Universidade de Aveiro
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Tiago Duarte Cordeiro Mateus

Estudo do metaboloma e medidas de força muscular para a caracterização de doentes com Distrofia Miotónica do tipo 1

Study of the metabolome and muscle strength measures for the characterization of patients with Myotonic Dystrophy type 1

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Molecular e Celular, realizada sob a orientação científica da Professora Doutora Sandra Maria Tavares da Costa Rebelo, Professora Auxiliar do Departamento de Ciências Médicas da Universidade de Aveiro, e coorientação científica da Professora Doutora Maria Teresa Ferreira Herdeiro, Professora Auxiliar do Departamento de Ciências Médicas da Universidade de Aveiro.

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o júri

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palavras-chave

Distrofia miotônica tipo 1, síndrome metabólica, dislipidemia, Lipina, resistência à insulina, perfil do metaboloma, espectroscopia ATR-FTIR, análise multivariada, medidas de resultados, força muscular, músculo cardíaco; músculo esquelético; músculo respiratório

resumo

A distrofia miotônica tipo 1 (DM1) é uma doença hereditária autossómica dominante causada por uma alteração que leva a uma expansão anormal de repetições instáveis de CTG na região 3' não traduzida do gene da proteína quinase da distrofia miotônica (DMPK). DM1 é caracterizado por miotonia, fraqueza muscular distal progressiva e por envolvimento multissistémica, nomeadamente cataratas, dores musculares, disfunções cardíacas e respiratórias, disfunções endócrinas (resistência à insulina, síndrome metabólica, dislipidemia), cancro e alterações no sistema nervoso central (SNC). Doentes com DM1 apresentam frequência de síndrome metabólica maior do que na população geral. Assim, o estudo do metaboloma é de grande importância, pois pode fornecer novos ideias sobre as vias moleculares afetadas nas doenças DM1, bem como discriminar entre os diferentes graus de gravidade em doentes com DM1 e também pode levar ao desenvolvimento de novas terapêuticas metabólicas.

Dadas as alterações metabólicas previamente descritas e observadas em doentes com DM1, consideramos que a avaliação do perfil metabólico destes doentes é de grande importância. Portanto, elaborou-se uma revisão da literatura para resumir as alterações metabólicas previamente descritas em doentes com DM1 e a relação da Lipina com as alterações metabólicas na DM1 (Capítulo I). Essencialmente, os estudos anteriores mostraram uma clara alteração metabólica entre os doentes com DM1 e os grupos controlo, nomeadamente o aumento dos níveis de colesterol total, lipoproteína de baixa densidade, triacilglicerol, insulina e resistência HOMA-insulina, o aumento dos níveis de glicose, assim como a diminuição dos níveis de lipoproteína de alta densidade. Esta revisão também demonstrou uma potencial relação entre a Lipina e a sua associação com as anormalidades metabólicas encontradas em doentes com DM1, nomeadamente os papéis metabólicos no tecido adiposo, músculo esquelético, fígado e a sua associação com a dislipidemia e a resistência à insulina, que é uma das características em doentes com DM1.

O perfil metabólico dos doentes com DM1 foi então avaliado pela técnica de espectroscopia ATR-FTIR, em conjunto com a análise multivariada, sendo que é adequada para fornecer um perfil (bio) químico dos doentes com DM1 e controlos. Essencialmente, fibroblastos derivados de DM1 e controlos foram utilizados, e os resultados demonstraram uma clara discriminação dentro de fibroblastos derivados de DM1 com diferentes repetições de CTG e idades de início da doença, o que significa que estes podem ter um perfil metabólico distinto. Esta discriminação pode ser atribuída principalmente ao metabolismo lipídico alterado na região 1800-1500 cm^{-1}. Também foi possível discriminar entre os grupos controlo e fibroblastos derivados de DM1 do Instituto Coriell e Centro Hospitalar do Tâmega e Sousa na região de 3000-2800 cm^{-1} (Capítulo II).

Além disso, foi feita uma revisão sistemática para reunir informações de todos os resultados e medidas utilizadas para avaliar a força muscular em doentes adultos com DM1 (Capítulo IV). Foi avaliada a força muscular cardíaca, esquelética e respiratória. Resumidamente, a revisão sistemática demonstrou uma utilização consistente da ecocardiografia, teste muscular quantitativo, teste muscular manual e manometria para avaliar a força muscular cardíaca, esquelética e respiratória. As medidas escolhidas para avaliar a força muscular foram: (1) fração de ejeção para a força do musculo cardíaco; (2) torque isométrico muscular, força de preensão e conselho de pesquisa médica (0-5 pontos e 0-60 pontos) para a força do músculo esquelético; (3) pressão inspiratória máxima e pressão expiratória máxima para a força dos músculos respiratórios. Em conclusão, os resultados sugerem que há uma necessidade de estudos adicionais relativamente ao metabolismo lipídico em doentes com DM1, não apenas para caracterizar melhor estes doentes, como também para compreender o mecanismo subjacente das anormalidades lipídicas e ter novas noções sobre a Lipina na DM1. A espectroscopia FTIR é uma ferramenta valiosa para caracterizar doentes com diferentes severidades da DM1, o que é crucial para um diagnóstico adequado e para estudos futuros. Reunimos com sucesso as medidas mais consensuais e importantes para avaliar a força muscular. Os resultados obtidos foram importantes e úteis, pois serão valiosos para avaliação da força muscular em futuros ensaios clínicos e estudos observacionais, principalmente para testar se um medicamento está a melhorar a força muscular nos doentes com DM1.

keywords

Myotonic dystrophy type 1, DMPK, metabolic syndrome, dyslipidemia, Lipin, insulin resistance, metabolome profile, ATR-FTIR spectroscopy, multivariate analysis, outcome measures, muscle strength, cardiac muscle; skeletal muscle; respiratory muscle

abstract

Myotonic dystrophy type 1 (DM1) is an autosomal dominant hereditary disease caused by an alteration leading to an abnormal expansion of unstable repetitions of CTG in the 3' untranslated region of *Myotonic Dystrophy Protein Kinase* (DMPK) gene. DM1 is characterized by myotonia, progressive distal muscle weakness and by multisystemic involvement namely cataracts, muscle pain, cardiac and respiratory dysfunctions, endocrine dysfunctions (insulin resistance, metabolic syndrome, dyslipidemia), cancer and alterations in the central nervous system (CNS). Patients with DM1 have a frequency of metabolic syndrome higher than in the general population. Thus, the study of the metabolome is of a great importance since it can give new insight regarding the molecular pathways affected in DM1 diseases as well as to discriminate between the different degrees of severities in patients with DM1 and may also, lead to the development of new metabolic therapeutics.

Given the previously reported metabolic alterations observed in patients DM1, we considered that the evaluation of the metabolic profile of those patients of paramount importance. Therefore, we started with the literature review for summarizing the metabolic alterations previously reported in patients with DM1 and the relationship of Lipin with the metabolic alterations in DM1 (Chapter I). Essentially, the previous studies showed a clear metabolic alteration between patients with DM1 and control groups, namely, increased total cholestrol, Low-density lipoprotein, triacylglycerol, insulin and HOMA-Insulin resistance levels, increased glucose levels and low levels of high-density lipoprotein. This review also showed a potential relationship between Lipin and its association with metabolic abnormalities found in patients with DM1, namely, the metabolic roles in adipose tissue, skeletal-muscle, liver and its association with dyslipidemia and insulin resistance, which is a characteristic feature in patients with DM1.

The metabolic profile of patients with DM1 then was evaluated using the ATR-FTIR spectroscopy technique, together with multivariate analysis, which is suitable for providing a (bio)chemical profile of patients with DM1 and controls. Essentially, DM1-derived fibroblast and controls were used, and the results showed a clear discrimination within DM1-derived fibroblast with different CTG repeat length and age at onset, meaning that they may have a distinct metabolic profile. This discrimination can be attributed mainly to the altered lipid metabolism in 1800-1500 region cm^{-1}. It was also possible to discriminate between the control groups and both DM1-derived fibroblast from Coriell institute and Centro Hospitalar do Tâmega e Sousa in 3000-2800 cm^{-1} region (Chapter II).

Additionally, a systematic review was made to gather information of all outcome and measurements used to assess muscle strength in adult patients with DM1 (Chapter IV). The cardiac, skeletal and respiratory muscle strength was evaluated. Briefly, the systematic review showed a consistent use of echocardiography, quantitative muscle test, manual muscle test and manometry to assess cardiac, skeletal and respiratory muscle strength. The measures of choice to assess muscle strength were: (1) ejection fraction in cardiac muscle; (2) muscle isometric torque, grip strength and medical research council (0-5 points and 0-60 points) in skeletal-muscle; (3) maximal inspiratory pressure and maximal expiratory pressure in respiratory muscles. In conclusion, our results suggest that there is a need to further research the lipid metabolism of patients with DM1, not only to better characterize these patients but also to understander the underlying mechanism of lipid abnormalities and to have new insights of Lipin in DM1. FTIR spectroscopy is a valuable tool to characterize patients with DM1 severities, which is crucial for a proper diagnosis and further studies. We successfully gather the more consensual and important measures to evaluate muscle strength. The results obtained were important and useful given that they will be valuable for muscle strength evaluation in future clinical trials and observational studies, particularly to test if a drug is improving muscle strength in patients with DM1.

This dissertation is organized in 5 Chapters: Chapter I, II, III, IV and V

I. Introduction, includes:

- Review article entitled ‘Metabolic alterations in Myotonic Dystrophy type 1 and their correlation with Lipin function’

II. Aims of the dissertation.

III. Evaluation of metabolic profile of patients with DM1, includes:

- Pilot study entitled ‘Fourier-transform infrared spectroscopy as a discrimination tool for patients with DM1: pilot study’

IV. Muscle strength measurements in patients with DM1, includes:

- Systematic review entitled ‘Muscular strength measures in Myotonic dystrophy type 1: A Systematic Review’

V. Concluding remarks and future perspectives

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List of Abbreviations

ATR	Attenuated total reflectance
CCTG	Cytosine-cytosine-thymine-guanine
CDP-DAG	Cytidine diphosphate-diacylglycerol
CNBP	Nucleic acid-binding protein
CTG	Cytosine-thymine-guanine
CUGBP1	CUG-Binding Protein-ELAV-Like family member1
DAG	Diacylglycerol
DM1	Myotonic dystrophy type 1
ER	Endoplasmic reticulum
FFA	Free fatty acid
FTIR	Fourier-transform infrared spectroscopy
HDL	High-density lipoprotein
IGFR	Insulin growth factor receptors
IL-1β	Interleukin-1β
IL-6	Interleukin-6
INSR	Insulin receptor
IP3K	PhosphoInositide 3-kinase
IR	Insulin binding receptor
IR	Infrared
IRS	insulin response elements
LDL	Low-density lipoprotein
LRD	Leucine rich Domain
MAPK	Mitogen-activated protein kinase
MBNL1	Muscleblind-like protein1
MS	Mass Spectrometry
mTOR	Mammalian target of rapamycin
MYPT1	Myosin phosphatase
NAFLD	Non-alcoholic fatty liver disease
NMR	Nuclear Magnetic Resonance
PA	Phosphatidic acid
PAP	Phosphatidate phosphatase
PC	Phosphatidylcholine
PC	Principal component
PCA	Principal component analysis
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PGC-1α	Peroxisome proliferator-activated receptor-gamma coactivator-1alpha
PI	Phosphatidylinositol
PKA	Protein Kinase A
PKB	Protein Kinase B
PKC	Protein Kinase C
RBP	RNA-binding proteins
SERCA	Sarco/Endoplasmic reticulum Ca^{2+}-ATPase
SREBP	Sterol regulatory element-binding protein
SRF	Serum response factor
TAG	Triacylglycerol
TNFα	Tumour-necrosis factor-α
3´UTR	3' untranslated region

Chapter I. Introduction

1. Metabolic alterations in Myotonic Dystrophy type 1 and their correlation with Lipin function

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1.1 Abstract

Myotonic dystrophy type 1 (DM1) is an autosomal dominant hereditary and multisystemic disease, characterized by progressive distal muscle weakness and myotonia. Despite huge efforts, the pathophysiological mechanisms underlying DM1 remain elusive. In this review, the metabolic alterations observed in patients with DM1 and their connection with lipin proteins are discussed. We start by briefly describing the epidemiology, the physiopathological and systemic features of DM1. The molecular mechanisms proposed for DM1 are explored and summarized. An overview of metabolic syndrome, dyslipidemia, and the summary of metabolic alterations observed in patients with DM1 are presented. Patients with DM1 present clinical evidence of metabolic alterations, namely increased levels of triacylglycerol and low-density lipoprotein, increased insulin and glucose levels, increased abdominal obesity, and low levels of high-density lipoprotein. These metabolic alterations may be associated with lipins, which are phosphatidate phosphatase enzymes that regulates the triacylglycerol levels, phospholipids, lipid signaling pathways, and are transcriptional co-activators. Furthermore, lipins are also important for autophagy, inflammasome activation and lipoproteins synthesis. We demonstrate the association of lipin with the metabolic alterations in patients with DM1, which supports further clinical studies and a proper exploration of lipin proteins as therapeutic targets for metabolic syndrome, which is important for controlling many diseases including DM1.

1.2 Introduction

Myotonic dystrophy type 1 (DM1) is a multisystemic and autosomal dominant hereditary disease mainly characterized by progressive distal muscle weakness and myotonia (sustained muscle contractions) [1–5]. DM1 is caused by the expansion of unstable repetitions of cytosine-thymine-guanine trinucleotide (CTG) in the 3'untranslated region (3'UTR) of the Myotonic Dystrophy Protein Kinase (DMPK) gene located at chromosome 19q13.3 [4,6,7].

Metabolic syndrome (MetS) in patients with muscular disorders are significantly higher than in general population due to abnormal lipid metabolism, such as insulin resistance, hypertriglyceridemia, increased fat mass, high levels of low-density lipoprotein (LDL), low levels of high-density lipoproteins (HDL) and abdominal obesity [8,9]. These abnormalities seem to be related to lipin, since it is a key enzyme that regulates lipid metabolism and signaling, and is a transcriptional co-activator of fatty acid β-oxidation [10–14]. Other important features associated to the effects of lipin alterations that have been studied in the last decade and demonstrated by previous studies will be detailed below.

Therefore, the purpose of this review is to provide an overview of the metabolic changes observed in patients with DM1 and to correlate them with current knowledge of lipin and their role in DM1.

We briefly summarize the DM1 epidemiology and the underlying molecular mechanism, after which we provide clinical evidence of abnormal lipid metabolism in patients with DM1. The role of lipin in DM1 is also discussed in this review.

1.3 Epidemiology and molecular characteristics of DM1

Muscular dystrophies are a group of inherited muscle disorders, in which one or more genes necessary for normal muscular function are mutated, resulting in progressive weakness and loss of muscle mass with a variety of severity degrees [15–17]. Concerning muscular dystrophies, there are nine major forms namely, Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), congenital muscular dystrophy (CMD), myotonic dystrophy (MD), facioscapulohumeral muscular dystrophy (FSHD), Emery–Dreifuss muscular dystrophy (EDMD), distal muscular dystrophy (DD), limb-girdle muscular dystrophy (LGMD) and oculopharyngeal muscular dystrophy (OPMD) [15–17].

DM1 also known as Steinert's disease, is the most common form of muscular dystrophy in adults, with a prevalence of 1 in 3000 and 8000 individuals worldwide (Table 1. 1) [1,4,18,19]. The main clinical feature of DM1 is myotonia (sustained muscle contractions) at the skeletal muscle level, progressive weakening of the distal muscles, and also affects other organ systems (e.g., eyes, heart, lungs) (Table 1. 1) [1,4,18]. Additionally, other multisystem characteristics are observed namely, insulin resistance, dyslipidemia, defects in cardiac conduction, gonodal atrophy, alterations in the central nervous system (CNS), and breathing problems (Table 1. 1) [1,2,20]. DM1 is caused by the expansion of unstable repetitions of CTG in the 3'UTR DMPK gene (Figure S1. 1). The latter gene gives rise through extensive alternative splicing to 6 major DMPK isoforms proteins (DMPK A, B, C, D, E, and F) in both humans and mice, and 1 isoform (DMPK G) only present in humans (Figure S1. 1) [6,20–22]. The expanded CUG repeats of the DMPK protein accumulates as nuclear foci compromising nuclear functions, and the expanded mRNA products are toxic to cells, affecting the normal processing of other genes in different tissues [4,6,7,18].

Table 1. 2. Summary of principal clinical features of DM1 [1,2,7,15,16,19]

Global Clinical Features	DM1
Heredity	Autosomal dominant
Prevalence	1 in 3000 and 8000 individuals
Cancer	Reproductive tract (endometrial, ovarian and testicular), thyroid and colorectal High risk of tumour development
Anticipation	Present
Life expectancy	Reduced
Mortality	70%, Caused by cardiorespiratory complications
Appearance	Forehead balding
	Myopathic face
	Temporal wasting
	Ptosis

	Nasal/slurred speech
	Dysphagia
Age at onset	Childhood to adulthood
Congenital form	Present
Facial dysmorphism	Present in congenital form
Male hypogonadism	Present
Insulin Resistance	Present
Dyslipidemia	Present
Metabolic syndrome	Present
Thyroid deficiency	More common
Diabetes mellitus	High risk of development
Creatine kinase, liver enzymes and cholesterol	Elevated
Hypogammaglobulinemia	High incidence
Muscle weakness	Extreme at 50 years of age (distal)
Muscle Pain/myalgia	Less frequent
Clinical myotonia	Evident in early adulthood
Myotonia	Handgrip, tongue (distal)
Atrophy	Distal (early)
Type 1 fiber atrophy	Present
Calf hypertrophy	Absent
Cardiac arrhythmias	Present
Sudden death	More common
Sleep disorders	Present
Cognitive decline	Prominent in congenital form
Central nervous system problems	Present
Cataracts	Present
Respiratory failure	Present
Gastrointestinal problems	Present
Abdominal pain	Present
Constipation	Present
Gene	*DMPK*, chromosome 19q13.3, CTG expansion at 3'UTR
DMPK	Reduced
CNBP/ZNF-9	Normal
CUGBP1	Upregulated
MBNL1	Down regulated/sequestered
Ribonuclear inclusion	Present
Spliceopathy	Present
Transcription dysregulation	Present
MicroRNA dysregulation	Present
RNA translation	Present

The number of CTG repeats in the DMPK gene is polymorphic and is correlated with the severity of DM1. In healthy population it varies between 5–37 repetitions. Individuals who have the DM1 pre-mutation have at least 38–49 repetitions being considered generally asymptomatic. However, they can pass the disease to the next generation. In contrast, individuals with repeats number comprised between 50–4000 are considered patients with DM1 [5,23]. In average, CTG

expansions increase an additional 200 repetitions when transmitted from one generation to the next, leading to the disease anticipation, meaning that the disease symptoms will appear earlier and with higher severity in the subsequent generation [5,23]. In addition, although DM1 is more common in early adulthood, it can also affect fetal development and postnatal growth in individuals with large number of CTG expansions. Due to DM1 clinical heterogeneity, it is routinely subdivided according to the severity of symptoms and the age of disease onset. These categories are important to provide adequate DM1 recognition, diagnosis, and prognosis. In other words, the phenotype is divided into three categories (mild, classic, and severe) and clinically categorized as congenital (<1 month and ≥1000 CTG repeat length), infantile (1 months to 10 years and >500 CTG repeat length), juvenile (>10 to 20 years and >400 CTG repeat length), adult-onset (>20 to 40 years and 150 to 1000 CTG repeat length), and late-onset (>40 years and 50 to 149 CTG repeat length) [23–26].

1.4 Physiological and systemic features of DM1

Besides progressive muscle weakness and myotonia, the most common features observed in patients with DM1 are endocrine and metabolic alterations, respiratory and cardiac dysfunctions (Table 1. 1). Endocrine dysfunctions have an increased incidence in patients with DM1, especially gonadal insufficiency (hypogonadism), diabetes, and thyroid disorders [27,28]. Hypogonadism is the most frequent feature and is characterized by low serum testosterone levels being more prevalent in men (Table 1. 1) [27]. Palpable thyroid gland abnormalities have been described in 20% of patients with DM1 [27,28]. These endocrine alterations are correlated with metabolic dysfunctions in these patients which will be detailed below.

Overall respiratory dysfunction (respiratory failure or aspiration) is the most common cause of death in patients with DM1, since respiratory disease has a complex etiology in these patients, combining both peripheral respiratory dysfunction and central respiratory drive dysfunction, as well as upper airway muscles dysfunction (obstructive sleep apnea and aspiration) and high risk of pulmonary infections [29,30]. The mortality rate involving respiratory dysfunction ranges from 51% to 76% specially in congenital form (Table 1. 1) [29,30]. Cardiac dysfunction is the second common cause of death, about 30% of mortality in adults with DM1. Cardiac involvement in DM1 includes conduction disease, atrial and ventricular tachyarrhythmias, atrioventricular block, left-ventricular systolic dysfunction, and myocardial fibrosis resulting in sudden death (Table 1. 1) [31–33].

These systemic features may be associated to DMPK gene alterations, given that DMPK mRNA is highly expressed in skeletal, cardiac, smooth muscle and in smaller amount in different areas of the brain. DMPK is highly expressed during muscle development between weeks 9–16 and is associated to myogenic differentiation, meaning that DMPK has an important role during myogenesis [6,34–36]. In addition to muscle development, DMPK is also involved with the maintenance of ion homeostasis (Dihydropyiridine-Voltage-Dependent L-type Calcium receptors) and sodium channels. Furthermore, DMPK also modulates the chloride channel phospholemman

(PLN), the activation or inhibition of Sarco/Endoplasmic reticulum Ca2+-ATPase (SERCA) through phosphorylation of phospholamban and sarcolipine. As such, it is possible to state that DMPK is involved in skeletal muscle contraction and relaxation and ion homeostasis. In the case of DM1 disease, DMPK may be related to myotonia and also could be involved indirectly or directly with the cytoskeleton myosin phosphatase target subunit (MYPT1), serum response transcription factor (SRF), and the nuclear envelope protein lamin A/C [6,37–39].

1.5 DM1 Underlying Molecular Mechanisms

To date the identification of the molecular mechanisms underlying this pathology is not yet fully understood. However, there are three hypotheses that are more consensual within the scientific community namely the rearrangement of DM1 locus, DMPK haploinsufficiency, and gain of toxic RNA function (Figure 1. 1)

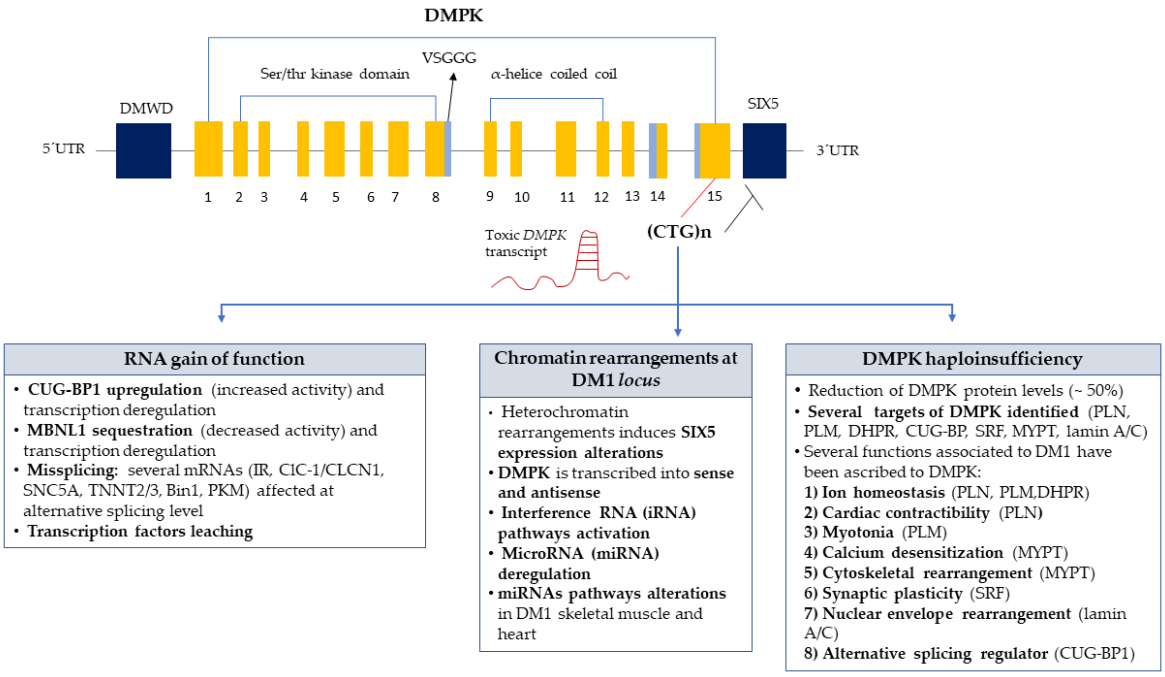

Figure 1. 1. Molecular Mechanisms in DM1. Toxic CUG-containing transcripts forms secondary structures and accumulates in the nucleus of DM1 cells, causing multisystemic effects of DM1 throughout RNA gain-of-function mechanism, Chromatin rearrangements at the DM1 locus and DMPK haploinsufficiency [6,21,40-48]. CUGBP1-CUGBP-Elav-like family member 1; MBLN1—muscleblind-like protein 1; Insulin receptor (IR); DHPR—Voltage-Dependent L-type Calcium; PLM—Phospholemman, PLN—Phospholamban; SRF—Serum response factor; MYPT—Myosin phosphatase; CIC/CLCN1—Voltage-gated chloride channel; SNC5A—Sodium channel protein type 5 subunit alpha; TNNT2/3—Troponin T 2/3; Bin1—Bridging integrator 1; PKM—Pyruvate kinase.

Regarding rearrangement of the DM1 locus, DMPK gene is transcribed into sense and anti-sense transcripts (Figure 1. 1) [7]. Anti-sense transcripts could be involved in chromatin ultrastructure regulation, since it is known that anti-sense DMPK transcripts extend into an insulator element located between DMPK and SIX5 genes [7,40]. The formation of double strand RNA structures due to the folding of CUG transcripts into hairpin structures, or due to complementary hybridization between sense and anti-sense DMPK transcripts might activate iRNA [22,39]. Furthermore,

microRNA (miRNA) deregulation could be an additional mechanism involved in DM1 biogenesis, since it has been found altered in skeletal muscle and heart of patients with DM1 [41,42].

Concerning DMPK haploinsufficiency (Figure 1. 1), it is also known that about ~50% of DMPK expression and activity are decreased due to CTG expansions (haploinsufficiency) and that DMPK is crucial for the phosphorylation of certain essential proteins for muscle contraction and relaxation, such as phospholamban (PLB), phospholemman (PLM), sarcolipine (SLN), and lipin [21,43,44]. PLB and SLN when phosphorylated interact with SERCA allowing muscle contraction, through calcium output. Due to the decrease in DMPK levels, phospholamban and sarcolipine are not phosphorylated, in turn SERCA is constantly inhibited, leading to an increase concentration of calcium in the cytoplasm, causing sustained muscle contraction (myotonia) [6,21,29,43,44].

Gain of toxic RNA function is another plausible DM1 mechanism (Figure 1. 1). The mutant DMPK mRNA with the CUGexp repeats accumulates in the nucleus as a ribonuclear foci folding into 'RNA hairpins', sequestering and deregulating RNA-binding proteins (RBP) crucial for alternative cell processing of other genes and/or mRNAs, leading to the complexity of the DM1 phenotype. Among the RBP associated with abnormal splicing mechanism related with DM1 are muscleblind-like protein1 (MBNL1) and CUG-BP-ELAV-Like family member1 (CUGBP1 or CELF1) [43,44]. MBNL1 protein is responsible for splicing regulation of several hundreds of transcripts, regulating mRNA transport and decay. MBLN1 has high affinity to the CUG repeats of the RNA, thus being sequestered in the nucleus by the DMPK CUGexp mRNA, reducing its activity and availability in the cell [5,43]. CUGBP1 is linked to a "short single-stranded CUG repeat", so it is not sequestered as MBNL1 [21]. It is hypothesized that hyperphosphorylation and stabilization of CUGBP1 occurs [21] through a PKC activation induced by a mutant mRNA through an unknown mechanism [21]. Both MBNL1 and CUGBP1 proteins may be responsible for some of the symptoms observed in patients with DM1 (Figure 1. 1) [6,21,45–48]. Additionally, being DMPK an important protein kinase is reasonable to deduce that protein phosphorylation is also an important regulatory mechanism in DM1. Aberrant protein phosphorylation is recognized as a critical step in the pathogenesis of several diseases, such as Alzheimer's disease and cancer [49–52].

1.6 Metabolic dysfunction in DM1

MetS represents an accumulation of at least three metabolic abnormalities out of the five conditions, which are hypertension, central obesity, insulin resistance, atherogenic dyslipidemia, and pro-inflammatory state [53]. Metabolic syndrome can lead to cardiovascular disease, diabetes mellitus, vascular, and neurological problems such as cerebrovascular accident [9,53,54]. Metabolic abnormalities can become a syndrome if a patient presents at least three of the following metabolic risk factors:

- Waist circumference (or abdominal obesity) more than 40 inches or ≥102 cm in men and 35 inches or ≥88 cm in women [9,53,54];

- Elevated triglycerides (TAG) ≥150 milligrams per deciliter of blood (mg/dL) or higher [9,53,54];
- Reduced high-density lipoprotein cholesterol (HDL) less than <40 mg/dL (1.0 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women that is associated with increased cholesterol levels [9,53,54];
- Normal value of fasting glucose <100 mg/dL. Elevated fasting glucose of ≥l00–125 mg/dL is considered pre-diabetes and 126 mg/dL or higher is considered diabetes [9,53,54];
- Blood pressure values of systolic ≥130 mmHg or higher and/or diastolic ≥85 mmHg or higher [9,53,54].

Literature review was conducted in Web of Science and PubMed. Data were gathered and extracted to a structured table (Table S1. 1) with authors, year of publication, number of participants (DM1 patients and controls), age and sex, CTG repeat length, body mass index (BMI) and waist circumferences, insulin metabolism, HOMA-IR, glucose metabolism, and lipid metabolism. Data regarding age, sex, CTG repeat length, BMI were collected to characterize the population. One summary table (Table 1. 2) was created to synthesize previously reported results of abnormal metabolism studies in patients with DM1. From the included studies, all data was gathered, and upon adequate calculations they were expressed as mean±standard deviation (SD; range) and median (Table 1. 2). From the literature review, 18 studies evaluated metabolic alterations in patients with DM1 (summarized in Table 1. 2), in which is it was possible to observe differences regarding lipid metabolism between patients with DM1 and control group. Clinically, insulin metabolism and HOMA-IR (Insulin resistance index) were significantly higher in patients with DM1 than control group [27,28,55–62] as demonstrated in Table 1. 2. Glucose levels were similar in both patients with DM1 and control group [27,28,55–58,60–67]. Total cholesterol values in patients with DM1 were higher than control group [27,28,55–60,62–65,67]. LDL was also higher in patients with DM1 when compared with control group [27,28,58–60,62–64,68,69]. Whereas HDL was similar in both patients and control group [27,28,58–60,62–66,68,69]. TAG levels were higher in patients with DM1 than control group [27,28,56–60,62–69]. Furthermore, from the 18 studies, 11 [27,55,57,59–62,64,65,68,70] showed a significant difference ($p \leq 0.05$) between patients with DM1 and controls (Table S1. 1), namely, higher levels of insulin [57,59,61,62], HOMA-IR [27,57,61,65,70], total cholesterol [55,57,60,65], LDL [60,68], TAG [27,57,59,60,65,68], whereas glucose levels [64] and HDL were significantly lower [59,60,65].

Table 1. 2. Summary of previously reported data concerning abnormal metabolism in patients with DM1.

