore included in the final analysis. All other participants completed the 12-week intervention and had an average adherence of 98%. Adherence rates for each patient can be found in Table 1.

To ensure our exercise training was safely tolerated, we measured circulating GLUF, CK, creatinine, bilirubin, ALT and GGT before and after exercise in DM1. There was no significant difference ($P > 0.05$) between Pre (240 ± 44) and Post (290 ± 49) plasma CK levels. Furthermore, creatinine, bilirubin, ALT or GGT did not significantly change following the exercise intervention (Table 2, $P > 0.05$). GLUF significantly increased from 5.1 ± 0.1 mmol/L to 5.4 ± 0.1 mmol/L in DM1 patients following exercise intervention, but all remained < 6.00 mmol/L. We next analyzed muscle histology sections for the rate of fibres containing centrally located nuclei to further confirm that our exercise protocol had no adverse effects on skeletal muscle of DM1 patients. At baseline, ~27 out of 100 fibres in DM1 muscle contained centrally located nuclei compared to only ~5 per 100 fibres in CON (Fig 2A and B, $P < 0.05$). Following exercise training, DM1 patients and CON group were no longer significantly different ($P > 0.05$). Despite the lack of significance in DM1 Pre and Post exercise, Patients 2, 3, 5, 6, 9 and 13 experienced >15% reduction in the prevalence of centrally nucleated fibres, patients 4 and 7 did not change while

Table 1: Individual characteristics of patients

Patient # (Sex)	Age (y)	Age at diagnosis	Weight (kg)	Height (m)	CTG repeats	Adherence
1 (M)	26	23	80.3	1.88	650	34/36
2 (F)	50	31	71.5	1.67	800	36/36
3 (M)	50	NA	53.3	1.76	650	36/36
4 (M)	43	34	77.9	1.76	400	36/36
5 (F)	27	16	78.9	1.66	500	18/36
6 (F)	49	44	50.9	1.53	900	36/36
7 (M)	47	42	52.6	1.63	900	36/36
8 (F)	39	19	91.6	1.71	300	34/36
9 (F)	40	10	47.3	1.60	900	36/36
12 (M)	53	42	85.3	1.82	800	36/36
13 (F)	45	NA	59.5	1.52	300	35/36
Mean	42.6 ± 3	-	68.1 ± 4.8	1.68 ± 0.03	645 ± 71	34/36
CON	42.5 ± 2	-	69.7 ± 2.7	1.72 ± 0.03	-	-

Note: only the 11 patients who completed the study and were included in the final analysis are presented here. Group values are the mean ± SEM.

Table 2: Blood work

Measures	DM1-PRE	DM1-POST	
GLUF (mmol/L)	5.1 ± 0.07	5.4 ± 0.09	$P < 0.05$
CK (units/L)	240 ± 44	290 ± 49	NS
Creatinine (µmol/L)	71.6 ± 3.4	74.1 ± 4.9	NS
Bilirubin (µmol/L)	8.4 ± 0.7	8.5 ± 1.0	NS
ALT (units/L)	39.3 ± 9.0	52.4 ± 12.2	NS
GGT (units/L)	94.9 ± 36.6	75.0 ± 22.1	NS

GLUF, fasting blood glucose. CK, creatine kinase. ALT, alanine transaminase. GGT, gamma-glutamyl transpeptidase. Values are mean ± SEM.

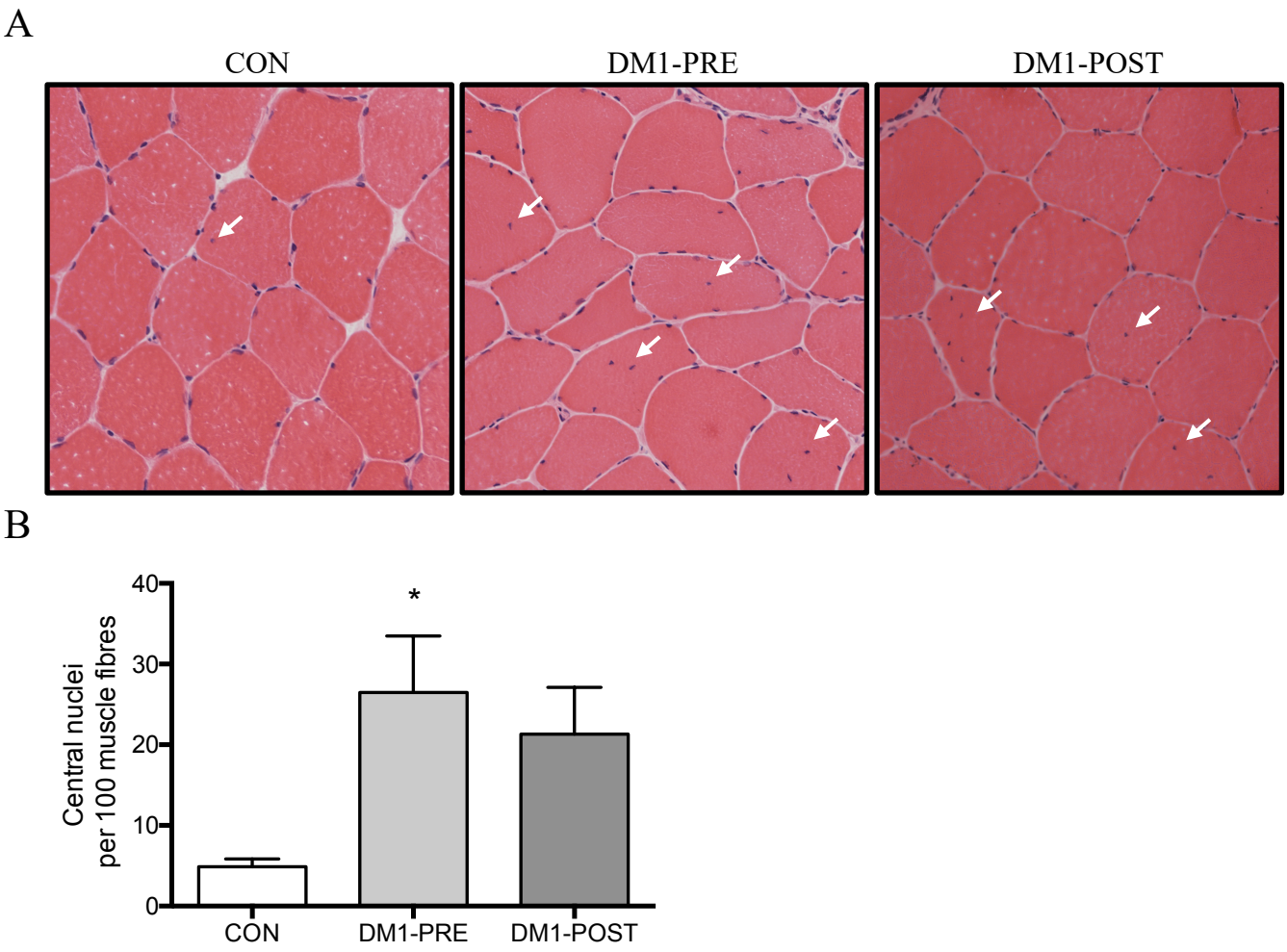

Figure 2. DM1 myopathy in response to exercise training. (A) Representative images of haematoxylin and eosin (H&E) staining of the *vastus lateralis* muscle from control (CON – left panel), DM1 patients before exercise (DM1-PRE – middle panel) and following exercise (DM1-POST – right panel). Central nucleated muscle fibres are indicated by white arrows. (B) Graphical representation of the number of myofibres containing centrally located nuclei expressed as per 100 fibres. * $P < 0.05$ vs CON.

only patients 1, 8 and 12 saw an increase. Thus, DM1 patients adhered well to our exercise protocol and training was safe and well tolerated based on blood and muscle analyses.

Spirometry and ECG. We first assessed pulmonary function through spirometry testing considering the well-known benefits of aerobic exercise on the respiratory system (Pelkonen et al. 2003; Angane and Navare 2016) and the impact of respiratory failure on DM1 mortality rates (Thijs et al. 1998). At baseline, FVC and FEV1 were significantly lower in DM1 (3.10 ± 0.3 L and 2.41 ± 0.2, respectively) when compared to CON (4.59 ± 0.4 L and 3.74 ± 0.4 L, respectively) as commonly seen with disease progression (Table 3). Following 12-weeks of physical activity, DM1 patients had no significant change in FVC (3.17 ± 0.3 L; $P > 0.05$). Furthermore, although not statistically significant, FEV1 showed a trending increase of ~125 mL with aerobic exercise training that has been considered clinically meaningful (P. W. Jones et al. 2014). To further elucidate the influence of training on FEV1, we performed a Pearson correlation to evaluate the relationship between relative FEV1 (% of predicted) and the percent change between Pre and Post exercise FEV1 values. As seen in Figure 3, there was a moderate correlation ($r = -0.62$, $P < 0.05$) between relative FEV1 values and exercise induced FEV1 increases. No correlation was seen between relative FVC and changes following training (data not shown). Subsequently, we examined the influence of exercise on QRS and PR duration (Table 3). No significant difference was observed in QRS complex duration following exercise ($P > 0.05$). Nonetheless, PR interval significantly increased from 190.0 ± 9.5 ms to 198.9 ± 9.6 ms following exercise training which is a commonly seen phenomenon following aerobic training in healthy individuals (Huston, Puffer, and Rodney 1985; Akinbiola, Adeniran, and Ogunlade 2019; Talan et al. 1982; Zehender et al. 1990).

Cycle ergometry training improved VO_{2peak} and W_{peak}. Expectedly, VO_{2peak} and W_{peak} of CON

Table 3: Electrocardiography and spirometry results

Measures	CON	DM1-PRE	DM1-POST
ECG			
PR interval	-	190.0 ± 9.5	198.9 ± 9.6#
QRS interval	-	104.5 ± 7.3	105.3 ± 5.5
Spirometry			
Max FVC (L)	4.59 ± 0.4	3.10 ± 0.3*	3.17 ± 0.3*
% of Predicted	101 ± 5	71 ± 4*	72 ± 4*
Max FEV1 (L)	3.74 ± 0.4	2.41 ± 0.2*	2.54 ± 0.2* †
% of Predicted	103 ± 5	69 ± 4*	74 ± 3* †

* P < 0.05 vs CON. # P < 0.05 vs DM1-PRE. † trending vs DM1-PRE. Values are mean ± SEM.

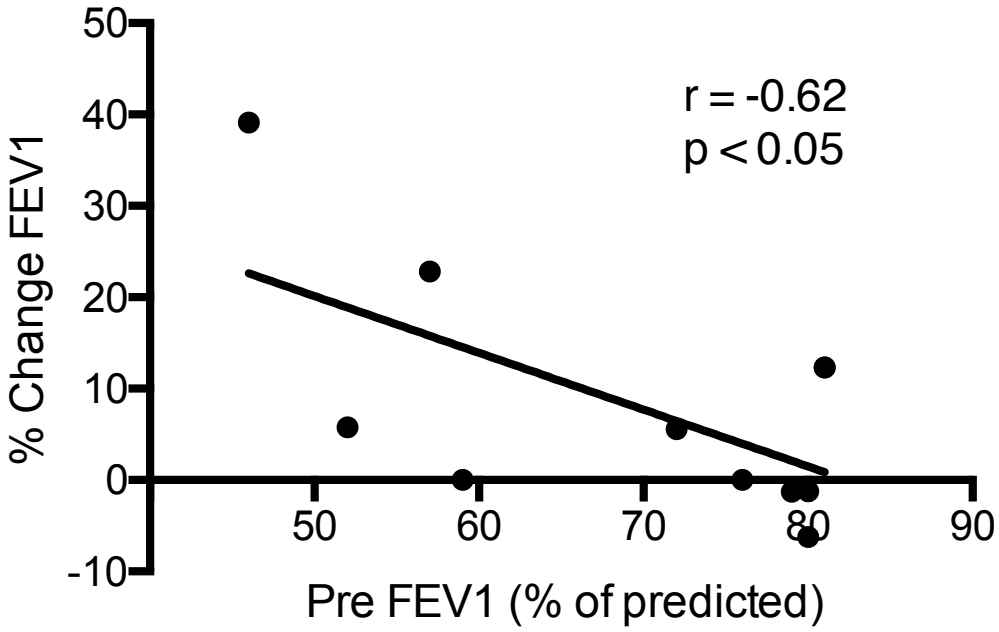

Figure 3. Improved functional respiratory measures in patients with lowest capacity after training. Pearson correlation between baseline scores of forced expiratory volume in one second (FEV1 – made as a percentage of predicted) and percent change before and after exercise of FEV1.

group were ~93 % and ~169 %, respectively, greater than that of the DM1 group at baseline (38.1 ± 2.9 mL/min/kg vs 19.7 ± 1.5 mL/kg/min; 232.5 ± 24.7 W vs 86.5 ± 8.7 W; $P < 0.05$). Interestingly, exercise improved cardiorespiratory fitness by ~32 % in DM1 patients from 19.7 mL/kg/min to 26.0 mL/kg/min ($P < 0.05$). Similarly, W_{peak} increased by ~35 % following training (Table 4; $P < 0.05$). However, after 12-weeks of aerobic exercise training VO_{2peak} and W_{peak} were still significantly lower than CON ($P < 0.05$).