Patients Characteristics	**DM1 (*n* = 623)** [27,28,63–70,55–62]	**Controls (*n* = 428)** [27,55,68,70,56,57,59–62,64,65]
Patients per study	44 ± 33; (8–115) [27,28,63–70,55–62]	96.8 ± 203.3; (3–734) [27,55,68,70,56,57,59–62,64,65]
Age (years)	41.7 ± 3.8; (34–47)	42.35 ± 6.17; (33–54.6)

	[27,28,63–70,55–62]	[27,55,68,70,56,57,59–62,64,65]
Sex	379 F, 417 M [27,28,63–70,55–62]	347 F, 815 M [27,55,68,70,56,57,59–62,64,65]
BMI (kg/m^2)	23.8 ± 1.75; (22.3–27.2); Median: 23.4 [27,28,66,67,70,55,57–59,61,63–65]	23 ± 0.72; (21.7–23.7); Median: 23.2 [27,55,57,59,61,64,65,70]
Waist circumference (cm)	95.8 ± 2.19; (94.3–97.4) Median: 93.43 [27,57–59]	84.9; Median: 87.5 [27,57,59]
CTG repeat length	555.22 ± 250.99; (355.9–973); Median: 413.75 [28,55,70,58–60,63,65–67,69]	ND
Central Obesity (%)	13.6 [63]	ND
Metabolic syndrome (%)	16.7–41.2 [58,63]	ND
Insulin metabolism (pmol/L)	125.4 ± 63.5; (51.38–186.11); Median: 73.5 **Male:** 127.8 ± 33.4; (104.26–151.45) **Female:** 119.8 ± 22,1; (104.16–135.48) [27,28,55–62]	69.9 ± 28.4; (45.8–86.0); Median: 44.29 **Male:** 56.25 **Female:** 63 [27,57,59–62]
HOMA-IR	3.03 ± 1.7; (1.9–6.4); Median: 2.25 **Male:** 4.7 **Female:** 3.7 [27,55–58,61,65,70]	1.43 ± 0.13; (1.3–1.6); Median: 1.42 [27,57,61,65]
Glucose metabolism (mg/dL)	91.84 ± 7.5; (82.7–108.5); Median: 91.4 **Male:**90.5 ± 6.3; (86–95) **Female:** 95.9 ± 24.1; (78.9–113.0) [27,28,64–67,55–58,60–63]	89.65 ± 5.11; (81.6–95.6); Median: 90 **Male:**83 **Female:** 88 [27,55,57,60–62,64,65]
Total cholesterol (mg/dL)	200.34 ± 15.88; (176–228); Median: 200 **Male:** 216.37 ± 50.02; (181.0–251.7) **Female:** 200.8 ± 13.9; (191.0–210.7) [27,28,64,65,67,55–60,62,63]	179.4 ± 24.5; (146.0–200.5); Median: 176.72 **Male:** 128 **Female:** 134 [27,55,57,59,60,62,64,65]
HDL (mg/dL)	52.42 ± 3.64; (48.2–58.0); Median: 51.4 **Male:** 44 **Female:** 61 [27,28,68,69,58–60,62–66]	51.4 ± 5.5; (45.7–56.7 51.82); Median: 51.82 **Male:** 67 **Female:** 55 [27,53,57,58,61,62,65,66]
LDL (mg/dL)	118.38 ± 14.65; (106.34–143.08); Median: 122.6 **Male:** 104 **Female:** 101 [27,28,58–60,62–64,68,69]	110.0 ± 17.7; (97.4–122.6); Median: 129.3 **Male:** 146 **Female:** 102 [27,59,60,62,64,68,69]
TAG (mg/dL)	184.9 ± 52.5; (108–274); Median: 172.4 **Male:** 194.3 ± 11.7; (186.0–202.6) **Female:** 134.1 ± 18.2; (121–147) [27,28,65–69,56–60,62–64]	115.9 ± 39.6; (77–168); Median: 95.2 **Male:** 75 **Female:** 105 [27,57,59,60,62,64,65,68,69]

Data expressed as mean ± SD (range). Abbreviations: NA—Not applicable; ND—Not Determined; F—Female; M—Male; BMI—Body Mass Index; HOMA-IR—Insulin resistance index HDL—High-density lipoprotein; LDL—Low-density lipoprotein; TAG—Triacylglycerol

Interestingly, the occurrence of MetS in patients with muscle disorders is significantly higher than in the general population [63]. Abnormal lipid metabolism is frequently observed in patients with DM1, particularly in skeletal tissue. Additionally, the main cause of this abnormality is insulin resistance (increased levels of insulin at plasma, hyperinsulinemia) and dyslipidemia (hypertriglyceridemia, low HDL cholesterolemia, high LDL cholesterolemia, and abdominal obesity) [8,9]. MetS in patients with DM1 may be associated with lipin deficiency levels. In fact, in previous studies LPIN1 gene was reported to be among the aberrant alternative splicing genes in DM1 mouse model, particularly in the biological function of lipid metabolism, indicating that LPIN1 gene seems to be dysregulated in DM1 [45]. Further, in a more recent study using human skeletal and heart muscle DM1-derived biopsies LPIN1 gene was also found associated to DM1 [71]. Thus, the study of lipin is of paramount importance and the underlying mechanisms should be extensively studied. Given that, lipin could be a key to understand the metabolic features in the DM1 disease, as described in the following section.

1.7 Lipin Protein Family

Lipins are phosphatidate phosphatase (PAP) enzymes that catalyze the conversion of phosphatidic acid (PA) to diacylglycerol (DAG) in TAG biosynthesis, phosphatidylethanolamine (PE) or phosphatidylcholine (PC) (Figure 1. 2) as well as phosphatidylinositol (PI), phosphatidylglycerol (PG) and cardiolipin (CL) via cytidine diphosphate diacylglycerol (CDP-DAG) [10,11,72,73]. Phosphatidic acid is an intermediate in TAG biosynthesis that has an important function in the Kennedy pathway for the component of cell membranes and dynamic effects on intracellular and intercellular signaling pathways (Figure 1. 2) [73].

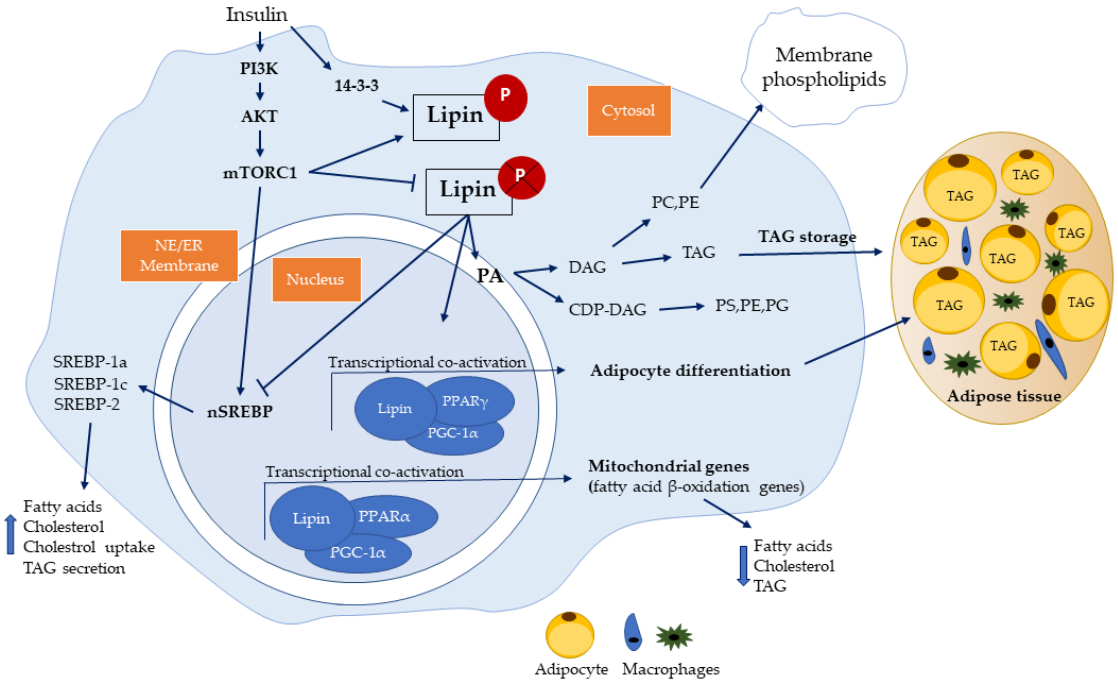

Figure 1. 2. Schematic representations of lipin pathway and signaling. PA—phosphatidic acid; DAG—diacylglycerol; TAG—triacylglycerol; PE—phosphatidylethanolamine; PC—phosphatidylcholine; PI—phosphatidylinositol; PG—phosphatidylglycerol; CL—cardiolipin; CDP-DAG—cytidine diphosphate diacylglycerol; PS—phosphatidylserine; AKT—protein kinase B; PI3K—phosphoInositide 3-kinase; mTORC1—mitogen-activated protein kinase complex 1; nSREBP—nuclear Sterol regulatory element-binding protein; NE—nuclear envelope; ER- endoplasmic reticulum; PPARα/γ—peroxisome proliferation-activated receptor α/γ; PGC-1α—peroxisome proliferator-activated receptor gamma coactivator 1-alpha [12,72,73,76].

It is also known that phosphatidic acid is the precursor of CDP-DAG used to produce phosphatidylglycerol and phosphatidylinositol, while DAG is the substrate for synthesis of other abundant phospholipids like phosphatidylcholine and phosphatidylethanolamine [73]. There are three main pathways to synthesize phosphatidic acid which are (1) *de novo* synthesis pathway through acylation of lysophosphatidic acid by lysophosphatidic acid acyltransferase; (2) phosphorylation of DAG; (3) hydrolysis of phospholipids by phospholipase D [74]. Some of phosphatidic acid targets are activation of NADPH, PKC-ζ, phosphatidylinositol 4-kinase, Raf proteins, phospholipase C-γ, Ras, and inhibition of protein phosphatase-1 (PP1) [75]. The effect of phosphatidic acid on Ras and Raf increases extracellular signal-related kinase activity and cell division [76]. The relative concentrations of lysophosphatidate/DAG and phosphatidic acid contributes to membrane curvature (through fusion and fission) and vesicle budding [76]. Phosphatidic acid has also been shown to activate the extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK) signaling cascades and suppressed protein kinase A (PKA) activity by a direct interaction with phosphodiesterase 4 (PDE4) and by activating mitogen-activated protein kinase (mTOR) signaling to enhance PDE activity and reduce cyclic adenosine monophosphate (cAMP) [77,78]. Additionally, phosphatidic acid can increase cell division through mTOR, and it stimulates stress fibers formation [75].

Lipin also has a transcriptional co-activator motif and can co-regulate the expression of fatty acid β-oxidation and inflammatory genes. Lipin subcellular localization is related to its dual role of transcriptional co-activator (nucleus), lipid metabolism, and lipid signaling (ER) and in cytoplasm when phosphorylated (not activated) (Figure 1. 2) [10–14].

Lipin family consists of three members, which are lipin-1, lipin-2, and lipin-3. Lipin-1 was the first lipin member identified and is encoded by LPIN1 gene [13]. Lipin-1 is a Mg^{2+} dependent protein, predominantly expressed in adipose tissue, skeletal muscle, and testis, being expressed at lower levels in other tissues such as liver, kidney, brain, heart, and lungs. There are three human lipin-1 isoforms (lipin1-α, lipin1-β, lipin1-γ) generated by alternative mRNA splicing (Figure 1. 3) [14,72,79,80]. Lipin-1α contains 890 amino acids, predominates in pre-adipocytes during early stages of adipocytes differentiation, but most of the α-isoform is located at the nucleus as a transcriptional co-activator of lipid metabolism genes [12,13,72,79]. Lipin-1β is the longest isoform and contains an additional β-specific region of 36 amino acids (242–277) and is mainly associated with ER membrane carrying the PAP activity function. Lipin-1β predominates in mature adipocytes and induces the expression of lipogenic genes and lipid storage, whereas lipin-1γ is highly expressed in human brain compared to lipin-1α and β (Figure 1. 3) [12,13,72,79]. Lipin-2, encoded by LPIN2 gene, is commonly expressed in the liver, kidney, brain, lungs, macrophages, and small intestine. Lipin-3, encoded by LPIN3 gene, has been reported that in vivo it cooperates with lipin-1 to influence adipose tissue PAP activity and adiposity, but it has the highest levels in small intestine, adipose tissue, and liver (Figure 1. 3) [12,81]. All three lipin proteins are important to activate the synthesis of both major storage phospholipids, the TAG and membrane phospholipids.

Lipin-1, lipin-2, and lipin-3 are also involved in many cellular process through intermediates that activate cellular signaling, including the regulation of lipid storage, lipoprotein synthesis, autophagy, inflammation, and gene expression [81]. Lipin proteins suffer post-translation modifications including phosphorylation, sumoylation, acetylation, and ubiquitination [81]. From the lipin family, only lipin-1 is regulated by insulin via mTOR phosphorylation. Dephosphorylation of lipin-1 allows to translocate freely from the cytoplasm to the nucleus and to membranes containing phosphatidic acid, such as endoplasmic reticulum, mitochondria, and autophagosomes/lysosomes (Figure 1. 2) [81]. Lipin defects in the formation of DAG may lead to lipodystrophy or block adipocyte differentiation [14]. Also, DAG is a regulator of signaling cascades including protein kinase C (PKC) and protein kinase D (PKD) [73,82]. Activation of PKC through accumulation of DAG has been linked to insulin-resistance in obesity [73,82–84]. Also, activation of PKC may results in the activation of the transcription factor activator protein-1 (AP-1), which has many sequence targets in inflammation-related genes (e.g., Ciclo-oxigenase-2 (Cox2)) [85–88]. Activation of PKD cascade through DAG at the surface of autophagosomes/lysosomes, leads to fusion of autophagosomes with lysosomes to form functional autolysosomes [81]. Additionally, lipin-1 can interact with other nuclear receptors, such as PPARγ [12,89], hepatocyte nuclear factor-4α (HNF-4α) [12,90], glucocorticoid receptor (GR) [12,91], as well as non-nuclear receptor transcription factors, including nuclear factor of activated Tcells c4 (NFATc4) [92,93] and myocyte enhancer factor 2 (MEF2) [94]. Lipin-1 also represses the activity of Sterol regulatory element-binding protein 1 and 2 (SREBP1, SREBP2), and NFAT4c by inhibiting the binding of these transcription factors to their respective promoters in hepatocytes. SREBP1, SREBP2, and NFAT4c have been identified to contribute to promotion of macrophage pro-inflammatory responses and inhibition of wound healing macrophage polarization [12,14,78,81,93]. Therefore, lipin-1 can serve as gene expression activator or repressor [12].

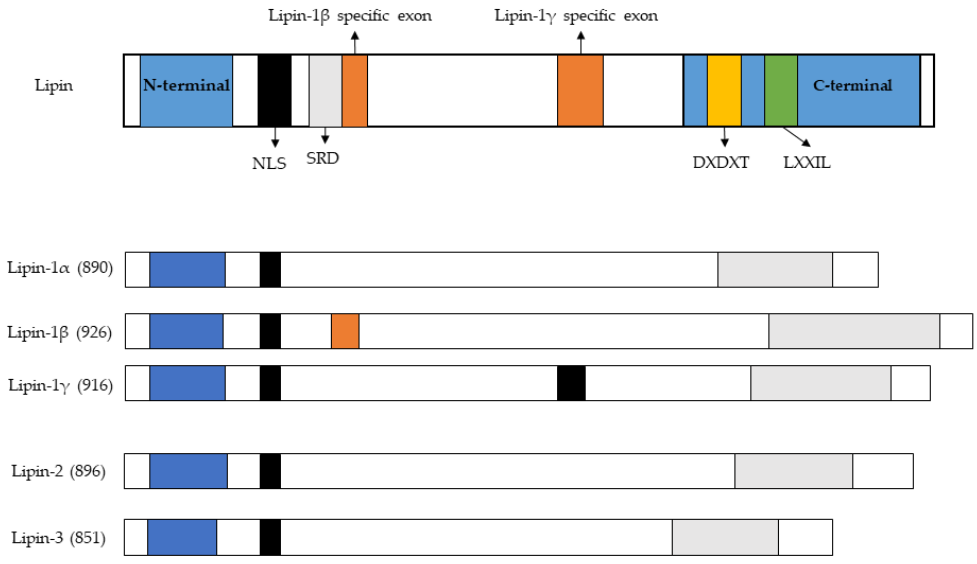

Figure 1. 3. Lipin family (lipin-1, lipin-2, and lipin-3) domains and motifs in humans. N-terminal C-terminal NLS—nuclear local-ization; SRD—serine rich domain; DXDXT—phosphatidate phosphatase enzyme; LXXIL—transcriptional co-activator motif [12,72,73,76,79].

1.7.1 Dyslipidemia and Lipin

Dyslipidemia is frequently observed in patients with DM1 [9,27,28,56–60,62–64,68,69,95]. It is a term used for lipid disorders from hyperlipidemia to hypolipidemia, which includes the elevation of plasma cholesterol, hypertriglyceridemia, low HDL, high LDL, and exceed visceral fat accumulation. It is a major risk factor for cardiovascular disease, cerebrovascular disease accident and peripheral arterial disease [9,53,54]. Dyslipidemia is strongly associated to MetS, nonalcoholic fatty liver disease (NAFLD) and is closely related with insulin resistance, since alteration of lipid metabolism and the increase of free fatty acid (FFA) flux inhibits insulin signaling leading to insulin resistance [8,9,53,56,63].

These alterations in lipid metabolism frequently observed in patients with DM1 (Table 1. 2) may be correlated with lipin metabolism, since lipin regulates adipocyte differentiation allowing TAG storage. Thus, with decreased lipin function there will be a decrease of adipocyte differentiation and an increase of TAG levels. Also, lipin has a major role in biosynthesis of phospholipids, fatty acid β-oxidation, inhibition of de *novo lipogenesis* and TAG secretion through lipoproteins. These features were also in accordance with Table 1. 2, as with the downregulation of lipin the *de novo* lipogenesis was not inhibited and therefore there will be an increase of total cholesterol, LDL, and TAG secretion, as well as fatty acid synthesis [14,76,81,96–101]. Furthermore, the lipin deficiency may also, lead to insulin resistance, obesity, and amplification of the proinflammatory factors, influencing abnormal lipid metabolism, cell growth, SERCA function, endoplasmic reticulum stress, mitochondrial dysfunction, autophagosome accumulation, and lipid accumulation in skeletal muscle and liver, among other organs [8,9,14,81,96]. Therefore, it would be important to understand the correlation of lipin alteration in patients with DM1 with the high frequency of dyslipidemia and MetS. Thus, more studies with a large cohort should be taken in consideration, particularly, to evaluate the involvement of lipin with the levels of TAG, total cholesterol, HDL, LDL (Figure 1. 4).

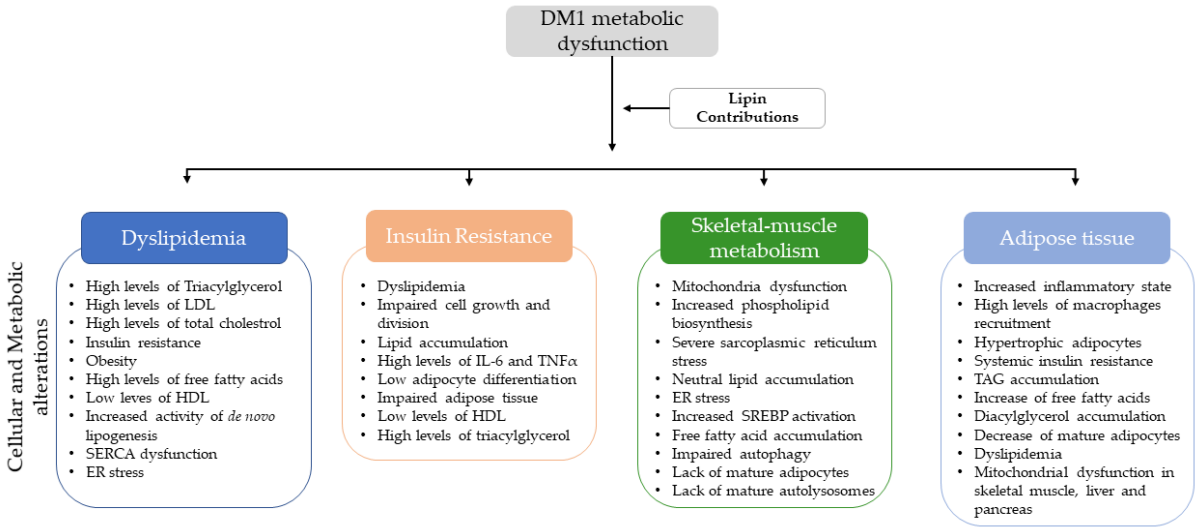

Figure 1. 4. Summary of the potential contributions of lipin dysfunction in DM1. LDL—low-density lipoprotein; HDL—high-density lipoprotein; SERCA—sarco/endoplasmic reticulum Ca^{2+}-ATPase; ER—endoplasmic reticulum; IL-6—interleukine-6; TNFα—tumour-necrosis factor-α; SREBP—Sterol regulatory element-binding protein; TAG—triacylglycerol.

1.7.2 Insulin Resistance Association with Lipin

Insulin is a metabolic hormone, produced by pancreatic beta cells to regulate the glucose uptake. The major targets of insulin are adipose tissue, muscle, liver, and heart. Insulin receptor can also be found in brain, lungs, kidneys, fibroblasts, and blood cells [8]. Insulin is also a key molecule in lipid metabolism and protein synthesis, and is involved in glucose uptake in skeletal muscle and adipocytes, promotion of glycogen synthesis, inhibiting lipolysis in adipocytes, reduction of hepatic gluconeogenesis process by lipolysis inhibition, regulation of muscle proteins synthesis, and promoting cell division and growth [8,70].

Insulin is produced by the cleavage of pro-insulin. After insulin production it interacts with insulin binding receptors (IR) and insulin growth factor 1 receptor (IGF1R). Insulin receptors (IR) belong to a super family of transmembranar receptor tyrosine kinases that are encoded by the insulin receptor (INSR) gene located on chromosome 19p13.2 [55,70,102]. Alternative splicing of INSR pre-mRNA originates two isoforms, IRA and IR-B. IR-A and IR-B are activated by auto-phosphorylation resulting in downstream cascades activation which interacts with insulin response elements 1 (IRS-1) or 2 (IRS-2), through tyrosine residues phosphorylation. IRS-1 activates the RAS/mitogen-activated protein kinase (MAPK) cascade and regulates the extracellular-signal-regulated kinase signaling (ERKS) [55,70,102,103]. Also, IRS-1 activates the phosphoInositide 3-kinase (IP3K) signaling pathway as well as protein kinase B (PKB or AKT) regulating the protein production and apoptosis via mitogen-activated protein kinase (mTOR) and glycogen synthesis via glycogen synthase kinase 3 beta (GSK-3β). Consequently, an increase of glucose transporter 4 (GLUT4) is observed enabling the glucose uptake [55,70,102–104]. Insulin signaling also enhances lipid storage in adipocytes, through stimulation of TAG synthesis and inhibition of lipolysis, as well as stimulation of glycogen synthesis through AKT2, glycogen synthase kinase 3 (GSK3) inhibition and glycogen synthase (GS) activation [70]. Insulin growth factor receptors (IGFR) are metabolic hormones that are structural homologs to insulin and share a common signaling pathway. There are two isoforms of IGFR namely IGF1R and IGF2R, which are produced in the liver, brain, pancreas, intestine, kidney, adipose tissue, and muscle [8].

Furthermore, it is known that insulin plays an important role in skeletal muscle growth, development, differentiation, and regeneration and in the absence of insulin stimulus, a loss in muscle mass and strength can be observed. Therefore, insulin plays a critical role controlling skeletal muscle mass through regulation of protein metabolism via two main downstream effectors, mTOR and FoxO [70]. Insulin induces the phosphorylation and activation of mTOR promoting protein synthesis, while insulin dependent phosphorylation of FoxO1 leads to a decrease in atrogenes expression [70,105], such as MuRF1 and Atrogin-1/MAFbx, thus inhibiting ubiquitin-dependent protein degradation [55,70]. The binding of insulin receptors leads to AKT/PKB activation and mTOR and FoxO phosphorylation. Phosphorylation of mTORComplex 1 allows the protein synthesis, autophagy, mitochondrial metabolism, and activation of SREBP, through lipin

inhibition, which regulates *de novo* lipogenesis [8,70,73,106,107], whereas mTORComplex 2 (mTORC2) negatively regulates insulin signaling, controls cell stress response, apoptosis, and cytoskeleton organization [73]. In addition insulin stimulates the interaction between lipin-1 and 14-3-3 proteins promoting cytoplasmic retention of lipin-1 [108]. Interestingly, loss of MBNL1 function leads to changes in mTOR pathway [109] and the reduction of cyclin D3 in DM1 muscles due to abnormal increase of GSK3β switches the CUGBP1ACT to CUGBP1REP leading to a miss regulating myogenic CUGBP1 targets [110].

Insulin resistance is the inability of exogenous or endogenous insulin to increase glucose uptake and use, basically the cells stop responding to the hormone [102]. Insulin resistance is a common MetS feature observed in patients with DM1 and more than 50% of the patients have insulin resistance after dyslipidemia (Table 1. 2) [8,63]. It is further involved in cardiovascular disease, left ventricular hypertrophy, type 2 diabetes mellitus (T2DM), atherosclerosis, hypertension, neuropathy, dyslipidemia, obesity, and loss of muscle mass and/or strength [8,70,102]. Due to high metabolic demand, insulin resistance has a significant impact in skeletal muscles, adipocytes and liver, since skeletal muscle and adipocytes account for 60–70% and 10% of insulin, respectively, for the uptake of glucose via GLUT4 [55,70,102,103]. The liver account for 30% of insulin-stimulating glucose and insulin resistance can lead to an increased triglycerides content and very-low-density-lipids (VLDL) secretion [55,70,102,103,111]. Insulin resistance also induces hyperglycemia, activation of oxidative stress (mithocondrial and endoplasmic reticulum dysfunction) and ectopic lipid accumulation [55,70].

Insulin resistance can be caused by lipid accumulation, inflammatory molecules (interleukine-6 (IL-6), tumor-necrosis factor-α (TNF-α)) secretion by adipocytes and macrophages, mitochondrial dysfunction related to fatty acid accumulation, reduced insulin mediated glucose uptake by GLUT4, and dyslipidemia (in terms of lipogenesis and lipolysis) [8,55,63,103,112]. Also, our findings summarized in Table 1. 2 have shown an abnormal lipid metabolism in patients with DM1, which are crucial for the development of insulin resistance, such as increased levels of TAG and LDL, as well as waist circumferences, and in some cases lower levels of HDL [59,60,65]. It is recognized that lipin altered levels may lead to insulin resistance, probably through a combination of factors including impaired glucose uptake in adipose tissue and muscle, and reduced levels of adipose-derived factors, such as leptin and adiponectin (Figure 1. 4) [113].

Interestingly, these features may be deeply involved with the lack of adipocyte stimulus and differentiation due to insulin resistance and lipin altered levels. Although, patients with DM1 have a normal glucose tolerance and a very low prevalence of diabetes, in spite of a marked insulin resistance (Table 1. 2) [59,61], raising many questions. Thus, it is important to further study the relation and connection of lipin with insulin resistance underlying pathways, particularly, the glucose uptake and adipose tissue in these patients.

1.7.3 Skeletal Muscle Metabolism and Lipin Role

Skeletal muscle accounts for almost half of the total body mass and energy expenditure. Additionally, it is the major site for fatty acid and glucose oxidation [114]. Lipin-1 is involved in almost all muscular PAP activity compared with lipin-2 and lipin-3 that are expressed at much lower levels in skeletal muscle. Thus, altered levels of lipin-1 affects the energy metabolism, mitochondrial enzymes [12] the glycolysis, glycogenolysis, and mitochondrial respiration, which leads to impaired skeletal muscle functions causing severe myopathies [114,115].

A previous study demonstrated a severe myopathy after deletion of lipin-1 in murine model skeletal muscle [114], and an increased phospholipid biosynthesis of PC, PE, PI, and PG, thus providing a tool for assessing lipin-1 role on skeletal muscle function and metabolism [114]. Interestingly in this study [114] the authors also found a severe sarcoplasmic reticulum (SR) stress, mitochondrial alteration, and neutral lipid accumulation of TAG, cholesterol, cholesterol esters, and increased levels of DAG, and increased SREBP activation [114].

There are three SREBP mammalian isoforms, namely, SREBP-1a, SREBP-1c, and SREBP-2 [116,117], in which, SREBP-1a regulates fatty acid and cholesterol synthesis, and cholesterol uptake [116,118,119], SREBP-1c regulates the expression of genes required for fatty acid synthesis [118,120] and is upregulated by insulin stimulation [116,119,120] and SREBP-2 regulates uptake and synthesis of cholesterol [116].

These findings are in accordance with previous studies described in Table 1. 2, since due to deficiency of lipin-1 levels there will be an increased activation of SREBP and therefore increased fatty acids synthesis, phospholipids biosynthesis, cholesterol, and TAG levels (Figure 1. 2) [97–101]. Interestingly, similar results were reported by another previous study [97] using Duchenne muscular dystrophy (DMD) mouse model, where lipin-1 knockdown lead to changes of lipid metabolism in the skeletal muscles, resulting in increased phospholipid biosynthesis and a reduction of SERCA function leading to a pronounced muscle weakness (myopathy) [97].

Previous studies also, showed mitochondria dysfunction in skeletal muscle of patients with DM1 indicating impaired oxidative skeletal muscle metabolism, low levels of ATP production and increased reactive oxygen species (ROS) production [121–124]. In addition, it was shown a 50% reduction of ATP production via mitochondrial oxidative phosphorylation system activity in patients with DM1 compared with controls [121]. As previous stated lipin-1 is a key regulator of transcriptional factors related to the fatty acid β-oxidation such as PPARα and PGC-1α promoting cellular energy consumption and the downregulation of nuclear SREBP protein, a master regulator of lipid synthesis (Figure 1. 2) [10,14,99]. Thus, lipin-1 deficiency may be involved in mitochondrial dysfunction, which was observed in previous studies with a lipin-1 deficiency mouse model that had impaired autophagy, accumulation of dysfunctional mitochondria in skeletal muscle, and lack of mature autolysosomes formation [81,125–128]. These findings are in accordance with Table 1. 2 data, since the lipin-1 altered levels seems to alter the mitochondrial function, and the amount of

free fatty acids accumulation will increase as well as the total cholesterol and LDL synthesis through SREBP increased activity.

Interestingly one additional study has suggested a connection between lipin-1 and mitochondria through a phosphoglycerate mutase family member 5 (PGAM5), a serine/threonine specific protein phosphatase that promotes lipin-1 dephosphorylation in the nucleus and therefore, promoting the cellular energy consumption and the control of lipid metabolism through PPARα and PGC-1α. More studies are needed to understand the consequences of lipin-1 dephosphorylation in skeletal muscle mitochondria of patients with DM1 [10] as well as the true impact of lipin in skeletal muscle mitochondria function (Figure 1. 4).

1.7.4 Lipin Association with Adipose Tissue

Adipose tissue is mainly composed by adipocytes and acts as an energy store for skeletal muscles. There are two types of adipocytes, the white and brown adipocytes. White adipocytes store energy in a highly concentrated form, namely as TAG, mostly in a single large lipid droplets [129]. Brown adipocytes are characterized by multiple, smaller droplets of TAG, which are accessible for rapid hydrolysis and rapid oxidation of the fatty acids [129]. Adipose tissue can secret a large number of peptide hormones, adipokines and activated lipids [130], affecting energy metabolism in liver muscles and neuroendocrine pathways (behaviors related to feeding) [129]. Adipokines, for example leptin, monocyte chemoattractant protein-1/chemokine (C–C motif) ligand-2 (MCP-1/CCL2) and TNFα, modulates an inflammatory response in adipose tissue and regulate the mitochondria present in adipose tissues playing a role in the regulation of lipid and glucose metabolism [129,131].

Several studies regarding non-DM1 obese individuals have reported a relation between hypertrophic adipocytes in white adipose tissue and MetS [132–134]. These patients had a higher activation of inflammation, increased TAG and accumulation of lipid species, such as diacylglycerol and ceramides and systemic insulin resistance, features presented in Table 1. 2. In addition, some of the gathered previous studies have also reported an altered adipose tissue function in patients with DM1 (Table 1. 2) [56,57,59,64,65]. Given this findings, we hypothesized the involvement of lipin in adipose tissue dysfunction, since lipin-1α can be found in the nucleus and stimulates expression of genes involved in adipocyte differentiation such as PPARγ and CCAAT/enhancer-binding protein α [12,96]. On the other hand, lipin-1β is predominantly localized on the cytoplasm and is associated with the expression of genes involved in lipogenesis and triglyceride storage in adipocytes, including fatty acid synthase and diacylglycerol acyltransferase [96]. Given this, both lipin-1α and lipin-1β are important for normal adipogenesis and lipogenesis, as observed in previous studies with a lipin-deficient mice models which developed decreased mature adipocytes [68,125]. However, in another study the overexpression of lipin in adipose tissue of a transgenic mice caused obesity, increased lipogenic gene expression, and hypertrophic adipocytes, which could lead to improved insulin sensitivity [96,127].

Furthermore, insulin resistance and dyslipidemia in skeletal muscle are associated with inflammatory state in adipose tissue through recruiting MCP-1, TNFα, and other cytokines leading to increased free fatty acids causing a decreased TAG synthesis, accumulation of TAG and activated lipids with a long-chain of fatty acyl-CoA esters. Consequently, a disruption of mitochondrial function, and glucose transport in the skeletal muscle, liver, and pancreas is observed, thus triggering insulin resistance and dyslipidemia [9,69,129]. Interestingly, macrophages can express lipin-1, lipin-2, and lipin-3, thus lipin also has an important role in macrophage physiology and pathophysiology [135,136].

Although, we hypothesize the role of lipin altered levels in adipose tissue (Figure 1. 4), there are evidences of adipose tissue alteration in patients with DM1, which have features of MetS summarized in Table 1. 2. Thus, more studies regarding lipin function in adipose tissue of patients with DM1 would give insight towards these metabolic alterations and may be give rise to new underlying metabolic pathways and/or therapeutics.

Over all our findings suggests that there is indeed a correlation between lipin dysfunction and DM1 altered metabolism, particularly, a relation of lipin with dyslipidemia, insulin resistance, skeletal muscle metabolism, and adipose tissue (Figure 1. 4). Therefore, there is a need to understand not only the underlying pathways of lipin in DM1 disease, but also, how are these pathways affected and how they potentially lead to DM1 metabolic dysregulation (Figure 1. 4).

1.8 Future Perspectives

Of note, there are several metabolic changes observed in patients with DM1 (Table 1. 2). These alterations are potentially involved with altered lipin expression levels and activity apparently leading to lipid metabolism abnormalities in patients with DM1. Therefore, all these aspects need further investigation. The underlying mechanisms and pathways associated to these lipid metabolic changes should be explored in DM1, since it poses interesting challenges and potential therapeutically opportunities for MetS and diabetes mellitus.

From the literature review, many questions were raised, such as: How is lipin regulated in patients with DM1? What conditions influence lipin activation and inhibition? What is the association between DMPK mRNA and/or other nuclear proteins with lipin? What are the consequences of downregulation (lipin deficiency) or overexpression in patients with DM1? Can lipin proteins be targets for the modulation of TAG storage and/or TAG secretion? These questions are in reach through studies of genetically modified mouse models, cell culture systems, transcriptome alterations, and metabolome analysis in patients with DM1 [45,71,81,127] with the aid of technology advancements and methods in measurement of lipids [107], including nuclear magnetic resonance (NMR), mass spectrometry (MS), and vibrational spectroscopy like Raman and Fourier-transform infrared (FTIR) that have the capability to analyze lipid abnormalities in a variety of biological samples [137–141]. In addition, there is a need to better understand and study the role of

lipin in DM1, since to our current knowledge there are few studies related to this subject. Also, it would be interesting to evaluate the metabolic alterations and their relations to DM1 phenotypes (congenital-onset, infantile-onset, juvenile-onset, adult-onset, late-onset), to observe if it is possible to discriminate patients with DM1 by disease severity through metabolome analysis as a screening tool.

1.9 Concluding remarks

In this review, we have consolidated the clinical and molecular evidence of metabolic alterations observed in patients with DM1 and the potential involvement of lipin in such metabolic alterations. In addition, evidence of altered lipin levels in DM1 mouse model and in humans has been reported [45,71], thus emphasizing the importance of this review, regarding the potential correlation between altered lipin levels and the DM1 metabolism. Also, alterations in lipidome composition have been observed in Duchenne muscular dystrophy, Becker muscular dystrophy, DM1, myotonic dystrophy type 2, among other skeletal muscle disorders [107]. These lipid alterations are important components of MetS, non-alcoholic fatty diseases, cardiovascular diseases, lipid storage disorders, vascular and neurologic disorders, diabetes mellitus, insulin resistance, atherogenic dyslipidemia, hypertension, central obesity, and pro-inflammatory state disorders [8,53,54].

Clear clinical metabolic alterations, namely, increased total cholesterol, LDL, TAG, insulin, and HOMA-IR have been demonstrated in this review between patients with DM1 and control groups. These clinical results showed not only the incidence of metabolic alterations in patients with DM1 but also, the urgent need to further study the underlying mechanisms and pathways leading to these metabolic alterations. One hypothesis is that altered levels of lipin may be associated with metabolic abnormalities found in patients with DM1, since lipin has a critical metabolic role in adipose tissue, skeletal muscle system, and liver, being localized in the cytoplasm, endoplasmic reticulum, and in the nucleus. Our findings are in accordance with previous studies [73,81,97,113,114,129,142], were alterations in lipin levels potentially led to impaired skeletal muscle functions, impaired mitochondrial function, severe sarcoplasmic reticulum stress, neutral lipid accumulation, insulin resistance, dyslipidemia, inflammatory state (macrophage) in adipose tissue, endoplasmic reticulum stress, and SREBP activation, which are common features observed in patients with DM1.

Interestingly, to our current knowledge patients with DM1 despite abnormal metabolism, rarely became glucose intolerant, and the incidence of diabetes ranges from 0 to 6.5%. Also, the blood pressure is not elevated as in patients with MetS [56,61,65,143]. Thus, patients with DM1 may be of interest for further studies on therapeutically mechanisms for diabetes and components of MetS.

Nonetheless, there is still a gap between the pathway and the mechanism underlying the molecular pathology of altered lipin levels' downstream events regulating lipid metabolism in patients with DM1. Although, the hypothesis that altered lipin levels is a hallmark of several dysfunction in

patients with DM1 is emerging. Therefore, understanding the different molecular mechanism underlying lipin dysfunction will significantly increase the knowledge of the physiological and pathological abnormal metabolism in patients with DM1.

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1.11 Attachments

Table S1. 1: Summary data of altered metabolism in patients with DM1 and controls.

Author, Country, Year	**Sample**	**CTG repeat length, BMI and Waist circumference**	**Insulin metabolism (pmol/L)**	**HOMA-IR**	**Glucose metabolism (mg/dL)**	**Lipid metabolism (mg/dL)**
Passeri, E., et al. [27], Italy, 2015	**DM1 (n):** 31 Sex: 31M Age (Median): 45y **Control** (n): 32 Sex: 32M Age (Median): 46y	Median (interquartile range): CTG=ND BMI (kg/m^2) **DM1=**24.3(22.2-27.7) **Controls=**25.5(24.1-28.0); p=Ns Waist circumference (cm) **DM1=**96(87–103) vs **Controls=**91(87–99); p=Ns	**DM1**=71.52±103.47 vs **Controls** (median (IQR))=39.58(29.86-62.50); p= Ns	**DM1=**2.03±2.7 vs **Controls=** 1.48±1.1; p=Ns	Median (interquartile range): **DM1=**76.0 (73.5-84.4) vs **Controls=** 85.5 (79.2-98.0); p=Ns	Median (interquartile): Total-Cholesterol **DM1=**208(181–227) vs **Controls=**200(180–224); p=Ns; TAG **DM1=**129(95-189) vs **Controls=**92(74-125); p=0.005 HDL-Cholesterol **DM1=**48(39–57) vs **Controls=**47(40–54); p=Ns; LDL-Cholesterol **DM1=**129(92-148) vs **Controls=**131(107-156), p=Ns Diabetes mellitus, % **DM1=**1/32 vs **Controls=**0/32 p=0.04
Ben Hamou, A.,et al. [28], France, 2019	**DM1 (n):**115 Sex: 71F, 44M Age:45.1y	CTG (media (IQR))=500(260-850) BMI (kg/m^2) **DM1=**26.4±6.5	**DM1=**51.38±42.36	ND	**DM1=**90.0 ± 15.0	Total cholesterol **DM1=**200.0 ± 40.0 TAG (median (IQR)) **DM1=**132 (97–192) LDL (median (IQR)) **DM1=**117 (96–140) HDL (median (IQR)) **DM1=**50 (43–59) Diabetes (n/N; %) **DM1=**30/115 (26.1);
Vujnic, M., et al [63], Bosnia and Herzegovina, 2015	**DM1 (n):** 66 Sex: 33F, 33M Age: 41.9y	CTG=751.9±280.6 BMI (kg/m^2) **DM1=**23.1±4.5	ND	ND	**DM1=**88.28±16.21	Total cholesterol **DM1=**228.15± 54.14 TAG **DM1=**194.86± 124 LDL **DM1=**143.08±50.27 HDL **DM1=**50.27± 11.6 Low HDL (%) **DM1=**34.8 Metabolic syndrome (n, %) **DM1=** 11,16.7 Central obesity- (n, %): **DM1=**9,13.6
Renna, L.V., et al. [70], Italy, 2019	**DM1 (n):** 8 Sex: 6F, 2M Age: 34y **Control (n):** 3 Sex: 2F, 1M Age: 43y	CTG=413,75 (230-800) BMI (kg/m^2) **DM1=**23.3; **Control=**23.7	ND	**DM1=**2.25; **Control=**1.6	ND	ND
Renna, L.V., et al. [55], Italy, 2017	**DM1 (n):** 8 Sex: 7F, 1M Age: 38y **Control (n):** 8 Sex: 4F, 4M Age: 35y	CTG=370,6±111.1 (220-560) BMI (kg/m^2) **DM1=**23.6 **Control=**22.7	**DM1=**65.62	**DM1=**1.945	**DM1=**82.75; **Control=**89.375	Total Cholesterol **DM1=**176; **Control=**146
Stojanovic, R.V., et al.	**DM1 (n):** 34 Sex: 18F, 16M	CTG: ND BMI (kg/m^2)	**DM1=**135.48±74.30F;	**DM1=**3.78±2.03F;	**DM1=**78.91±8.28F; 86.12±20.18M	Total Cholesterol **DM1=**210.75±55.68F;

[56], Serbia, 2010	Age: 43y **Control (n):** 34 Sex: 18F, 16M Age: 43y	**DM1=**23.5±4.3 (15.6-30.1); 23±4.4F; 24.1±4.2M; **Control=** 23.5±4.	151.45±59.16M; p= Ns	4.77±2.81M		251.74±62.65M TAG **DM1=**52.98±19.335F; 88.55±37.9M
Daniele, A., et al [57], Italy, 2011	**DM1 (n):** 21 Sex: 8F, 13M Age: 44.5y **Control (n):** 82 Sex: 43F, 39M Age: 39.2y	CTG=ND BMI (kg/m^2) **DM1=**25.7±3.6, Median=25.2 **Control=**23.2±2.9, Median=23.4; p=0.001 Waist circumference(cm) **DM1=**97.4 (8.6); Median=95 **Controls=**84.9 (11.6); Median=89; p<0.001	**DM1=**95.83±63.19, Median=75 vs **Control=**45.83±12.50, Median=41.6; p<0.001	**DM1=**3.6±2.2, Median=3.0 vs **Control=**1.3±0.2Median=1.3; p<0.001	**DM1=**108.5±42.2, Median=100.0 vs **Control=**81.6±11, Median=80.0; p<0.001	Total cholesterol **DM1=**210.8±51.3; median=198.5 vs **Control=**194.5±38.9; median=190.0; p<0.001 TAG **DM1=**244.1±186, median=146 vs **Control=**95.2±53.9 Median=82.5; p<0.001
Shieh, K., et al. [58], USA, 2010	**DM1 (n):** 36 Sex: 20F, 16M Age: 42.2y	CTG=ND BMI (kg/m^2) **DM1=**27.2(19-38) Waist circumference (cm) **DM1=**94.3(71 – 119)	**DM1=**186.11 (13.8–1229.16)	**DM1=**6.4 (0.4–35.0)	**DM1=**97.0 (78–138)	Total cholesterol **DM1=**195.2(129-286) TAG **DM1=**162.8(29–320) LDL cholesterol **DM1=**111.2(59–194) HDL cholesterol **DM1=**51.4(28–93) Diabetes (n, %) **DM1=**4, 11.8 Metabolic syndrome (%) **DM1=**14 (41.2%)
Johansson, A., et al. [59], Sweden, 2001	**DM1 (n):** 42 Sex: 20F, 22M Age (median): 41.5y (IQR:28.5-58.7) **Control (n):** 50 Sex: 23F, 27M Age (median): 42y (IQR: 27.0-56.9)	Median (10th and 90th percentil) CTG=679(152-1142) BMI (kg/m^2) **DM1=**23.3(18.6-29.2) vs **Control=**24.0 (20.7-30.0); p=Ns Waist circumference (cm) DM1=89.3(69.1–108.9) vs Controls=82.5(70.0–103.0); p=Ns	Median (10th and 90th percentil) **DM1=**64.58(33.33–155.55) vs **Control=**40.27(20.83–75.69); p<0.001	ND	ND	Median (10th and 90th percentil) Total cholesterol **DM1=**208.8(139.2–278.4) vs **Control=**193.35; (158.55–239.75); p= Ns TAG **DM1=**168.3(88.57–310) vs **Control=**83.26(52.26–150.58) p<0.001 LDL cholesterol **DM1=**127.6(69.6–185.6) vs **Control=**127.6 (85.07–166.28) p=Ns HDL cholesterol **DM1=**47.56(34.38–66.13) vs **Control=**51.04(40.6–76.18) p <0.05
Perseghin, G., et al. [64], Italy, 2004	**DM1 (n):** 10 Sex: 8F, 2M Age: 38y **Control (n):** 10 Sex: 8F, 2M Age: 33y	CTG= ND BMI (kg/m^2) **DM1=**22.3±1.4 **Control=**21.7±1.2	ND	ND	**DM1=**82.70±4.14 vs **Control=**91.71±2.34; p<0.05	Total cholesterol **DM1=**186±11.21 vs **Control=**176.72±11.21; p=Ns TAG **DM1=**108.06±15.94 vs **Control=**77.06±28.3; p=Ns LDL cholesterol **DM1=**106.34±13.14 vs **Control=**97.45±8.50; p=Ns HDL cholesterol **DM1=**58.39±3.09 vs **Control=**51.82±2.32; p=Ns
Spaziani, M., et al. [60], Italy, 2020	**DM1 (n):** 63 Sex: 27F, 36M Age: 43y **Control (n):** 100 Sex: All male Age (mean): 42	CTG Range= 50 to >1000	**Male** **DM1=**104.16±97.22 **Controls**=56.25±43.75; p=Ns **Female** **DM1=**104.16±76.38 **Controls=**63.19±18.75; p=Ns	ND	**Male:** **DM1=**95±17 vs **Control=**83±7.9; p< 0.005; **Female:** **DM1=**113±63 vs **Controls=**88±9.0; p< 0.0001	**Male** Total cholesterol **DM1=**181±43 vs **Control=**128 (20) p< 0.0001; TAG **DM1=**186± 87 vs **Controls=**75 (32); p< 0.0001

						LDL cholesterol **DM1=**104±29 vs **Control=**46 (12); p< 0.0001 HDL cholesterol **DM1=**44±9.1 vs **Control=**67 (19) p< 0.0001; **Female:** Total cholesterol **DM1=**191±27 vs **Controls=**134 (20); p< 0.0001 TAG **DM1=**147±82 vs **Controls=**105 (20); p< 0.05 HDL cholesterol **DM1=**61±21 vs **Controls=**55 (8.9); p=NS LDL cholesterol **DM1=**101±25 vs **Controls=**102 (21); p=NS
Johansson, A., et al. [61], Sweden, 2002	**DM1 (n):** 18 Sex: all male Age (median): 39y (percentil: 22-90) **Control (n):** 18 Sex: all male Age (median): 38y, (percentil 23-62)	Median 10th-90th percentiles CTG=614.5 (347-1088) BMI (Kg/m^2) **DM1=**24.7(18.9-30.3) vs **Control=**24.6(19.8-30.7); p=Ns	Median 10th-90th percentiles **DM1=**83.0(55.6–210.4) vs **Control=**51.0(34.0–106.7); p<0.01	Median 10th-90th percentiles **DM1=**2.3(1.4–5.5) vs **Control=**1.4(0.90–3.5); p<0.001	Median 10th-90th percentiles **DM1=**82.88(72.07–95.49) vs **Control=** 79.28 (68.46–91.89); p=Ns	ND
Hudson, A.J., et al. [62], England, 1987	**DM1 (n):** 10, Sex: 6F; 4M Age: 45y **Control (n):** 10; Sex: 6F; 4M Age: 45 y	CTG=ND	**DM1=**181.1±25.5 vs **Control=**86.0±3.7; p<0.001	ND	**DM1=**93.69±1.8 vs **Control=**90.09±1.80; p=Ns	**Mean (5th and 95th percentil)** Total cholesterol **DM1=**210(183-237) TAG **DM1=**274(127-421) VLDL **DM1=**180(32-328) LDL cholesterol **DM1=**120(98-143) HDL cholesterol **DM1=**57(46-68) LDL apolipoprotein B **DM1=**102.0+ 8.4 vs **Control=** 84.3 ±4.0; p<0.05
Moorjani, S., et al. [68], Canada, 1989	**DM1 (n):** 70 Sex: 35F, 35M Age: 36 y **Control (n):** 81 Sex: 50F, 31M Age: 39y	CTG=ND	ND	ND	ND	TAG **DM1=** 216± 17 vs **Controls** =168 ±9; p<0.01 VLDL cholesterol **DM1=** 32.4±4.0 vs **Controls=** 21.0±1.3; p< 0.01 LDL cholesterol **DM1=** 111.3±4.4t vs Controls= 122.6 ±3.8; p< 0.05 HDL cholesterol **DM1=** 48.2 ±1.4 vs **Controls=** 45.7 ±1.3; p=Ns
Takada, H., et al [69], Japan, 2016	**DM1 (n):** 91 Sex: 41F, 50M Age (median): 47y	CTG (Median)=1075	ND	ND	ND	TAG (abnormality) 38 cases (42%) **DM1 (median):** 129 mg/dL LDL cholesterol (abnormality) 19 cases (21%) **DM1 (median):** 117 mg/dL HDL cholesterol (abnormality) 14 cases (15%) **DM1 (median):** 51
Matsumura, T., et al. [65],	**DM1 (n):** 95 Sex: 45F, 50M Age: 43y **Control (n):**	CTG= 973±744 BMI (Kg/m^2) **DM1:** 21.4±4.9	Insulinogenic index **DM1:** 1.71±0.29 vs **Control:** 0.77±0.12; p=	**DM1:** 1.96± 0.11 vs **Control:** 1.36±0.04;	**DM1:** 94.2±1.7 vs **Control:** 95.6± 0.6;	Total cholesterol **DM1:** 208.7±3.7 vs **Control:** 200.5±1.2; p= 0.038

Japan, 2009	734 Sex: 193F, 541M Age: 54.6±8.5	**Control:** 23.2±2.8	0.004	p <0.001	p=0.455	TAG **DM1:** 172.4±91 vs **Control:** 123.4±3.0; p < 0.001 HDL **DM1:** 52.2±2.0 vs **Control:** 56.7±0.6; p= 0.035
Heatwole, R.C., et al. [66], USA, 2011	**DM1 (n):** 15 Sex: 8F, 7M Age: 42.7±10.4	CTG: 355.9±209.6 BMI (Kg/m^2): 23.0±3.9			**DM1:** 88.57±10.12	TAG **DM1:** 140.27± 60.65 HDL **DM1:** 49.93±15.64
Perna, A., et al., [67], Italy, 2020	**DM1 (n):** 61 Sex: 26F, 35M Age: 47.2±13.8	CTG (n=53): 466.17±269.32 BMI (Kg/m^2): 24.21±4.78 BMI (Kg/m^2) ≥ 30: n= 8			**DM1:** 91.8±26.69	Total cholesterol **DM1 (n=59):** 188.22±40.62 TAG **DM1 (n=59):** 152.41±71.14 Diabetes type II: **DM1:** n= 6 Hypercholesterolemia (>200 md/dL) **DM1:** n= 21

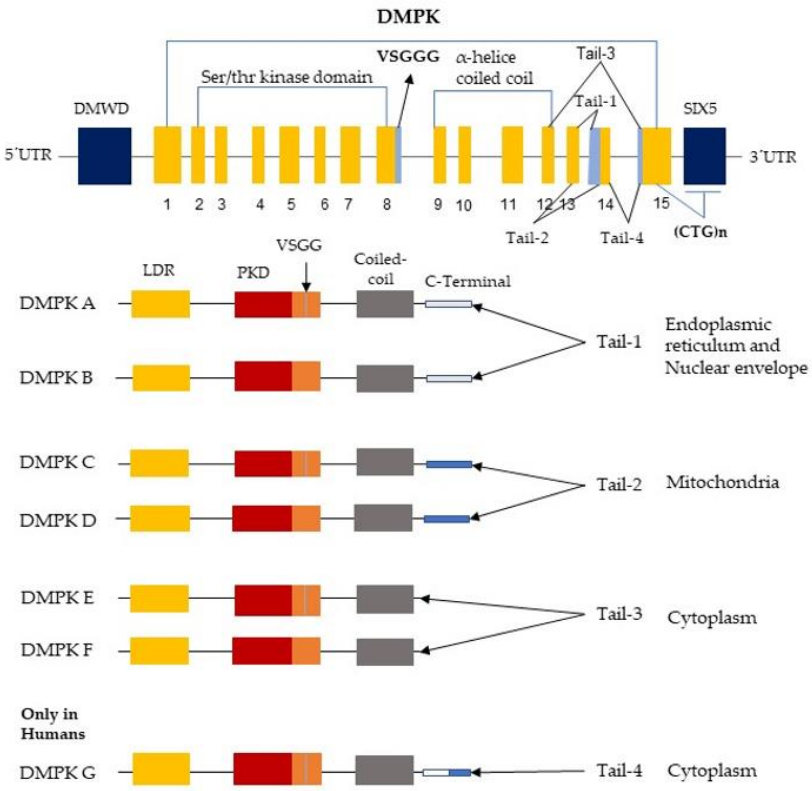

Figure S1. 1. Schematic representation of DM1 gene and isoforms. (A) DMPK gene and major DMPK isoforms in humans and their domains and localization [5,6,7]. DMPK subcellular localization is confined to either endoplasmic reticulum (ER) or nuclear envelope (DMPK A and B), to mitochondria (DMPK C and D) or to cytoplasm (DMPK E, F, and G). [6,36,20]. UTR, untranslated region; DMWD, Dystrophia myotonica WD repeat-containing protein (represented as Blue); DMPK, Myotonic Dystrophy Protein Kinase; CTG, cytosine-thymine-guanine trinucleotide; LDR-Leucine rich Domain; PKD, Serine/threonine protein kinase domain. Alternative splicing sites of VSGGG and Tail (1, 2, 3, and 4) are represented as light blue.

Chapter II: Aims of the dissertation

2. Aims

This present work aimed to provide an overview of the metabolic alterations in patients with DM1 and establish its correlation between Lipin, metabolic dysfunction and DM1 pathology; to determine the metabolic profile of patients with DM1, FTIR spectroscopy analysis was performed using four DM1-derived fibroblasts and control purchased from Coriell Institute with different CTG repeat length (DM1_1000 and DM1_2000) and 5 DM1-derived fibroblasts and one control established at the iBiMED laboratory from Centro Hospitalar do Tâmega e Sousa, EPE (Penafiel, Portugal) with different age at onset (congenital-onset, infantile-onset, juvenile-onset, adult-onset and late-onset) in order to verify if FTIR spectroscopy is as suitable technique to provide a novel biochemical insight into the characterization and discrimination of the different severities in patients with DM1; to systematically review and gather information about the outcomes and measures used to assess muscle strength in adult patients with DM1 to contribute for clinical practice and guidance.

With this work, we intended to:

- Review the metabolic alterations observed in patients with DM1;
- Review the current knowledge of Lipin and their relationship with metabolic alterations evidenced in DM1;
- Determine the metabolic profile using ATR-FTIR spectroscopy in the different DM1-derived fibroblasts and control purchased from Coriell Institute and DM1-derived fibroblasts and control established at iBiMED.
- Identify (bio)chemical differences between the DM1-derived fibroblasts and control from both Coriell Institute and DM1-derived fibroblasts and control established at iBiMED.
- Verify if ATR-FTIR can characterize and discriminate between the different forms of severities in patients with DM1;
- Summarize the most common outcomes and measurements previously reported to assess muscle strength in patients with DM1;
- Propose the consensual measures to access the muscle strength to be used in further clinical studies.

Chapter III. Evaluation of metabolic profile of patients with DM1

3. Fourier-transform infrared spectroscopy as a discrimination Myotonic dystrophy type 1 metabolism: pilot study

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3.1 Abstract

Myotonic dystrophy type 1 (DM1) is an autosomal dominant multisystemic hereditary disease, characterized by progressive distal muscle weakness and myotonia. Patients with DM1 have abnormal lipid metabolism and a high propensity to develop a metabolic syndrome, in comparison to the general population. Therefore, metabolome evaluation in these patients is crucial and may contribute to better characterization and discrimination between disease phenotype and severities in patients with DM1.

Fourier-transform infrared spectroscopy (FTIR) is a metabolic technique that discriminates samples through the biochemical composition. In this study, FTIR spectra were acquired using cell models of skin DM1-derived fibroblasts and controls as and analysed by multivariate analysis (Principal Component Analysis). DM1-derived fibroblasts and controls were obtained from both Coriell Institute and Institute of Biomedicine of Aveiro (iBiMED).

Our results showed a clear discrimination between both DM1-derived fibroblasts with different CTG repeat length and age at onset, indicating that they have a distinct metabolic profile. This discrimination could be attributed mainly to the altered lipid metabolism and proteins in the 1800-1500 cm^{-1} spectral region. These results suggest that FTIR spectroscopy is a valuable tool to discriminate both DM1-derived fibroblasts with different CTG repeat length and age at onset and to study the metabolomic profile of the patients with DM1.

3.2 Introduction

Myotonic dystrophy type 1 (DM1) is an hereditary disease mainly characterized by progressive distal muscle weakness and myotonia (sustained muscle contractions) [1–5]. DM1 is caused by the expansion of unstable repetitions of the cytosine-thymine-guanine trinucleotide (CTG) in the 3' untranslated region (3´UTR) of the Myotonic Dystrophy Protein Kinase (DMPK) gene located at chromosome 19q13.3 [4–7]. Due to DM1 clinical heterogeneity, disease severity can be subdivided according to symptoms severity and the age of onset [5–8]. In other words, DM1 is subdivided into three different phenotypes (mild, classic and severe) and clinically categorized as congenital-onset (<1 month and ≥1000 CTG repeat length), infantile-onset (1 month to 10 years and >500 CTG repeat length), juvenile-onset (10-20 years and >400 CTG repeat length), adult-onset (20-40 years and 150 to 1000 CTG repeat length) and late-onset (>40 years and 50 to 149 CTG repeat length) [6–9].

The frequency of Metabolic Syndrome (MetS) in patients with muscle disorders is significantly higher than in the general population [10,11] due to abnormal lipid metabolism particularly observed in skeletal muscle tissue of patients with DM1. The main causes of metabolic syndrome are insulin resistance, hypertriglyceridemia, increased fat mass, high levels of low-density lipoprotein (LDL), low levels of high-density lipoproteins (HDL), hypertension, elevated levels of glucose and abdominal obesity [10,12–17]. Also, it is interesting that patients with DM1 have a

normal glucose tolerance and a very low prevalence of diabetes, in spite of a marked insulin resistance [17,18]. Previous study showed a significant difference between patients with DM1 and controls regarding the lipid metabolism, in which triacylglycerol levels were higher, as well as total cholestrol levels, low-density lipoprotein levels, insulin levels and HOMA-Insulin resistance (IR) were also higher, and high-density lipoproteins were lower than the controls [11]. Due to these features, patients with DM1 have a high propensity to develop a metabolic syndrome. Therefore, the evaluation of the metabolome these patients represents an important step, since it provides a rapid, sensitive and reproducible data to understand the differences between the observed phenotypes in patients with DM1, allowing to better characterize and distinguish the different phenotypes within DM1patients [19,20].

The study of the lipidome (lipidic metabolome) has evolved in the last years due to development of techniques such as Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS) and vibrational spectroscopy like Raman and Fourier-transform infrared spectroscopy (FTIR) [19,20]. Although, FTIR is not as selective or sensible as NMR and MS, it is able to identify the presence of carbohydrates, amino acids, lipids, and fatty acids, as well as proteins and polysaccharides in the sample in one single experiment, being recognized as a valuable tool for metabolic fingerprinting [19,21]. Infrared (IR) spectroscopy is a non-invasive and, in some cases, non-destructive type of techniques based on the vibration of molecules covalent bonds as result of the interaction of infrared radiation with a sample (tissues, body fluids or cells) [22–24]. Vibrational spectroscopy techniques are known as relatively simple and reproducible; spectra are obtained in few minutes and has spectroscopic bands are relatively narrow, easy to resolve, and sensitive to molecular structure, conformation, and environment [25]. Additionally requires a minimal sample preparation and small amount of biological material (micrograms to nanograms) for analysis [25–27]. IR can be divided into three regions: near-IR region (13000–4000 cm−1), mid-IR region (4000–400 cm−1), and far-IR region (<400 cm−1). Mid-IR region spectrum is currently considered the most promising spectroscopic techniques for application in biomedical diagnostics [21,28] and can be divided into four regions, and the nature of a group frequency is related to the region in which it is located. These regions are defined as follows: X–H stretching region (4000–2500 cm−1), triple bond region (2500–2000 cm−1), double bond region (2000–1500 cm−1), and fingerprint region (1500–600 cm−1) [22].

One disadvantage of FTIR spectroscopy is the strong absorption of water in mid-IR region. To overcome this disadvantage, experiments are carried out with dried samples [29]. A common method for biological samples analysis is the Attenuated Total reflectance FTIR spectroscopy (ATR-FTIR) [23,27], which is one of the approaches for FTIR Spectroscopy sampling that allows to measure directly the sample in the ATR crystal (crystal with a high refractive index) [30]. The ATR reduces the dispersion of the incident IR beam, increasing the sensitivity and reproducibility of the technique [26,30]. The resultant attenuated radiation is measured and plotted as a function of wavelength and gives rise to the absorption spectra characteristics of the sample [22,26,30].

FTIR spectroscopic data can be analysed by direct spectra analyses or with the support of statistical tools (multivariate analysis). Multivariate analysis uses mathematical, statistical, and computer sciences to efficiently extract useful information from data generated [28]. One of the most used unsupervised methods is Principal Component Analysis (PCA), which does not require previous knowledge about the samples [28]. PCA reduces a large number of variables characteristic of a spectrum to a few variables or Principal Components that reflects the most relevant information and assists in resolving overlapping spectral features [28].

As the contribution of the metabolic dysfunction for DM1 is not fully elucidated. Therefore, the aim of the present study is to verify if ATR-FTIR spectroscopy, together with multivariate analysis is a suitable technique to provide a novel molecular insight into the characterization and distinction of the biochemical profile of patients with DM1 with different severities and matched controls.

3.3 Material and Methods

3.3.1 DM1-derived fibroblast and controls

DM1-derived fibroblasts and controls were obtained from two different origins, namely the Coriell Institute and at our institute of iBiMED research unit; and two distinct data sets were gathered.

DM1-derived and control-derived fibroblasts from skin biopsies donors were obtained from Coriell Institute. Five samples of fibroblast were obtained and used: one was a control and four were diagnosed with DM1. Control-derived fibroblasts (GMO2673) comprise a CTG repeat length between 5 and 27. The age at sampling of donor GMO2673 was 33 years. Two different DM1-derived fibroblasts with approximately 1000 CTG repeat length (GMO4033 and GMO4647) were also analysed. The age of the donor GMO4033 was 48 years and the donor GMO4647 was 23 years. Finally two different DM1-derived fibroblasts with approximately 2000 CTG repeat length (GMO3989 and GMO3759) were also used. The age at sampling of donor GMO3989 was 38 years and donor GMO3759 was 33 years. All donors were male and Caucasian. From this point, and all over the manuscript, the cell lines GMO4033 and GMO4647 will be denominated as DM1_1000 (1) and DM1_1000 (2), respectively. When analysed together they will be denominated as DM1_1000. The same will happen with the cell lines GMO3759 and GMO3989, which will be denominated as DM1_2000 (1) and DM1_2000 (2), respectively. When analysed together they were denominated as DM1_2000. Also, the cell line GMO2673 will be denominated as control. All cell lines were age and sex matched.

Through a collaborative work developed by University of Aveiro (iBiMED) and the Centro Hospitalar do Tâmega e Sousa, EPE (Penafiel, Portugal) skin biopsies of adult patients with previous genetically confirmed DM1 and adult healthy volunteers without any neuromuscular disorder for the

control group were collected. This study was approved by the Ethics Committee for Health of the Centro Hospitalar do Tâmega e Sousa, EPE (obtained at 14-08-2019 with approval number 31-2019).

Skin punch biopsies were collected from the patients with DM1 and from the controls and skin-derived fibroblasts were cultured at iBiMED for either patients with DM1 and controls. For the present study 5 male DM1-derived fibroblasts and one control were chosen for ATR-FTIR spectroscopy analysis. These 5 DM1-derived fibroblasts were characterized by different age at onset (congenital-onset, infantile-onset, juvenile-onset, adult-onset and late-onset), the CTG repeat length for these DM1-derived fibroblasts were not evaluated. From this point, and all over manuscript the DM1-derived fibroblasts were denominated as: congenital-onset (cDM1); infantile-onset (iDM1); juvenile-onset (jDM1); adult-onset (aDM1); late-onset (lDM1) and the control. All cell lines were age and sex matched.

3.3.1.1 Cell culture

Fibroblasts obtained from Coriell Institute were cultured in complete DMEM (Dulbecco's Modified Eagle Medium, high glucose) (GibcoTM) supplemented with 15% FBS (Fetal Bovine Serum, Origin South America) (GibcoTM) in T75 flasks. The culture medium was changed every two days. All washes were performed using Phosphate-buffered saline (PBS), without Ca2+ and Mg2+ (BioConcept Ltd. Amimed®). Whenever fibroblasts reach 80-90% confluence the were subcultured using 0.05% trypsin-EDTA and transferred to the new T75 flasks and maintained at 37ºC in a humidified 5% CO2 atmosphere.

Skin-derived fibroblasts from iBiMED, were cultured in complete DMEM (Dulbecco's Modified Eagle Medium, high glucose) (GibcoTM) supplemented with 20% FBS (Fetal Bovine Serum, Origin South America) (GibcoTM) and 1% penicillin/streptomycin in T75 flasks. The culture medium was changed every two days. All washes were performed using Phosphate-buffered saline (PBS), without Ca^{2+} and Mg^{2+} (BioConcept Ltd. Amimed®). Whenever fibroblasts reach 80-90% confluence the were subcultured using 0.05% trypsin-EDTA and transferred to the new T75 flasks and maintained at 37ºC in a humidified 5% CO2 atmosphere.