Improved body composition and increased muscle mass with cycle ergometry training. Although resistance exercise is considered the primary mode of training for muscle growth, there is evidence to support the role of aerobic training on hypertrophy (Ozaki et al. 2015). Therefore, we sought to investigate the influence of aerobic training on TLM in DM1 patients. In line with the muscle wasting nature of the disease, DM1 patients (36.9 ± 3.1 kg) had significantly lower lean mass than CON (50.7 ± 3.7 kg) as measured by DEXA (Table 5, $P < 0.05$). Exercise successfully enhanced TLM by an average of ~1.6 kg in DM1 patients ($P < 0.05$). Furthermore, training sufficiently improved body composition in DM1 by reducing total body fat from 41.6 ± 3.5 % to 39.9 ± 3.6. % ($P < 0.05$). To further confirm that our exercise protocol increased the skeletal muscle mass component of TLM, we employed MHC staining to determine the muscle fibre specific increase in CSA. Fibre distribution analysis revealed that DM1 muscle contains a greater proportion of type IIA glycolytic fibres compared to CON (Fig 4A and B, $P < 0.05$). Following training, type IIX frequency was significantly reduced relative to CON ($P < 0.05$). Fibre type specific analysis of CSA showed a non-statistically significant increase of type I ($P = 0.06$) and type IIA ($P = 0.1$) following the cycling intervention (Fig 4C). The overall mean increase in muscle fiber CSA across all fiber types was ~30 % (5981 ± 530 μm^2 to 7925 ± 1060 μm^2) (Data not shown, $P < 0.05$). When considering the fibre size distribution, exercise notably reduced ($P < 0.05$) the frequency of smaller

Table 4: Individual patient VO_{2PEAK} and W_{PEAK} values

Patient # (Sex)	VO_{2PEAK} (ml/kg/min)		W_{PEAK} (Watts)	
	DM1-PRE	**DM1-POST**	**DM1-PRE**	**DM1-POST**
1 (M)	23.2	34.6	90	150
2 (F)	18.3	25.3	60	105
3 (M)	28.0	33.7	90	120
4 (M)	26.5	34.7	150	180
6 (F)	13.9	20.1	60	90
7 (M)	18.5	25.6	75	105
8 (F)	14.2	16.6	105	120
9 (F)	19.9	26.8	65	95
12 (M)	17.3	19.7	100	115
13 (F)	17.4	23.1	70	85
Mean	19.7 ± 1.5*	26.0 ± 2.1*#	86.5 ± 8.7*	116.5 ± 9.2*#
CON	38.1 ± 2.9	-	232.5 ± 24.7	-

* P < 0.05 vs CON. # P < 0.05 vs DM1-PRE. Values are mean ± SEM.

Table 5: DEXA results

Measures	**CON**	**DM1-PRE**	**DM1-POST**
BMD (g/cm^2)	1.26 ± 0.04	1.29 ± 0.02	1.27 ± 0.02
Total Lean Mass (kg)	50.7 ± 3.7	36.9 ± 3.1*	38.5 ± 3.1*#
Fat Mass (kg)	16.4 ± 2.0	26.9 ± 3.4*	26.4 ± 3.3
Fat Percentage (%)	24.9 ± 3.2	41.6 ± 3.5*	39.9 ± 3.6*#

BMD, bone mineral density. * P < 0.05 vs CON. # P < 0.05 vs DM1-PRE. Values are mean ± SEM.

atrophic type I fibres and increased larger hypertrophic fibres (Fig 4D), with no statistically significant changes seen for type IIA (Fig 4E). These results suggested that moderate intensity cycling elicited increases in skeletal muscle fibre size and improved overall body composition in DM1 patients.

Cycle ergometry training augmented functional capacity but not strength. We then implemented 6-MWT, TUG and 5X STS tests to assess muscular endurance, mobility and functional strength of the lower limbs, respectively. As anticipated, DM1 patients had a significantly lower functional capacity compared to healthy controls with CON outperforming DM1 by 57 % during 6-MWT, 32 % during TUG test and 34 % during 5X STS (Fig. 5A-C, $P < 0.05$), prior to aerobic training. After exercise, DM1 patients travelled ~47 m further during 6-MWT to cover a total of 468 ± 21 m after training (Fig. 5A, $P < 0.05$). Moreover, TUG and 5X STS tests significantly improved by 14 % and 21 %, respectively (Fig. 5 B and C, $P < 0.05$). Post exercise scores of 6-MWT and TUG were still significantly lower than CON ($P < 0.05$), but 5X STS was performed at a comparable level to that seen for CON ($P > 0.05$). Despite improvements in function following exercise, training had no effect on maximal knee isometric strength, grip strength or pinch grip (Fig. 5 D and E, $P > 0.05$).

Cycle ergometry training modestly altered RNABPs expression. To assess the influence of aerobic training on basic DM1 pathology, we investigated the expression of several RNABPs dysregulated in DM1 biology. At baseline, no difference in total protein expression of MBNL1, MBNL2 and CUGBP1 between DM1 patients and CON (Fig 6A and B, $P > 0.05$). Exercise training resulted in an increase in MBNL2 protein expression ($P < 0.05$) but no changes were observed in MBNL1 or CUGBP1 protein abundance.

GSK3β has been shown to increase CUGBP1 activity through cyclin D3-dependent kinase 4 (CDK4) (K. Jones et al. 2012; Wei et al. 2018). Therefore, we aimed to assess phosphorylated

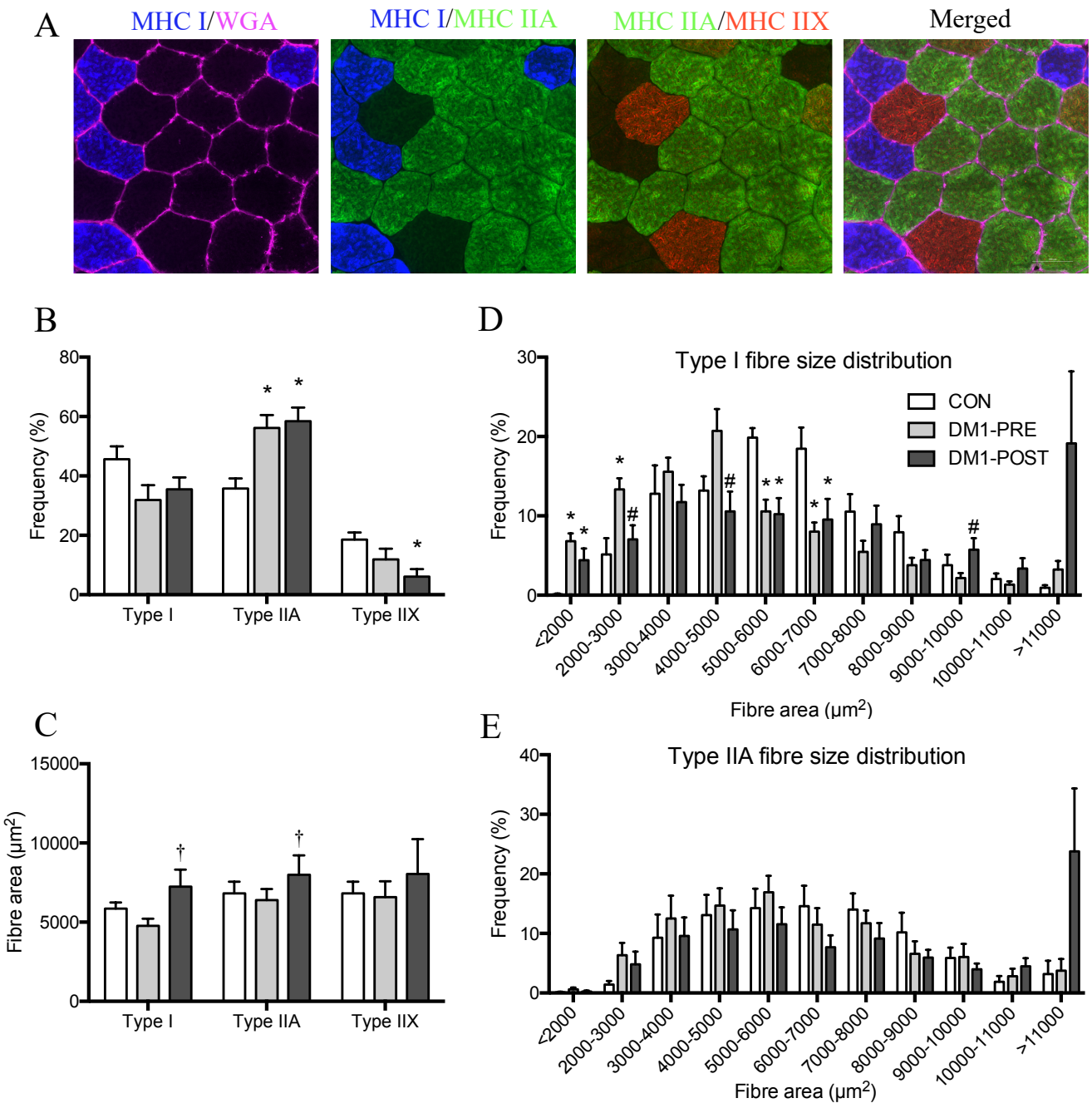

Figure 4. Fibre cross-sectional area in response to exercise training in DM1 patients. (A) Representative image of immunofluorescence staining for wheat germ agglutinin (WGA – cyan) myosin heavy chain (MHC) type I (blue), type IIA (green) and type IIX (red) (B) Average fibre type distribution analyzed from MHC immunofluorescence staining. (C) Average fibre type specific cross-sectional area analysis. (D & E) Size distribution analysis of fibre cross-sectional area for type I and type IIA fibres, respectively. * $P < 0.05$ vs CON. # $P < 0.05$ vs DM1-PRE. † trending vs DM1-PRE.

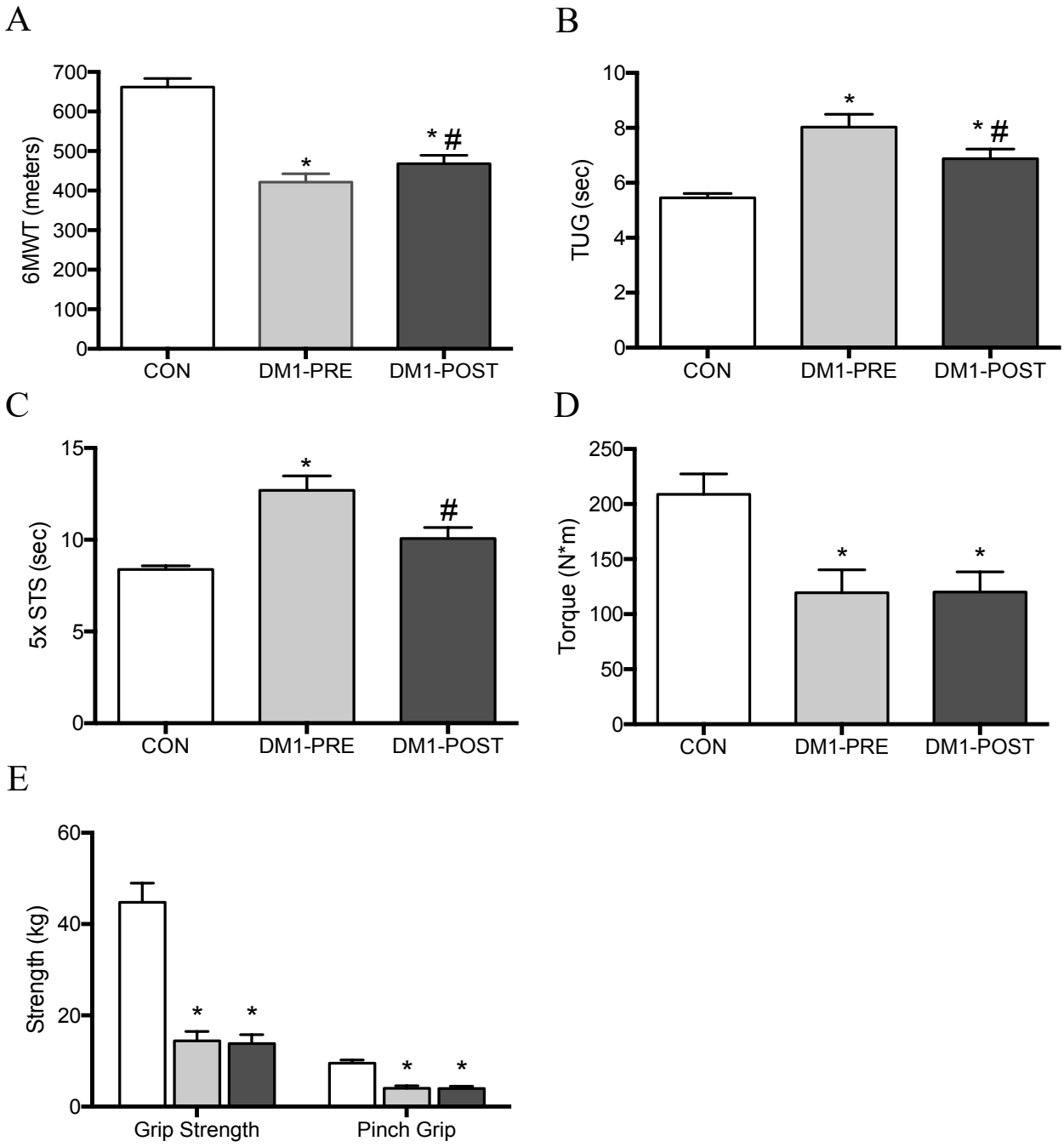

Figure 5. Exercise adaptations on functional and strength outcomes. (A) Scores of 6-minute walk test (6MWT) presented in meters. (B) Average time, in seconds, to complete timed up-and-go test. (C) Average time, in seconds, to complete a 5 times sit-to-stand test. (D) Torque values of maximal isometric knee extension. (E) Strength values (kg) of maximal grip strength and lateral pinch grip. * $P < 0.05$ vs CON. # $P < 0.05$ vs DM1-PRE.

GSK3β (Ser9) and total GSK3β protein levels as an indirect indication of CUGBP1 activity. Inhibition status (phospho/total) of GSK3β was significantly lower ($P < 0.05$) at baseline for DM1 participants compared to DM1 patients as expected (Fig 6C and D). Exercise training had no further effect on expression on either phosphorylated or total GSK3β protein levels. In summary, these results suggest that exercise had a modest influence on MBNL2 expression but did not alter total protein expression of other mis-regulated RNABP in DM1.

Mitochondrial proteins and respiration in DM1 are lower than healthy individuals but are augmented with exercise. Given recent evidence of impaired oxygen consumption rates in DM1 fibroblasts (García-puga et al. 2020), we aimed to investigate mitochondrial function and content in DM1 patients as compared to healthy men and women. We first measured phosphorylated (Thr172) and total AMPK protein content due to its critical role in regulating mitochondrial protein content. DM1 patients showed ~20 % reduction in phosphorylated AMPK protein expression compared to CON (Fig 7A and B, $P < 0.05$). Independent of PGC-1α protein expression (Fig 7A, $P > 0.05$), mitochondrial protein content was significantly lower in DM1 patients. More specifically, complex I, III, IV and V protein were lower by ~63 %, 32 %, 44 % and 23 % in DM1 relative to CON (Fig 7C and D, $P < 0.05$). Lower protein abundance was accompanied with reduced ADP stimulated respiration of complex I (~35 %) and complex I+II (~31 %) in DM1 patients at baseline (Fig 8A, $P < 0.05$).