For all FTIR spectroscopy experiments a fibroblasts density of 0.9x106 was used. Briefly, primary fibroblasts were cultured and after reaching around 90% of confluency (T75 flasks), the fibroblast were PBS washed, trypsinazed using 0.05%trypsin-EDTA (GibcoTM) solution and then resuspended in 3 mL of fresh complete medium and cell density determined using the Trypan Blue assay. A total of 0.9x106 fibroblasts were used for each replicate.

3.3.2 FTIR Measurements

The DM1-derived fibroblasts and controls spectra were acquired using a FTIR spectrometer (Alpha Platinum ATR, ©Bruker Optics Ltd.) equipped with a diamond ATR crystal, and pre-processed using OPUS software version 7.0 (©Bruker Optics Ltd.). Five µL of the DM1-derived fibroblasts were placed in the crystal. To overcome the water interference, DM1-derived fibroblasts were air-dried before spectra were acquired. The spectral were obtained in the wavenumber range 4000-600 cm^{-1}, with a resolution of 8 cm^{-1} and 64 co-added scans. Three replicates were obtained for DM1-derived fibroblasts and controls. Between different samples reading the crystal was cleaned with 70% ethanol and distilled water and a background spectrum was acquired with the crystal empty (cleaned), to eliminate possible interference from fluctuations in the conditions of the room. All spectra acquisition was performed in a controlled room with a temperature of 23 º C and humidity of 35%.

3.3.2.1 FTIR Data Analysis

All spectra were processed using The Unscrambler X® software (v.10.4, CAMO Analytics, Oslo, Norway). All spectra were baseline corrected and area normalized. The normalized (duplicated matrix) spectra were derivatized, using the second derivate and Savitzky-Golay method with 3 smoothing points, to resolve the overlapping peaks and maximize the differences between the spectra. Principal Component Analysis was applied to the normalized second-derivative spectra of all DM1-derived fibroblasts. For multivariate analysis, three spectral region (mid-IR) 3000-2800 cm^{-1}, 1800-1500 cm^{-1} and 1200-900 cm^{-1} were chosen, and all spectral assignments were made according to cited literature references

3.3.2.2 Intensity ratio evaluation

In order to study possible disease-induced variation of the lipid content between control and DM1-derived fibroblasts with different CTG repeat length and age at onset, the long hydrocarbon chains in lipids (CH2/CH3), lipid peroxidation (Carbonyl/Total lipid) and the unsaturated/saturated ratios were calculated by measuring peak intensity of the second derivative spectra [31,32]. Peak intensity for CH2 (~2922 cm^{-1} - asymmetric vibration of the CH2 groups) and CH3 (~2959 cm^{-1} - asymmetric vibration of the CH3) were used to calculate CH2 and CH3 ratio [31]. Peak intensity for C=O stretching (~1747 cm^{-1}) and total lipid (CH2 sum of the saturated lipid bands (~2922 cm^{-1} - asymmetric vibration of the CH2 groups and ~2851 cm^{-1} - symmetric vibration of the CH2 groups)) were used to calculate Carbonyl/total lipid ratio [31,32]. Peak intensity for olefinic band (~3013 cm^{-1} - olefinic band, C=CH stretching) and CH2 sum of the saturated lipid bands (~2922 cm^{-1} - asymmetric vibration of the CH2 groups and ~2851 cm^{-1} - symmetric vibration of the CH2 groups) were used to calculate unsaturated/saturated ratio [31,32].

Data were evaluated using a normality test, to decide between parametric or nonparametric tests to use. The normal distribution could not be tested due to the number of samples. To identify the differences in the lipid composition between groups the ANOVA nonparametric statistical test was

used, followed by the Dunnett´s test for multiple comparisons between groups. The data obtained were expressed as mean ± standard deviation, and the results were considered statistically significant for a p value ≤0.05 [31,33]. Also, Mann-Whitney U nonparametric test was used to evaluate if the independent groups had significant differences. The results obtained were expressed as mean ± standard deviation and were considered statistically significant for a p value ≤0.05. All statistical analysis was performed using GraphPad Prism v.6.01 (GraphPad Software, Inc.).

In a first part results of DM1-derived fibroblasts and control obtained from Coriell institute will be presented and, in a second part, the spectral and statistical analysis of DM1-derived fibroblasts and control at iBiMED will be shown. A comparison between the two data sets, using PCA will also be presented.

3.4 Results

3.4.1 FTIR spectral analysis of fibroblast from Coriell Institute

In the current study, ATR-FTIR spectroscopy coupled with multivariate analysis (PCA) was performed to characterize and discriminate DM1-derived fibroblasts with different CTG repeat length. Figure 3. 1 shows ATR-FTIR spectral analysis in the region between 4000-600 cm^{-1}. DM1-derived fibroblasts and control were analysed in the 3000-2800 cm^{-1}, 1800-1500 cm^{-1} and 1200-900 cm^{-1} spectral regions. In 3000-2800 cm^{-1} spectral region are located some of the bands assignments of lipids [30,32,34], namely vibrational asymmetric (υas) and symmetric (υs) stretching modes of -CH3 and CH2 [30,32,34] (figure 3. 1B). In the 1800-1500 cm^{-1} region are located bands associated mostly to proteins secondary structures namely amide I band between 1700-1600 cm^{-1} and amide II band between 1600-1500 cm^{-1} region [27] and carbonyl band around 1747 cm^{-1} [22,27]. The amide I band is attributed to 80% of υs(C=O), 10% N–H bending and 10% υs(C–N). The amide II band is attributed to 60% of N–H bending and 40% υs(C–N) [22,27,32] (figure 3. 1C). Additionally, in the region 1800-1500 cm^{-1}, the band between located around 1747-1736 cm^{-1} corresponds to an assignment of triacylglycerol, cholestrol esters and glycerophospholipids with a vibrational υs(C=O) mode [27,30,32,34], which may be used to observe the degree of lipid peroxidation [33]. In the 1200-900 cm^{-1} region the major bands are associated to carbohydrates and phosphates associated to nucleic acids [22,30,32] (figure 3. 1D).

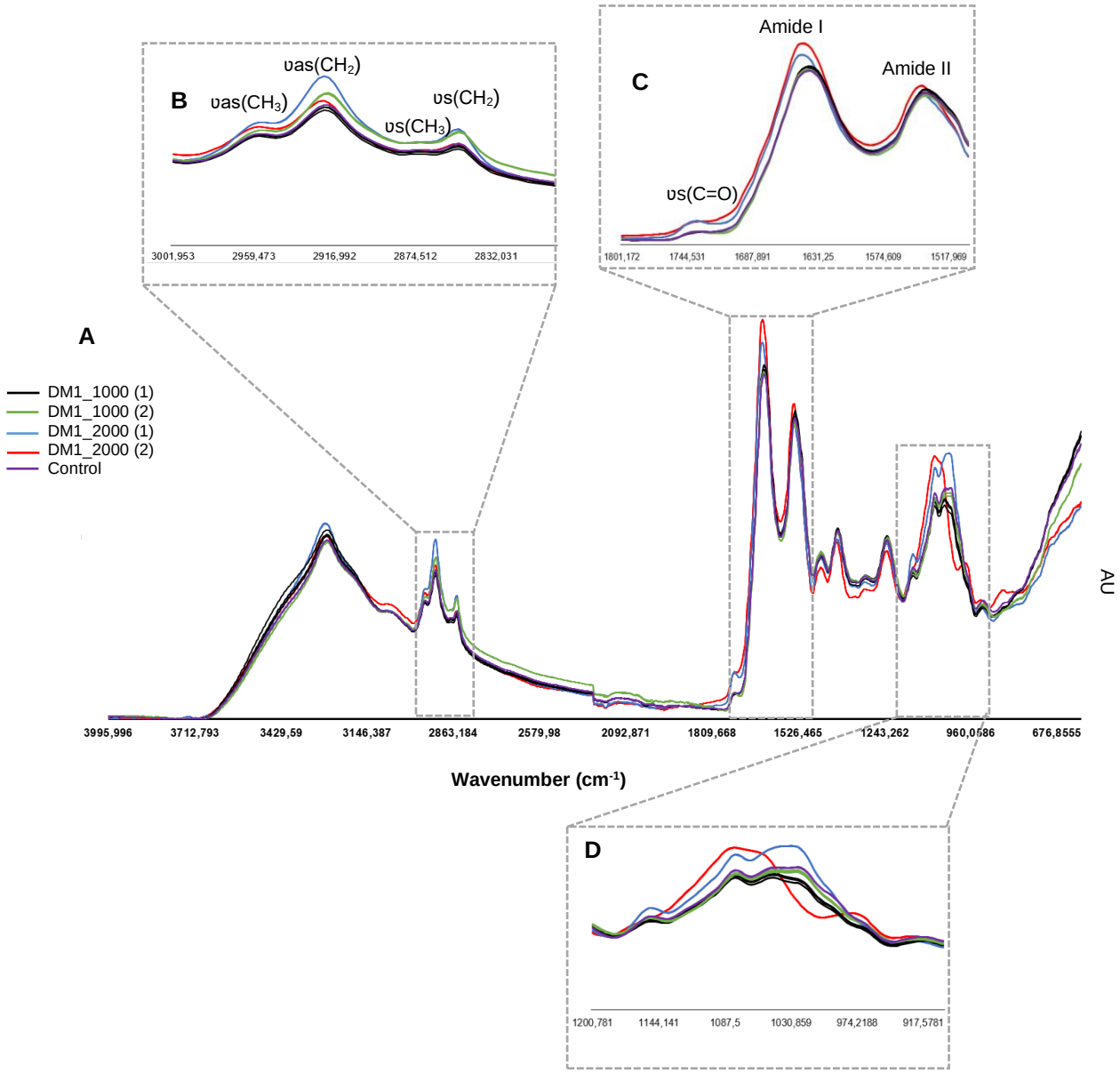


Figure 3. 1. FTIR spectra of DM1-derived fibroblasts and control. (A) Baseline-corrected and area normalized spectra of DM1-derived fibroblasts and control in 4000-600 cm^{-1} region. **(B)** Amplification of the region 3000-2800 cm^{-1}, **(C)** amplification of the region 1800-1500 cm^{-1}, **(D)** amplification of the 1200-900 cm^{-1} region. X-axis: wavenumber cm^{-1}, Y-axis: arbitrary units (AU) [21,26,29,31,33].

3.4.1.1 Multivariate analysis of the spectroscopic data of fibroblast from Coriell Institute

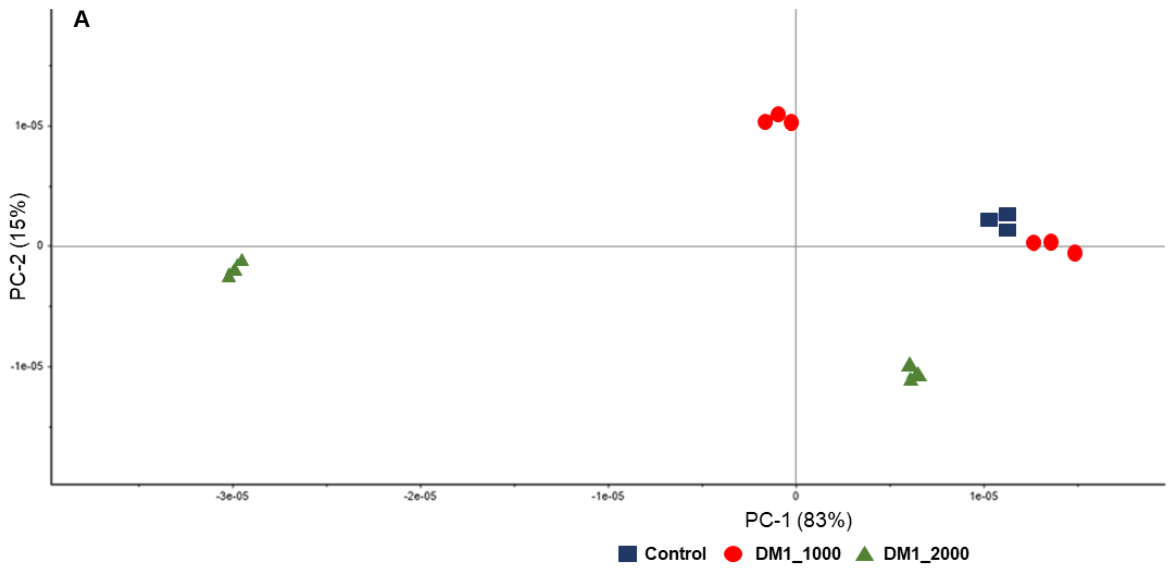
The first approach used in the present study was to assess whether this methodology is able to discriminate between DM1-derived fibroblasts and controls from the different groups (control, DM1_1000 (1 and 2) and DM1_2000 (1 and 2)) as well as to discriminate between DM1-derived fibroblasts (DM1_1000 (1 and 2) and DM1_2000 (1 and 2)) according to the increase of CTG length repeat. For that purpose, PCA analysis of the FTIR spectra were used.

PCA results from the region 3000-2800 cm^{-1} were illustrated in figure 3. 2. In the PCA score it was possible to discriminate by principal component-2 (PC-2), where DM1_2000 was in the negative side of the PC-2 while control and DM1_1000 were in the positive side of PC-2 (figure 3. 2A) (Table 3. 1), showing a clear separation between DM1_2000 and control and DM1_1000. According to the loading profile (figure 3. 2A), DM1_2000 are characterized by the spectroscopic signals located at 2953 cm^{-1} (CH3 asymmetric stretching), 2916 cm^{-1} (CH2 and CH3 stretching of phospholipids) and 2849 cm^{-1} (CH2 symmetric stretching), while control and DM1_1000 were characterized by the peaks 2925 cm^{-1} (CH2 asymmetric stretching) and 2874 cm^{-1} (CH3 symmetric stretching) [30,32,34] (figure 3. 2B) (Table 3. 1).

Table 3. 1. DM1-derived fibroblast (Coriell Institute) and control spectroscopic signals, assignments and vibrational mode obtained by PCA in the 3000-2800 cm^{-1}, 1800-1500 cm^{-1} and 1200-900 cm^{-1} regions [22,30,32,34]. **Q -** Quadrant; **– -** Negative PC region; **+ -** Positive PC region; **DM1_2000 (1 and 2) -** DM1_2000 (1) and DM1_2000 (2); **DM1_1000 (1 and 2) -** DM1_1000 (1) and DM1_1000 (2).

3000-2800 cm^{-1} region			
Discrimination across PC-2	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
PC-2 – DM1_2000 (1 and 2)	2953	CH_3 asymmetric stretching	Lipid (long chain fatty acids, phospholipids)
	2916	CH_2 and CH_3 stretching of phospholipids	
	2849	CH_2 symmetric stretching	
PC-2 + Control and DM1_1000 (1 and 2)	2925	CH_2 asymmetric stretching	
	2874	CH_3 symmetric stretching	
1800-1500 cm^{-1} region			
Discrimination across PC-1	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
PC-1 – Control and DM1_1000 (1 and 2)	1747	C=O stretching	Triacylglycerol, cholestrol esters, glycerophospholipids
	1736		
	1696	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: anti-parallel β-sheets (peptide, protein)
	1682		Amide-I: anti-parallel β- sheets (peptide, protein)
	1651		Amide-I: α- helices
	1554	60% N–H bending, 40% C–N stretching	Amide II (proteins)
	1523		
PC-1 + DM1_2000 (1 and 2)	1628	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: parallel β- sheets (peptide, protein)
	1537	60% N–H bending, 40% C–N stretching	Amide II (proteins)
	1509	CH_2 bending	Lipid, protein
1800-1500 cm^{-1} region (Q2 and Q3)			
Discrimination across Quadrant-2	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
DM1_1000 (1 and 2)	1693	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: anti-parallel β-sheets (peptide, protein)

	1639		Amide-I: parallel β- sheets (peptide, protein)
Discrimination across Quadrant-3	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
Control samples	1747-1743	C=O stretching	Triacylglycerol, cholestrol esters, glycerophospholipids
	1682	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: anti-parallel β- sheets (peptide, protein)
	1651		Amide-I: α- helices
	1554	60% N–H bending, 40% C–N stretching	Amide II (proteins)
	1543		
1200-900 cm^{-1} region			
Discrimination across PC-2	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
PC-2 – DM1_2000 (1 and 2)	1172	C–O stretching	Carbohydrates/glycogen, nucleic acids
	1013	C-O stretching and C-OH bending	DNA and RNA, oligosaccharides, polysaccharides (e.g., glucose)
	991	C-O stretching	DNA and RNA ribose
	914	$C\text{-}N^{+}\text{-}C$ stretching	DNA and RNA ribose-phosphate chain vibration of RNA
PC-2 + Control and DM1_1000 (1 and 2)	1152	C–O stretching, C-O-H bending	Carbohydrates
	1104	PO^{2-} symmetrical stretching	DNA, RNA, phospholipid, phosphorylated protein
	1079		
	1053	C-O stretching and C-OH bending	DNA and RNA, oligosaccharides, polysaccharides (e.g., glucose)
	968	PO_3^{2-} stretching	DNA and RNA ribose
	928	$C\text{-}N^{+}\text{-}C$ stretching	DNA and RNA ribose-phosphate chain vibration of RNA



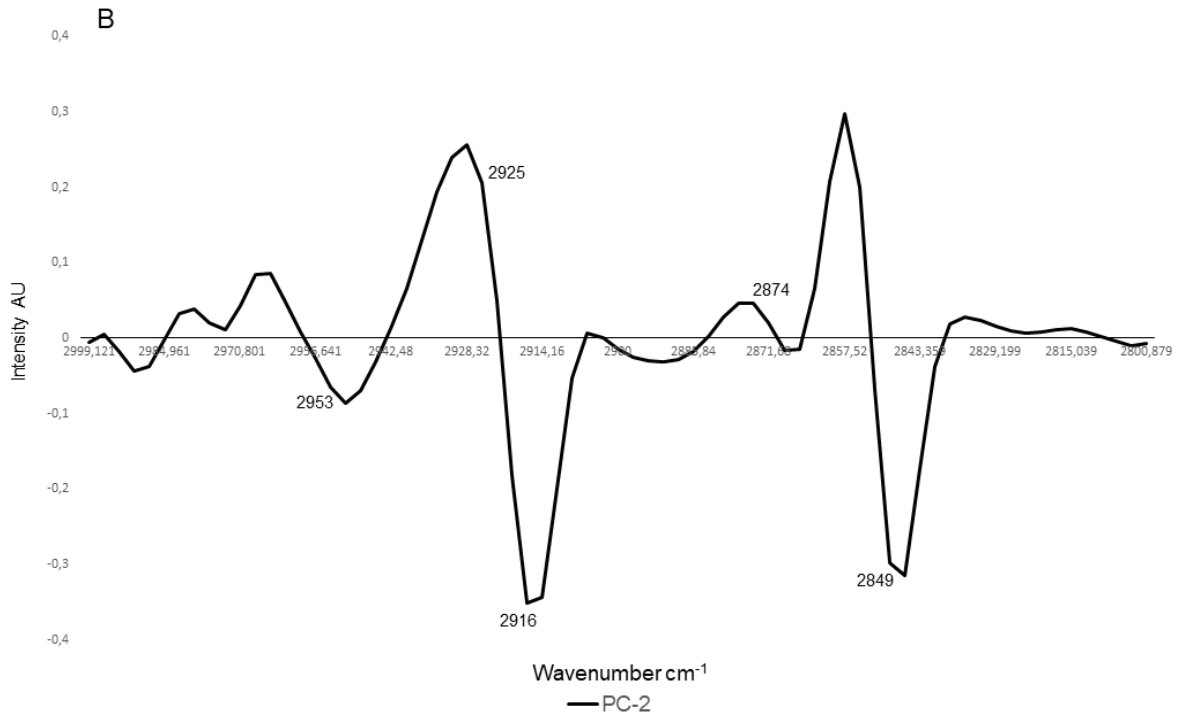


Figure 3. 2. PCA scores and loading profile of DM1-derived fibroblasts and control in the 3000-2800 cm^{-1} region. **(A)** PCA scores. **(B)** PC-2 loading profile plot.

PCA results from the region 1800-1500 cm^{-1} were presented in figure 3. 3. It was possible to discriminate between DM1-derived fibroblasts and controls by PC-1, where DM1_2000 sample was in the positive PC-1 separated from control and DM1_1000 sample, which were in the negative PC-1 (figure 3. 3A). The loading plot showed that DM1_2000 is mainly characterized by the following spectral assignments: 1628 cm^{-1} (Amide-I: parallel β-sheets (peptide, protein)), 1537 cm^{-1} (Amide II (proteins)) and 1509 cm^{-1} (CH2 bending (lipid, protein)), whereas the control and DM1_1000 sample are characterized by the following spectral assignments: 1747 cm^{-1} and 1736 cm^{-1} (C=O stretching (Triacylglycerol, cholestrol esters, glycerophospholipids)), 1696 cm^{-1} and 1682 cm-1 (Amide-I: anti-parallel β-sheets (peptide, protein)), 1651 cm^{-1} (Amide-I: α-helices), 1554 cm^{-1} and 1523 cm^{-1} (Amide II (proteins)) [22,30,32] (figure 3. 3B) (Table 3. 1).

In addition, DM1_1000, located in Q2 (both negative PC-1 and positive PC-3) were discriminated from control were in Q3 (both negative PC-1 and PC-3) (figure 3. 3A). DM1_1000 were characterized by the following spectroscopic signals: 1693 cm^{-1} (Amide-I: anti-parallel β-sheets (peptide, protein)) and 1639 cm^{-1} (Amide-I: parallel β-sheets (peptide, protein)) while the control was characterized by the following assignments 1747 cm^{-1} and 1743 cm^{-1} (C=O stretching (triacylglycerol, cholestrol esters, glycerophospholipids)), 1682 cm^{-1} (Amide-I: anti-parallel β-sheets (peptide, protein)), 1651 cm^{-1} (Amide-I: α-helices), 1554 cm^{-1} and 1543 cm^{-1} (Amide II (proteins)) [22,30,32] (figure 3. 3B) (Table 3. 1).

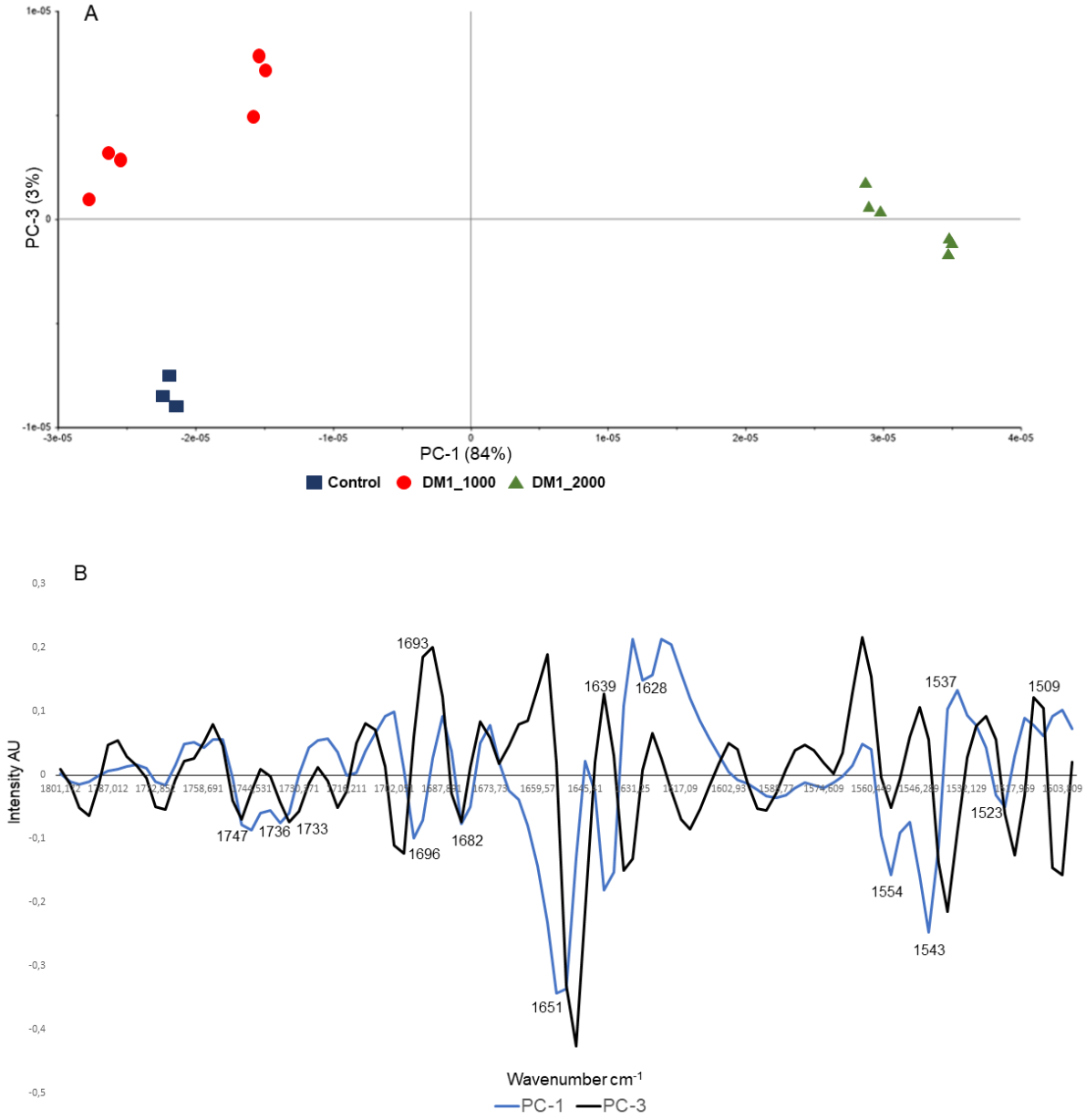


Figure 3. 3. PCA scores and loading profile of DM1-derived fibroblasts and control in the 1800-1500 cm^{-1} region. **(A)** PCA scores. **(B)** PC-1 and PC-3 loading profile plot.

PCA results from the region 1200-900 cm^{-1} was illustrated in figure 3. 4. In the PCA scores it is possible to discriminate, across PC-2, DM1_2000 sample from DM1_1000 sample. DM1_2000 was located in the negative side of the PC-2 while control and DM1_1000 sample were located in the positive side of PC-2 (figure 3. 4A). PC-2 shows a clear discrimination between DM1_2000 sample and control and DM1_1000 sample. According to the loading profile, DM1_2000 was characterized by the spectroscopic signals located at 1172 cm^{-1} (C–O stretching (Carbohydrates/glycogen, nucleic acids)), 1013 cm^{-1} (C-O stretching and C-OH bending (DNA and RNA, oligosaccharides, polysaccharides)), 991 cm^{-1} (C-O stretching (DNA and RNA ribose)) and 914 cm^{-1} (C-N+-C

stretching (DNA and RNA ribose-phosphate chain vibration)), while control and DM1_1000 sample were characterized by the following spectroscopic signals: 1152 cm^{-1} (C–O stretching, C-O-H bending (Carbohydrates)), 1104 cm^{-1} and 1079 cm^{-1} (PO2− symmetric stretching (DNA, RNA, phospholipid, phosphorylated protein)), 1053 cm^{-1} (C-O stretching and C-OH bending (DNA and RNA, oligosaccharides, polysaccharides)) and 968 cm^{-1} (PO32- stretching (DNA and RNA ribose)), 928 cm^{-1} (C-N+-C stretching (DNA and RNA ribose-phosphate chain vibration of RNA)) [22,30,32,34] (figure 3. 4B) (Table 3. 1).

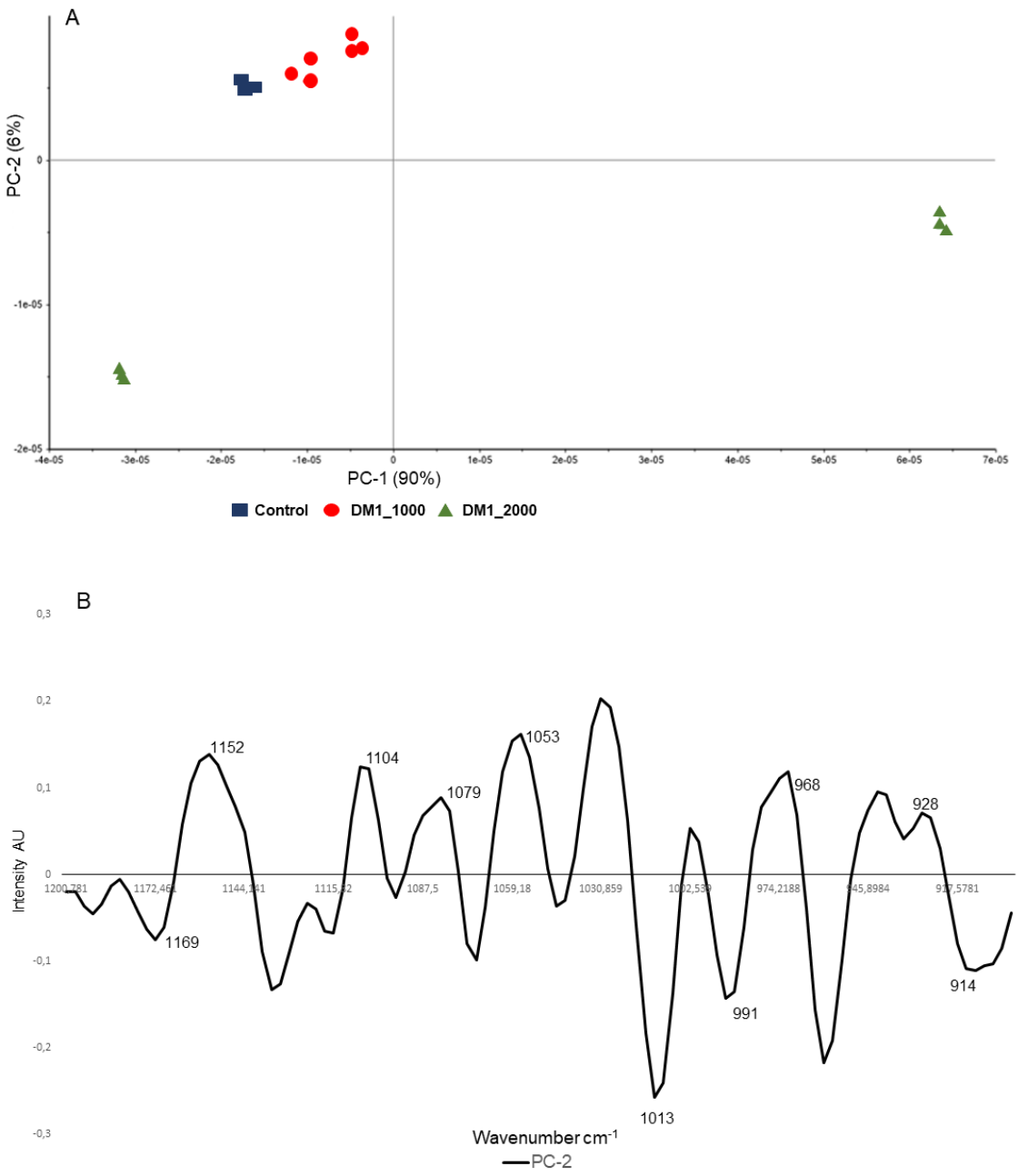


Figure 3. 4. PCA scores and loading profile of DM1-derived fibroblasts and control in the 1200-900 cm^{-1} region. **(A)** PCA scores. **(B)** PC-2 loading profile plot.

3.4.1.2 Intensity ratios obtianed from spectroscopic data of DM1-derived fibroblasts from Coriell institute

The CH2/CH3 ratio that translate the length of lipidic chains, was not significantly different between both DM1-derived fibroblast (DM1_1000 and DM1_2000) and between the DM1-derived fibroblast and respective control, also, there was not a significant difference between the values when applying Kruskal-Wallis test (p= 0.1439) and Dunnett's test was also not significative (figure 3. 5A).

The Unsaturated/Saturated ratio that translate the content of double bonds in the lipid structure, was not within the DM1-derived fibroblast and between the DM1-derived fibroblast and the control, also, there was not a significant different when applying Kruskal-Wallis test (p= 0.2571) and Dunnett's test was also not significative (figure 3. 5B).

The Carbonyl/Total lipid ratio that translate the carbonyl ester concentration in lipids, showed significant result between control and DM1_1000 sample (p=0.0476), between the control and DM1_2000 sample (p=0.023), and between DM1_1000 and DM1_2000 samples (p=0.0022). In addition, Kruskal-Wallis test showed a significant result between the whole group with a p<0.0001 and Dunnett's test was also significant between control and DM1_2000 sample (figure 3. 5C).