Following exercise training, both phosphorylated and total AMPK protein levels were increased (Fig 7A and B, $P < 0.05$), but training had no effect on PGC-1α levels (Fig 7A, $P > 0.05$). Aerobic training increased protein content of all mitochondrial complexes (Fig 7C and D, $P < 0.05$), to levels comparable with CON ($P > 0.05$). Furthermore, both complex I, I+II and II maximal respiration were enhanced in DM1 patients after exercise training (Fig 8A, $P < 0.05$).

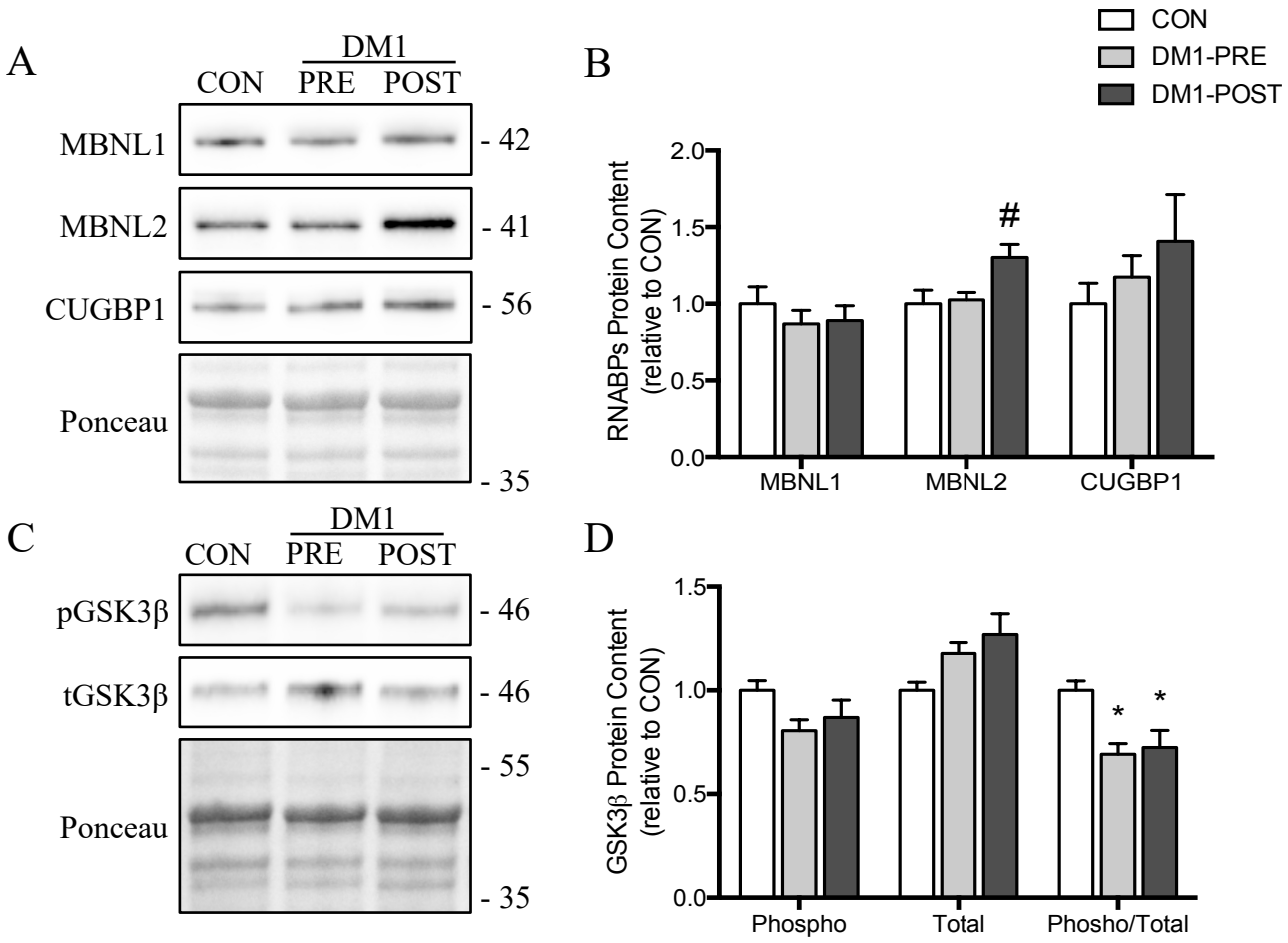

Figure 6. Expression of RNABPs following exercise intervention. (A) Representative western blot of RNABPs muscleblind like protein 1 (MBNL1), muscleblind like protein 2 (MBNL2) and CUGBP Elav-like family member 1 (CUGBP1). Ponceau stain included to indicate equal protein loading of each sample. (B) Graphical summary of MBNL1, MBNL2 and CUGBP1 expression relative to CON. (C) Representative western blot of phosphorylated (Serine 9) and total expression of glycogen synthase kinase 3 beta (GSK3b). (D) Graphical summary of pGSK3b and tGSK3b expression relative to CON. Inhibition status was calculated by making pGSK3b relative to tGSK3b. * $P < 0.05$ vs CON. # $P < 0.05$ vs DM1-PRE.

Taken together, DM1 is associated with reduced mitochondrial protein abundance and function, which were effectively improved with exercise training.

We further assessed mitochondrial respiration at a submaximal ADP concentration to mimic physiological levels. DM1 patients at baseline had lower submaximal ADP stimulated respiration relative to CON (Fig 8B, $P < 0.05$). Although, submaximal respiration rates of DM1 patients after exercise did not increase significantly from baseline ($P = 0.13$), they were no longer different than CON (Fig 8B, $P > 0.05$). To obtain predicted values of ADP sensitivity, we employed Michaelis-Menten analysis and calculated an estimate apparent Km for ADP. There was no significant difference ($P > 0.05$) in apparent Km between CON, DM1 at baseline or DM1 following exercise (Fig 8C). In conclusion, impaired mitochondrial respiration at a submaximal ADP concentration in DM1 patients was enhanced to CON levels following aerobic training independent of changes ADP sensitivity.

Exercise alters expression of proteins important for mitochondrial dynamics. Considering the strong influence of mitochondrial morphology on oxygen consumption rates, we examined expression of proteins critical for mitochondrial fusion and fission. At baseline, DM1 patients showed lower OPA1 (Fig 9A and B, $P < 0.05$) and MFN-2 ($P = 0.062$) protein levels. MFN-1 protein levels were similar at baseline between DM1 and CON ($P > 0.05$), as were fission related proteins (DRP-1 and FIS1, Fig 9C and D). As a consequence of aerobic exercise training, OPA1, MFN-1, MFN-2, DRP1 and FIS1 protein levels all significantly increased in DM1 patients (Fig 9A-D, $P < 0.05$). Collectively, this data suggests that there was an imbalance between fusion and fission related proteins in DM1 patients that was restored following 12-weeks of aerobic training.

Increased mitochondrial superoxide dismutase in DM1 patients following training. DMPK protein has been implicated in reducing production of reactive oxygen species (ROS) (Pantic et al., 2013),

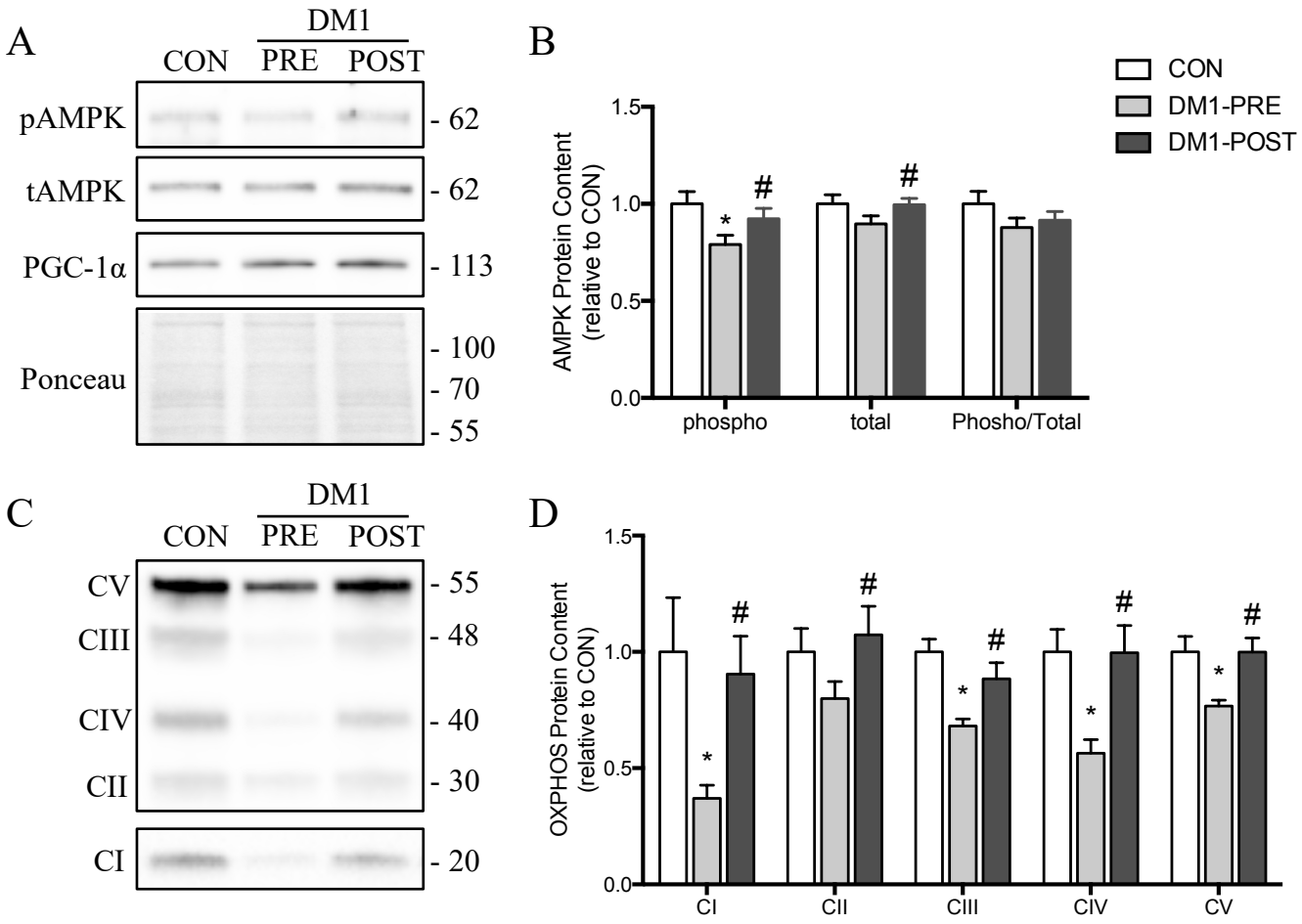

Figure 7. Training alters expression of mitochondrial proteins in DM1 patients. (A) Representative western blot of phosphorylated (Threonine 172) AMP activated kinase (AMPK), total AMPK and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a). (B) Graphical summary of pAMPK, tAMPK and PGC-1a expression relative to CON. (C) Representative western blot of mitochondrial protein complexes (CI-CV). Full blot was overexpressed and cropped for better visualization of CI. (D) Graphical representation of CI-CV protein expression relative to CON. * $P < 0.05$ vs CON. # $P < 0.05$ vs DM1-PRE.

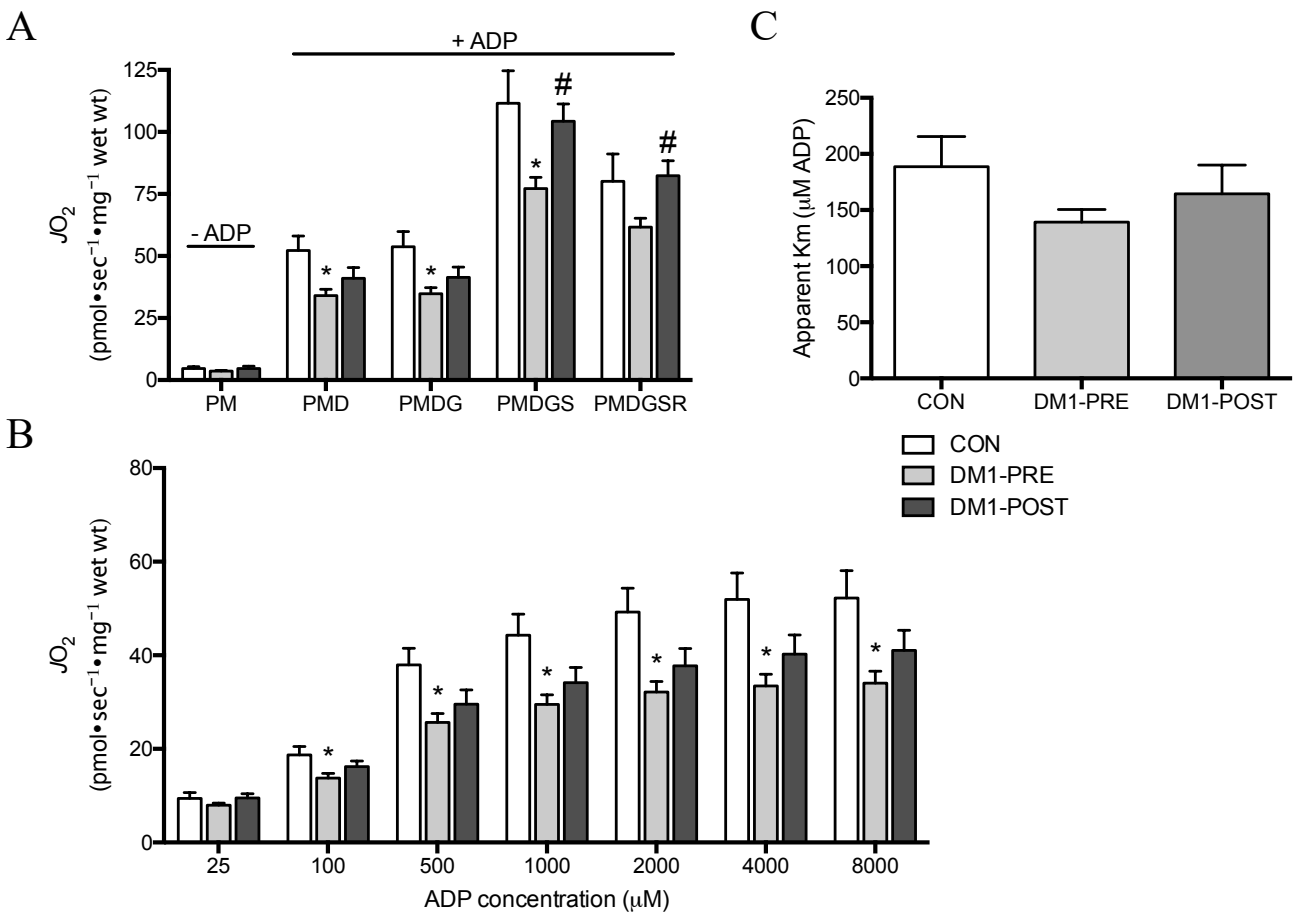

Figure 8. Maximal and submaximal mitochondrial respiration in response to aerobic exercise. (A) ADP-supported maximal respiration of complex I and II. Pyruvate + malate, PM; PM + ADP, PMD; PMD + glutamate, PMDG; PMDG + succinate, PMDGS; PMDGS + rotenone; PMDGSR. (B) Submaximal ADP titration (25, 100, 500, 1000, 2000, 4000 and 8000; mM) curve with PM. (C) Apparent ADP Km calculated from ADP titration curve using Michaelis-Menten. * $P < 0.05$ vs CON. # $P < 0.05$ vs DM1-PRE.

and some have found that DM1 fibroblasts displayed higher levels of ROS (García-puga et al. 2020). Therefore, we measured markers of oxidative damage and antioxidants in whole muscle homogenates. Protein levels of 4HNE in DM1 patients at baseline was similar to CON and following the exercise period (Fig 10A and B, $P > 0.05$). SOD2 levels were significantly lower in DM1 patients at baseline (Fig 10C and D, $P < 0.05$) but no difference was detected for SOD1 levels ($P > 0.05$). Following aerobic training, SOD2 levels were significantly increased ($P < 0.05$) in DM1 skeletal muscle back to levels similar to CON. In contrast, SOD1 levels were significantly reduced in DM1 patients following exercise period.