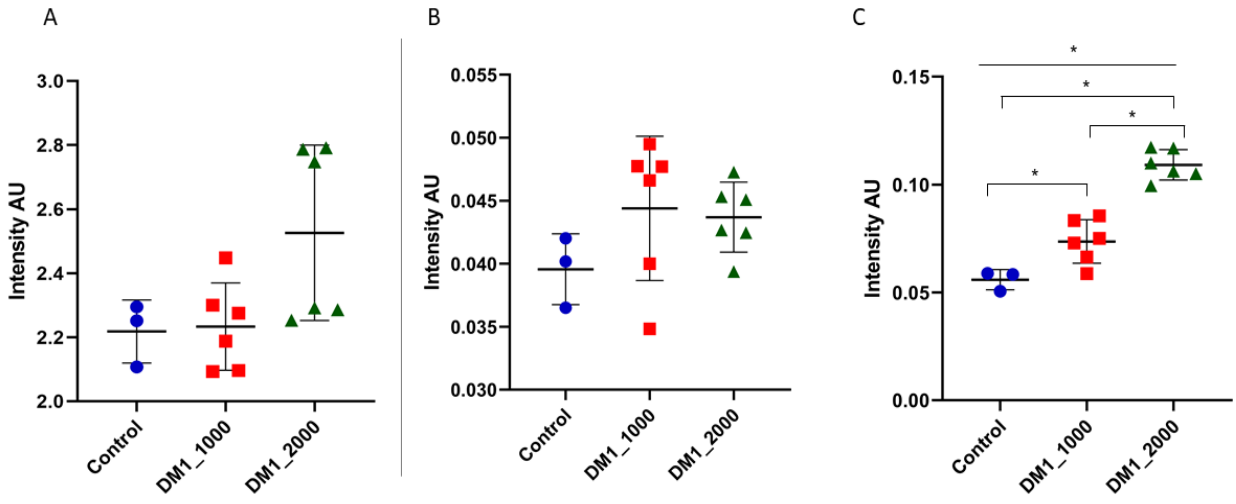


Figure 3. 5. Scatter dot plot of DM1-derived fibroblasts and control ratios. (A) CH_2/CH_3 ratio of DM1-derived fibroblasts and control. **(B)** Unsaturated/Saturated ratios of DM1-derived fibroblasts and control **(C)** Carbonyl/Total lipid ratio of DM1-derived fibroblasts and control [30,31,32]**.** * p≤0.05. **AU:** arbitrary units

Since previous results regarding Coriell fibroblast demonstrated a clear discrimination and statistical significance within the DM1-derived fibroblast (DM1_1000 and DM1_2000) and between DM1-derived fibroblasts and control, the same analytic procedure, both PCA and intensity ratio evaluation, was applied to the fibroblast spectroscopic data obtained from fibroblasts isolated according to experimental procedure at our iBiMED laboratory.

However, due to variables that were not possible to control in Coriell samples the two data sets (DM1-derive fibroblasts from Coriell and at iBiMED) were not possible to analyse together. The difference in the two data sets was confirmed by PCA results presented in (figure 3. 6).

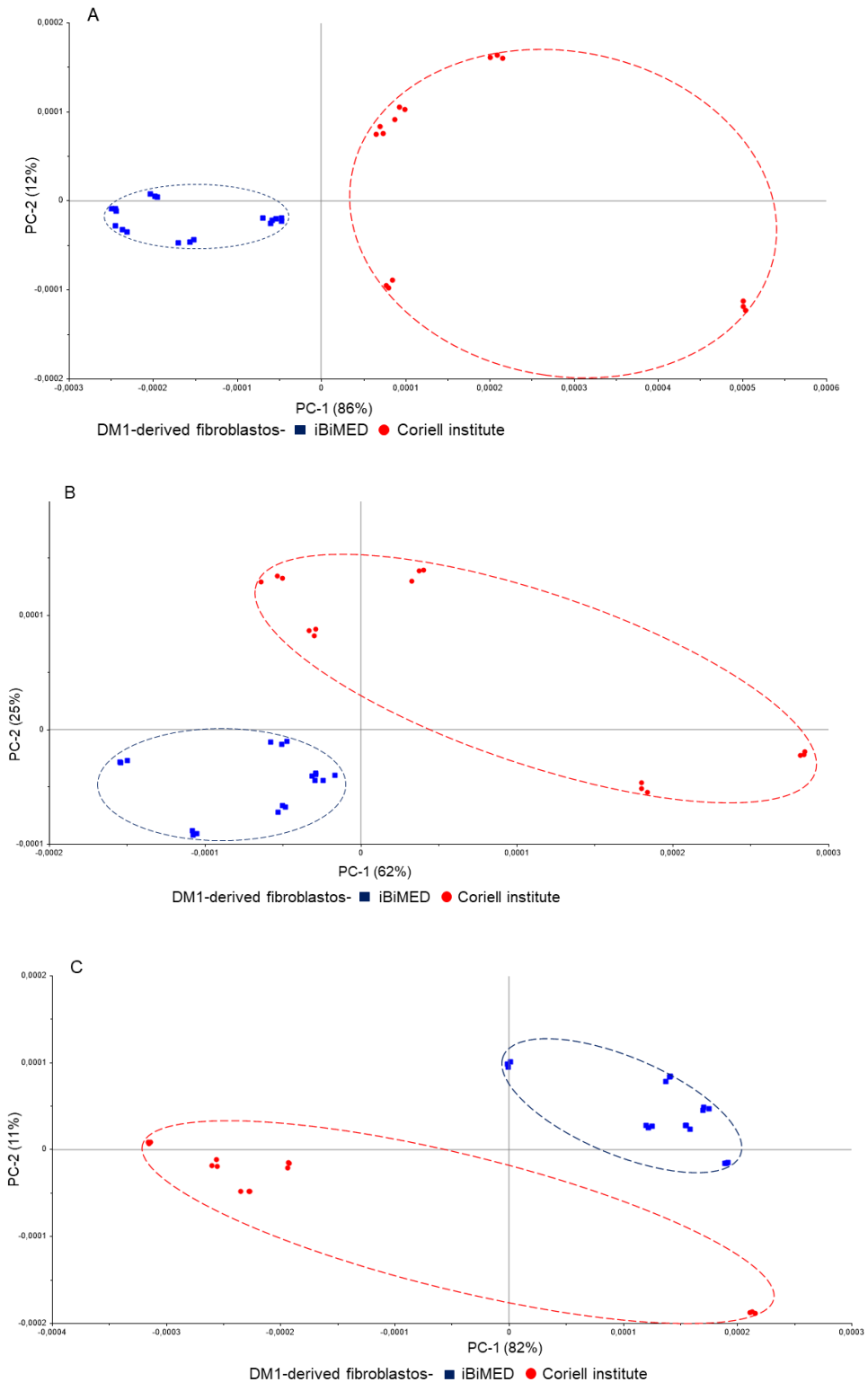


Figure 3. 6. PCA scores of Coriell Institute and iBiMED DM1-derived fibroblasts and controls. (A) 3000-2800 cm^{-1} region. **(B)** 1800-1500 cm^{-1} region. **(C)** 1200-900 cm^{-1} region.

3.4.2 FTIR spectral analysis of DM1-derived fibroblasts established at iBiMED laboratory

ATR-FTIR spectroscopy and multivariate analysis (PCA) were performed to characterize and discriminate between DM1-derived fibroblasts according to the age of onset. Figure 3. 7 shows ATR-FTIR spectral analysis in the region between 4000-600 cm^{-1}. Briefly, the DM1-derived fibroblasts were analysed in the 3000-2800 cm^{-1} (figure 3. 7B), 1800-1500 cm^{-1} (figure 3. 7C) and 1200-900 cm^{-1} (figure 3. 7D) region, which were already discussed and explained previously. The same strategy was followed when analysed the iBiMED control and DM1-derived fibroblasts.

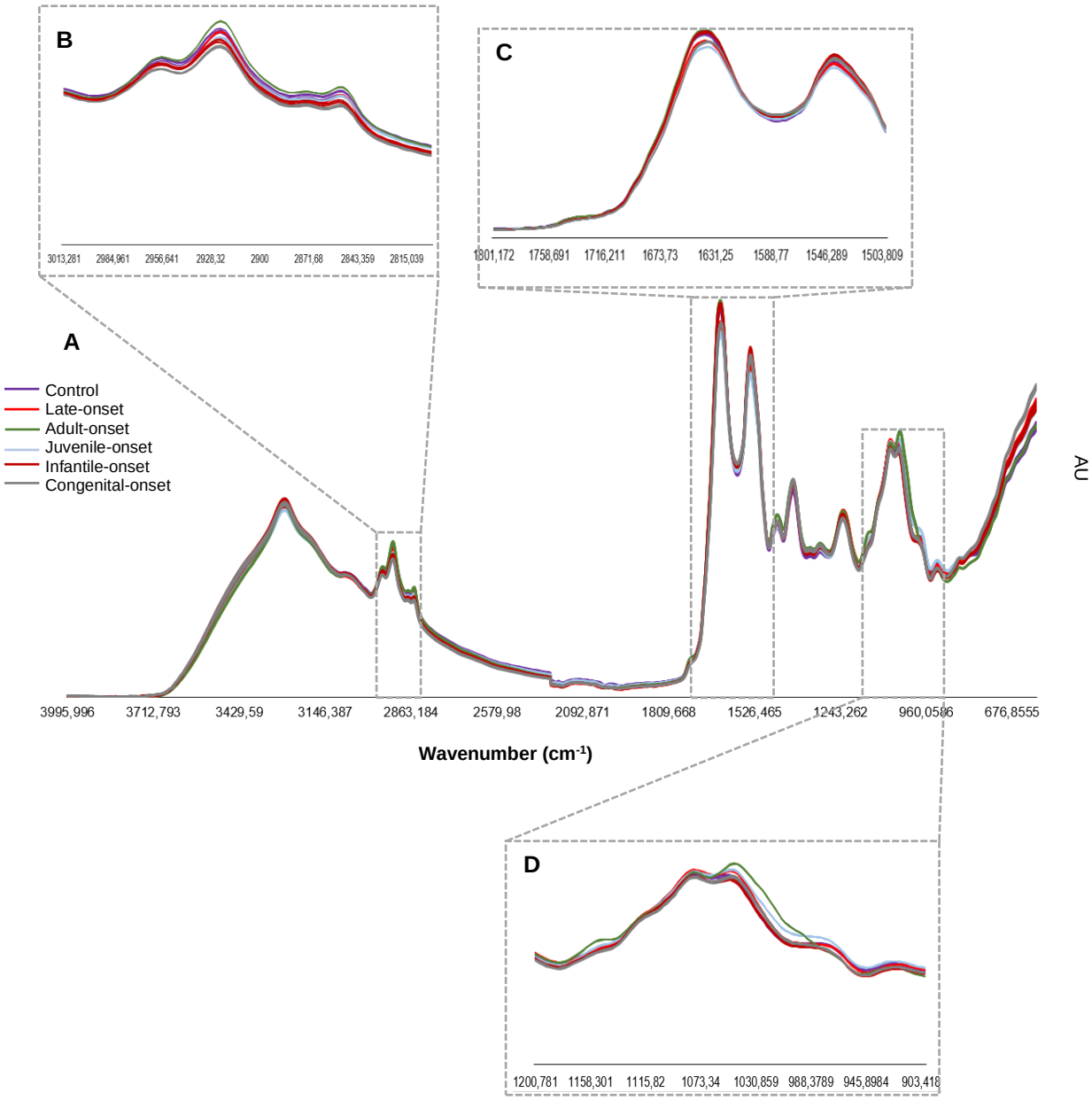


Figure 3. 7. FTIR spectra of DM1-derived fibroblasts and controls. (A) Baseline-corrected and area normalized spectra of Patients' cell lines (late-onset, adult-onset, juvenile-onset, infantile-onset and congenital-onset) and control in 4000-600 cm^{-1} region. **(B)** Amplification of the region 3000-2800 cm^{-1}, **(C)** amplification of the region 1800-1500 cm^{-1}, **(D)** amplification of the 1200-900 cm^{-1} region. X-axis: wavenumber cm^{-1}, Y-axis: arbitrary units (AU) [21,26,29,31,33].

3.4.2.1 Multivariate analysis of the spectroscopic data of DM1-derived fibroblasts established at iBiMED

The first approach of this study was to assess whether the DM1-derived fibroblasts, abbreviated as late-onset (lDM1), adult-onset (aDM1), juvenile-onset (jDM1), infantile-onset (iDM1) and congenital-onset (cDM1) as well as the control could be discriminated from each other through spectroscopic analysis and multivariate analysis.

PCA results from 3000-2800 cm^{-1} was illustrated in figure 3. 8. Interestingly, using PCA score it was possible discriminate across PC-1, control, lDM1 and aDM1 that are located in the PC-1 negative region and jDM1, iDM1 and cDM1 that are located in the PC-1 positive region (figure 3. 8A). According to the loading profile plot, control, lDM1 and aDM1 were characterized by the spectroscopic signals at 2871 cm^{-1} (CH3 symmetric stretching), while jDM1, iDM1 and cDM1 were characterized by the peaks 2959 cm^{-1} (CH3 asymmetric stretching), 2919 cm^{-1} (CH2 asymmetric stretching) and 2851 cm^{-1} (CH2 symmetric stretching) [30,32,34] (figure 3. 8B) (Table 3. 2). In addition, iDM1 and cDM1 located at the Q4 are characterized by the following spectroscopic signals: 2956 cm^{-1} (CH3 asymmetric stretching), 2922 cm^{-1} (CH2 asymmetric stretching) and 2854 cm^{-1} (CH2 symmetric stretching) [30,32,34] (figure 3. 8B) (Table 3. 2).

Table 3. 2. iBiMED DM1-derived fibroblast and control spectroscopic signals, assignments and vibrational mode obtained by PCA in the 3000-2800 cm^{-1}, 1800-1500 cm^{-1} and 1200-900 cm^{-1} regions [22,30,32,34]. **Q -** Quadrant; **-** - Negative quadrants; **+ -** Positive quadrants. DM1-derived fibroblast (congenital-onset (cDM1); infantile-onset (iDM1); juvenile-onset (jDM1); adult-onset (aDM1); late-onset (lDM1)).

3000-2800 cm^{-1} region			
Discrimination across PC-1	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
PC-1 – Control, lDM1, aDM1	2871	CH_3 symmetric stretching	Lipid (long chain fatty acids, phospholipids)
PC-1 + jDM1, iDM1 and cDM1	2959	CH_3 asymmetric stretching	
	2919	CH2 asymmetric stretching	
	2851	CH_2 symmetric stretching	
3000-2800 cm^{-1} region (Q1, Q3 and Q4)			
Quadrant-1	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
jDM1	No peaks	**NA**	Lipid (long chain fatty acids, phospholipids)
Quadrant-3	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
aDM1	No peaks	**NA**	Lipid (long chain fatty acids, phospholipids)
Quadrant-4	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
iDM1 and cDM1	2956	CH_3 asymmetric stretching	Lipid (long chain fatty acids, phospholipids)
	2922	CH_2 asymmetric stretching	
	2854	CH_2 symmetric stretching	
Mid-IR bands at 1800-1500 cm^{-1} region			
PC-2	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
PC-2 – Control, lDM1, aDM1 and jDM1	1648	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: α- helices
	1628	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: parallel β- sheets (peptide, protein)
	1551	60% N–H bending, 40% C–N stretching	Amide II (proteins)
	1537	60% N–H bending, 40% C–N stretching	Amide II (proteins)
	1512	CH_2 bending	Lipid, protein
PC-2 + iDM1 and cDM1	1747	C=O stretching	Triacylglycerol, cholestrol esters, glycerophospholipids
	1696	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: anti-parallel β-sheets (peptide, protein)
	1682	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: anti-parallel β-sheets (peptide, protein)
	1662	80% C=O stretching, 10% N–H	Amide-I: α- helices

		bending, 10% C–N stretching	
	1639	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: parallel β- sheets (peptide, protein)
	1523	60% N–H bending, 40% C–N stretching	Amide II (proteins)
1800-1500 cm^{-1} (Q1, Q2, Q3 and Q4)			
Quadrant-1	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
iDM1	No peak	**NA**	**NA**
Quadrant-2	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
cDM1	1747	C=O stretching	Triacylglycerol, cholestrol esters, glycerophospholipids
	1736		
	1696	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: anti-parallel β-sheets (peptide, protein)
	1682		
	1639		Amide-I: parallel β- sheets (peptide, protein)
Quadrant-3	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
lDM1 and jDM1	No peak	**NA**	**NA**
Quadrant-4	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
Control and aDM1	1631	80% C=O stretching, 10% N–H bending, 10% C–N stretching	Amide-I: parallel β- sheets (peptide, protein)
	1534	60% N–H bending, 40% C–N stretching	Amide II (proteins)
	1515	CH_2 bending	Lipid, protein
1200-900 cm^{-1} region			
PC-4	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
PC-4 – Control, lDM1, aDM1, iDM1	1155	C–O stretching, C-O-H bending	Carbohydrates
	1076	PO^{2-} symmetrical stretching	DNA, RNA, phospholipid, phosphorylated protein
	1025	C-O stretching and C-OH bending	DNA and RNA, oligosaccharides, polysaccharides (e.g. glucose)
	923	C-N^+-C stretching	DNA and RNA ribose-phosphate chain vibration of RNA
PC-4 + jDM1 and cDM1	1121	Phosphodiester groups of PO^{2-}	RNA
	1084	PO^{2-} symmetrical stretching	DNA, RNA, phospholipid, phosphorylated protein
	1047	C-O stretching and C-OH bending	DNA and RNA, oligosaccharides, polysaccharides (e.g. glucose)
	994	C-O stretching	DNA and RNA ribose
	968	PO_3^{2-} stretching	DNA and RNA ribose
	931	C-N^+-C stretching	DNA and RNA ribose-phosphate chain vibration of RNA
	914	C-N^+-C stretching	DNA and RNA ribose-phosphate chain vibration of RNA
1200-900 cm^{-1} region (Q1, Q2, Q3 and Q4)			
Quadrant-1	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
jDM1	1124	Phosphodiester groups of PO^{2-}	RNA
	996	C-O stretching	DNA and RNA ribose
Quadrant-2	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
cDM1	914	C-N^+-C stretching	DNA and RNA ribose-phosphate chain vibration of RNA
Quandrant-3	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
aDM1	1112	Phosphodiester groups of PO^{2-}	RNA
Quadrant-4	**Wavenumber (cm^{-1})**	**Vibrational mode**	**Assigenments**
Control, lDM1 and iDM1	1155	C–O stretching, C-O-H bending	Carbohydrates
	1079	PO^{2-} symmetrical stretching	DNA, RNA, phospholipid, phosphorylated protein
	1042	C-O stretching and C-OH bending	DNA and RNA, oligosaccharides, polysaccharides (e.g. glucose)
	1022		
	926	C-N^+-C stretching	DNA and RNA ribose-phosphate chain vibration of RNA

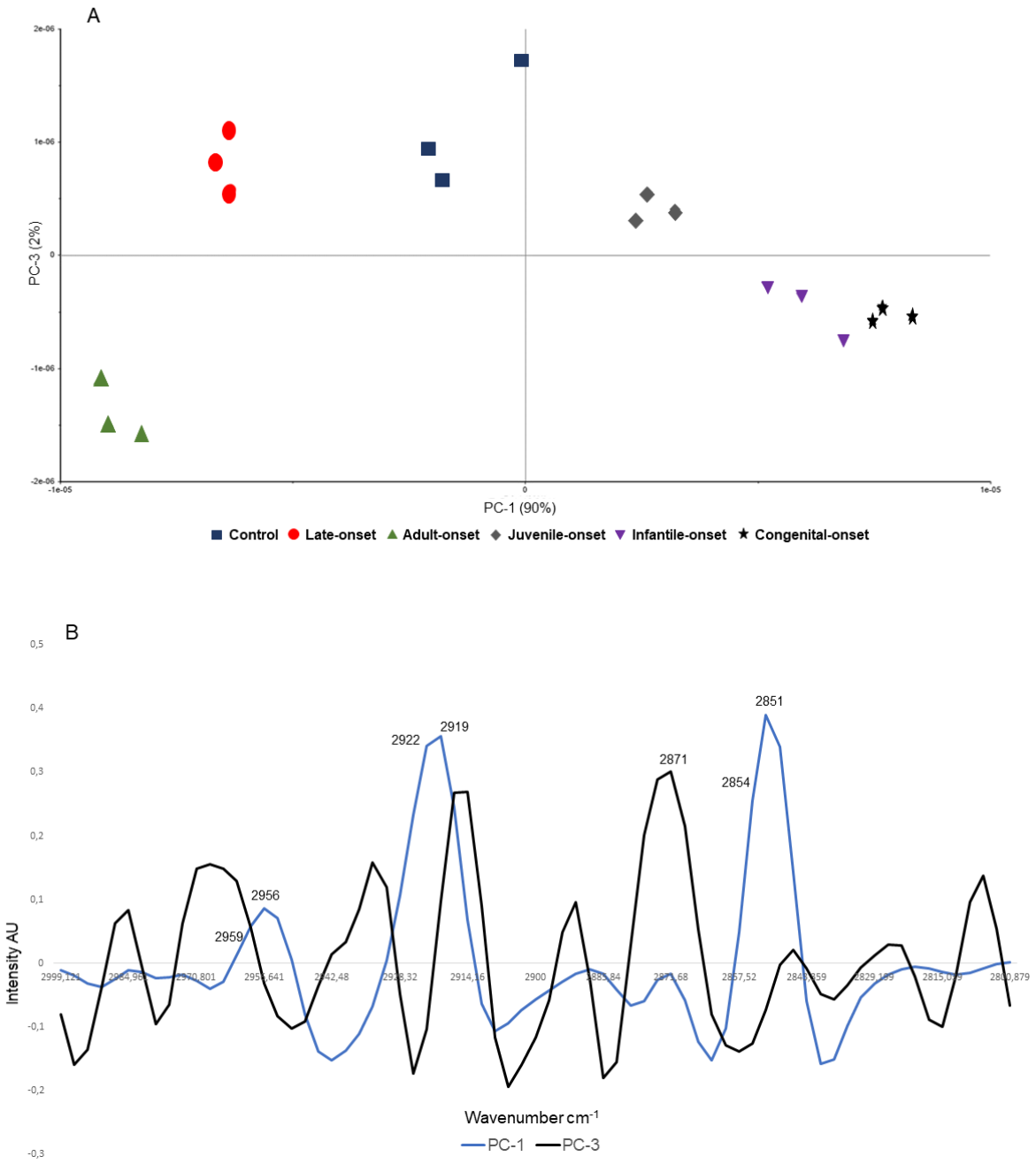
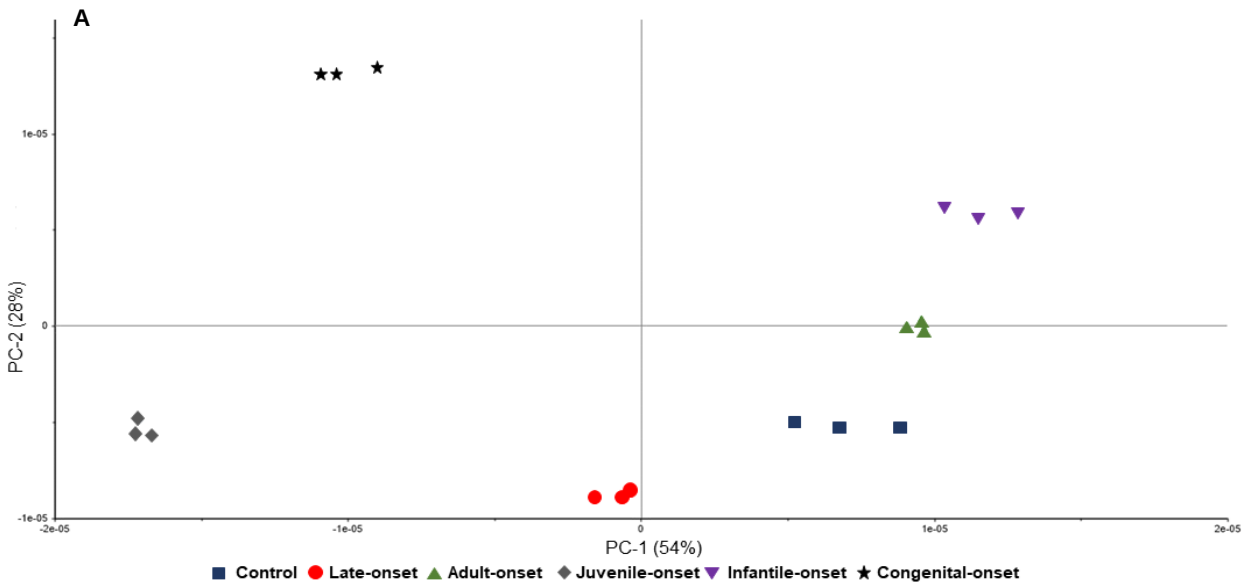


Figure 3. 8. PCA scores and loading profile of DM1-derived fibroblasts and control in the 3000-2800 cm^{-1} region. **(A)** PCA scores. **(B)** PC-1 and PC-3 loading profile plot.

PCA results from 1800-1500 cm^{-1} was illustrated in figure 3. 9. In this case it was possible to discriminate the samples thought PC-2, where iDM1 and cDM1 were located in the positive PC-2, lDM1, aDM1 and jDM1 were located in the negative PC-2 (figure 3. 9A). The loading plot showed that iDM1 and cDM1 was characterized by 1747 cm^{-1} (C=O stretching), 1696 cm^{-1} and 1682 cm^{-1} (Amide-I: anti-parallel β-sheets (peptide, proteins)), 1662 cm^{-1} (Amide-I: α-helices), 1639 cm^{-1} (Amide-I: parallel β- sheets (peptide, protein)) and 1523 cm^{-1} (Amide II (proteins)), while control, lDM1, aDM1 and jDM1 were characterized by the following spectral assignments 1648 cm^{-1}

(Amide-I: α-helices), 1628 cm^{-1} (Amide-I: parallel β-sheets (peptide, protein)), 1551 cm^{-1} and 1537 cm^{-1} (Amide II (proteins)) and 1512 cm^{-1} (Lipid, protein) [22,30,32] (figure 3. 9B) (Table 3. 2).

In addition, cDM1 could be discriminated from control and aDM1, lDM1 and jDM1, and iDM1. In fact, iDM1 was in the Q1 (positive PC-1 and negative PC-2), cDM1 was in the Q2 (negative PC-1 and positive PC-2), lDM1 and jDM1 were located at Q3 (negative PC-1 and PC-2) and control and aDM1 were located at Q4 (positive PC-1 and negative PC-2) (figure 3. 9B). cDM1 was characterized by: 1747 and 1736 cm^{-1} (C=O stretching), 1696 cm^{-1} and 1682 cm^{-1} (Amide-I: anti-parallel β-sheets (peptide, protein)) and 1639 cm^{-1} (Amide-I: parallel β-sheets (peptide, protein)). Control and aDM1 were characterized by the peaks located at 1747 cm^{-1} and 1743 cm^{-1} (C=O stretching), 1682 cm^{-1} (Amide-I: anti-parallel β-sheets (peptide, protein)), 1651 cm^{-1} (Amide-I: α-helices), 1554 cm^{-1} (Amide II (proteins)) and 1543 cm^{-1} (Amide II (proteins)) [22,30,32] (figure 3. 9B) (Table 3. 2).



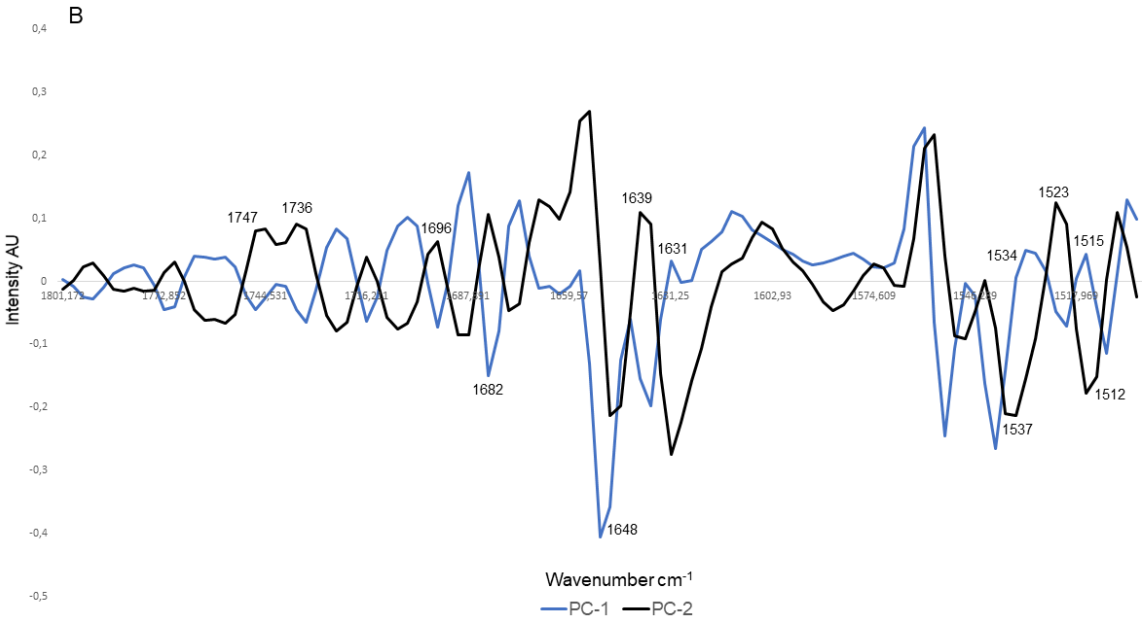


Figure 3. 9. PCA scores and loading profile of DM1-derived fibroblasts and control in the 1800-1500 cm^{-1} region. **(A)** PCA scores. **(B)** PC-1 and PC-2 loading profile plot

PCA results from 1200-900 cm-^{1} region was illustrated in figure 3. 10. In the PCA score it is possible discriminate by PC-4, where jDM1 and cDM1 were in the positive side of the PC-4 while control, lDM1, aDM1 and iDM2 were in the negative side of PC-4 (figure 3. 10A). PC-4 allowed a clear separation between jDM1 and cDM1 samples and control, lDM1, aDM1 and iDM2 samples. According to the loading plot, jDM1 and cDM1 samples were characterized by the spectroscopic signals located 1121 cm^{-1} (Phosphodiester groups of PO2− (RNA)), 1084 cm^{-1} (PO2− symmetrical stretching (DNA, RNA, phospholipid, phosphorylated protein)), 1047 cm^{-1} (C-O stretching and C-OH bending (DNA, RNA, oligosaccharides, polysaccharides)), 994 cm^{-1} (C-O stretching (DNA and RNA ribose)), 968 cm^{-1} (PO32- stretching (DNA and RNA ribose)) and 914 cm^{-1} (C-N+-C stretching (DNA and RNA ribose-phosphate chain vibration)), while control, lDM1, aDM1 and iDM2 samples were characterized by the following spectroscopic signals 1155 cm^{-1} (C–O stretching, C-O-H bending (Carbohydrates)), 1076 cm^{-1} (PO2− symmetrical stretching (DNA, RNA, phospholipid, phosphorylated protein)), 1025 cm^{-1} (C-O stretching and C-OH bending (DNA, RNA, oligosaccharides, polysaccharides)), 923 cm^{-1} (C-N+-C stretching (DNA and RNA ribose-phosphate chain vibration)) [22,30,32,34] (figure 3. 10B).

Also, control, lDM1 and iDM1 samples could be discriminated from aDM1, jDM1 and cDM1 samples. jDM1 sample was in the Q1 (positive PC-1 and negative PC-2), cDM1 was in the Q2 (negative PC-1 and positive PC-2), aDM1 samples was located at Q3 (negative PC-1 and PC-2) and control, lDM1and iDM1 sample were located at Q4 (positive PC-1 and negative PC-2) (figure

3. 10C). jDM1 was characterized by the peaks: 1124 cm^{-1} (Phosphodiester groups of PO2− (RNA)) and 996 cm^{-1} (C-O stretching (DNA and RNA ribose)). cDM1 sample was characterized by the peak at 914 cm^{-1} (C-N+-C stretching (DNA and RNA ribose-phosphate chain vibration)). aDM1 sample was characterized by the peak at 1112 cm^{-1} (Phosphodiester groups of PO2− (RNA)). Control, lDM1 and iDM1 sample are characterized by the following peaks: 1155 cm^{-1} (C–O stretching, C-O-H bending (Carbohydrates)), 1079 cm^{-1} (PO2− symmetrical stretching (DNA, RNA, phospholipid, phosphorylated protein)), 1042 cm^{-1} and 1022 cm^{-1} (C-O stretching and C-OH bending (DNA, RNA, oligosaccharides, polysaccharides)) and 926 cm^{-1} (C-N+-C stretching (DNA and RNA ribose-phosphate chain vibration)) (figure 3. 10C) [22,30,32,34].

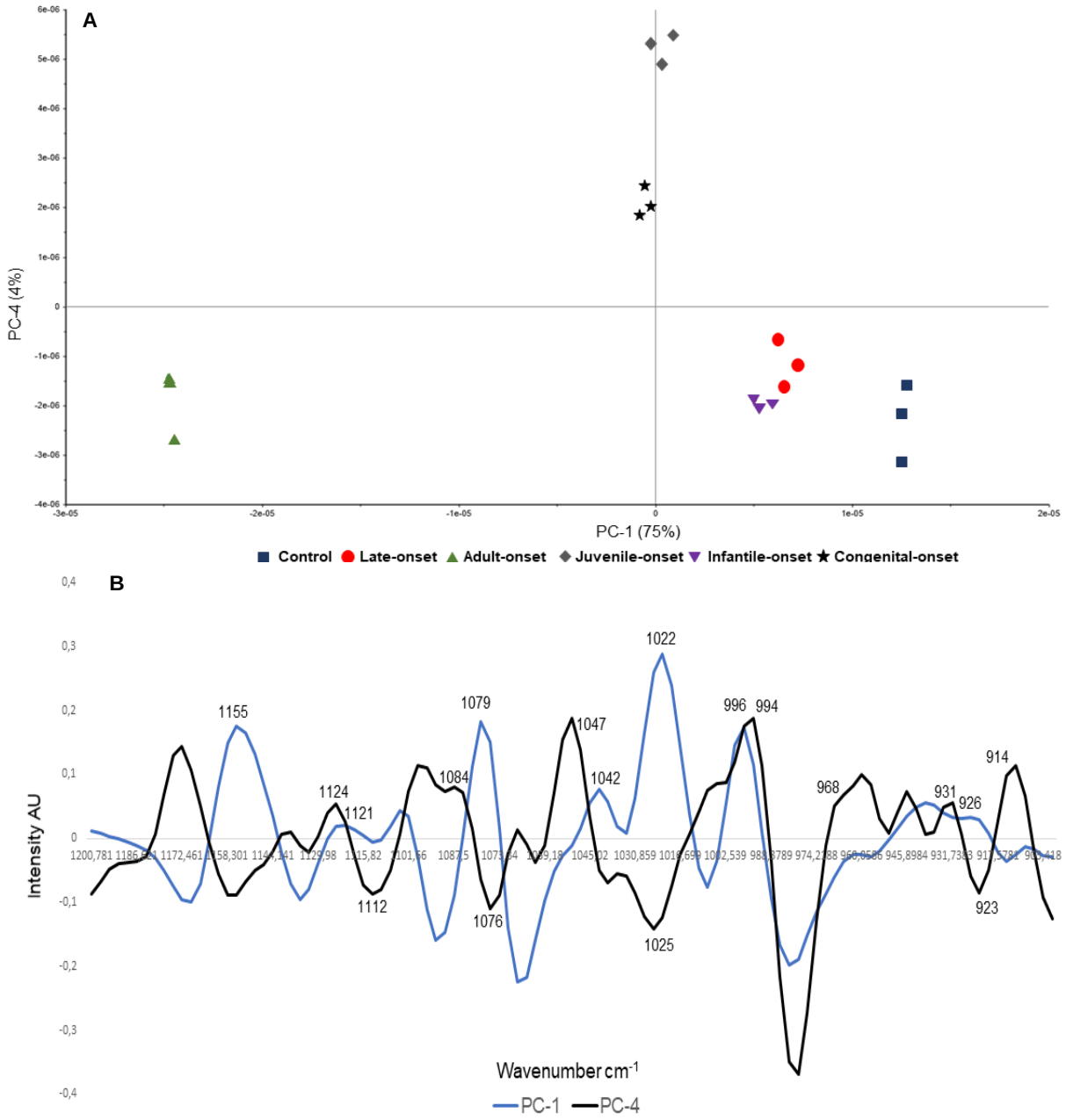


Figure 3. 10. PCA scores and loading profile of DM1-derived fibroblasts and control in the 1200-900 cm^{-1} region. **(A)** PCA scores. **(B)** PC-1 and PC-4 loading profile plot.

3.4.2.2 Intensity ratios obtained from spectroscopic data of DM1-derived fibroblasts established at iBiMED

The CH2/CH3 ratio that translate the length of lipidic chains, was not significantly different between control and all DM1-derived fibroblasts tested and within DM1-derived fibroblast (aDM1 and jDM1, iDM1 and cDM1), also, Kruskal-Wallis test (p = 0.3538) and Dunnett's test were not significative (figure 3. 11A).

The Unsaturated/Saturated ratio that translate the content of double bonds in the lipid structure, was not significantly different between the control and DM1-derived fibroblasts tested and within the DM1-derived fibroblast (aDM1 and jDM1, iDM1 and cDM1). Whereas when comparing the whole group using the Kruskal-Wallis test the results were statistically significant (p= 0.0459) as well when Dunnett's test was used (figure 3. 11B).

The Carbonyl/Total lipid ratio that translate the carbonyl ester concentration in lipids, was not significantly different between the control and all DM1-derived fibroblasts tested and within the DM1-derived fibroblast (aDM1 and jDM1, iDM1 and cDM1). Whereas Kruskal-Wallis test showed a significant difference between the whole group (p=0.0135). Dunnett's test was not significant (figure 3. 11C).

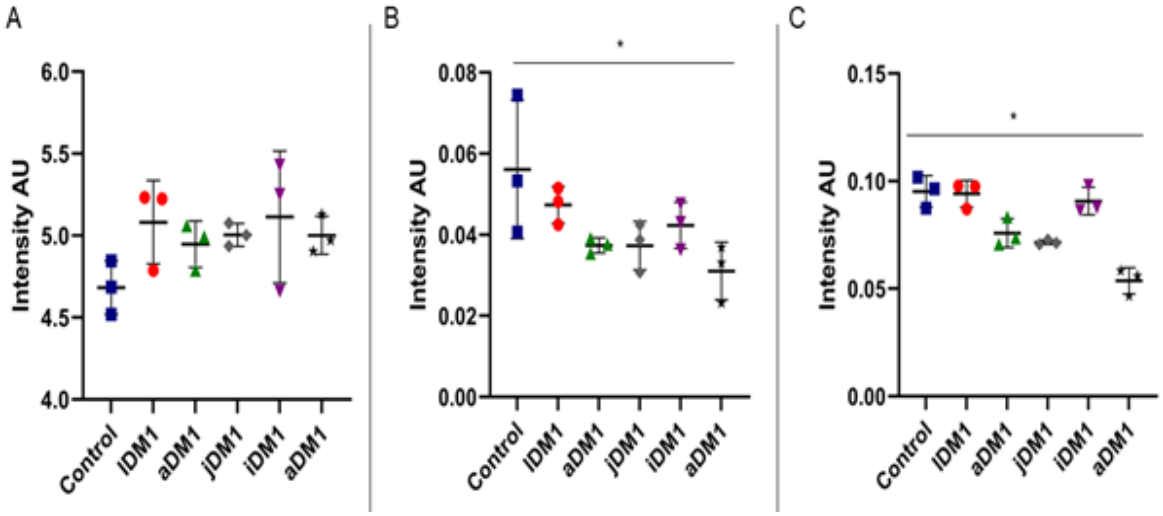


Figure 3.11. Scatter dot plot of DM1-derived fibroblasts and control ratios. (**A**) CH_2/CH_3 ratio (**B**) Unsaturated/Saturated ratios of (**C**) Carbonyl/Total lipid ratio [31,32,33]. * p≤0.05. AU: arbitrary units

3.5 Discussion:

Patients with DM1 present several metabolic alterations, namely, insulin resistance, hypertriglyceridemia, increased fat mass, high levels of low-density lipoprotein (LDL), low levels of high-density lipoproteins (HDL), hypertension, and abdominal obesity [10,12–17]. Given these metabolic dysfunctions observed in patients with DM1, the evaluation of the metabolic profile of

those patients is important. For that purpose, in the present study, we performed ATR-FTIR spectroscopy analysis coupled with PCA (an exploratory tool of multivariate analysis) to characterize and discriminate the biomolecular profile within skin DM1-derived fibroblasts and control established either by Coriell Institute or by does at iBiMED laboratory.

The analysis of the DM1-derived fibroblasts from Coriell Institute showed clear alterations between DM1-derived fibroblasts with different CTG repeat length (DM1_1000 with DM1_2000) at the molecular level and the same happened with does at iBiMED. These alterations were mainly observed in the 1800-1500 cm^{-1} region. In both spectral regions of 3000-2800 cm^{-1} and 1800-1500 cm^{-1} there was also, possible to discriminate between DM1-derived fibroblasts and control group for both Coriell Institute and iBiMED fibroblasts.

In the DM1-derived fibroblasts (Coriell Institute), there was a clear discrimination, provided by PCA analysis, between both DM1-derived fibroblasts and controls across PC-2. This discrimination is due to spectral assignments in the 3000-2800 cm^{-1} spectroscopic region in which, DM1_2000 were characterized by a higher contribution of CH3 stretching signal, which may be associated with shorter lipidic stretching, while control and DM1_1000 were characterized by CH2 stretching signal, which can be associated with longer lipid chains [31,33].

Regarding iBiMED DM1-derived fibroblasts, there was a clear discrimination between the most severe forms of DM1 (congenital-onset, infantile-onset and juvenile-onset) from control, late-onset and adult-onset in the 3000-2800 cm^{-1} region and across PC-1. The most severe forms of DM1 were characterized by both CH3 and CH2 asymmetric stretching, meaning that, these forms may have a higher number of lipid content in comparison to the control, late-onset and adult-onset form. This higher number of shorter lipidic stretching may be associated with deficient in lipin protein. Lipin is a key enzyme that regulates transcriptional co-activators of fatty acid β-oxidation, lipid metabolism and signaling through the conversion of phosphatidic acid into diacylglycerol, in which diacylglycerol formations will be converted into triacylglycerol, phosphatidylethanolamine and/or phosphatidylcholine [11,35–38]. In a previous study was shown common features of DM1 abnormal metabolism and the potential role of lipin with dyslipidemia, insulin resistance, skeletal-muscle impairment, immune system (macrophages) and adipose tissues [11], demonstrating once again the importance of further studies related to the lipid metabolism of these patients. Also, our results could be associated with the increase of lipid peroxidation, consistent with the decrease in the olefinic content in the cellular membrane of DM1-derived fibroblasts (unsaturated/saturated ratio) due to the oxidation and degradation of lipids, since lipid peroxidation modifies the physical properties of lipids as demonstrated in previous studies, as described below [31,33].

In the spectral region of 1800-1500 cm^{-1}, it was possible to discriminate DM1_2000 sample from control and DM1_1000 (Coriell Institute), across PC-1. DM1_2000 sample were characterized by the peak assigned to Amide-I whose location may indicate the parallel β-sheet associated with protein aggregates [30,39]. Amide-I: parallel β-sheet are energetically less stable than Amide-I:

anti-parallel β-sheets and Amide-I: α-helices, and occur more rarely in proteins [40]. Also, this protein aggregates may be related to the abnormal size of transcribed mutant RNA (CTG expansion). These mutant RNA with CUG repeats accumulates in the nucleus as a ribonuclear foci, sequestering and deregulating muscleblind-like protein1 (MBNL1) and CUG-BP-ELAV-Like family member1 (CUGBP1 or CELF1), which are important regulators of alternative splicing of other genes and core proteins in DM1 disease complexity [11,41,42]. As for control and DM1_1000 were identified mainly by C=O stretching (Triacylglycerol, cholestrol esters, glycerophospholipids) and by Amide-I: anti-parallel β-sheets and Amide-I: α-helices. Despite the differences in the spectral assignments of Amide I group, which may be due to the protein mixture of the secondary structure, the increase of C=O stretching band corresponds to the lipid peroxidation seems to be the principal reason of discrimination between the control and DM1_1000 [31,33].

As for iBiMED DM1-derived fibroblast in the spectral region of 1800-1500 cm^{-1}, it was possible to discriminate between the congenital and infantile-onset from the control, late-onset, adult-onset and juvenile-onset provided by PC-2. The congenital-onset and infantile-onset fibroblast were mainly identified by the C=O stretching (Triacylglycerol, cholestrol esters, glycerophospholipids), which may be related to the lipid peroxidation. Lipid peroxidation is an oxidative degradation of lipids (e.g. membrane lipids, such as glycolipids, phospholipids and cholestrol), in which there is increased degradation of polyunsaturated fatty acids, giving rise to peroxides primary products, and then broken down into secondary products with shorter hydrocarbon chains and carbonyl compounds [31,33,43,44]. The increased lipid peroxidation may lead to atherosclerosis, Alzheimer's disease, rheumatic arthritis, cancer, immune disorders, among other features, since it causes uncontrolled oxidative stress, alterations in cell signalling, protein and DNA damage and cytotoxicity [31,43,45].

In the 1200-900 cm^{-1}, there is a clear discrimination of DM1_2000 from control and DM1_1000 through PC-2 analysis. However, it is difficult to infer biochemical differences between the samples, since this region is characterized by many different biomolecules, namely fatty acids, sugars, amino acids, nucleic acids, among other [23,25,31,33]. As for the spectral region of 1200-900 cm^{-1}, there is a clear discrimination of congenital-onset and juvenile-onset from control group, late-onset, adult-onset and infantile-onset provided by PC-4. As described above, it is difficult to infer biochemical differences between the samples, since this region is characterized by many different biomolecules [23,25,31,33].

The statistically analysis also showed significant results between DM1_2000 and control samples and DM1_1000 sample, in the 1800-1500 cm^{-1} region, once again showing the importance of the lipid peroxidation as a valuable target to discriminate between patients with different severities and to further studies.

Interestingly, changes in lipid absorption (3000-2800 cm^{-1} region) and ester cholestrol, phospholipids and proteins absorption (1800-1500 cm^{-1} region) occur in both cell lines of patients

with DM1 and control from Coriell Institute and does cultured at our iBiMED laboratory. However, the 1800-1500 cm^{-1} region clearly discriminates the most severe forms of DM1 disease (congenital-onset and infantile-onset) and DM1_2000 samples from the other forms of DM1 disease (late-onset, adult-onset and juvenile-onset) and DM1_1000 samples. Also, it was possible to discriminate between from the most severe forms of DM1 disease and DM1_2000 samples from the control groups. These discriminations mean that there are changes in lipid content and in lipid peroxidation, and that ATR-FTIR spectroscopy analysis of 1800-1500 cm^{-1} region may be the region of choice to help discriminate between the different severity forms of DM1 disease.

Although the promising results, this study have some limitations, due to the small sample-size it was difficult to make the statistical analysis, which may lead to bias results. Also, the age at onset of the DM1-derived fibroblasts obtained from Coriell Institute were not known, as well as, other variables of the protocol, which may be present on the heterogeneity seen in previously (figure 6). Therefore, it is important to establish a large cohort with more heterogeneity of the severities of the DM1 disease, to properly evaluate the usefulness of ATR-FTIR spectroscopy, to take in consideration of all variabilities, meaning that a strict protocol must be followed, in which all variables are known, in order to obtain a consistent homogeneity of the samples. Also, it is important to statistically analyse the differences of the lipidic profile between the different severities DM1 disease. It would be interesting for future studies the analysis of DM1-derived fibroblasts through other metabolic techniques such as NMR and MS to understand deeply which classes and species are responsible for changes in the lipid metabolism in patients with DM1, since ATR-FTIR spectroscopy is uncapable of detecting specific metabolites that are responsible for causing metabolic changes.

3.6 Conclusion:

This work demonstrated that ATR-FTIR spectroscopy with the aid of multivariate analysis (PCA) is a valuable and useful technique as a screening tool for initial confirmation of the severity of the disease between patients with DM1, particularly in the 1800-1500 cm^{-1} region, as well, it can give important biochemical insight of the lipid metabolism alterations, allowing the understanding of the DM1 disease complexity and further research and the development of new therapeutics.

Although, the small sample size hampers the building of evidence of FTIR spectroscopy technique in such a fundamental domain as lipid metabolism in patients with DM1 the results obtained highlight the need for further research with a well characterized and larger cohort.

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Chapter IV. Muscle strength measurements in patients with DM1

4. Muscular strength measures in Myotonic dystrophy type 1: A Systematic Review

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4.1 Abstract

Background: Measurement of muscle strength is fundamental for the management of myotonic dystrophy type 1 (DM1). Nevertheless, guidance on this topic is somewhat limited due to heterogeneous outcomes and measures used.

Objectives: This systematic literature review aimed to summarise the most common outcomes and measurements previously reported to assess muscle strength in patients with DM1.

Methods: We searched Pubmed, Web of Science and Embase databases. Studies using measures of muscle strength assessment in adult patients with DM1 were included.

Results: From a total of 80 included studies, 24 studies measured cardiac muscle strength indirectly, 45 measured skeletal muscle strength and 23 measured respiratory muscle strength. Echocardiography (n=22), quantitative muscle test (n=39), manual muscle test (n=15) and manometry (n=17) were the most common methods to assess cardiac, skeletal and respiratory muscle strength, respectively. Measures of choice to assess muscle strength were (1) ejection fraction (n=21) for cardiac muscle; (2) muscle isometric torque (n=10) and medical research council (n=12) for skeletal muscle and (3) maximal inspiratory (n=15) and expiratory (n=17) pressure for respiratory muscles.

Conclusion: We successfully gather the more consensual and important measures to evaluate muscle strength. Results will be valuable for muscle strength evaluation in future studies, particularly to test muscle strength response to treatments in patients with DM1. Consensus on a minimum set of measures to evaluate muscle strength (Core Outcome Set), would be important in patients with DM1.

4.2 Introduction

Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults with a prevalence of 1 in 8,000 individuals worldwide [1]. DM1 is an autosomal dominant hereditary disease caused by the abnormal expansion of unstable repetitions of cytosine-thymine-guanine trinucleotide (CTG) in the 3' untranslated region of Myotonic Dystrophy Protein Kinase (DMPK) gene and present different phenotypes according to the age of onset and size of CTG repeat expansion [2,3]. Accordingly, the phenotypes of patients with DM1 can be categorized as follows: normal (5-37 repeats), pre-mutation (38-49 repeats), late-onset (>40 years; 50-149 CTG repeats), adult-onset (20-40 years; 150-1000 CTG repeats), juvenile (10-20 years; >400 CTG repeats), infantile (1 month-10 years; >500 CTG repeats) and congenital (<1 month; ≥1000 CTG repeats) [4–7]. These patients experience muscular and non-muscular manifestations and in more severe cases prematurely death can occur [2,8,9]. Muscle dysfunction is clinically characterized by myotonia, progressive distal muscle weakness, muscle pain, and muscle atrophy [2,3,10], which affects skeletal muscles but also the muscles associated with the cardiac and respiratory systems.

Cardiac dysfunction is the second most common cause of death (30% of mortality) in adults with DM1 [11]. Cardiac conduction abnormalities and myocardial fibrosis are the most common features observed in patients with DM1, resulting in sudden death [11–13]. Respiratory dysfunction is the most common cause of death in patients with DM1 (51-76%), especially in the congenital form [11,14]. Respiratory disease is the combination of both peripheral respiratory dysfunction and central respiratory drive dysfunction, upper airway muscle dysfunction and high risk of pulmonary infections [11,14,15].

Therefore, an increased interest in assessing and developing tailored interventions for muscle strength (skeletal, cardiac, and respiratory) in this population has been observed. Nevertheless, knowing what to measure and how to measure muscle strength is currently challenging as the evidence is widespread in the literature [16]. Therefore, the aim of this systematic review was to systematically gather information about the most common outcomes and measures used to assess muscle strength in adult patients with DM1 to contribute for future clinical practice guidance and research.

4.3 Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) checklist for systematic reviews and meta-analysis [17]. The protocol used for this review was registered in the International prospective registry for systematic reviews (PROSPERO) (CRD42020143429).

4.3.1 Search Strategy

Searches were conducted in Web of Science, PubMed and EMBASE and were complemented by weekly automatic updates retrieved from 01 January 2000 until 18 September 2020 (search strategies in supplementary material).

Studies were considered eligible if: (1) Included adult patients with DM1(≥ 18 years old), (2) were written in English, Portuguese, Spanish or French, (3) were original quantitative studies published in scientific journals or in conference proceedings after the year 2000, to ensure that the method and outcome measures of muscle strength reflect the current standard practices. Studies were excluded if they met any of the following criteria: (1) abstract and title not available, (2) Meta-analysis, systematic review, qualitative studies, news, research protocols, theses, dissertations, abstracts/conference abstract, letters to the editor, unpublished work, editorials, commentaries, books, book chapters, systematic reviews (references checked), guidelines, statements and position paper and case reports, (3) focuses on research with animals in vitro or molecular studies (4) studies not addressing DM1-related muscle strength (5) studies involving proxy versions, (6) interventional studies.

Three independent researchers (T.M, A.C., D.V.) screened studies' titles and abstracts and subsequently reviewed the full text of the selected records for quantitative analysis. Potential disagreements were solved with consensus and, when needed, with the involvement of a fourth investigator (A.M., T.H., S.R.).

4.3.2 Quality assessment

The quality of each included study was assessed by 2 investigators independently using the National Institute of Health, National Heart, Lung and Blood Institute, Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies [18]. This tool includes 14 different questions to assess "research question", "selection of study population", "risk of bias", "groups recruited from the same population and uniform eligibility criteria", "sample size justification", "exposure assessed prior to outcome measurement", "sufficient timeframe to see an effect", "different levels of exposure of interest", "exposure measured and assessment", "repeated exposure assessment", "outcomes measures", "blinding", "follow-up rate", and "statistical analyses", which are used to evaluate the quality of the studies as "Good", "Fair" or "Poor". For each criterion, the following answers were given "Yes", "Not applicable (NA)" or "Cannot Determine (CD)". One point was given to a "Yes", "Not applicable (NA)" and "Cannot Determine (CD)" answer, whereas a "No" and "Not Reported (NR)" was graded with zero. After rating all the 14 different criteria, the quality assessment was given by the sum of all criteria according to following studies rating scores: if the studies scored between 0-4 it would be considered as "Poor", between 5-9 as "Fair" and between 10-14 as "Good".

Inter-rater agreement was assessed using Cohen's kappa coefficient to explore the consistency of the quality assessment performed by the two reviewers. Cohen's kappa was interpreted as: i) 0-0.2: slight agreement; ii) 0.21-0.4: fair agreement; iii) 0.41-0.6 moderate agreement; iv) 0.61-0.8: substantial agreement; v) 0.81-1: almost perfect agreement [19].

4.3.3 Data extraction, synthesis, and analysis

From the final included studies, only data assessing muscle strength of cardiac, skeletal and respiratory muscles were extracted. Additionally, functional measures which can indirectly influence muscle strength measures were also retrieved and can be found in supplementary table 4.1 (online resource) (volumes, diameters, and mechanics of breathing parameters).

Data were gathered and extracted to a structured table (supplementary table 4.1 (online resource)) with the following parameters: authors, year of publication, number of participants (patients and controls), age and sex, CTG repeat length, measures and main findings. Data regarding age, sex, CTG repeat length, Body Mass Index and degree of muscle impairment were collected to characterize the population. Three summary tables (table 4.1, table 4.2 and table 4.3) were created to synthetize results of the outcome and measures of cardiac muscle strength, skeletal-muscle strength and respiratory muscle strength.

The following definitions and interpretations of the muscle strength measures were used for the purpose of this systematic literature review.

Echocardiography is a test which uses ultrasound to produce an echocardiogram (cardiac imaging), allowing the monitorization of the heart and valves function [20]. Echocardiography results were considered abnormal if there was, left ventricular ejection fraction ≤50% and global longitudinal strain > -15,9% [21–23]. Additional cardiac function measures corresponding to volumes and diameter can be found in supplementary table 4.1, since those are used to calculate ejection fraction, stroke volume and shortening fraction.

Ejection fraction measures the percentage of blood pumped out from the left ventricle during each contraction of the systole (stroke volume) in relation to the total volume of blood present in the left ventricle at end of diastole. Stroke volume is calculated by the difference between left ventricular end-diastolic volume and left ventricular end-systolic volume. Ejection fraction is calculated by (stroke volume / left ventricular end-diastolic volume) x 100 [21].

Shortening fraction measures the heart's muscle contractility by calculating the percentage of the ventricle diameter change in between diastole and systole and it is calculated by ([left ventricle end-diastolic diameter - left ventricular end-systolic diameter] / left ventricle end-diastolic diameter) x 100 [22].

Global longitudinal strain is a parameter which measures longitudinal shortening as a percentage of change in length during systole in relation to the length during diastole [23].

Cardiac magnetic resonance is a highly sensitive non-invasive medical imaging tool to assess the strength and structure of the cardiovascular system. Abnormal cardiac magnetic resonance results were considered if: left ventricular ejection fraction < 55% and/or right ventricular ejection fraction < 45% [24].

Muscular impairment rating scale (MIRS) is a 5-point rating scale that categorizes patients according to the progression of distal to proximal muscle weakness, based on manual muscle tests [25]. MIRS ranges from I-V, in which MIRS-I represents no muscle impairment and MIRS-V represents severe proximal weakness [25].

Manual muscle test is a test which evaluates qualitatively the maximum isometric muscle strength of muscle groups and results are commonly presented as a Medical Research Council (MRC) scale [26].

Quantitative muscle test is a test that assesses the maximum isometric muscle strength26. The muscle isometric torque (corresponds to the effect of rotation of a force) is a muscle strength parameter that is obtained by multiplying the units of force (Newtons) by the length of the corresponding member (m) and it is expressed in Nm [27,28]. Muscle isometric strength consists of the measurement of the strength of a given muscle without changing the length of the muscle and

the biomechanical circumstances of the joint involved during contraction. This parameter is normally expressed in N [29]. Maximal voluntary isometric contraction is a method that safely provides interval data (typically in units of Kg or N of force) to assess muscle strength and has been widely used to study neuromuscular disorders [30].

Maximal inspiratory pressure and maximal expiratory pressure are measures that evaluate respiratory muscle strength. In these cases, patients need to perform a maximum forceful inspiration or expiration, against an occluded mouthpiece, reflecting inspiratory and expiratory muscle strength [31]. Both maximal inspiratory pressures and maximal expiratory pressure are measured in cmH2O or can be interpreted as predicted normal values for age, sex, height, and mass (% of predicted) [31]. Sniff nasal inspiratory pressure is similar to the measure previously described but can be easily performed by patients that have low levels of coordination via s nasal sniff [31]. In clinical practice, inspiratory muscle weakness can be excluded if the individuals have a transdiaphragmatic pressure during sniff greater than ≥80-100 cmH2O in males and ≥70-80 cmH2O in females and/or sniff nasal inspiratory pressure ≥70 cmH2O in males and ≥60 cmH2O in females [31,32,33].

Peak cough flow is a parameter measured in a peak flow meter and evaluates expiratory muscle strength through the performance of a maximal cough after complete inhalation and can be evaluated through % of predicted or L/min. While in healthy adults the peak cough flow measures can be approximately 470-600 L/min, values of less than 270 L/min can be a sign of pulmonary complication in neuromuscular patients and values as lower as 160 L/min can be associated with extubation failure [31].

Respiratory measures that correspond to mechanics of breathing parameters can be found in supplementary table 4.1, since those do not refer to respiratory strength directly, but are used to acquire maximal inspiratory pressure and maximal expiratory pressure.

4.4 Results

Database search generated 4499 studies. After removal of duplicates, 3410 articles remained for screening and 3084 articles were excluded following title and abstract screening. The full-text of 326 studies was analysed and 246 were excluded due to the following reasons: not being related to DM1 and DM1-related muscle strength (n=151); included paediatric patients with DM1 (n=41); were interventional studies (n=22); the research was conducted with animals, in vitro or in vivo and molecular studies (n=8); were not original quantitative studies (n=10); were not written in English, Portuguese, Spanish or French (n=9); the full text was not available (n=5). Eighty studies were included in the systematic review [34-113]. The PRISMA flow diagram can be found in Figure 4.1, showing the screening process in detail.

4.4.1 Quality assessment

Thirty-three (41.25%) studies were classified as “Good”, 46 (57.5%) were classified as “Fair”, and 1 (1.25%) was classified as “Poor”. Parameters most absent from studies were “sample size justification”, “sufficient timeframe to see an effect” and “blinding”.

Agreement between two independent reviewers was substantial (k = 0.75, 95%IC [0.61-0.89], p =0.0001).

4.4.2 Participants characterization

A total of 5204 patients with DM1 were included in the 80 studies included (supplementary table 4.1). Sample size ranged from 6 to 406 patients. Participants were 43±4 (mean±SD) years old (31-53), equally represented in terms of sex (50.3% female and 49.7% male) and were normal weight (body mass index =25±2 kg/m2 [19.5-29 kg/m2]) although 38 studies did not report the later (supplementary table 4.1). The reported CTG repeat length of patients, mainly evaluated through peripheral blood leukocytes, was 647±211 repeats (387-1338), although 53 studies did not report a mean of CTG repeat length (supplementary table 4.1). MIRS was not reported in 45 studies nevertheless, muscle impairment grades I-III was reported in 996 patients with DM1 and IV-V was reported in 1064 patients with DM1.

Twenty-six studies compared patients with DM1 with healthy controls. In total 743 healthy volunteers were included. Sample sizes ranged from 6 to 71. Healthy volunteers were 40±3 years old (32 to 50) equally represented in terms of sex (45.9% female and 54.1% male) and normal weight (body mass index = 23±1 kg/m2 [20 to 26 kg/m2]), although this variable was not reported in 16 studies (supplementary table 4.1).

4.4.3 Cardiac Muscle Strength

Twenty-four studies assessed cardiac muscle strength [34-52,104-106,112,113]. Twenty-two studies used echocardiography [34-37,39-52,105,106,112,113] and 3 used cardiac magnetic resonance [38,104,106] for the indirect evaluation of cardiac muscle strength. Main findings of the measures used to assess cardiac muscle strength for each study can be found in table 4.1 and Figure 4.2.

4.4.3.1 Echocardiography

Left ventricular ejection fraction (n=21) [34–37,39–47,49–52,105,106,112,113], endocardial shortening fraction (n=2) [47,48], left ventricular shortening fraction (n=4) [39,46,51,52], left ventricular global longitudinal strain (n=3) [43,44,106] and stroke volume (n=1) [46] were evaluated (table 4.1). Stroke volume was 58±16 mL and 72±15 mL for patients with DM1 and controls, respectively (table 4.1) [46]. The range of left ventricular ejection fraction values obtained for patients with DM1 was 56%-70% and 61%-77% for controls [34-37,39-47,49-52,105,112,113]

(table 4.1). The range of endocardial shortening fraction was 31-37 cm and 31.6-37.4 cm for patients with DM1 and controls, respectively (table 4.1) [47,48]. Left ventricular shortening fraction [39,46,51,52], values ranged between 31.5-39% for patients with DM1 and 33.8-40% for controls (table 4.1). Values of left ventricular global longitudinal strain were of -13.55±1.82% (median: -17.3%) [43] in patients with DM1 and of -16.11±1.33% in healthy controls (table 4.1).

4.4.3.2 Cardiac Magnetic Resonance

Left ventricular ejection fraction [38,104,106] was evaluated in three studies, right ventricle ejection fraction [38,104] was evaluated in two studies, stroke volume [38] and right ventricle stroke volume was evaluated in one study [38] (table 4.1 and Figure 4.2).

Patients with DM1 had a median of 58% [104] and mean 57.6±8% [38] for left ventricular ejection fraction and in the control group left ventricle fraction had a mean value of 59.12±6% [38]. Right ventricle ejection fraction in patients with DM1 had a median of 64% [104] and mean 45.8±9% [38], as for the control group the mean was 50.0±7% [38]. Stroke volume was 57.5±15 mL and 64.73±19 mL for patients with DM1 and controls, respectively (table 4.1) [38]. Right ventricle stroke volume was 48.7±13 mL for patients with DM1and 60.3±19 mL for the control group [38].

4.4.4 Skeletal muscle strength

Forty-five studies assessed skeletal muscle strength. Thirty-nine used quantitative muscle testing [53-87,104,106-113] and fifteen used manual muscle test [55,64,73,74,80,81,83,84,104,106,109,111-113] (Figure 4.3 and table 4.2). Main findings of the skeletal muscle measures for each study can be found in the supplementary table 4.1.

4.4.4.1 Manual Muscle Test

Manual muscle test was used to assess the strength of ankle dorsiflexors [55,64,74,83,106], ankle plantar flexors [55,64], back extensors [83], elbow extensors [55,64,83], elbow flexors [55,64,83], grip strength [106], hip flexors [55,64,74,83], knee extensors [55,64,74,83], knee flexors [55,74,83], neck flexors [55,83], shoulder abductors [55,64,83], trunk extensors [111], trunk flexors [83,111], wrist extensors [55,64,83] and total muscle groups [64,73,80,81,84,104,105,109,112,113] (Figure 4.3A).

Manual muscle test results in patients with DM1 were between 1.5 points to 2.9 points (MRC scale of 0 to 3 points) [83,111] and between 3.3 points to 4.8 points (MRC scale of 0 to 5 points) [55,73]. Manual muscle test for total muscle groups were between 49.8 points to 50.2 points (MRC scale of 0-60 points) [109,112,113] and 123 points to 134.5 points (MRC scale of 0-150 points) [80,105] in patients with DM1 and 60±0.01 points (MRC scale of 0-60 points) in controls [109] (table 4.2).

4.4.4.2 Quantitative Muscle Test

test, 17 studies did not report the method used [57,68-70,73,75-79,81,82,85,86,107,108,110], 10 studies assessed maximum isometric torque [53,59,62,63,65,70-72,79,108], 7 studies assessed maximal voluntary isometric contraction [54,55,58,66,67,73,87], and 5 studies assessed maximum isometric force [60,61,74,81,84] (table 4.2).

Quantitative muscle test was used to assess the strength of ankle dorsiflexors [57,68], grip strength [55,57,69,70,73,75,76,77,78,81,107,108,110], hip flexors [57,68], knee extensors [57,68], lip strength [82], maximum bit strength [86], pinch strength [70,76,77,108], tongue strength [85] and wrist extensors [57] (Figure 4.3A).

Muscle strength results assessed by quantitative muscle test (table 4.2) in patients with DM1 were between 14 to 47.8 lb [57,68], 5.2 to 12.8 kg [69,70,73,75,76,78,107,108], 12 N to 82.6 N [82,86], 31.7% to 41.3% of predicted [75,78] and a mean of 132 kPa [85]. In controls quantitative muscle test results were 2.5 kg [73] and 29 N [82].