DISCUSSION

This is the first study to demonstrate the functional, clinical and cellular benefits of 12-weeks of moderate intensity cycling in patients with DM1. Aerobic exercise training safely improved functional respiration, physical function, cardiorespiratory fitness and muscle mass. Moreover, at a mechanistic level, training increased MBNL2 protein levels while augmenting mitochondrial content and function in DM1 muscle. Collectively, the cycle ergometry protocol described herein is a safe and effective mode of exercise training that can be prescribed to DM1 patients to mitigate muscle wasting and improve overall health.

A recent review by Roussel and colleagues (M. Roussel et al. 2019) summarized the small body of literature regarding the effect of aerobic, strength or mixed exercise training in DM1 patients, suggesting that the evidence for the benefits of exercise training are equivocal due to the small sample size and the heterogeneity of exercise modalities, intensities and outcomes employed in these studies. Furthermore, a large number of those studies were focused on the efficacy of strength training while aerobic training studies are scarce. Nevertheless, the majority of studies

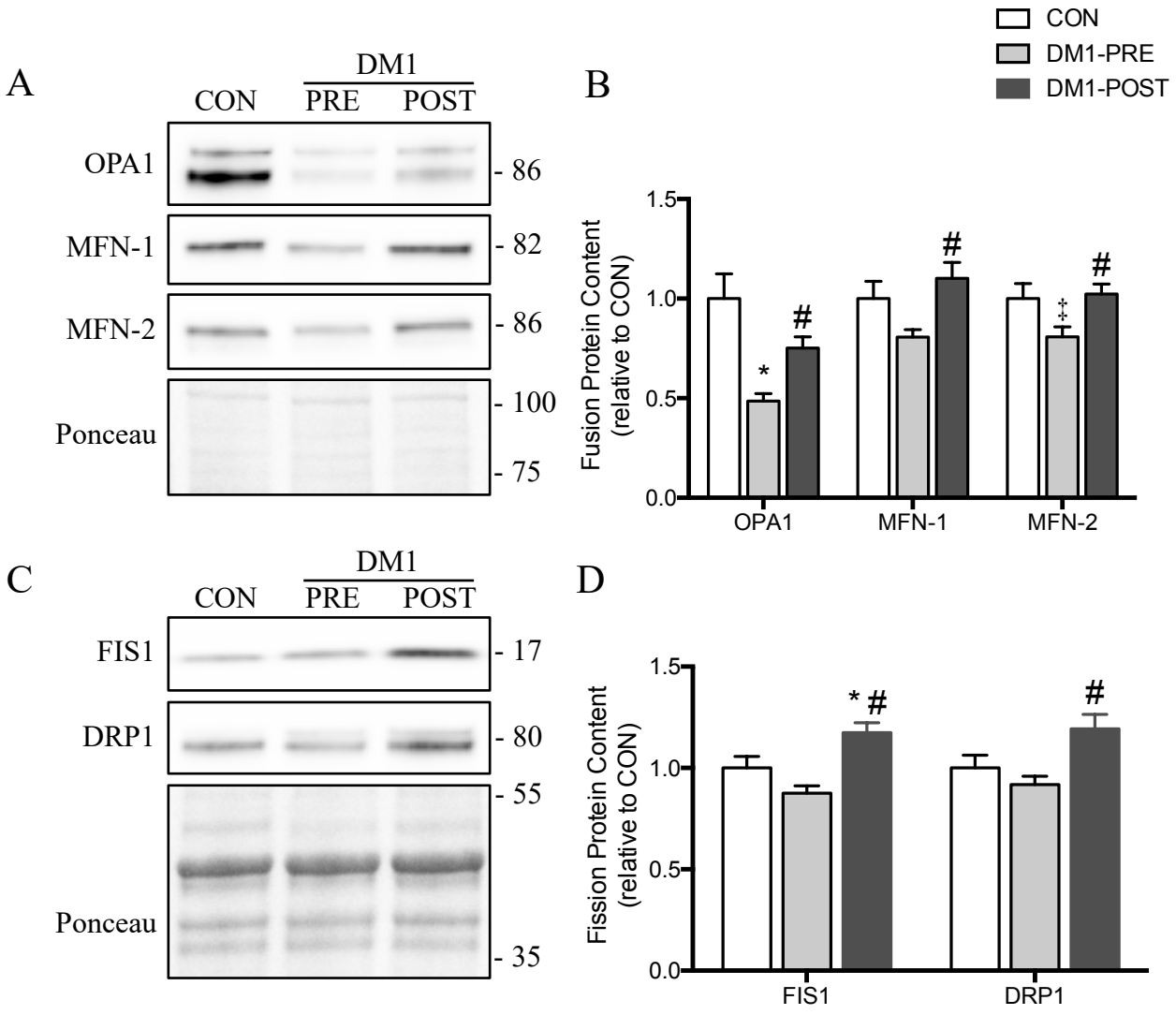

Figure 9. Mitochondrial dynamics related proteins following exercise training. (A) Representative western blot of mitochondrial fusion related proteins optic atrophy 1 (OPA1), mitofusin 1 (MFN-1) and MFN-2. (B) Graphical summary of OPA1, MFN-1 and MFN-2 expression relative to CON. (C) Representative western blot of mitochondrial fission related proteins mitochondrial fission 1 (FIS1) and GTPase dynamin-related protein 1 (DRP1). (D) Graphical summary of FIS1 and DRP1 expression relative to CON. * $P < 0.05$ vs CON. # $P < 0.05$ vs DM1-PRE. ‡ trending vs CON.

concluded that resistance-based training increased overall strength and improved fibre CSA resulting enhanced functionality (Aldehag, Jonsson, and Ansved 2005; Aldehag et al. 2013; Tollbäck et al. 1999; M. P. Roussel, Hébert, and Duchesne 2020). Additionally, our laboratory has previously shown that DM1 patients who participated in regular physical activity outperformed those who were sedentary in several strength outcomes (Brady, MacNeil, and Tarnopolsky 2014). Others, however, have shown no improvements, but importantly no detriments following strength training (Lindeman et al. 1995, 1999). The limited evidence in support of aerobic training demonstrates superior cardiorespiratory fitness and myofiber CSA in response to a similar 12-weeks aerobic exercise protocol as the one employed in this trial (Ørngreen, Olsen, and Vissing 2005). Evidence of the beneficial effects of aerobic training on DM1 biology are more apparent in rodent models. Recently, in three independent investigations, physical activity in DM1 mice effectively improved spliceopathy of several genes, reduced myotonic discharge, increased mitochondrial content and reduced MBNL1 sequestration (Manta et al. 2019; Ravel-chapuis et al. 2018; Sharp, Cox, and Cooper 2019). To date, exercise-induced molecular signaling and mitochondrial adaptions in DM1 patients have yet to be investigated. Therefore, it's critical to extensively understand the role of aerobic training on functional and clinically relevant outcomes but also, to further investigate its influence on molecular mechanisms in DM1 muscle.

In the present study, all participants, with the exemption of patient 5, adhered well to the exercise protocol similar to previous exercise training trials in DM1 patients (Wright et al. 1996; Ørngreen, Olsen, and Vissing 2005). We reported an average adherence rate of 98% in 10/11 participants while patient 5 completed 75% of training sessions in an 8-week period (Table 1). Despite lower attendance, patient 5 exhibited similar improvements in all outcomes as compared to the rest of the study cohort and was included in analysis. More importantly, our exercise protocol

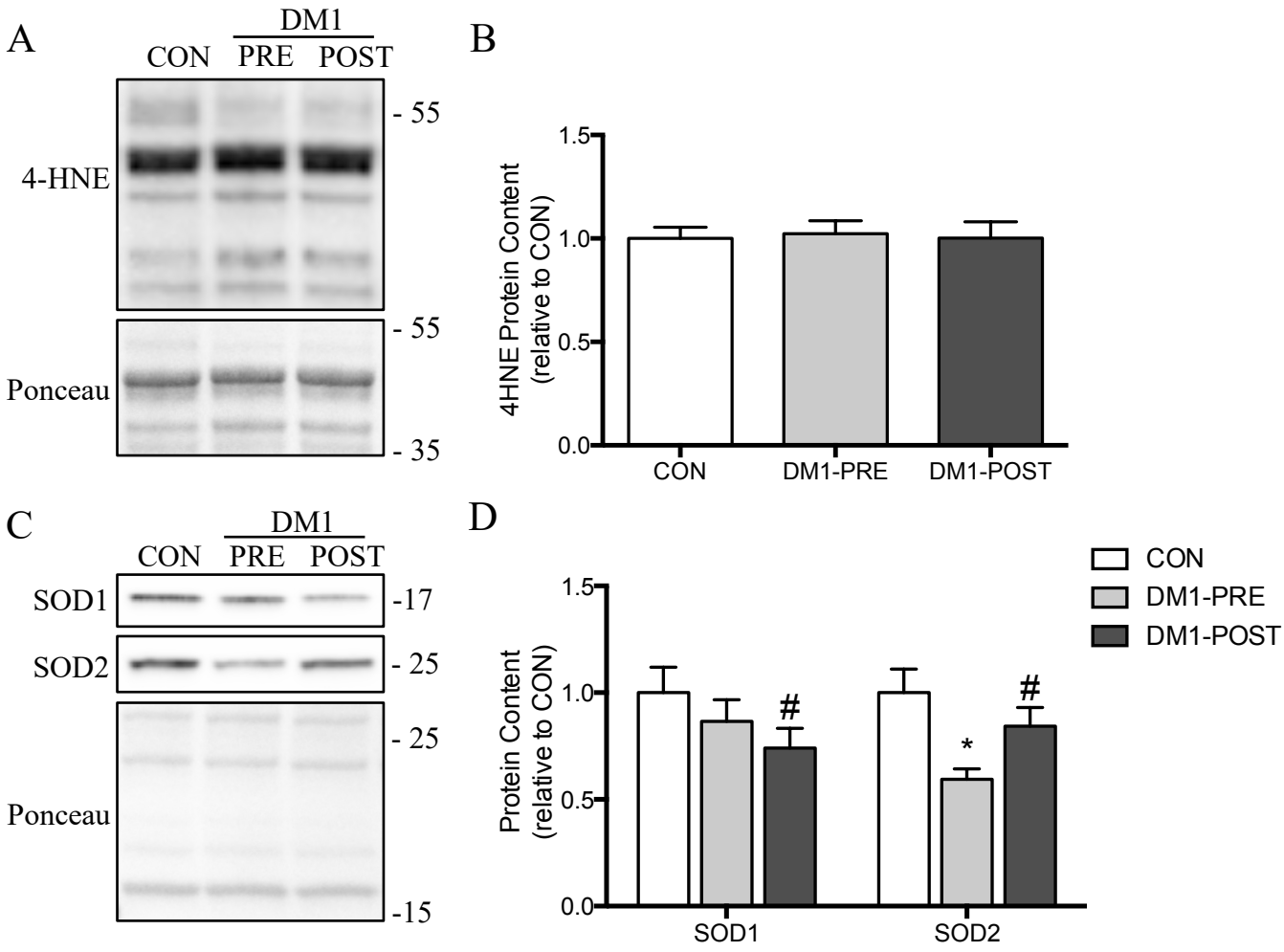

Figure 10. Markers of oxidative stress and antioxidants in response to aerobic training. (A) Representative western blot of lipid peroxidation marker 4-hydroxynonenal (4HNE). (B) Graphical summary of 4HNE expression relative to CON. (C) Representative western blot of antioxidants superoxide dismutase 1 (SOD1) and SOD2. (B) Graphical summary of SOD1 and SOD2 expression relative to CON. * $P < 0.05$ vs CON. # $P < 0.05$ vs DM1-PRE.

had no detrimental concerns as indicated by the lack of change in circulating levels of CK, creatinine, bilirubin, ALT and GGT (Table 2). There was an increase of ~0.3 mmol/L in GLUF levels following training intervention but all patients remained within clinically normal ranges (< 6.0 mmol/L). As a more comprehensive measure of skeletal muscle damage, centrally nucleated fibres were analysed to further reiterate the safety of cycling in DM1 patients (Fig 2A and B). Although speculative, our exercise protocol partially ameliorated this aspect of the myopathy for the majority of patients showed a reduction (6/11) or no change (2/11) in the frequency of centrally nucleated fibres.

Skeletal muscle atrophy is inevitable with DM1 progression, specifically in those muscles assisting in respiration, and is the main contributor to mortality in DM1 patients (Thijs et al. 1998). Fat infiltration and atrophy of the trunk muscles have also been reported in DM1 patients (Solbakken et al. 2019) which likely contributes to low respiratory function measures (Hawkins et al. 2019). Our data conforms with previously reported FVC and FEV1 predictive values with DM1 patients scoring an average of 71% and 69%, respectively, indicating a restrictive ventilatory defect (Table 3). During aerobic exercise training the minute ventilation rate is dramatically elevated requiring extensive involvement of respiratory muscles. Minimal clinically important differences have been established and validated in those with chronic obstructive pulmonary disease. In that population, benefits of >100 mL in FEV1 were considered to be the threshold for clinically relevant improvements (P. W. Jones et al. 2014). Our results demonstrated a mean increase of ~125 mL in FEV1 volume following 12-weeks of cycle ergometry. Interestingly, Pearson correlation revealed a modest and statistically significant negative correlation between FEV1 values made relative to predicated and exercise induced changes in FEV1. Thus, those with low respiratory function (FEV1 <70 % of predicted) experience the greatest improvements

following exercise while patients with normal lung function (>70%) had little to no changes in FEV1 values (Fig 3). This data should be interpreted with caution as the authors recognize the low sample size used for the correlation analysis; however, there appears to be greater respiratory benefits from aerobic exercise training in DM1 patients with low respiratory function. Second to respiratory dysfunction, cardiac arrhythmias considerably limit the life expectancy of DM1 patients (Thijs et al. 1998). During the clinical trial, ECG readings of DM1 patients displayed an increase in PR intervals by ~8.9 ms with no changes in QRS complex (Table 3). This was not unexpected as first-degree antrioventricular blocks are commonly seen in healthy individuals who participant in regular aerobic training (Huston, Puffer, and Rodney 1985; Talan et al. 1982; Zehender et al. 1990). In addition, similar increase in PR intervals were seen in response to 8-weeks of moderate intensity aerobic training in healthy adult males and females (Akinbiola, Adeniran, and Ogunlade 2019; Malandish et al. 2020). An electrophysiology cardiologist with extensive experience in DM1 was consulted and indicated that the increase is far below the threshold of 240 ms to be considered an abnormal ECG (Ha et al. 2012). Therefore, the authors believe that the increase in PR interval was not pathological but a naturally occurring adaptation of aerobic training.

Previously, 12-weeks of cycling improved VO_{2peak} and W_{peak} in nine DM1 patients by ~15 % and ~11 %, respectively (Ørngreen, Olsen, and Vissing 2005). Our results demonstrate superior benefits in VO_{2peak} (~32 %) and W_{peak} (~35 %) despite similar exercise protocols and lower number of training sessions (50 vs 36 sessions within 12 weeks). Discrepancies in cardiorespiratory benefits seen between both trials are likely due to the lower baseline aerobic fitness status of the participants in our study (Table 4), which may be attributed to larger average of ‘CTG’ repeats and older age of our DM1 patients. Improvements of cardiorespiratory fitness are only partly explained

by improved body composition and increased TLM (Table 5). Although CON and DM1 groups were evenly matched for BMI, body fat percentage of DM1 participants was far greater than fat percentage of CON putting them a significantly higher risk of several metabolic disorders. Cycle ergometry significantly reduced overall body fat percentage and elicited skeletal muscle hypertrophy, measured at the whole-body (DEXA) and muscle fibre (CSA) level. Preferential atrophy of type I slow oxidative fibres has been previously reported in DM1 muscle (Pisani et al. 2008; Vihola et al. 2003). Although the underlying mechanism is unknown, DMPK protein content was found in greater abundance within type II fibres as compared to type I fibres in DM1 muscle biopsies suggesting an increased translational capacity of *DMPK* in type II glycolytic fibres or a greater accumulation of the kinase within myonuclei of type I fibres (Salvatori et al. 2005). Nevertheless, we observed a greater frequency of atrophic type I oxidative fibres in DM1 patients at baseline in comparison to healthy individuals but no significant differences in type IIA fibres (Fig 4D and E). Size distribution analysis was not performed in type IIX due to their low frequency in human muscle biopsies. Exercise training remarkably stimulated growth in type I muscle fibres reducing the number of atrophic fibres and increasing the frequency of hypertrophic fibres (Fig 4D). Analysis of fibre type specific average CSA revealed a non-statistical increase of both type I and type IIA fibres (Fig 4C). Exercise mediated increases in fibre CSA translated into exceptional improvements in physical ability of DM1 patients. Muscular endurance, mobility and functional strength as measured by 6MWT, TUG and 5X STS, respectively, all significantly improved in response to the training program; however, no changes were observed in knee extension or hand/finger strength measures, likely due to the non-weight bearing nature of the exercise protocol and the lack of specific hand exercises, respectively (Fig 5A-E).

To our knowledge, this was the first study to investigating exercise induced molecular and

mitochondrial adaptions in patients with DM1. RNA toxicity is the primary hypothesis behind DM1 pathology where alternation of downstream RNABPs result in hundreds of missplicing events in DM1 tissue (Mankodi et al. 2001; Masayuki Nakamori et al. 2013; Kuyumcu-Martinez, Wang, and Cooper 2007). We examined the protein expression of several RNABP that are known to play a key role in DM1 pathology in response to cycle ergometry. Contrary to recently published data in DM1 mice (Sharp, Cox, and Cooper 2019), we observed an increase in the total expression of MBNL2 with no concomitant alterations detected in MBNL1 or CUGBP1 content (Fig 6A and B). MBNL2 is a homologue to MBNL1 but is primarily expressed in the brain and to a lesser extend in skeletal muscle (Kanadia, Urbinati, et al. 2003) and as such the observed increased in the isoform is intriguing. Transgenic knockouts of MBNL2 recapitulate cognitive impairments regularly reported in DM1 patients. Therefore, future studies should investigate the relationship between training induced increases in skeletal muscle MBNL2 protein content and cognitive function in DM1 patients. We further assessed inhibition status of GSK3β as in indirect indicator of CUGBP1 activity. As previously reported, GSK3β inhibition was detected to a greater extend in healthy muscle as compared to DM1 muscle (K. Jones et al. 2012). However, cycle ergometry had no effects on GSK3β protein expression, or phosphorylation (Fig 6C and D).

Several markers of mitochondrial dysfunction have been reported in fibroblasts, blood and muscle samples of patients with DM1 (García-puga et al. 2020; Gramegna et al. 2018), yet direct measures of mitochondrial function and content have yet to be reported. Here we have utilized high resolution respirometry and immunoblotting techniques to examine mitochondrial function, content and dynamics. Consistent with previous literature (Ravel-chapuis et al. 2018; Brockhoff et al. 2017), AMPK phosphorylation was significantly blunted in DM1 patients at baseline. In turn, maximal mitochondrial respiration of complex I and I+II in DM1 muscle were significantly lower

(Fig 8A) which is likely due to the extreme deficiency in protein content of complex I, III, IV and V (Fig 7C and D). Furthermore, submaximal ADP-stimulated respiration is blunted in DM1 patients (Fig 8B). OPA1, a key regulator of mitochondrial fusion, has been show to play a critical role in mitochondrial respiration capacity and ATP production (Cogliati et al. 2013). In DM1 muscle at baseline, OPA1 protein levels were ~50 % lower relative to CON (Fig 9 A and B). Although not directly measured in this study, one could speculate that DM1 mitochondria are likely to be fragmented and of simple morphology as indicated by an imbalance in fusion related and fission related proteins. Additionally, reduced mitochondrial function could likely be a secondary contributor to muscle wasting and weakness phenotype (Hyatt et al. 2019).

Following aerobic exercise training, phosphorylated and total AMPK levels were augmented. In addition, both submaximal and maximal respiration (Fig 8A and B) along with mitochondrial oxidative protein content dramatically increased to levels similar to healthy muscle (Fig 7C and D). Additionally, fusion related proteins were restored to healthy levels along with minor but significant increases in fission related proteins (Fig 9 A-D). Therefore, with exercise training the balance between fusion and fission processes was restored and likely contributed to improved mitochondrial quality underpinning some of the physiological adaptions reported. Lastly, we assessed the oxidative status of whole muscle homogenates in DM1 patients as the DMPK protein has been implicated in regulating ROS (Pantic et al. 2013). 4HNE, a marker of lipid peroxidation, showed no difference between CON or DM1 patients Pre or Post exercise (Fig 10A and B). Reduction in SOD1 levels may suggest a reduction in oxidative stress within the cytoplasm while the increase in mitochondrial specific SOD2 are in line with overall increases in mitochondrial content (Fig 10C and D). As the primary source of ROS production, assessing mitochondrial fractions for markers of oxidative stress and antioxidants is a necessary step to shed

insight on the changes in SOD protein expression observed. As a result, no direct conclusions can be made regarding the influence of aerobic training on ROS production or handling in DM1 muscle.

In summary, 3 months of cycle ergometry were sufficient to elicit physiological, functional and cellular benefits in patients with classical DM1. The novel findings of this study emphasized the importance of exercise prescription in clinical setting to mitigate/delay DM1 disease progression. Here we demonstrated that aerobic exercise elicited multisystemic benefits that ameliorated aspects of DM1 pathology including; enhanced lung function, cardiorespiratory fitness, overall functional capacity and mitochondrial function. Future studies are still needed to investigate exercise mediated changes to DM1 spliceopathy and MBNL1 sequestration. Furthermore, clinical trials with a larger patient population and longer interventions are needed to determine the parameters (type, intensity, time and frequency) of exercise for optimal and safe benefits in the DM1 population.

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REFERENCES

Agtmaal, Ellen L. van, Laurène M. André, Marieke Willemse, Sarah A. Cumming, Ingeborg D.G. van Kessel, Walther J.A.A. van den Broek, Geneviève Gourdon, et al. 2017. "CRISPR/Cas9-Induced (CTG·CAG)n Repeat Instability in the Myotonic Dystrophy Type 1 Locus: Implications for Therapeutic Genome Editing." *Molecular Therapy* 25 (1): 24–43. https://doi.org/10.1016/j.ymthe.2016.10.014.

Akinbiola, O O, S A Adeniran, and O Ogunlade. 2019. "Effects of an 8 Week Continuous Exercise Training on the Electrocardiogram and Physiological Parameters of Institutional Security Personnel in Nigeria." *Asian J. Biol. Sci.* 12 (2): 342–48. https://doi.org/10.3923/ajbs.2019.342.348.

Aldehag, Anna Sandin, Hans Jonsson, and Tor Ansved. 2005. "Effects of a Hand Training Programme in Five Patients with Myotonic Dystrophy Type 1." *Occupational Therapy International* 12 (1): 14–27. https://doi.org/10.1002/oti.12.

Aldehag, Anna Sandin, Hans Jonsson, Jan Lindblad, Anders Kottorp, Tor Ansved, and Marie Kierkegaard. 2013. "Effects of Hand-Training in Persons with Myotonic Dystrophy Type 1-a Randomised Controlled Cross-over Pilot Study." *Disability and Rehabilitation* 35 (21): 1798–1807. https://doi.org/10.3109/09638288.2012.754952.

Angane, Esha Y, and Amit A Navare. 2016. "Effects of Aerobic Exercise on Pulmonary Function Tests in Healthy Adults." *International Journal of Research in Medical Sciences* 4 (6): 2059–63.

Ashizawa, Tetsuo, M. Anvret, M. Baiget, J. M. Barceló, H. Brunner, A. M. Cobo, B. Dallapiccola, et al. 1994. "Characteristics of Intergenerational Contractions of the CTG Repeat in Myotonic Dystrophy." *American Journal of Human Genetics* 54 (3): 414–23.

Barbé, Lise, Stella Lanni, Arturo López-Castel, Silvie Franck, Claudia Spits, Kathelijn Keymolen, Sara Seneca, et al. 2017. "CpG Methylation, a Parent-of-Origin Effect for Maternal-Biased Transmission of Congenital Myotonic Dystrophy." *American Journal of Human Genetics* 100 (3): 488–505. https://doi.org/10.1016/j.ajhg.2017.01.033.

Bassez, Guillaume, Etienne Audureau, Jean-yves Hogrel, Sandrine Baghdoyan, Hamza Bhugaloo, Philippe Le Corvoisier, and Marc Peschanski. 2018. "Improved Mobility with Metformin in Patients with Myotonic Dystrophy Type 1 : A Randomized Controlled Trial." *Brain* 141 (10): 2855–65. https://doi.org/10.1093/brain/awy231.

Batra, Ranjan, David A. Nelles, Elaine Pirie, Steven M. Blue, Ryan J. Marina, Harrison Wang, Isaac A. Chaim, et al. 2017. "Elimination of Toxic Microsatellite Repeat Expansion RNA by RNA-Targeting Cas9." *Cell* 170 (5): 899-912.e10. https://doi.org/10.1016/j.cell.2017.07.010.

Brady, Lauren I., Lauren G. MacNeil, and Mark A. Tarnopolsky. 2014. "Impact of Habitual Exercise on the Strength of Individuals with Myotonic Dystrophy Type 1." *American Journal of Physical Medicine & Rehabilitation / Association of Academic Physiatrists* 93 (9): 739–50. https://doi.org/10.1097/PHM.0000000000000088.

Braida, Claudia, Rhoda K.A. Stefanatos, Berit Adam, Navdeep Mahajan, Hubert J.M. Smeets, Florence Niel, Cyril Goizet, et al. 2010. "Variant CCG and GGC Repeats within the CTG Expansion Dramatically Modify Mutational Dynamics and Likely Contribute toward Unusual Symptoms in Some Myotonic Dystrophy Type 1 Patients." *Human Molecular Genetics* 19 (8): 1399–1412. https://doi.org/10.1093/hmg/ddq015.

Brockhoff, Marielle, Nathalie Rion, Kathrin Chojnowska, Tatiana Wiktorowicz, Christopher

Eickhorst, Beat Erne, Stephan Frank, et al. 2017. "Targeting Deregulated AMPK / MTORC1 Pathways Improves Muscle Function in Myotonic Dystrophy Type I." *Journal of Clinical Investigation* 127 (2): 549–63.

Brook, J D, M E McCurrach, H G Harley, A J Buckler, D Church, H Aburatani, K Hunter, V P Stanton, J P Thirion, and T Hudson. 1992. "Molecular Basis of Myotonic Dystrophy: Expansion of a Trinucleotide (CTG) Repeat at the 3' End of a Transcript Encoding a Protein Kinase Family Member." *Cell* 68 (4): 799–808.