Maximal voluntary isometric contraction was used to assess the strength of Abductor digiti minimi [54], ankle dorsiflexors [58,87], ankle plantar flexors [87], grip strength [56,66,67], elbow flexors [73], elbow extensors [73], hip abductors [87], hip extensors [87], knee extensors [58,73,87], knee flexors [73,87] and pinch strength [56]. Maximal voluntary isometric contraction results were between 42.2 N to 303 N [56,58,67,87] in patients with DM1 and between 143.7 N to 371.5 N [58,67,87] in controls (Figure 4.3B).

Maximum isometric torque was used to assess the strength of ankle dorsiflexors [53,59,62,63,65,70,71,72,108], ankle evertors [62,63], ankle plantar flexors [53,71,72], elbow extensors [59], elbow flexors [59], hip extensors [71,72], hip flexors [53,59,70-72], knee extensors [53,59,70,71,72,79,108] knee flexors [59,70,71,72], lower limb [70], neck flexors [53], shoulder abductors [59,108], and wrist extensors [59]. Maximum isometric torque results were between 5.1 N to 139.78 N [53,59,70-72,108] in patients with DM1 and 19.7 Nm to 129.4Nm [53, 59,70,71, 72,108] in controls (table 4.2 and Figure 4.3B).

Maximum isometric force was used to assess the strength of ankle dorsiflexors [60,61,74,81,84] elbow flexors [84], hip flexors [60,61,74], knee extensors [60,61,74,84], knee flexors [60,61,74] and pinch strength [84]. Maximum isometric force results were between 49 N to 277 N [60,61,74,81,84] in patients with DM1 (table 4.2). No data was reported in controls (Figure 4.3B).

4.4.5 Respiratory Muscle Strength

Twenty-three studies assessed respiratory muscle strength. Seventeen used manometry [85-100,102,103,108-110] (supplementary table 4.1).

4.4.5.1 Maximal Inspiratory and Expiratory Pressures

Fifteen studies analysed Maximal expiratory pressure [88-93,96-100,102,103,108,110] and 17 analysed maximal inspiratory pressure [88-93,95-100,102,103,108,109,110]. Maximal expiratory pressure mean values were between 35.5 cmH2O to 71 cmH2O in patients with DM1 and controls presented a mean value of 133.8±28 cmH2O [88,90-92,97-100,102,103,108,110]. Maximal inspiratory pressure mean values were between 34 to 76 cmH2O in patients with DM1 and controls presented a mean value of 77.8±44 cmH2O [88,90-92,95,97-100,102,103,108,110] (table 4.3 and Figure 4.4).

4.4.5.2 Sniff Inspiratory Pressure

Four studies analysed Sniff nasal inspiratory pressure [91,96,103,109] and one study evaluated transdiaphragmatic pressure following a sniff maneuver (SniffPdi) and both transdiaphragmatic pressure following supramaximal cervical stimulation of the phrenic nerves (twPdi) and gastric pressure following stimulation of the abdominal muscle nerve roots at the tenth vertebra (twPgas) [92] (supplementary table 4.1). Sniff nasal inspiratory pressure mean values were between 46% to 80% in patients with DM1 and 70.64±19.6% in controls [91,109] (table 4.3). SniffPdi mean value was 50.4±30.5 cmH2O in patients with DM1 and 92.9±28.9 cmH2O in the control group [92]. Mean value of twPdi in patients with DM1 was 10.8±8.3 cmH2O and 21.4±10.1 cmH2O in the control group [92]. As for twPgas mean value for patients with DM1 was 19.7±15.5 cmH2O and 22.5±13.3 cmH2O in the control group [92].

4.4.5.3 Peak Cough Flow

Three studies analysed peak cough flow [89,92,93] and one study evaluated cough Pgas [92] (supplementary table 4.1). Mean results obtained for peak cough flow were between 280 L/min to 284.7 L/min in patients with DM1 and 547.4±156.6 L/min in controls [92] (table 4.3). Cough Pgas mean value was 71.9±43.2 cmH2O in patients with DM1 and 102.4±35.5 cmH2O in the control group.

4.5 Discussion

In the current literature, there is a high variability and heterogeneity of outcomes and measures, which can hamper the synthesis and comparison of data across studies. Consequently, there was urgent need to synthetize the most common outcomes and measures to evaluate muscle strength in patients with DM1. In fact, previous studies emphasized the heterogeneity in the protocols and methods used to evaluate muscle strength, referring it as a limitation, and muscle strength assessment in relation to DM1 characterization was deemed questionable due to its high variability and undocumented methodology [118-120].

In this systematic review, although high heterogeneity of measures and units of measure is present, some important conclusions could be retrieved regarding the most common measures used to evaluate muscle strength in patients with DM1 in further clinical studies.

Cardiac conduction abnormalities are the most common cardiac manifestations in patients with DM1, being the second most common cause of death among these patients [12]. Myocardial systolic and diastolic dysfunctions can also be observed in patients with DM1, manifesting as impaired early myocardial relaxation or heart failure [12]. Bearing our objective in mind, and not devaluing the important functional cardiac changes reported in these patients, our results focused exclusively on measures that allow assessment of cardiac muscle strength, however indirectly.

Previous studies [114,115] showed that cardiac magnetic resonance is more reproducible and accurate to evaluate left ventricular volume and ejection fraction than echocardiography. Also, echocardiography is thought to underestimate left ventricular ejection fraction values compared with cardiac magnetic resonance, regardless of the technique used [114,115]. However, our results clearly indicate that echocardiography was chosen preferably over cardiac magnetic resonance (table 4.1). This may be due to the cost of cardiac magnetic resonance procedure being 4 to 10 times higher than echocardiography [114]. More studies are needed to compare between echocardiography and cardiac magnetic resonance in patients with DM1 to understand the differences of both techniques more clearly in cardiac muscle strength. In addition, ejection fraction was the most common measure used to indirectly evaluate cardiac muscle strength (table 4.1 and Figure 4.2).

Despite being the most commonly used measure to indirectly assess cardiac muscle strength, the majority of studies that evaluated ejection fraction between patients with DM1 and control group (n=14) did not report a significant difference (n=11/14). In addition, in all the studies that reported a statistically significant difference, left ventricular ejection fraction in patients with DM1 presented values above 50% (table 4.1 and Figure 4.2).

Although the results of ejection fraction observed in patients with DM1 were normal in comparison to controls, one study correlated CTG repeat length with patients with DM1 with left ventricle ejection fraction below 50%, in which they showed that the relative risk of left ventricle systolic dysfunction significantly increases with CTG repeat length [35] (supplementary table 4.1).

In addition, endocardial shortening fraction [47,48], left ventricular shortening fraction [39,46,51,52], left ventricular global longitudinal strain [43,44,106] and stroke volume [38,46], did not show unanimous results of a significant difference between patients with DM1 and control group. Due to the findings regarding cardiac muscle strength measures between patients with DM1 and the control group, we recommend that more studies are needed to accurately assess cardiac muscle strength between a well characterized cohort of patients with DM1 and a healthy control group.

DM1 phenotypes and MIRS rating scale were not correlated in any of the 21 cardiac selected studies. It would be interesting to correlate left ventricle ejection fraction with both following parameters, DM1 phenotype and MIRS rating scale (supplementary table 4.1).

In skeletal muscle strength, there were 8 proximal muscle groups, 7 distal muscle groups and 5 axial muscle groups evaluated throughout the studies. However, there were more studies evaluating distal muscle groups (n=39) than proximal (n=26) and axial (n=5) muscle groups, which are in accordance to DM1's symptomatology of prominent distal muscle weakness [3].

Although manual muscle test (n=15) was a frequently used measure, it has limitations, even when applied by experienced clinicians, since tester judgment and strength can influence the results [118,119]. The most common manual muscle test scale in our studies were the 0 to 5 points (n=5), followed by 0 to 60 points (n=3), 0 to 3 points (n=2), 0-110 points (n=2) and 0-150 points (n=2) (table 4.2 and Figure 4.3A). It was not possible to observe if the results of all manual muscle test scales were significant between patients with DM1 and the control group. However, 2 studies showed a negative correlation between the 0-5 and 0-60 points scale values with the CTG repeat length [73,113] (supplementary table 4.1). In addition, 1 study also compared the rating scale of 0-60 points between patients with DM1 and control group, in which it was shown a significant difference [109] (supplementary table 4.1). Therefore, more studies are necessary to evaluate not only the correlation between de manual muscle test scale with CTG repeat length (increasing disease severity), but also to compare between patients with DM1 and control group, to observe if there is a change in muscle strength.

The quantitative muscle test is considered to be a more precise measurement of muscle strength to discriminate between healthy and patients with DM1 with different levels of impairment using manual or fixed dynamometers [118,119].

Seventeen out of 39 studies that used quantitative muscle test did not describe the type of quantitative muscle test used, which hampered the conclusion regarding the measurement of choice between maximum isometric force, maximum isometric torque and maximal voluntary isometric contraction (supplementary table 4.1) [57,68-70,73,75-79,81,82,85,86,107,108,110]. Nonetheless, grip strength in quantitative muscle tests was the measure of choice (n=13/17). Three studies showed a negative correlation between grip strength muscles and CTG repeat length [55,73,108], also another study showed a negative correlation between grip strength muscles and MIRS rating scale [75] (supplementary table 4.1). Two other studies also showed a statistically significant difference between adult-onset and late-onset patients and between patients with MIRS I-III and MIRS IV-V rating scale [76,107]. Comparison between patients with DM1 and control group was also statistically significant [73]. Therefore, grip strength can be a suitable measure to discriminate between patients with DM1 with different forms of severities and to properly evaluate the degree of severity in these patients.

Muscle isometric torque was the most reported skeletal-muscle measure in this systematic review.

Three studies compared muscle isometric torque between patients with DM1 and control participants [53,63,65]. All of them detected a decrease in DM1 compared to the control group, but

only 1 reported the p-value, which was statistically significant in several muscle groups (ankle dorsiflexors, ankle plantar flexors, Hip flexors, Knee extensors and Neck flexors) [53] (table 4.2). Also, a substantial decline in muscle isometric torque was observed in patients with DM1 during a 9-year follow-up [59] (ankle dorsiflexors, elbow extensors and flexors, hip flexors, knee extensors and flexors, shoulder abductors and wrist extensors) and 5-year follow-up [62] (ankle dorsiflexors and evertors) (supplementary table 4.1). These results are positive indicators that muscle isometric torque is a useful measure to evaluate the evolution and change in muscle strength in future clinical trials, observational studies and clinical practice.

Lastly, our findings for expiratory and inspiratory muscle strength in patients with DM1 demonstrated a preference for the use of maximal expiratory pressure (n=15/23) and maximal inspiratory pressure (n=17/23) (Figure 4.4), even though there is not a consensus regarding the unit of measurement between cmH2O and % of predicted as seen in table 4.3, which made comparisons across studies difficult. Regarding maximal expiratory pressure, one study showed a negative correlation between maximal expiratory pressure and CTG repeat length [88], and another two studies compared maximal expiratory pressure between patients with DM1 and control group, which statistically significantly decreased [91,92] (supplementary table 4.1). As for maximal inspiratory pressure, 2 studies showed a negative correlation between maximal inspiratory pressure and CTG repeat length in patients with DM1 [88,108], and another 3 studies showed a statistically significant decrease between maximal inspiratory pressure between patients with DM1 and control group [91,92,109] (supplementary table 4.1).

However, maximal inspiratory pressure has been reported to lead to falsely low values in patients with neuromuscular disorders, due to the challenges of maintaining the mouth seal and keeping maximal inspiratory effort [116]. As an alternative, sniff nasal inspiratory pressure may be adequate to evaluate inspiratory and diaphragm muscle strength, since it is also a non-invasive test and easier to perform by patients that have low levels of coordination [31,116,117,118]. Two out of 4 studies that evaluated sniff nasal inspiratory pressure showed a significant decrease of sniff nasal inspiratory pressure values in patients with DM1 compared with the control group [91,109] (supplementary table 4.1). Although maximal inspiratory and expiratory pressure can be considered useful measures, more studies are necessary to evaluate which measure is the most suitable to assess respiratory muscle strength in DM1.

Strengths of this review include a thorough systematic search of three databases (Web of Science, PubMed and Embase) and a broad range of search keywords, which resulted in a wide selection of studies according to the PRISMA guidelines methodology. Also, even though there was some ambiguity related to the scale used for quality assessment between the independent reviewers the calculation of Cohen's kappa coefficient score showed a substantial agreement.

Limitations

The limitations of this systematic review are: (1) only peer-reviewed publications were included, even though the grey literature may likely have merit publications that were not included; (2) interventional studies were excluded, so data published on muscle strength in patients with DM1 in this type of studies have not been evaluated. However, it can be argued that their baseline assessment could have been used for the description of outcomes and measures used to assess muscle strength in patients with DM1; (3) some degree of bias may have occurred when performing the qualitative assessment of studies. To minimise this risk, two reviewers assessed each article and agreement between both was calculated. (4) due to high heterogeneity and lack of patient characterization it was not possible to correlate between cardiac, skeletal and respiratory muscle strength.

Significance in clinical practice and future directions

Researchers and clinicians may now consider to start using the measures described in this systematic review, to contribute to high quality research in this field, especially to observe response to in muscle strength after an intervention in future clinical trials. We recommend, for future clinical trials, clinical practice and/or research the use of handgrip strength and muscle isometric torque for the assessment of skeletal muscle strength and maximal expiratory and inspiratory pressure for the assessment of respiratory muscle strength. For cardiac muscle strength, cardiac magnetic resonance seems to be a good candidate, however additional studies to evaluate and compare ejection fraction in patients with DM1 and healthy participants, given that the present did not show a clear and significant discrimination between patients and the control group.

Muscle strength in DM1 has been frequently assessed, however key aspects need improvement and consensus, such as standardized methodology regarding muscle strength. As a future direction, it is necessary to develop core outcome sets (COMET initiative) for assessing muscle strength in DM1 in clinical trials, clinical practice and research.

Characterization of disease severity measures and phenotype of patients with DM1 were not taken into consideration, which reflects the absence of consensus in the literature regarding the matter, as reported and in accordance with previous studies [16,118-122]. Since different disease severities affect the results of muscle strength, it is necessary that studies include severity measurements of their population, along with more robust methodologies to correlate the differences of muscle strength with the 5 DM1 phenotypes (congenital, infantile, juvenile, adult and late-onset), as well as with CTG repeat length. We then suggest a better characterization of patients with DM1 to have a proper evaluation and comparison within the different phenotypes of DM1 and CTG repeat length. Additionally, it is necessary more studies that compare patients with DM1 with control groups, since the lack of control groups hinders the correct assessment of muscle strength in people with DM1, as previously reported [122].

4.6 Conclusion

Major conclusions of this systematic review are:

(1) Echocardiography is the most common method used to evaluate cardiac muscle strength and left ventricular ejection fraction was the preferential measure. Results were not however, significant or conclusive.

(2) Quantitative and manual muscle strength assessment were the most common methods to evaluate skeletal-muscle muscle strength, in which grip strength, maximum isometric torque and the medical research council (0-5 points and 0-60 points) scale were the measures of choice;

(3) Manometry was the most common method to evaluate respiratory muscle strength, in which maximal expiratory pressure and maximal inspiratory pressure were the measures of choice;

(4) Almost half the studies did not address disease severity, since MIRS and/or CTG repeat length were not reported, which challenges the understanding of the results;

Although high heterogeneity was found in this systematic literature review, hampering the building of evidence in such a fundamental domain as muscle strength in patients with DM1, the most common measures used to assess each outcome were successfully gathered and proposed for further clinical studies.

Results of this systematic review were of paramount importance, given the importance of muscle strength evaluation during future clinical trials to assess response to intervention aiming at improving muscle strength. For that all clinicians need to use the same tool. Further, for the management of patients with DM1, a Core Outcome Set to assess muscle strength in this population is urgently needed.

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4.8 Attachments

Table 4.1. Summary of cardiac muscle strength measures [34-52,104-106,112,113].

Sample	CTG repeat length	Muscle impairment (MIRS), n	Measures	Methods and Findings	
DM1= 1403, Sex: 629F; 743M; 31 NR [36]; Age: 32.2-50 y **Controls**=544; Sex: 206F; 234M; 104 NR [36,37,42]; Age: 32.2-50 y	386.7-815.6	I-III: 89 IV-V: 41 1273 NR		**ECHO [34-37,39-52, 105, 106, 112, 113]**	**CMR [38,104,106]**
			ESF [47,48]	**DM1:** 31-37.6cm; **Controls:** 31.6-37.4cm	
			LVEF [34-47, 49-52, 104-106,111,112]	**DM1:** 56-70%; **Controls:** 61-77% **DM1:** Median: 66%	**DM1:** 57.6±8%* vs **Control:** 59.12±6%* **DM1:** Median: 58%*
			LV GLS [43,44,106]	**DM1:** -13.55±1.82%*; **Controls:** -16.11±1.33%* Median: -17.3 %	
			LVSF [39,46,51,52]	**DM1:** 31.5-39.0%; **Controls:** 33.8-40.0%	
			RVEF [38,104]		**DM1:** 45.8±9%* vs **Controls:** 50.0±7%* **DM1: Median:** 64%*
			RVSV [38]		**DM1:** 48.7±13 mL vs **Control:** 60.3±19 mL
			SV [38,46]	**DM1:** 58±16 mL*; **Controls:** 72±15 mL*	**DM1:** 57.5±15 mL*; **Controls:** 64.73±19 mL*

Table 4.1 footnote: Abbreviations: **ESF-** Endocardial shortening fraction**; LVEF–** Left Ventricular Ejection Fraction; **LVSF-** Left Ventricular Shortening Fraction; **RVEF-** Right ventricle ejection fraction; **SV-** Stroke volume; Note: Data are presented as mean range, unless otherwise stated; In case the data is represented by only one study the corresponding mean±standard deviation or median are presented. *data reported in 1 study only; In this table only the indirect outcome measures of cardiac muscle strength are presented making reference to the articles that mention them.

Table 4.2. Summary of skeletal muscle strength measures [53-87, 104-113].

Sample	CTG repeat length	Muscle impairment (MIRS), n	Muscle groups	Outcome measures and Findings				
DM1=2764; Sex: 1452F; 1296M; 16NR [54]; Age: 27.1-52.8 y **Controls**=247; Sex: 107F; 134M; 6NR [65]; Age: 34-45.7 y	386.67-1337.5	I-III: 623 IV-V: 712 1429 NR		**MMT: MRC scale (points)** [55,64,73,74,80,81,83,84,105,104,106,109,111-113]	**QMT** [57,68-70,73,75-79,81,82,85,86,107,108,110]	**QMT: MIT** [53,59,62,63,65,70,71,72,79,108]	**QMT: MIF** [60,61,74,81,84]	**QMT: MVIC** [54,56,58,66,67,73,87]
			Abductor digiti minimi [54]					**DM1:** 1.21±0.76 kg* **DM1:** Median: 0.98 kg*
			Ankle dorsiflexors strength [53,55,57-65,68,70-72,74,81,83,84,87,108]	**DM1:** 2.4±0.68 (0-3)* **DM1:** 3.6±1.3 (0-5)*	**DM1:** 19.3-26.0 lb	**DM1:** 14.2-46.8 Nm; **Controls:** 111.6±32.2 Nm* **DM1:** Median: 15.1-20.5Nm **DM1:** 43±29*; **Controls:** 102±26%*	**DM1:** 56-130 N **DM1:** Median: 110-111 N **DM1:** 61.3±29.2 % of pred.* **DM1:** Median: 37.8-52.0% of pred.; **Controls:** Median: 52%*	**DM1:** 54.3-217 N; **Controls:** 180.6-475 N
			Ankle plantar flexors strength [53,55,64,71,72,87]	**DM1:** 4.1±1.3 (0-5)*		**DM1:** 7.7-19.02 Nm; **Controls:** 28.1±10.2 Nm* **DM1:** Median:18.0-27.25Nm* **DM1:** 26±18%*; **Controls:** 94±26%*		**DM1:** 109.4±65.8 N*; **Controls:** 313.1±126.3 N*
			Back extensors strength [83]	**DM1:** 2.2±0.53 (0-3)*				
			Digit flexors [55]	**DM1:** 3.3±1.3 (0-5)*				
			Elbow extensors strength [55,59,83]	**DM1:** 2.4±0.5 (0-3)* **DM1:** 4.7±0.6)0-5)*		**DM1:** 21.2±8.8 Nm*		
			Elbow flexors strength [55,59,83,84]	**DM1:** 2.7±0.46 (0-3)* **DM1:** 4.6±0.6 (0-5)*		**DM1:** 33.9±12.6 Nm*	**DM1:** 105±31N*	

			Grip strength [55-57,66,67,69,70,73,75-78,81,106-108,110]		**DM1:** 8.6-12.8 Kg; **Controls:** 2.5 kg* **DM1:** Median: 8.0-11.kg **DM1:** 31.7-41.3% of pred. **DM1:** 14.0±9.3 lb*			**DM1:** 42.2-125.2 N; **Controls:** 371.5±103.8 N* **DM1:** 35.4±19.4% of pred.*; **Controls:** 101±13.8% of pred.*
			Hip abductors strength [87]					**DM1:** 96.5±53.8 N*; **Controls:** 194.8±91.6 N*
			Hip extensors strength [71,72,87]			**DM1:** 62.36-128.51Nm **DM1:** Median:128.4 Nm*		**DM1:** 141.0±57.8 N*; **Controls:** 262±145 N*
			Hip flexors strength [53,55,57,59-61,68,70-72,74,83]	**DM1:** 2.6±0.49 (0-3)* **DM1:** 4.5±0.6 (0-5)*	**DM1:** 29.0-33.7 lb	**DM1:** 51.2-69.42Nm; **Controls:** 87.5±33.6 Nm* **DM1:** Median: 50.8Nm* **DM1:** 66±19% of pred.*; **Controls:** 103±38% of pred.*	**DM1:** 169-171N **DM1:** Median: 172N* **DM1:** 69.5-85% of pred.	
			Knee extensors strength [53,55,57-61,68,70-72,74,79, 83,84,87,108]	**DM1:** 2.90±0.34 (0-3)* **DM1:** 4.7±0.6 (0-5)*	**DM1:** 46.0-47.8 lb	**DM1:** 73.60-139.78 Nm; **Controls:** 129.4±46.3 Nm* **DM1:** Median: 79.5 Nm* **DM1:** 75±36% of pred.*; **Controls** 131±44% of pred.*	**DM1:** 113-277 N **DM1:** 67.1-86.0% of pred.	**DM1:** 169.5-303.0 N; **Controls:** 274-612 N
			Knee flexors strength [55,59-61, 70-72,74, 83,87]	**DM1:** 2.80±0.39 (0-3)* **DM1:** 4.8±0.5 (0-5)*		**DM1:** 57.32-68.14 Nm **DM1:** Median: 48.9 Nm*	**DM1:** 100-101 N **DM1:** 53.0-86.1% of pred.	**DM1:** 95.6±50.7 N*; **Controls:** 143.7±63.6 N*
			Lip strength [81]		**DM1:** 12.0±5.5N*; **Controls:** 29±9N*			
			Lower Limb] [70]			**DM1:** Median: 390 Nm*		
			Maximum bit strength [86]		**DM1:** 82.6±47.9 N*			
			Neck flexors strength [53,55,83]	**DM1:** 1.6±0.55(0-3)* **DM1:** 4.1±0.6 (0-5)*		**DM1:** 5.3±2.9 Nm*; **Controls:** 19.7±8.7 Nm* **DM1:** 25±11 %*; **Controls:** 87±25%*		
			Pinch strength		**DM1:** 5.2-6.1 kg		**DM1:** 49±27 N*	**DM1:** 43.4-46.2 N

			[56,70,76,77,108]		**DM1:** Median: 5.7 kg*			
			Shoulder abductors strength [55.59,83,108]	**DM1:** 2.5±0.5 (0-3)* **DM1:** 4.6±0.6 (0-5)*		**DM1:** 40.8-41.9 Nm		
			Tongue Strength [85]		**DM1:** 13.2±6.0 kPa*			
			Total muscle groups strength [64,73,80,83,104,109,112,113]	**DM1:** 3.9 (0-5)* **DM1:** 35.0±2.4 (0-40)* **DM1:** 49.8-50.2 (0-60); **Controls:** 60.0±0.01 (0-60)* **DM1:** Median: 98 (0-110)* **DM1:** 134.2-134.5 (0-150)				
			Trunk extensors strength [111]	**DM1:** Median: 2 (0-3)*				
			Trunk flexors strength [83,111]	**DM1:** 1.5±0.6 (0-3)* **DM1:** Median: 1.5*; **Controls:** 3 (0-3)*				
			Wrist extensors strength [55,57,59,83]	**DM1:** 2.30±0.51 (0-3)* **DM1:** 4.4±0.8 (0-5)*	**DM1:** 14.4±9.7 lb*	**DM1:** 5.1±2.8 Nm*		

Table 4.2 footnote: Abbreviations: **MMT**- Manual Muscle Test; **MRC**- Medical Research Council; **MIT**- Maximum isometric torque; **MIF**- Maximum isometric force; **MVIC**- Maximal voluntary isometric contraction; **QMT**- Quantitative Muscle Testing; Note: Data are presented as mean range, unless otherwise stated; In case the data is represented by only one study the corresponding mean±standard deviation or median are presented. *data reported in 1 study only; In this table only the outcome measures of skeletal muscle strength are presented making reference to the articles that mention them.

Table 4.3. Summary of respiratory muscle strength measures [88-103,107-113].

Sample	CTG repeat length	Muscle impairment (MIRS), n	Measures	Method and Findings
DM1=1513 Sex: 686F; 707M; 120NR [85]; Age: 37.7-49.7 y **Controls:** 63; Sex: 25F; 38M; Age: 37-42 y	386.67-868	I-III: 241 IV-V: 240 1032 NR		**Manometry [88-93, 95-100,102,103,108-110]**
			Cough Pgas [92]	**DM1:** 71.9±43.2 cmH2O*; **Controls:** 102.4±35.5 cmH2O*
			PCF [89,92,93]	**DM1:** 280-284.7 L/min; **Controls:** 547.4±156.6 L/min*
			PEmax [88-93,96-100,102,103,108,110]	**DM1:** 35.5-71.0 cmH_2O; **Controls:** 133.8±28.0 cmH_2O* **DM1:** Median: 42.0-43.5 cmH_2O; **Controls:** Median: 114 cmH_2O* **DM1:** 36.0-64% of pred. **DM1:** Median: 39.7 % of pred.*; **Controls:** Median: 102% of pred.*
			PImax [88-93,95-100,102,103,108,109,110]	**DM1:** 34.0-76.0 cmH_2O; **Controls:** 77.8±44 cmH_2O **DM1:** Median: 48 cmH_2O* **DM1:** 45.1-70.0% of pred. **DM1:** Median: 48% of pred. *
			SniffPdi [92]	**DM1:** 50.4±30.5 cmH2O*; **Controls:** 92.9±28.9 cmH2O*
			SNIP [91,96,103,109]	**DM1:** 46-80%; **Controls:** 70.64±19.6%* **DM1:** 46-79 cmH_2O; **Controls:** 72 cmH_2O*
			SNP [95]	**DM1:** 47±23 cmH_2O*
			twPdi [92]	**DM1:** 10.8±8.3 cmH2O*; **Controls:** 21.4±10.1 cmH2O*
			twPgas [92]	**DM1:** 19.7±15.5 cmH2O*; **Controls**: 22.5±13.3 cmH2O*

Table 4.3 footnote: Abbreviations: **Cough Pgas-** Gastric pressure during a maximum cough; **PEmax-** Maximal expiratory Pressure; **PImax**- Maximal Inspiratory Pressure; **PCF**- Peak cough flow; **SniffPdi-** Transdiaphragmatic pressure following a sniff maneuver; **SNIP**- Sniff nasal inspiratory pressure; **SNP**- Sniff Negative Pressure; **twPdi-** Transdiaphragmatic pressure following supramaximal cervical stimulation of the phrenic nerves; **twPgas-** Gastric pressure following stimulation of the abdominal muscle nerve roots at the tenth vertebra; Note: Data are presented as mean range, unless otherwise stated; In case the data is represented by only one study the corresponding mean±standard deviation or median are presented. *data reported in 1 study only; In this table only the outcome measures of respiratory muscle strength are presented making reference to the articles that mention them

Supplementary material

(“Myotonic dystrophy type 1” OR “Congenital Myotonic dystrophy” OR “Dystrophia Myotonica 1” OR “Myotonic Dystrophy 1” OR “Steinert Syndrome” OR “Steinert’s disease” OR “Steinert myotonic dystrophy” OR “Myotonic dystrophy of Steinert” OR “Steinert’s congenital myotonic dystrophy")

AND

(“respiratory muscles” OR “respiratory muscle strength” OR “respiratory strength” OR “respiratory weakness” OR “respiratory muscle weakness” OR “inspiratory muscles” OR “inspiratory strength” OR “expiratory strength” OR “expiratory muscles” OR “respiratory pressures” OR “maximum inspiratory pressure” OR “maximal inspiratory pressure” OR “maximum expiratory pressure” OR “maximal expiratory pressure” OR “maximum pressures” OR “maximal pressures” OR “sniff nasal pressure” OR “peripheral muscle strength” OR “quadriceps muscle strength” OR “maximum voluntary contraction” OR “maximal repetition” OR “trunk muscle strength” OR “biceps muscle strength” OR “lower limb muscle strength” OR “handgrip strength” OR “upper limb muscle strength” OR “function*” OR “physical functional performance” OR “activities of daily living” OR “activities of daily life” OR “gait speed” OR “balance” OR “postural control” OR “postural balance” OR “equilibrium” OR “stability” OR “fall*” OR “accidental falls” OR “muscle resistance” OR “muscle power” OR “muscle volume” OR “muscle density” OR “muscle contractibility” OR “contractility” OR “muscle fiber” OR “muscle thickness” OR “muscle cross sectional area” OR “ecograph*” OR “muscle imaging” or “muscle electrical activity” OR “neurotransmission” OR “neurotransmitter” OR “neurotransmitter agents” OR “synaptic transmission“ OR “neuromuscular transmission” OR “muscular neurotransmission” OR “neuromuscular dysfunction” OR “neuromuscular monitoring”)

Figures and legends

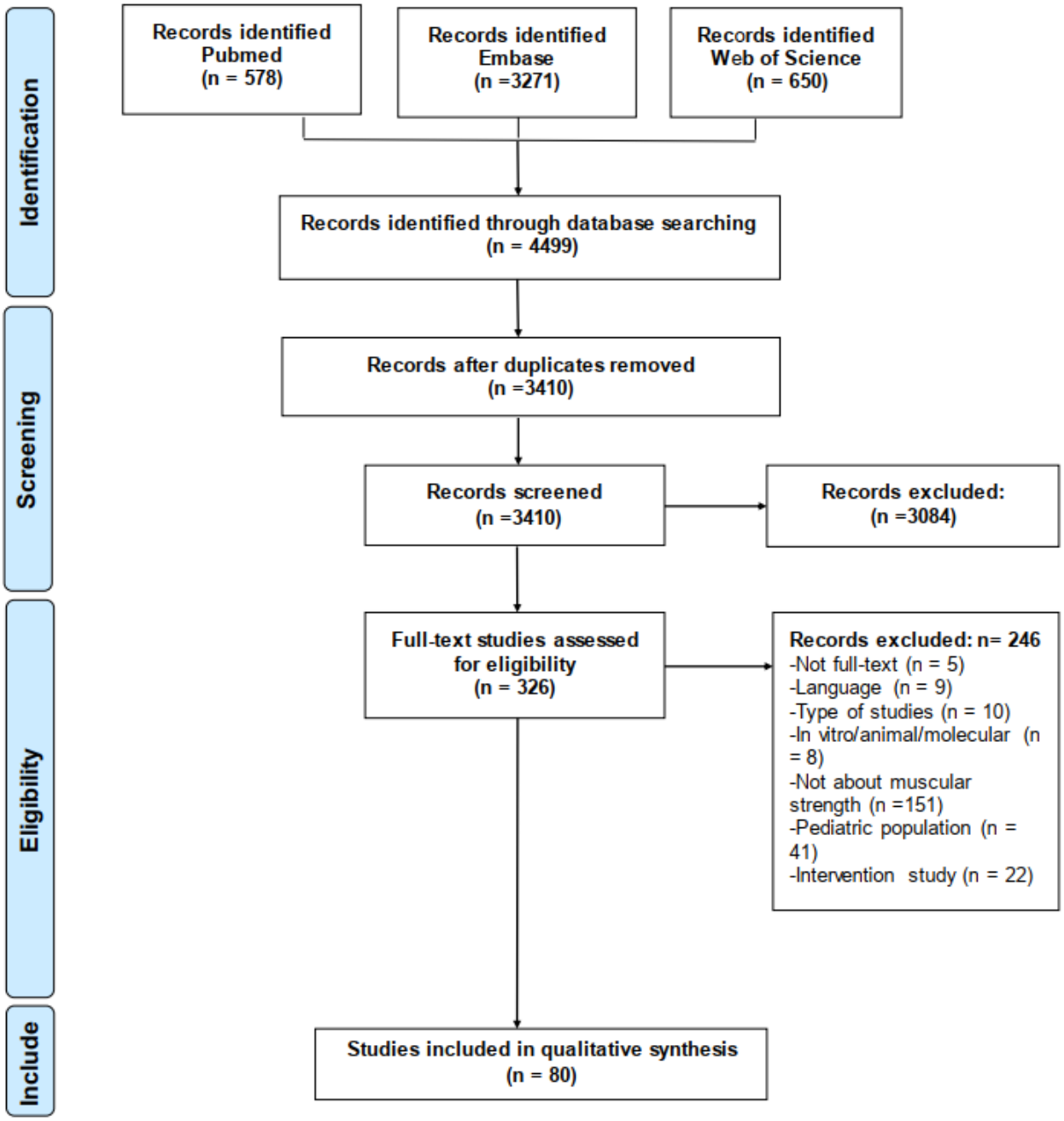

Figure 4.1. PRISMA flow diagram indicating inclusion and exclusion criteria of papers at each stage of screening.