Chau, Anthony, and Auinash Kalsotra. 2015. "Developmental Insights into the Pathology of and Therapeutic Strategies for DM1: Back to the Basics." *Developmental Dynamics* 244 (3): 377–90. https://doi.org/10.1002/DVDY.24240.

Chen, Zhi-Ping, Terry J Stephens, Sid Murthy, Benedict J Canny, Mark Hargreaves, Lee A Witters, Bruce E Kemp, Glenn K Mcconell, and Glenn Mcconell. 2003. "Effect of Exercise Intensity on Skeletal Muscle AMPK Signaling in Humans." *Diabetes* 52 (9): 2205–12. http://diabetes.diabetesjournals.org.libaccess.lib.mcmaster.ca/content/diabetes/52/9/2205.full.pdf.

Cho, Diane H., and Stephen J. Tapscott. 2007. "Myotonic Dystrophy: Emerging Mechanisms for DM1 and DM2." *Biochimica et Biophysica Acta - Molecular Basis of Disease* 1772 (2): 195–204. https://doi.org/10.1016/j.bbadis.2006.05.013.

Clawson, Lora L., Merit Cudkowicz, Lisa Krivickas, Benjamin R. Brooks, Mohammed Sanjak, Peggy Allred, Nazem Atassi, et al. 2018. "A Randomized Controlled Trial of Resistance and Endurance Exercise in Amyotrophic Lateral Sclerosis." *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration* 19 (3–4): 250–58. https://doi.org/10.1080/21678421.2017.1404108.

Cogliati, Sara, Christian Frezza, Maria Eugenia Soriano, Tatiana Varanita, Ruben Quintana-Cabrera, Mauro Corrado, Sara Cipolat, et al. 2013. "Mitochondrial Cristae Shape Determines Respiratory Chain Supercomplexes Assembly and Respiratory Efficiency." *Cell* 155 (1): 160–71. https://doi.org/10.1016/j.cell.2013.08.032.

Coonrod, Leslie A., Masayuki Nakamori, Wenli Wang, Samuel Carrell, Cameron L. Hilton, Micah J. Bodner, Ruth B. Siboni, et al. 2013. "Reducing Levels of Toxic RNA with Small Molecules." *ACS Chemical Biology* 8 (11): 2528–37. https://doi.org/10.1021/cb400431f.

Davis, Brigid M, Mila E Mccurrach, Krishan L Taneja, Robert H Singer, and David E Housman. 1997. "Expansion of a CUG Trinucleotide Repeat in the 3 ′ Untranslated Region of Myotonic Dystrophy Protein Kinase Transcripts Results in Nuclear Retention of Transcripts." *Proceedings of the National Academy of Sciences* 94 (14): 7388–93.

Day, J. W., K. Ricker, J. F. Jacobsen, L. J. Rasmussen, K. A. Dick, W. Kress, C. Schneider, et al. 2003. "Myotonic Dystrophy Type 2: Molecular, Diagnostic and Clinical Spectrum." *Neurology* 60 (4): 657–64. https://doi.org/10.1212/01.WNL.0000054481.84978.F9.

Dial, Athan G, Sean Y Ng, Alexander Manta, and Vladimir Ljubicic. 2018. "The Role of AMPK in Neuromuscular Biology and Disease." *Trends in Endocrinology & Metabolism* 29 (5): 300–312. https://doi.org/10.1016/j.tem.2018.02.010.

Du, Hongqing, Melissa S. Cline, Robert J. Osborne, Daniel L. Tuttle, Tyson A. Clark, John Paul Donohue, Megan P. Hall, et al. 2010. "Aberrant Alternative Splicing and Extracellular Matrix Gene Expression in Mouse Models of Myotonic Dystrophy." *Nature Structural and Molecular Biology* 17 (2): 187–93. https://doi.org/10.1038/nsmb.1720.

Dunne, Patrick W, E Timothy Walch, and Henry F Epstein. 1994. "Phosphorylation Reactions of Recombinant Human Myotonic Dystrophy Protein Kinase and Their Inhibition."

Biochemistry 33 (35): 10809–14. https://doi.org/10.1021/bi00201a031.
Enright, Paul L. 2003. "The Six-Minute Walk Test." *Respiratory Care* 48 (8): 783–85.
García-puga, Mikel, Ander Saenz-antoñanzas, Roberto Fernández-torrón, Adolfo Lopez De, and Ander Matheu. 2020. "Myotonic Dystrophy Type 1 Cells Display Impaired Metabolism and Mitochondrial Dysfunction That Are Reversed by Metformin." *Aging* 12 (7): 6260–75.
Gramegna, Laura Ludovica, Maria Pia Giannoccaro, David Neil Manners, Claudia Testa, Stefano Zanigni, Stefania Evangelisti, Claudio Bianchini, et al. 2018. "Mitochondrial Dysfunction in Myotonic Dystrophy Type 1." *Neuromuscular Disorders* 28 (2): 144–49. https://doi.org/10.1016/j.nmd.2017.10.007.
Ha, Andrew H, Mark A Tarnopolsky, T Graham Bergstra, Girish M Nair, Atif Al-qubbany, and Jeff S Healey. 2012. "Predictors of Atrio-Ventricular Conduction Disease , Long-Term Outcomes in Patients with Myotonic Dystrophy Types I and II" 35 (October): 1262–69. https://doi.org/10.1111/j.1540-8159.2012.03351.x.
Harley, Helen G, J David Brook, Shelley A Rundle, Stephen Crow, William Reardon, Alan J Buckler, Peter S Harper, David E Housman, and Duncan J Shaw. 1992. "Expansion of an Unstable DNA Region and Phenotypic Variation in Myotonic Dystrophy" 355 (November 1991): 1991–92.
Hawkins, A. M., C. L. Hawkins, K. Abdul Razak, T. K. Khoo, K. Tran, and R. V. Jackson. 2019. "Respiratory Dysfunction in Myotonic Dystrophy Type 1: A Systematic Review." *Neuromuscular Disorders* 29 (3): 198–212. https://doi.org/10.1016/j.nmd.2018.12.002.
Ho, Thai H, Nicolas Charlet-b, G Michael, Gopal Singh, S Maurice, and Thomas A Cooper. 2004. "Muscleblind Proteins Regulate Alternative Splicing" 23 (15): 3103–12. https://doi.org/10.1038/sj.emboj.7600300.
Hofmann-Radvanyi, Helen, and Claudine Junien. 1994. "MYOTONIC DYSTROPHY: OVER-EXPRESSION OR/AND UNDER-EXPRESSION? A CRITICAL REVIEW ON A CONTROVERSIAL POINT." *Neuromuscular Disorders* 3 (5): 497–501.
Horrigan, Joseph, Tiago Bernardino Gomes, Mike Snape, Nikoletta Nikolenko, Alison McMorn, Stuart Evans, Alex Yaroshinsky, Oscar Della Pasqua, Sean Oosterholt, and Hanns Lochmüller. 2020. "A Phase 2 Study of AMO-02 (Tideglusib) in Congenital and Childhood Onset Myotonic Dystrophy Type 1 (DM1)." *Pediatric Neurology*. https://doi.org/10.1016/j.eplepsyres.2019.106192.
Huston, Tim P., James C. Puffer, and WM. MacMillan Rodney. 1985. "The Athletic Heart Syndrome." *The New England Journal of Medicine* 313 (1): 24–32.
Hyatt, Hayden, Rafael Deminice, Toshinori Yoshihara, and Scott K. Powers. 2019. "Mitochondrial Dysfunction Induces Muscle Atrophy during Prolonged Inactivity: A Review of the Causes and Effects." *Archives of Biochemistry and Biophysics* 662 (November 2018): 49–60. https://doi.org/10.1016/j.abb.2018.11.005.
Ihara, Yuetsu, Akitane Mori, Reiko Namba, and Keigo Nobukuni. 1995. "Free Radicals , Lipid Peroxides and Antioxidants in Blood of Patients with Myotonic Dystrophy." *Neurology* 242 (4): 119–22.
Isashiki, Y, E Kawabata, N Ohba, I Higuchi, M Nakagawa, M Osame, E Kawabata, et al. 1989. "Mitochondrial Abnormalities in Extraocular Muscles in Myotonic Dystrophy." *Neuro-Opthalmology* 9 (2): 115–22. https://doi.org/10.3109/01658108909007466.
Jansen, G., P. Willems, M. Coerwinkel, W. Nillesen, H. Smeets, L. Vits, C. Howeler, H. Brunner, and B. Wieringa. 1994. "Gonosomal Mosaicism in Myotonic Dystrophy Patients: Involvement of Mitotic Events in (CTG)(n) Repeat Variation and Selection against Extreme

Expansion in Sperm." *American Journal of Human Genetics* 54 (4): 575–85.
Jansen, Gert, Patricia J T A Groenen, Dietmar Bachner, Paul H K Jap, Frank Oerlemans, Walther Van Den Broek, Barbel Gohlsch, et al. 1996. "Abnormal Myotonic Dystrophy Protein Kinase Levels Produce Only Mild Myopathy in Mice." *Nature Genetics* 13 (3): 316–24. https://doi.org/1061-4036 (Print).
Jansen, Merel, Nens Van Alfen, Alexander C.H. Geurts, and Imelda J.M. De Groot. 2013. "Assisted Bicycle Training Delays Functional Deterioration in Boys with Duchenne Muscular Dystrophy: The Randomized Controlled Trial 'No Use Is Disuse.'" *Neurorehabilitation and Neural Repair* 27 (9): 816–27. https://doi.org/10.1177/1545968313496326.
Jones, Karlie, Christina Wei, Polina Iakova, Enrico Bugiardini, Christiane Schneider-Gold, Giovanni Meola, James Woodgett, James Killian, Nikolai A. Timchenko, and Lubov T. Timchenko. 2012. "GSK3β Mediates Muscle Pathology in Myotonic Dystrophy." *Journal of Clinical Investigation* 122 (12): 4461–72. https://doi.org/10.1172/JCI64081.
Jones, Paul W, Kai M Beeh, Kenneth R Chapman, Marc Decramer, Donald A Mahler, and Jadwiga A Wedzicha. 2014. "Minimal Clinically Important Differences in Pharmacological Trials." *Am J Respir Crit Care Med.* 189 (3): 250–55. https://doi.org/10.1164/rccm.201310-1863PP.
Kalsotra, Auinash, Xinshu Xiao, Amanda J. Ward, John C. Castle, Jason M. Johnson, Christopher B. Burge, and Thomas A. Cooper. 2008. "A Postnatal Switch of CELF and MBNL Proteins Reprograms Alternative Splicing in the Developing Heart." *Proceedings of the National Academy of Sciences of the United States of America* 105 (51): 20333–38. https://doi.org/10.1073/pnas.0809045105.
Kanadia, Rahul N., Carl R. Urbinati, Valerie J. Crusselle, Defang Luo, Young Jae Lee, Jeffrey K. Harrison, S. Paul Oh, and Maurice S. Swanson. 2003. "Developmental Expression of Mouse Muscleblind Genes Mbnl1, Mbnl2 and Mbnl3." *Gene Expression Patterns* 3 (4): 459–62. https://doi.org/10.1016/S1567-133X(03)00064-4.
Kanadia, Rahul N, Karen A Johnstone, Ami Mankodi, Codrin Lungu, Charles A. Thornton, Douglas Esson, Adrian M Timmers, William W Hauswirth, and Maurice S. Swanson. 2003. "A Muscleblind Knockout Modet for Myotonic Dystrophy." *Science* 302 (5): 1978–80.
Kierkegaard, Marie, Karin Harms-Ringdahl, Lars Edström, Lotta Widén Holmqvist, and Anna Tollbäck. 2011. "Feasibility and Effects of a Physical Exercise Programme in Adults with Myotonic Dystrophy Type 1: A Randomized Controlled Pilot Study." *Journal of Rehabilitation Medicine* 43 (8): 695–702. https://doi.org/10.2340/16501977-0833.
Klesert, Todd R, Diane H Cho, John I Clark, James Maylie, John Adelman, Lauren Snider, Eric C. Yuen, Philippe Soriano, and Stephen J Tapscott. 2000. "Mice Deficient in Six5 Develop Cataracts : Implications for Myotonic Dystrophy." *Nature Genetics* 25 (1): 105–9.
Klesert, Todd R, Anne D Otten, Thomas D Bird, and Stephen J. Tapscott. 1997. "Trinucleotide Repeat Expansion at the Myotonic Dystrophy Locus Reduces Expression of DMAHP." *Nature Genetics* 16 (5): 1–5.
Kumar, A., V. Kumar, S. K. Singh, S. Muthuswamy, and S. Agarwal. 2014. "Imbalanced Oxidant and Antioxidant Ratio in Myotonic Dystrophy Type 1." *Free Radical Research* 48 (4): 503–10. https://doi.org/10.3109/10715762.2014.887847.
Kuyumcu-Martinez, N Muge, Guey-shin Wang, and Thomas A Cooper. 2007. "Increased Steady-State Levels of CUGBP1 in Myotonic Dystrophy 1 Are Due to PKC-Mediated Hyperphosphorylation." *Molecular Cell* 28 (1): 68–78.

https://doi.org/10.1016/j.molcel.2007.07.027.

Lam, L T, Y C N Pham, Nguyen Man, and G E Morris. 2000. "Characterization of a Monoclonal Antibody Panel Shows That the Myotonic Dystrophy Protein Kinase , DMPK , Is Expressed Almost Exclusively in Muscle and Heart." *Human Molecular Genetics* 9 (14): 2167–74.

Légaré, Cécilia, Gayle Overend, Simon Pierre Guay, Darren G. Monckton, Jean Mathieu, Cynthia Gagnon, and Luigi Bouchard. 2019. "DMPK Gene DNA Methylation Levels Are Associated with Muscular and Respiratory Profiles in DM1." *Neurology: Genetics* 5 (3). https://doi.org/10.1212/NXG.0000000000000338.

Lindeman, Eline, Pieter Leffers, Frank Spaans, Jan Drukker, Jos Reulen, Maria Kerckhoffs, and Albere Köke. 1995. "Strength Training in Patients with Myotonic Dystrophy and Hereditary Motor and Sensory Neuropathy: A Randomized Clinical Trial." *Archives of Physical Medicine and Rehabilitation* 76 (7): 612–20. https://doi.org/10.1016/S0003-9993(95)80629-6.

Lindeman, Eline, Frank Spaans, Jos Reulen, Pieter Leffers, and Jan Drukker. 1999. "Progressive Resistance Training in Neuromuscular Patients. Effects on Force and Surface EMG." *Journal of Electromyography and Kinesiology* 9 (6): 379–84. https://doi.org/10.1016/S1050-6411(99)00003-6.

Liquori, C. L., K. Ricker, M. L. Moseley, J. F. Jacobsen, W. Kress, S. L. Naylor, J. W. Day, and L. P.W. Ranum. 2001. "Myotonic Dystrophy Type 2 Caused by a CCTG Expansion in Intron I of ZNF9." *Science* 293 (5531): 864–67. https://doi.org/10.1126/science.1062125.

MacDonald, J. R., J. D. Hill, and Mark A. Tarnopolsky. 2002. "Modafinil Reduces Excessive Somnolence and Enhances Mood in Patients with Myotonic Dystrophy." *Neurology* 59 (12): 1876–80. https://doi.org/10.1212/01.WNL.0000037481.08283.51.

Madsen, Karen Lindhardt, Regitze Sølling Hansen, Nicolai Preisler, Frank Thøgersen, Martin Peter Berthelsen, and John Vissing. 2015. "Training Improves Oxidative Capacity, but Not Function, in Spinal Muscular Atrophy Type III." *Muscle & Nerve* 52 (2): 240–44. https://doi.org/10.1002/mus.24527.

Mahadevan, Mani, Catherine Tsilfidis, Luc Sabourin, Gary Shutler, Gert Jansen, Catherine Neville, Monica Narang, et al. 1992. "Myotonic Dystrophy Mutation : An Unstable CTG Repeat in the 3 ′ Untranslated Region of the Gene." *Science* 255 (5049): 1253–55.

Malandish, Abbas, Bakhtyar Tartibian, Zeinab Sheikhlou, Roghaiyeh Afsargharehbagh, and Mohammad Rahmati. 2020. "The Effects of Short-Term Moderate Intensity Aerobic Exercise and Long-Term Detraining on Electrocardiogram Indices and Cardiac Biomarkers in Postmenopausal Women." *Journal of Electrocardiology* 60: 15–22. https://doi.org/10.1016/j.jelectrocard.2020.03.004.

Mankodi, Ami, Carl R Urbinati, Qiu-ping Yuan, Richard T Moxley, Valeria Sansone, Matt Krym, Donald Henderson, Martin Schalling, Maurice S Swanson, and Charles A Thornton. 2001. "Muscleblind Localizes to Nuclear Foci of Aberrant RNA in Myotonic Dystrophy Types 1 and 2." *Human Molecular Genetics* 10 (19): 2165–70.

Manta, Alexander, Derek W Stouth, Donald Xhuti, Leon Chi, Irena A Rebalka, Jayne M Kalmar, Thomas J Hawke, and Vladimir Ljubicic. 2019. "Chronic Exercise Mitigates Disease Mechanisms and Improves Muscle Function in Myotonic Dystrophy Type 1 Mice." *Journal of Physiology* 597 (5): 1361–81. https://doi.org/10.1113/JP277123.

Mcdonald, Craig M. 2012. "Clinical Approach to the Diagnostic Evaluation of Hereditary and Acquired Neuromuscular Diseases." *Physical Medicine and Rehabilitation Clinics of N Am* 23 (3): 495–563. https://doi.org/10.1016/j.pmr.2012.06.011.

Miller, Jill W, Carl R Urbinati, Patana Teng-umnuay, Myrna G Stenberg, Barry J Byrne, Charles A Thornton, and Maurice S Swanson. 2000. "Recruitment of Human Muscleblind Proteins to (CUG) n Expansions Associated with Myotonic Dystrophy." *EMBO J.* 19 (17): 4439–48.

Moller, David E., Atsushi Yokota, Jose F. Caro, and Jeffrey S. Flier. 1989. "Tissue-Specific Expression of Two Alternatively Spliced Insulin Receptor Mrnas in Man." *Molecular Endocrinology* 3 (8): 1263–69. https://doi.org/10.1210/mend-3-8-1263.

Moon, Jae Ho, Young Moo Na, Seong Woong Kang, and Han Soo Lee. 1996. "The Changes in Muscle Strength and Relaxation Time after a Comprehensive Rehabilitation Program for Patients with Myotonic Dystrohy." *Yonsei Medical Journal* 37 (4): 237–42.

Morales, Fernando, Melissa Vásquez, Patricia Cuenca, Domingo Campos, Carolina Santamaría, Gerardo Del Valle, Roberto Brian, Mauricio Sittenfeld, and Darren G. Monckton. 2015. "Parental Age Effects, but No Evidence for an Intrauterine Effect in the Transmission of Myotonic Dystrophy Type 1." *European Journal of Human Genetics* 23 (5): 646–53. https://doi.org/10.1038/ejhg.2014.138.

Moxley, Richard T, Emma Ciafaloni, and Debra Guntrum. 2015. "Myotonic Dystrophy." In *Neuromuscular Disorders of Infancy, Childhood, and Adolescence 2e*, Second Edi, 697–718. Elsevier Inc. https://doi.org/10.1016/B978-0-12-417044-5.00037-8.

Mulders, Susan A M, Walther J A A Van Den Broek, Thurman M Wheeler, Huib J E Croes, Derick G Wansink, and Charles A Thornton. 2009. "Triplet-Repeat Oligonucleotide-Mediated Reversal of RNA Toxicity in Myotonic Dystrophy." *Proceedings of the National Academy of Sciences* 106 (33): 13915–20.

Nakamori, M., T. Kimura, T. Kubota, T. Matsumura, H. Sumi, H. Fujimura, M. P. Takahashi, and S. Sakoda. 2008. "Aberrantly Spliced Alpha-Dystrobrevin Alters Alpha-Syntrophin Binding in Myotonic Dystrophy Type 1." *Neurology* 70 (9): 677–85. https://doi.org/10.1212/01.wnl.0000302174.08951.cf.

Nakamori, Masayuki, Krzysztof Sobczak, Araya Puwanant, Katy Eichinger, Shree Pandya, Jeannne Dekdebrun, R Heatwole, et al. 2013. "Splicing Biomarkers of Disease Severity in Myotonic Dystrophy." *Annals of Neurology* 74 (6): 862–72. https://doi.org/10.1002/ana.23992.Splicing.

Nakamori, Masayuki, Katarzyna Taylor, Hideki Mochizuki, Krzysztof Sobczak, and Masanori P. Takahashi. 2016. "Oral Administration of Erythromycin Decreases RNA Toxicity in Myotonic Dystrophy." *Annals of Clinical and Translational Neurology* 3 (1): 42–54. https://doi.org/10.1002/acn3.271.

Nederveen, Joshua P., George Ibrahim, Stephen A. Fortino, Tim Snijders, Dinesh Kumbhare, and Gianni Parise. 2020. "Variability in Skeletal Muscle Fibre Characteristics during Repeated Muscle Biopsy Sampling in Human Vastus Lateralis." *Applied Physiology, Nutrition and Metabolism* 45 (4): 368–75. https://doi.org/10.1139/apnm-2019-0263.

Ng, Sean Y, Alexander Manta, and Vladimir Ljubicic. 2018. "Exercise Biology of Neuromuscular Disorders." *Applied Physiology, Nutrition, and Metabolism* 43 (11): 1194–1206. https://doi.org/10.1139/apnm-2018-0229.

Ng, Sean Y, Andrew Mikhail, and Vladimir Ljubicic. 2019. "Mechanisms of Exercise-Induced Survival Motor Neuron Expression in the Skeletal Muscle of Spinal Muscular Atrophy-like Mice." *Journal of Physiology* 587 (18): 4757–78. https://doi.org/10.1113/JP278454.

Ono, Seiitsu, Hiroshi Kurisaki, Kiyoharu Inouye, and Toru Mannen. 1986. "'Ragged-Red' Fibres in Myotonic Dystrophy." *Journal of the Neurological Sciences* 74 (2–3): 247–55.

https://doi.org/10.1016/0022-510X(86)90110-3.
Ørngreen, Mette C., David B. Olsen, and John Vissing. 2005. "Aerobic Training in Patients with Myotonic Dystrophy Type 1." *Annals of Neurology* 57 (5): 754–57. https://doi.org/10.1002/ana.20460.
Otten, A.D., and S.J. Tapscott. 1995. "Triplet Repeat Expansion in Myotonic Dystrophy Alters the Adjacent Chromatin Structure." *Proceedings of the National Academy of Sciences* 92 (12): 5465–69.
Ozaki, H, J P Loenneke, R S Thiebaud, and T Abe. 2015. "Cycle Training Induces Muscle Hypertrophy and Strength Gain : Strategies and Mechanisms (Review)." *Acta Physiol Hung.* 102 (1): 1–22. https://doi.org/10.1556/APhysiol.102.2015.1.1.
Pantic, B, E Trevisan, A Citta, M P Rigobello, O Marin, P Bernardi, S Salvatori, and A Rasola. 2013. "Myotonic Dystrophy Protein Kinase (DMPK) Prevents ROS-Induced Cell Death by Assembling a Hexokinase II-Src Complex on the Mitochondrial Surface." *Cell Death and Disease* 4 (10): e858. https://doi.org/10.1038/cddis.2013.385.
Pelkonen, Margit, Irma-leena Notkola, Timo Lakka, Hannu O Tukiainen, Paula Kivinen, and Aulikki Nissinen. 2003. "Delaying Decline in Pulmonary Function with Physical Activity." *Am J Respir Crit Care Med.* 168 (4): 494–99. https://doi.org/10.1164/rccm.200208-954OC.
Philips, A V, L T Timchenko, and T A Cooper. 1998. "Disruption of Splicing Regulated by a CUG-Binding Protein in Myotonic Dystrophy." *Science (New York, N.Y.)* 280 (5364): 737–41.
Pisani, Valerio, Maria Beatrice Panico, Chiara Terracciano, Emanuela Bonifazi, Giovanni Meola, Giuseppe Novelli, Giorgio Bernardi, Corrado Angelini, and Roberto Massa. 2008. "Preferential Central Nucleation of Type 2 Myofibers Is an Invariable Feature of Myotonic Dystrophy Type 2." *Muscle and Nerve* 38 (5): 1405–11. https://doi.org/10.1002/mus.21122.
Podsiadlo, Diane, and Sandra Richardson. 1991. "The Timed "Up & Go": A Test of Basic Functional Mobility for Frail Elderlv Persons." *Journal of the American Geriatrics Society*, 142–48.
Poulton, J., H. G. Harley, J. Dasmahapatra, G. K. Brown, C. G. Potter, and B. Sykes. 1995. "Mitochondrial DNA Does Not Appear to Influence the Congenital Onset Type of Myotonic Dystrophy." *Journal of Medical Genetics* 32 (9): 732–35. https://doi.org/10.1136/jmg.32.9.732.
Provenzano, Claudia, Marisa Cappella, Rea Valaperta, Rosanna Cardani, Giovanni Meola, Fabio Martelli, Beatrice Cardinali, and Germana Falcone. 2017. "CRISPR/Cas9-Mediated Deletion of CTG Expansions Recovers Normal Phenotype in Myogenic Cells Derived from Myotonic Dystrophy 1 Patients." *Molecular Therapy - Nucleic Acids* 9 (December): 337–48. https://doi.org/10.1016/j.omtn.2017.10.006.
Ravel-chapuis, Aymeric, Ali Al-rewashdy, Guy Bélanger, and Bernard J Jasmin. 2018. "Pharmacological and Physiological Activation of AMPK Improves the Spliceopathy in DM1 Mouse Muscles." *Human Molecular Genetics* 27 (19): 3361–76. https://doi.org/10.1093/hmg/ddy245.
Reddy, Sita, Daniel B J Smith, Mark M Rich, John M Leferovich, Patricia Reilly, Brigid M Davis, Khoa Tran, et al. 1996. "Mice Lacking the Myotonic Dystrophy Protein Kinase Develop a Late Onset Progressive Myopathy Sita." *Nature Genetics* 13 (3): 325–35.
Redman, Joy B., Raymond G. Fenwick, Ying Hui Fu, Antonio Pizzuti, and C. Thomas Caskey. 1993. "Relationship Between Parental Trinucleotide GCT Repeat Length and Severity of Myotonic Dystrophy in Offspring." *JAMA: The Journal of the American Medical*

Association 269 (15): 1960–65. https://doi.org/10.1001/jama.1993.03500150072029.
Rojas, Jose M., Marianne Subleski, Juan J.R. Coque, Carmen Guerrero, Rosana Saez, Bao Qun Li, Eva Lopez, et al. 1999. "Isoform-Specific Insertion near the Grb2-Binding Domain Modulates the Intrinsic Guanine Nucleotide Exchange Activity of HSos1." *Oncogene* 18 (9): 1651–61. https://doi.org/10.1038/sj.onc.1202483.
Roussel, Marie-pier, Marika Morin, Cynthia Gagnon, and Elise Duchesne. 2019. "What Is Known about the Effects of Exercise or Training to Reduce Skeletal Muscle Impairments of Patients with Myotonic Dystrophy Type 1 ? A Scoping Review." *BMC Musculoskelet Disord* 20 (1): 101.
Roussel, Marie Pier, Luc J. Hébert, and Elise Duchesne. 2020. "Strength-Training Effectively Alleviates Skeletal Muscle Impairments in Myotonic Dystrophy Type 1." *Neuromuscular Disorders* 30 (4): 283–93. https://doi.org/10.1016/j.nmd.2020.02.015.
Roussel, Marie Pier, Marika Morin, Mélina Girardin, Anne Marie Fortin, Mario Leone, and Jean Mathieu. 2019. "Training Program - Induced Skeletal Muscle Adaptations in Two Men with Myotonic Dystrophy Type 1." *BMC Research Notes*, 1–6. https://doi.org/10.1186/s13104-019-4554-z.
Sahashi, Ko, Masashi Tanaka, Michinari Tashiro, Kinji Ohno Tohru Ibi, Akira Takahashi, and Takayuki Ozawa. 1992. "Increased Mitochondrial DNA Deletions in the Skeleal Muscle of Myotonic Dystrophy." *Gerontology* 38: 18–29.
Salvatori, Sergio, M. Fanin, C. P. Trevisan, S. Furlan, S. Reddy, J. I. Nagy, and C. Angelini. 2005. "Decreased Expression of DMPK: Correlation with CTG Repeat Expansion and Fibre Type Composition in Myotonic Dystrophy Type 1." *Neurological Sciences* 26 (4): 235–42. https://doi.org/10.1007/s10072-005-0466-x.
Santoro, M., M. Masciullo, G. Silvestri, G. Novelli, and A. Botta. 2017. "Myotonic Dystrophy Type 1: Role of CCG, CTC and CGG Interruptions within DMPK Alleles in the Pathogenesis and Molecular Diagnosis." *Clinical Genetics* 92 (4): 355–64. https://doi.org/10.1111/cge.12954.
Sarkar, Partha S, Binoy Appukuttan, Jennifer Han, Yoshihiro Ito, Cuiwei Ai, Wenli Tsai, Yang Chai, J Timothy Stout, and Sita Reddy. 2000. "Heterozygous Loss of Six5 in Mice Is Sufficient to Cause Ocular Cataracts." *Nature Genetics* 25 (1): 110–14.
Savkur, R S, A V Philips, and T A Cooper. 2001. "Aberrant Regulation of Insulin Receptor Alternative Splicing Is Associated with Insulin Resistance in Myotonic Dystrophy." *Nature Genetics* 29 (1): 40–47. https://doi.org/10.1038/ng704.
Savouret, Cédric, Corinne Garcia-Cordier, Jérôme Megret, Hein te Riele, Claudine Junien, and Geneviève Gourdon. 2004. "MSH2-Dependent Germinal CTG Repeat Expansions Are Produced Continuously in Spermatogonia from DM1 Transgenic Mice." *Molecular and Cellular Biology* 24 (2): 629–37. https://doi.org/10.1128/mcb.24.2.629-637.2004.
Sharp, Lydia, Diana C. Cox, and Thomas A. Cooper. 2019. "Endurance Exercise Leads to Beneficial Molecular and Physiological Effects in a Mouse Model of Myotonic Dystrophy Type 1." *Muscle and Nerve* 60 (6): 779–89. https://doi.org/10.1002/mus.26709.
Siboni, Ruth B., Masayuki Nakamori, Stacey D. Wagner, Adam J. Struck, Leslie A. Coonrod, Shanee A. Harriott, Daniel M. Cass, Matthew K. Tanner, and J. Andrew Berglund. 2015. "Actinomycin D Specifically Reduces Expanded CUG Repeat RNA in Myotonic Dystrophy Models." *Cell Reports* 13 (11): 2386–94. https://doi.org/10.1016/j.celrep.2015.11.028.
Siciliano, G., M. Mancuso, D. Tedeschi, M. L. Manca, M. R. Renna, V. Lombardi, A. Rocchi, F. Martelli, and L. Murri. 2001. "Coenzyme Q10, Exercise Lactate and CTG Trinucleotide

Expansion in Myotonic Dystrophy." *Brain Research Bulletin* 56 (3–4): 405–10. https://doi.org/10.1016/S0361-9230(01)00653-0.

Solbakken, Gro, Bård Bjørnarå, Eva Kirkhus, Bac Nguyen, Gunnar Hansen, Jan C. Frich, and Kristin Ørstavik. 2019. "MRI of Trunk Muscles and Motor and Respiratory Function in Patients with Myotonic Dystrophy Type 1." *BMC Neurology* 19 (1): 1–11. https://doi.org/10.1186/s12883-019-1357-8.

Steinberg, Gregory R. 2019. "AMP-Activated Protein Kinase : The Current Landscape for Drug Development." *Nature Reviews Drug Discovery* 18 (7): 527–51. https://doi.org/10.1038/s41573-019-0019-2.

Talan, D. A., R. A. Bauernfeind, W. W. Ashley, C. Kanakis, and K. M. Rosen. 1982. "Twenty-Four Hour Continuous ECG Recordings in Long-Distance Runners." *Chest* 82 (1): 19–24. https://doi.org/10.1378/chest.82.1.19.

Taneja, Krishan L, Mila McCurrach, Martin Schalling, David Housman, and Robert H Singer. 1995. "Foci of Trinucleotide Repeat Transcripts in Nuclei of Myotonic Dystrophy Cells and Tissues" 128 (6): 995–1002.

Tarnopolsky, Mark A., Erin Pearce, Katelyn Smith, and Boleslaw Lach. 2011. "Suction-Modified Bergström Muscle Biopsy Technique: Experience with 13,500 Procedures." *Muscle and Nerve* 43 (5): 717–25. https://doi.org/10.1002/mus.21945.

Temmerman, Nele De, Karen Sermon, Sara Seneca, Martine De Rycke, Pierre Hilven, Willy Lissens, André Van Steirteghem, and Inge Liebaers. 2004. "Intergenerational Instability of the Expanded CTG Repeat in the DMPK Gene: Studies in Human Gametes and Preimplantation Embryos." *American Journal of Human Genetics* 75 (2): 325–29. https://doi.org/10.1086/422762.

Thijs, C, J F Mirandolle, H B Anten, H J M Smeets, K E Chandler, and J P M Geraedts. 1998. "Age and Causes of Death in Adult-Onset Myotonic Dystrophy," 1557–63.

Thornton, Charles A. 2014. "Myotonic Dystrophy." *Neurologic Clinics* 32 (3): 705–19. https://doi.org/10.1016/j.ncl.2014.04.011.

Thornton, Charles A., Keith Johnson, and Richard T. Moxley. 1994. "Myotonic Dystrophy Patients Have Larger CTG Expansions in Skeletal Muscle than in Leukocytes." *Annals of Neurology* 35 (1): 104–7. https://doi.org/10.1002/ana.410350116.

Thornton, Charles A, James P Wym, Zachary Simmons, Carolyn Mcclain, and Richard T Moxley III. 1997. "Expansion of the Myotonic Dystrophy CTG Repeat Reduces Expression of the Flanking DMAHP Gene." *Nature Genetics* 16 (4): 407–9.

Timchenko, L, W Nastainczykt, T Schneidert, B Patel, F Hofmannt, and C T Caskey. 1995. "Full-Length Myotonin Protein Kinase (72 KDa) Displays Serine Kinase Activity." *Proceedings of the National Academy of Sciences* 92 (12): 5366–70.

Tollbäck, Anna, Stefan Eriksson, Anna Wredenberg, Göran Jenner, Roberto Vargas, Kristian Borg, and Tor Ansved. 1999. "Effects of High Resistance Training in Patients with Myotonic Dystrophy." *Scandinavian Journal of Rehabilitation Medicine* 31 (1): 9–16. https://doi.org/10.1080/003655099444678.

Tomé, Stéphanie, Elodie Dandelot, Céline Dogan, Alexis Bertrand, David Geneviève, Yann Péréon, Marie Simon, et al. 2018. "Unusual Association of a Unique CAG Interruption in 5′ of DM1 CTG Repeats with Intergenerational Contractions and Low Somatic Mosaicism." *Human Mutation* 39 (7): 970–82. https://doi.org/10.1002/humu.23531.

Toscano, A, S Messina, G M. Campo, R Di Leo, O Musumeci, C Rodolico, M Aguennouz, G Annesi, C Messina, and G Vita. 2005. "Oxidative Stress in Myotonic Dystrophy Type 1."

Free Radical Research 39 (7): 771–76.

Vihola, A., G. Bassez, G. Meola, S. Zhang, H. Haapasalo, A. Paetau, E. Mancinelli, et al. 2003. "Histopathological Differences of Myotonic Dystrophy Type 1 (DM1) and PROMM/DM2." *Neurology* 60 (11): 1854–57. https://doi.org/10.1212/01.WNL.0000065898.61358.09.

Vita, G, A Toscano, A Prelle, A Bordoni, N Checcarelli, N Bresolin, A Baradello, and C Messina. 1993. "Muscle Mitochondria Investigation in Myotonic Dystrophy Abstract." *Eur Neurol* 33: 423–27.

Wang, Eric T., Daniel Treacy, Katy Eichinger, Adam Struck, Joseph Estabrook, Hailey Olafson, Thomas T. Wang, et al. 2019. "Transcriptome Alterations in Myotonic Dystrophy Skeletal Muscle and Heart." *Human Molecular Genetics* 28 (8): 1312–21. https://doi.org/10.1093/hmg/ddy432.

Wang, Eric T, Amanda J Ward, Jennifer M Cherone, Jimena Giudice, Thomas T Wang, Daniel J Treacy, Nicole J Lambert, et al. 2015. "Antagonistic Regulation of MRNA Expression and Splicing by CELF and MBNL Proteins." *Genome Res.* 25 (6): 858–71. https://doi.org/10.1101/gr.184390.114.Freely.

Wang, Guey-shin, Xander H T Wehrens, A Thomas, Guey-shin Wang, Muge N Kuyumcu-martinez, Satyam Sarma, and Nitin Mathur. 2009. "PKC Inhibition Ameliorates the Cardiac Phenotype in a Mouse Model of Myotonic Dystrophy Type 1 Find the Latest Version : PKC Inhibition Ameliorates the Cardiac Phenotype in a Mouse Model of Myotonic Dystrophy Type 1." *Journal of Clinical Investigation* 119 (12): 3797–3806. https://doi.org/10.1172/JCI37976.for.

Warf, M. Bryan, Masayuki Nakamori, Catherine M. Matthys, Charles A. Thornton, and J. Andrew Berglund. 2009. "Pentamidine Reverses the Splicing Defects Associated with Myotonic Dystrophy." *Proceedings of the National Academy of Sciences of the United States of America* 106 (44): 18551–56. https://doi.org/10.1073/pnas.0903234106.

Wei, Christina, Lauren Stock, Leila Valanejad, Zachary A. Zalewski, Rebekah Karns, Jack Puymirat, David Nelson, et al. 2018. "Correction of GSK3β at Young Age Prevents Muscle Pathology in Mice with Myotonic Dystrophy Type 1." *FASEB Journal* 32 (4): 2073–85. https://doi.org/10.1096/fj.201700700R.

Wheeler, Thurman M., Andrew J. Leger, Sanjay K. Pandey, A. Robert Mac Leod, Thurman M. Wheeler, Seng H. Cheng, Bruce M. Wentworth, C. Frank Bennett, and Charles A. Thornton. 2012. "Targeting Nuclear RNA for in Vivo Correction of Myotonic Dystrophy." *Nature* 488 (7409): 111–17. https://doi.org/10.1038/nature11362.

Wheeler, Thurman M, John D Lueck, Maurice S Swanson, Robert T Dirksen, and Charles A Thornton. 2007. "Correction of ClC-1 Splicing Eliminates Chloride Channelopathy and Myotonia in Mouse Models of Myotonic Dystrophy" 117 (12). https://doi.org/10.1172/JCI33355.3952.

Whiting, Elisabeth J., James D. Waring, Katsuyuki Tamai, Martin J. Somerville, Maxwell Hincke, William A. Staines, Joh-e Ikeda, and Robert G Korneluk. 1995. "Characterization of Myotonic Dystrophy Kinase (DMK) Protein in Human and Rodent Muscle and Central Nervous Tissue." *Human Molecular Genetics* 4 (6): 1063–72.

Whitney, Susan L., Diane M. Wrisley, Gregory F. Marchetti, Michael A. Gee, Mark S. Redfern, and Joseph M. Furman. 2005. "Clinical Measurement of Sit-to-Stand Performance in People with Balance Disorders: Validity of Data for the Five-Times-Sit-to-Stand Test." *Physical Therapy* 85 (10): 1034–45. https://doi.org/10.1093/ptj/85.10.1034.

Wright, Nancy C., David D. Kilmer, Megan A. McCrory, Susan G. Aitkens, Bryan J. Holcomb,

and Edmund M. Bernauer. 1996. "Aerobic Walking in Slowly Progressive Neuromuscular Disease: Effect of a 12-Week Program." *Archives of Physical Medicine and Rehabilitation* 77 (1): 64–69. https://doi.org/10.1016/S0003-9993(96)90222-1.

Zehender, Manfred, Thomas Meinertz, Joseph Keul, and Hansjörg Just. 1990. "ECG Variants and Cardiac Arrhythmias in Athletes: Clinical Relevance and Prognostic Importance." *American Heart Journal* 119 (6): 1378–91. https://doi.org/10.1016/S0002-8703(05)80189-9.

Table S1: List of primary antibodies

Western Blotting					
Protein	**Manufacture**	**Product #**	**Host**	**Primary Dilution**	**Secondary Dilution**
AMPK	Cell Signaling Technology	2532	Rabbit	1:1000	1:20,000
pAMPK	Cell Signaling Technology	2531	Rabbit	1:1000	1:20,000
PGC-1α	EMD Millipore	ST1202	Mouse	1:1000	1:20,000
OXPHOS	Abcam	ab110413	Mouse	1:1000	1:20,000
OPA1	Abcam	ab42364	Rabbit	1:1000	1:20,000
MFN-1	Cell Signaling Technology	14739	Rabbit	1:1000	1:20,000
MFN-2	Abcam	ab56889	Mouse	1:1000	1:20,000
DRP-1	Cell Signaling Technology	8570	Rabbit	1:1000	1:20,000
Fis1	Proteintech	10956-1-AP	Rabbit	1:1000	1:20,000
4HNE	Abcam	ab46544	Rabbit	1:1000	1:20,000
SOD1	Abcam	ab16831	Rabbit	1:1000	1:20,000
SOD2	Abcam	ab13533	Rabbit	1:1000	1:20,000
MBNL1	Abnova	H00004154-M02	Mouse	1:1000	1:20,000
MBNL2	Santa Cruz Biotechnology	sc-136167	Mouse	1:1000	1:20,000
CUGBP1	Santa Cruz Biotechnology	sc-20003	Mouse	1:1000	1:20,000
GSK-3β	Cell Signaling Technology	9315	Rabbit	1:10,000	1:20,000
pGSK-3β	Cell Signaling Technology	9323	Rabbit	1:10,000	1:20,000
Immunoflorescence					
Protein	**Manufacture**	**Product #**	**Host**	**Primary Dilution**	**Secondary Dilution**
MHC I	DHSB	BA-F8	Mouse (IgG2b)	1:100	1:500
MHC IIA	DHSB	SC-71	Mouse (IgG1)	1:600	1:500
MHC IIX	DHSB	6H1	Mouse (IgM)	1:25	1:500
Wheat Germ Agglutinin	Thermo Fisher Scientific	W11263	Conjugated	1:300	NA