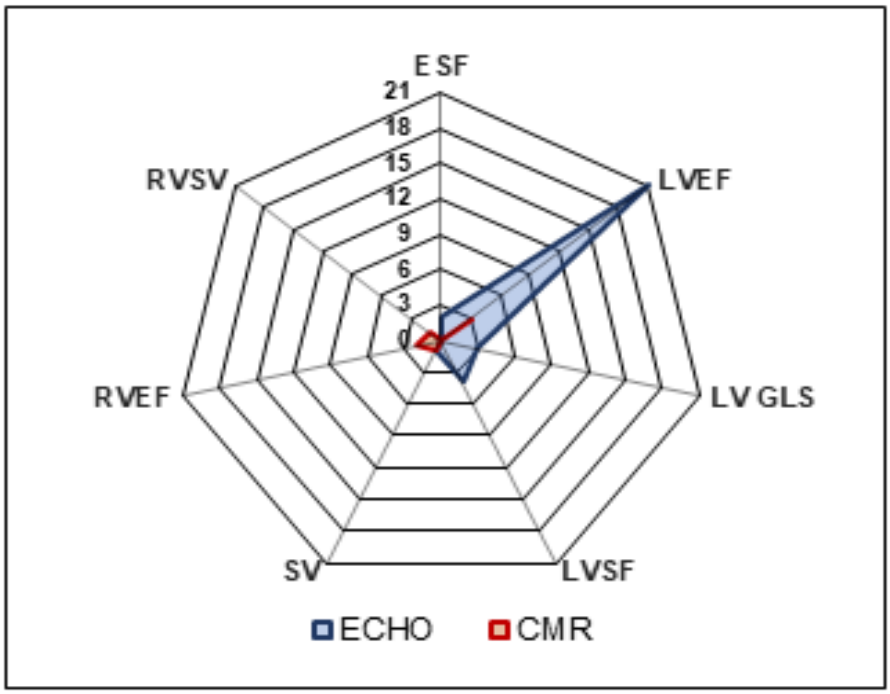

Figure 4.2. Radial chart representing indirect cardiac muscle strength measures, indicating the number of studies in which each measure was represented. ECHO was clearly more represented than CMR and LVEF was the most common measure to indirectly evaluate cardiac muscle strength. Abbreviations: **ECHO**- Echocardiography; **CMR**- Cardiac Magnetic Resonance; **ESF-** Endocardial shortening fraction; **LVEF-** Left Ventricular Ejection Fraction; **LV GLS-** Left Ventricular Global Longitudinal Strain; **LVSF**- Left Ventricular Shortening Fraction; **SV-** Stroke Volume; **RVEF-** Right Ventricle Ejection Fraction; **RVSV -** Right ventricle stroke volume.

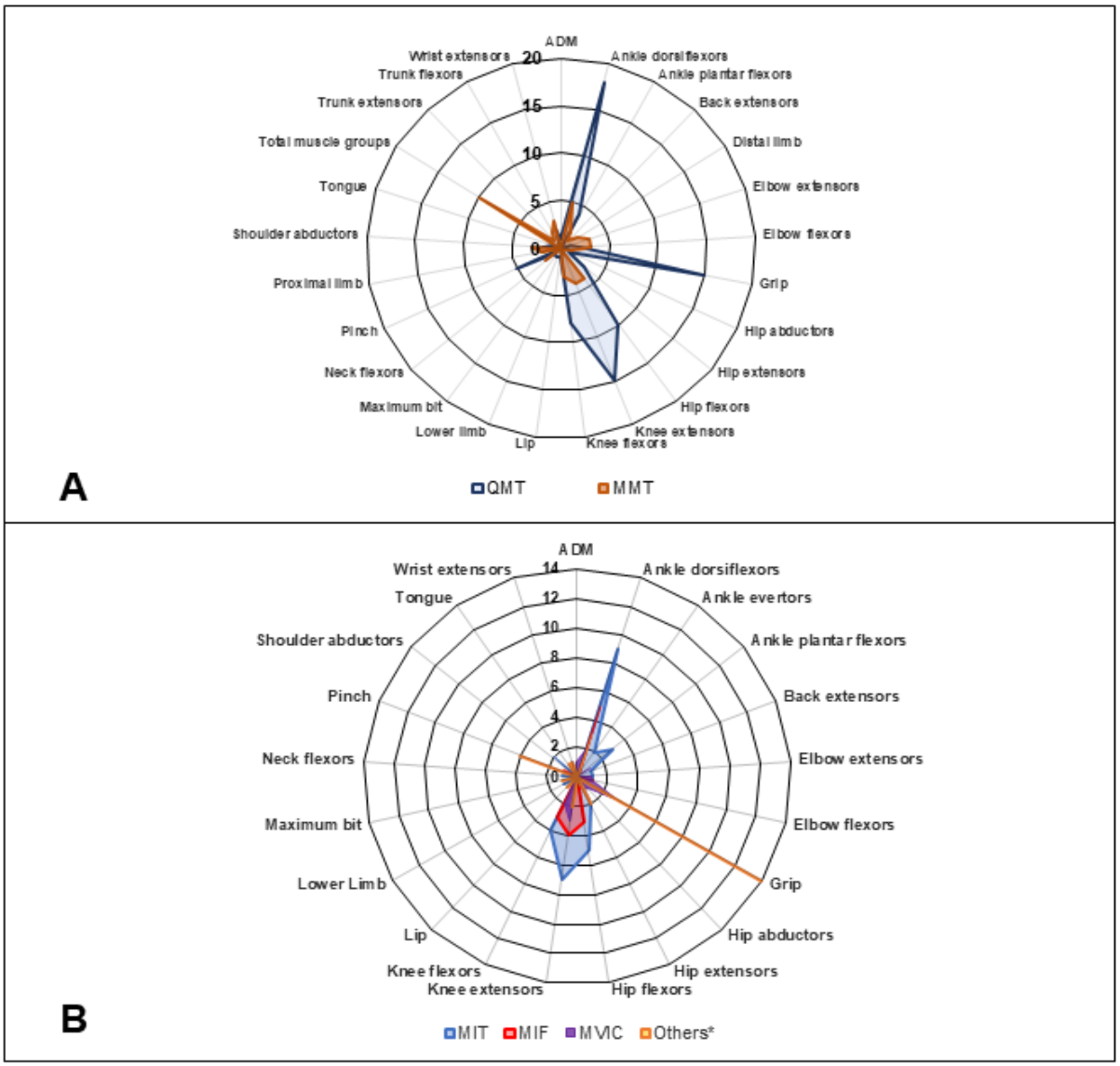

Figure 4.3. Radial chart representing skeletal muscle strength measures and muscle groups, indicating the number of studies in which each measure was represented. **(A)** Skeletal muscle strength comparison between QMT and MMT. QMT was clearly more represented than MMT and the most common muscle groups evaluated in QMT were ankle dorsiflexors, grip and knee extensors and, in MMT, the scale for total muscle groups was the most common. (**B**) Skeletal muscle quantitative strength measures. MIT and MIF were the most common described measures and ankle dorsiflexors, grip, knee extensors and pinch were the most common muscle groups. *QMT measure was not described. Abbreviations: **QMT**- Quantitative Muscle Testing; **MMT**- Manual Muscle Testing; **MIT**- Maximum Isometric Torque; **MVIC**- Maximal Voluntary Isometric Contraction; **MIF-** Maximum Isometric Force.

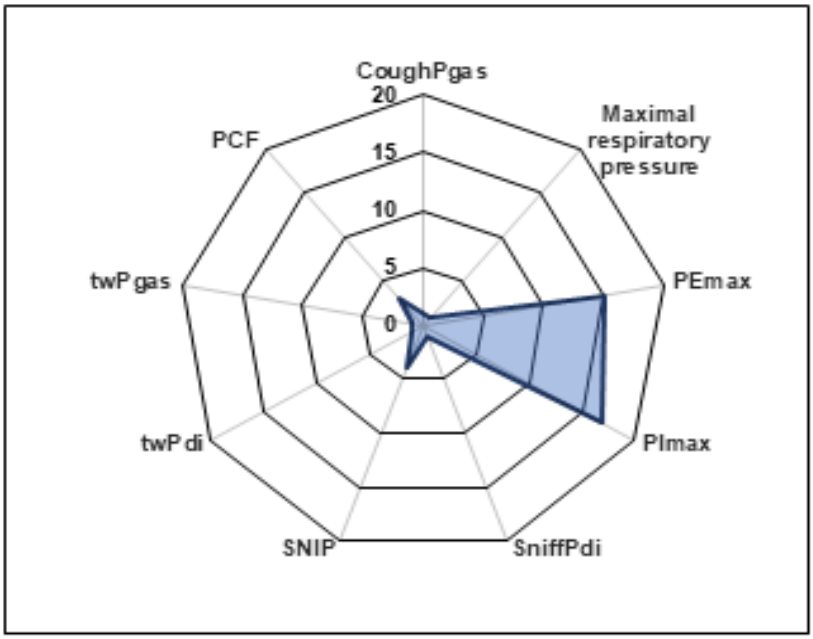

Figure 4.4. Radial chart representing respiratory muscle strength manometry measures, indicating the number of studies in which each measure was represented. PImax and PEmax were greatly more evaluated than the remaining measures. Abbreviations: **PEmax**- Maximal Expiratory Pressure; **PImax**- Maximal Inspiratory Pressure; **SniffPdi-** Transdiaphragmatic Pressure Following a Sniff Manoeuvre; **SNIP-** Sniff Nasal Inspiratory Pressure; **twPdi-** Transdiaphragmatic Pressure Following Supramaximal Cervical Stimulation of the Phrenic Nerves; **twPgas-** Gastric Pressure Following Stimulation of the Abdominal Muscle Nerve Roots at the Tenth Vertebra; **PCF-** Peak cough flow; **Cough Pgas-** Cough gastric pressure.

Supplementary table 4.1. Summary data of studies included in review.

Author, Year, Country	Sample	CTG repeat length	Muscle Impairment	Outcome and Outcome measures	Findings	Quality assessment
Cardiac Studies						
Bhakta et al (2010), USA [34]	**DM1**=406; Sex: 201F; 205M; Age: 42±12; **DM1 LVSD/HF (-) (Group A)**=365; Sex: 187F; 178M; Age: 42±12; **DM1 LVSD/HF (+) (Group B)**=41; Sex: 14F; 27M; Age: 47±10	**Group A:** 623±384 **Group B:** 690±400	MIRS Group A: 3.2±1 Group B: 3.4±1	ECHO: LVEDD	**Group A:** 45 mm vs **Group B:** 51 mm, p<0.001	Fair
				ECHO: LVEF	**Group A:** 57% vs **Group B:** 36%, p<0.001	
Bhakta et al. (2004), USA [35]	**DM1**=100; Sex: 46F; 54M; Age: 43.3±12.1	627±384 (54-1965)	MDRS 3.2±1	ECHO: LVEF	56±13% (20-88) Left ventricular systolic dysfunction (LVEF <50%): n= 14; 14% Left ventricular systolic dysfunction vs Age - RR: 1.9 per decade; 95%CI: 1.1-3.2; p=0.02 Left ventricular systolic dysfunction vs CTG Repeat Length - RR: 2.8 per 500 repeats; 95%CI: 1.3-6.3; p=0.01	Good
Bienias et al. (2017), Poland [36]	**DM1**=44; Sex: 16F; 28M; Age: 34.9±11.5; BMI: 22.8±5.8 **Controls**=35; Sex: 15F; 20M; Age: 35.1±8; BMI: 23.9±3.2	NR	MIRS I-III (n): 19 MIRS IV-V (n): 25	ECHO: LVEF	**DM1:** 64±3% vs **Controls:** 66±2%; p=0.08	Fair
Bienias et al. (2016), Poland [37]	**DM1**=51; Sex: 16F; 35M; Age: 37.3±12.5 **Controls**= 45; Sex: 24F; 21M; Age: 42.2±11.4	NR	MIRS III-V (n): 41	ECHO: LVEF	**DM1:** Median: 65% (20-70) vs **Controls**: Median: 65% (60-70), p=0.006	Fair
Choudhary et al. (2016), Australia [38]	**DM1**=41; Sex: 24F; 17M; Age: 45±14 **Controls**=36; Sex: 17F; 19M; Age: 42±14	428±365 <150 (n): 10 150-1000 (n): 27 >1000 (n): 3	MDRS I (n): 9 MDRS II (n): 11 MDRS III (n): 11 MDRS IV (n): 9	CMR: LVEDV	**DM1:** 101.1±29 mL vs **Controls:** 111±36 mL, p=0.17	Fair
				CMR: LVEDV/BSA	**DM1:** 57±17mL/m² vs **Controls:** 60.2±19mL/m², p=0.43	
				CMR: LVEF	**DM1:** 57.6±8% vs **Controls:** 59.12±6%, p=0.34	
				CMR: LVESV	**DM1:** 43.7±18 mL vs **Controls:** 46.3±19 mL, p=0.53	
				CMR: LVESV/BSA	**DM1:** 24.7±10 mL/m² vs **Controls:** 25.0±10mL/m², p=0.87	
				CMR: SV	**DM1:** 57.5±15 mL vs **Controls:** 64.73±19 mL, p=0.06	
				CMR: RVEF	**DM1:** 45.8±9% vs **Controls:** 50.0±7%, p=0.02	
				CMR: RVEDV	**DM1:** 108.8±29 mL vs **Controls:**121.6±38 mL, p=0.09	
				CMR: RVEDV/BSA	**DM1:** 61.3±17mL/m² vs **Controls:** 65.9±20mL/m², p=0.26	
				CMR: RVESV	**DM1:** 60.3±22mL vs **Controls:** 61.3±22mL, p=0.84	
				CMR: RVESV/BSA	**DM1:** 33.8±12mL/m² vs **Controls:** 33.1±11mL/m², p=0.81	
				CMR: RVSV	**DM1:** 48.7±13mL vs **Controls:** 60.3±19mL p=0.004	
Di Cori et al. (2009), Italy [39]	**DM1**=31; Age: 42±12; BMI: 25±5.7 **Controls**=31; Age: 41±10;	≤500 (n): 18 500-1000 (n): 10	MDRS 2 (n): 13 MDRS 3 (n): 18	ECHO: LVEDD	**DM1:** 48±4.3 mm vs **Controls:** 49±4.2 mm; AUC: 0.59; 95%CI= 0.46-0.72; p=Ns	Fair
				ECHO: LVEDV	**DM1**: 73±15 cm^3 vs **Controls**: 75±16 cm^3; AUC: 0.54; 95%CI: 0.4-0.68; p=Ns	
				ECHO: LVEF	**DM1:** 61±5% vs **Controls:** 61±5.2%; AUC: 0.50; 95%CI: 0.38-0.64; p=Ns	

	BMI: 23±2.5	1001-1500 (n): 3		ECHO: LVESV	**DM1:** 28±7.2 cm^3 vs **Controls:** 29±7.8 cm^3; AUC: 0.53; 95%CI: 0.39-0.67; p=Ns	
				ECHO: LVSF	**DM1:** 39±6.1% vs **Controls:** 40±5.2%; AUC: 0.56; 95%CI: 0.42-0.69; p=Ns	
Fayssoil et al. (2014), France [40]	**DM1**=26; Sex: 12F; 14M; Age: 45.1±10.9 **Control**=13; Age: 42.1±11	NR	NR	ECHO: LVEDD	**DM1:** 42.6±8 mm vs **Controls:** 46.6±4 mm; p=0.09	Fair
				ECHO: LVEF	**DM1:** 58±13% vs **Controls:** 66.2±4.7%; p=0.03	
Fung et al. 2003, Australia [41]	**DM1**= 22; Sex: 11F; 11M; Age: 39±13; **Controls**= 22; Sex: 9F; 13M; Age: 39±18	E1≤500 (n):4 E2=500 to 1000 (n):8 E3=1001 to 1500 (n):4 E4≥ 1500(n):3	NR	ECHO: LAD	**DM1:** 34±6 mm vs **Controls:** 34±4 mm; p= 0.8	Fair
				ECHO: LVEDD	**DM1:** 47±4 mm vs **Controls:** 47±4 mm; p=0.75	
				ECHO: LVEF	**DM1:** 60±5% vs **Controls:** 60±4%; p=0.86	
				ECHO: LVESD	**DM1:** 28±5 mm vs **Controls:** 30±5 mm; p=0.17	
Garcia et al. (Feb. 2017), France [42]	**DM1**=33; Sex: 16F; 17M; Age: 38.2±12.9 **Controls**=33; Sex: 16F;17M; Age: 38.5±13.1	416±56	NR	ECHO: LVEDD	**DM1:** 25.7±2.2 mm/m^2 vs **Controls:** 26.8±3.6 mm/m^2	Good
				ECHO: LVESD	**DM1:** 17.5±1.7 mm/m^2 vs **Controls:** 16.9±3.2 mm/m^2	
				ECHO (2D): Simpson-LVEF	**DM1:** 65.5±5.3% vs **Controls:** 64.3±4.9%	
				ECHO (2D): Triplane-LVEF	**DM1:** 64.6±5.2% vs **Controls:** 64.4±5.3%	
				ECHO (3D): LVEF	**DM1:** 61.6±3% vs **Controls:** 63.4±5.2%	
Garcia et al. (Sep. 2017), France [43]	**DM1**=46; Sex: 21F; 25M; Age: Median: 40 (29-49); BMI: Median: 25 (20-28)	Median:370; [80-700]	NR	ECHO: LVGLS	Median: -17.3% [-19; -15.5] GLS vs CTG Repeat Length: rho=-0.119; p=0.53 GLS vs Age: rho=0.246; p=0.1	Good
				ECHO: LVEDD	Median: 26.1 mm/m^2 [23.8;27.5]	
				ECHO: LVESD	Median:17.2 mm/m^2 [15.5;18.6]	
				ECHO: LVEF	Median:66% [61;70]	
Guedes et al (2018), Portugal [44]	**DM1**=25; Sex: 14F; 11M; Age: 36.9±16 **Controls**=25; Sex: 13F; 12M; Age: 40.3±12.4	NR	NR	ECHO: LVEDD	**DM1:** 45.12±4.94 mm vs **Controls:**47.53±3.99 mm; p=0.064	Fair
				ECHO: LAEF	**DM1:** 42.13±8.53% vs **Controls:**46.37±11.21%; p=0.139	
				ECHO: LAEDV/BSA	**DM1:** 22.7±4.75 mL/m^2 vs **Controls:**23.6±6.19 mL/m^2; p=0.677	
				ECHO: LAESV/BSA	**DM1:**13.12±4.35 mL/m^2 vs **Controls:**13.12±3.5 mL/m^2; p=0.569	
				ECHO: LA LS	**DM1:** 22.85±5.06% vs **Controls:** 26.82±5.15%; p=0.008 LA LS vs Age: r=-0.771; p<0.001	
				ECHO: LVEDV/BSA	**DM1:** 55.85±23.14 mL/m^2 vs **Controls:** 54.53±15.05 mL/m^2; p=0.814	
				ECHO: LVEF	**DM1:** 62.88±3.27% vs **Controls:** 65.64±6.65%; p=0.8	
				ECHO: LVESV/BSA	**DM1:** 24.42±12.54 mL/m^2 vs **Controls:** 25.16±9.87 mL/m^2; p=0.819	
				ECHO: LV GLS	**DM1:** -13.55±1.82% vs **Controls:** -16.11±1.33%; p<0.001	
Gomes et al. (2014),	**DM1**=31; Sex: 20F; 11M; 42±13.1 (18-66); BMI:	≤900 (n): 15 >900 (n): 16	NR	ECHO: LVEF	**DM1 (F):** 64.33±11.03 vs **DM1 (M):** 65.82±1.02, p=0.68 **Group A:** 65.48±10.81 vs **Group B**: 63.93±8.09, p=0.58	Good

Portugal [45]	27.14±4.72 **DM1 with ≤900 repeats (Group A):** 15; Sex:9F; 6M; Age: 39.4±15.38; BMI: 26.61±5.36 **DM1 with >900 repeats (Group B)**=16; Sex: 11F; 5M; Age: 44.44±10.46; BMI: 29.45±4.4					
Lindqvist et al. (2009), Sweden [46]	**DM1**=36; Sex: 14F; 22M; Age: 45±10 **Controls**=16; Sex: 8F; 8M; Age: 50±6	NR	NR	ECHO: CO	**DM1:** 4.3±0.9 l/min vs **Controls:** 4±1.2 l/min; p=Ns	Fair
				ECHO: SV	**DM1:** 58±16 mL vs **Controls:** 72±15 mL; p<0.05	
				ECHO: Ejection Index	**DM1:** 0.59±0.07 vs **Controls:** 0.71±0.12; p<0.001	
				ECHO: LVDD	**DM1:** 47±6 mm vs **Controls:** 50±4 mm; p=Ns	
				ECHO: LVEF	**DM1:** 70±8% vs **Controls:** 77±6%; p<0.001	
				ECHO: LVSF	**DM1:** 34±1% vs **Controls:** 40±5%; p<0.01	
				ECHO: LVSD	**DM1:** 31±6 mm vs **Controls:** 30±4 mm; p=Ns	
Ozyigit et al. (2010), Turkey [47]	**DM1**= 21; Sex: 6F;15M; Age: 32.2±12.3; BMI: 21±4.22; **Controls**= 21; Sex: 8F;13M; Age: 32.2±7.8; BMI: 22.9±2.64	NR	NR	ECHO: ESF	**DM1:** 37.6±4.7 vs **Controls:** 37.4±3.4; p=0.909	Good
				ECHO: LAD	**DM1:** 3.04±0.29 cm vs **Controls**: 3.24±0.36 cm; p=0.06	
				ECHO: LVED	**DM1:** 4.5±0.52 cm vs **Controls:** 4.73±0.31 cm; p=0.081	
				ECHO: LVEF	**DM1:** 61±6% vs **Controls:** 60±4%; p=0.68	
				ECHO: LVES	**DM1:** 2.82±0.48 cm vs **Controls:** 2.96±0.25 cm; p=0.235	
				ECHO: RV diameter	**DM1:** 2.28±0.23 cm vs **Controls:** 2.38±0.24 cm; p=0.205	
Parisi et al. 2007, Italy [48]	**DM1**= 36; Sex: 16F;20M; Age: 36.4±15; BMI: 24.3±4.6 **Controls**= 36; Sex: 16F; 20M; Age: 36.2±14.8; BMI: 22.6±3.2	663±406 (90-1500)	NR	ECHO: ESF	**DM1:** 31.0±7.2 cm vs **Controls:** 31.6±9.3 cm; p=Ns	Fair
				ECHO: LAD	**DM1:** 3.35±0.61 cm vs **Controls:** 3.23±0.43 cm, p=Ns	
				ECHO: LVEDD	**DM1:** 4.59±0.49 cm vs **Controls:** 4.75±0.61 cm; p=Ns	
Paunic et al (2016), Serbia [49]	**DM1=**111; Sex: 61F; 50M; Age: 42.2±10.9 **Controls**= 71; Sex: 35F; 36M; Age: 42 3±11.6	815.6±805.8 (177-1534); Median:748	3.3±1 MIRS I-III (n): 70 MIRS IV-V (n): 41	ECHO: LVEF	**DM1:** 61.3±7.5 % vs **Controls**: 62.2±3.4, r=0.35; p=ns **DM1 decreased <55%**: 7.5% vs **Controls decreased <55%**: 0, p=0.03 **DM1 decreased <45%**: 5.6% vs **Controls decreased <45%:** 0, p=0.04	Good
Russo et al. (2015), Italy [50]	**DM1**=60; Sex: 11F; 49M; Age: 51±4; BMI: 22±4 **Controls**=60; Age: 50±5; BMI: 23±3	NR	NR	ECHO: LVEF	**DM1:** 63.39±8.1% vs **Controls:** 64.56 ± 5.1%; p=0.1	Fair
				ECHO: LVEDD	**DM1:** 41.70±8 mm vs **Controls:** 45.22±4 mm; p=0.09	
Russo et al. (Oct. 2016), Italy [51]	**DM1**=50; Sex: 21F; 29M; Age: 44±5; BMI: 21±4 **Controls**=50; Sex: 21F; 29M; Age: 44±5; BMI: 20±5	NR	NR	ECHO: LVEF	**DM1:** 60.4±7.1% vs **Controls:** 62.6±4.2%; p=0.2	Good
				ECHO: LVEDD	**DM1:** 43.5±8.2 mm vs **Controls:** 43.3±6.4 mm; p=0.2	
				ECHO: LVESD	**DM1:** 25.3±3.1 mm vs **Controls:** 26.3±2.7 mm; p=0.4	
				ECHO: LVSF	**DM1:** 33.3±5.2% vs **Controls:** 33.8±4.3%; p=0.3	

Russo et al. (Jan 2016), Italy [52]	**DM1**=50; Sex: 22F; 28M; Age: 34.2±11.4; BMI: 19.5±3.6 **Controls**=50; Sex: 24F; 26M; Age: 33.5±8.9; BMI: 20.1±2.1 **DM1 with atrial fibrillation (Group A)**=15; Sex: 6F; 9M; Age: 22.2±2.1; BMI: 20.4±2.2 **DM1 without atrial fibrillation (Group B)**=35; Sex: 16F; 19M; Age: 30.5±6.5; BMI: 20.5±2.3	NR	NR	ECHO: LAEF	**DM1:** 32.8±7.5 mm vs **Controls:** 33.2±7.1 mm; p=0.4	Fair
				ECHO: LVEF	**DM1:** 62.6±5.9% vs **Controls:** 65.1±3.2%; p=0.2 **Group A:** 63.6±4.7% vs **Group B:** 62.2±4.2%; p=0.2	
				ECHO: LVEDD	**DM1:** 50.5±1.1 mm vs **Controls:** 47.2±1.9 mm; p=0.4 **Group A:** 50.1±3.2 mm vs **Group B:** 49.5±2.9 mm; p=0.4	
				ECHO: LVEDV	**DM1:** 60.5±19.2 mL vs **Controls:** 58.7±20.1 mL; p=0.4 **Group A:** 61.3±18.2 mL vs **Group B:** 60.4±18.2 mL; p=0.4	
				ECHO: LVESD	**DM1:** 30.1±3.1 mm vs **Controls:** 28.7±2.5 mm; p=0.4 **Group A:** 31.2±4.2 mm vs **Group B:** 29.5±1.5 mm; p=0.4	
				ECHO: LVESV	**DM1:** 28.2±12.5 mL vs **Controls:** 26.8±13.2 mL; p=0.4 **Group A:** 27.1±12.5 mL vs **Group B:** 26.2±11.5 mL; p=0.4	
				ECHO: LVSF	**DM1:** 31.5±3.2% vs **Controls:** 33.8±1.9%; p=0.3 **Group A:** 30.5±4.2% vs **Group B:** 31.3±1.9%; p=0.3	
Skeletal Studies						
Bachasson et al. (2016), France [53]	**DM1**=22; Sex:11F; 11M; Age 41.9±7.1; BMI 25.1±5.0 **Controls=**20; Sex: 9F; 11M; Age: 40.5±8.4; BMI: 25.1±3.7	657 ± 273 (162–1030)	MIRS III (n): 14 MIRS IV (n): 7	QMT (belt stabilizer dynamometry): MIT - Ankle dorsiflexors	**DM1:** 46.8±33.9 Nm vs **Controls:** 111.6±32.2 Nm; p<0.001 **DM1:** 43±29% vs **Controls:** 102±26%; p<0.001	Fair
				QMT (belt stabilizer dynamometry): MIT - Ankle plantar flexors	**DM1:** 7.7±6.3 Nm vs **Controls:** 28.1±10.2 Nm; p<0.001 **DM1:** 26±18% vs **Controls:** 94±26%; p<0.001	
				QMT (belt stabilizer dynamometry): MIT - Hip flexors	**DM1:** 52.6±18 Nm vs **Controls:** 87.5±33.6 Nm; p<0.001 **DM1:** 66±19% vs **Controls:** 103±28%; p<0.001	
				QMT (belt stabilizer dynamometry): MIT - Knee extensors	**DM1:** 73.6±38.3 Nm vs **Controls:** 129.4±46.7 Nm; p<0.001 **DM1:** 75±36% vs **Controls**:131±44%; p<0.001	
				QMT (belt stabilizer dynamometry: MIT - Neck flexors	**DM1:** 5.3±2.9 Nm vs **Controls:** 19.7±8.7 Nm; p<0.001 **DM1:** 25±11% vs **Controls:** 87±25%; p<0.001	
Boërio et al. (2007), France [54]	**DM1**=16; Sex:11F; 5M; Age: 45.9±10.4	700±413 (93-1330); Median:755	NR	QMT (Force transduction): MVIC - ADM	1.21±0.76 Kg; Median: 0.98 Kg (0.24-2.75)	Fair
Bouchard et al. (2015), Canada [55]	**DM1**=204; Sex: 108F; 96M; Age: Median: 48 (21-69); BMI: Median 23.5 (17-35)	Median: 600 (90-1500)	NR	MMT: MRC scale	Change per y: -0.193 MMT dominant side vs age at onset: p<0.0001 MMT dominant side vs duration of disease: p<0.0001 MMT dominant side vs CTG repeat length: p<0.0001	Fair
				MMT: MRC scale (0-5 points) - Ankle Dorsiflexors	**DM1**:3.6±1.3; Change (% change) per y 0.0235 (0.65%)	

				MMT: MRC scale (0-5 points) - Ankle Plantar flexors	**DM1**:4.1±1.3; Change (% change) per y: 0.0222 (0.5%)	
				MMT: MRC scale (0-5 points) - Digit flexors	**DM1**:3.3±1.3; Change (% change) per y: 0.026 (0.8%)	
				MMT: MRC scale (0-5 points) - Elbow extensors	**DM1**:4.7±0.6; Change (% change) per y: 0.009 (0.2%)	
				MMT: MRC scale (0-5 points) - Elbow flexors	**DM1**:4.6±0.6; Change (% change) per y: 0.0075 (0.16%)	
				MMT: MRC scale (0-5 points) - Hip flexors	**DM1**:4.5±0.6; Change (% change) per y: 0.0116 (0.25%)	
				MMT: MRC scale (0-5 points) - Knee extensors	**DM1**:4.7±0.6; Change (% change) per y: 0.00166 (0.03%)	
				MMT: MRC scale (0-5 points) - Knee flexors	**DM1**:4.8±0.5 ; Change (% change) per y: 0.0052 (0.1%)	
				MMT: MRC scale (0-5 points) - Neck flexors	**DM1**:4.1±0.6; Change (% change) per y: 0.012 (0.3%) Neck flexors vs duration of disease: r=-0.417; p<0.0001 Neck flexors vs CTG repeats length: r=-0.417; p<0.0001	
				MMT: MRC scale (0-5 points) - Shoulder abductors	**DM1**:4.6±0.6; Change (% change) per y: 0.01 (0.2%)	
				MMT: MRC scale (0-5 points) - Wrist extensors	**DM1**:4.4±0.8; Change (% change) per y 0.0125 (0.3%)	
				QMT (hand dynamometry): Peak Force - Grip strength	**DM1 (F):** 9.1±6 kg (0-26) vs **DM1 (M)**: 15.5±14 kg (0-66) Change (% change) per y: -0.24 (2.4%) Grip strength vs CTG repeat length: r=-0.34993; p=0.0053 Grip strength vs disease duration: p=0.0104 Grip strength (F) vs Grip strength (M): p=0.0138	
Cutellè et al. (2018), Italy [56]	**DM1**=50; Sex: 24F; 26M; Age: 45.5±10.2	NR	MIRS I-II (n): 11 MIRS III (n): 21 MIRS IV-V (n): 18	QMT (Handheld dynamometry): MVIC - Grip strength	Avg. Dominant Hand: 45.1±32 N (7-151) vs Avg. Non-dominant Hand: 42.2±32.9 N (7-130) Best Dominant Hand: 47.2±34 N (7-154) vs Best Non-dominant Hand: 44.5±33.3 N (8-135)	Good
				QMT (Handheld dynamometry): MVIC - Pinch strength	Avg. Dominant Hand: 45.5±35.6 N (7.5-132) vs Avg. Non- dominant Hand: 42.6±33.7 N (5.5-124.5) Best Dominant Hand: 46.2±33.8 N (8-38) vs Best Non-dominant Hand: 43.4±31.9 N (6-128)	
DiPaolo et al. (2017), UK [57]	**DM1**=51; Sex: 21F; 30M; Age 47.7±12.6 (18-77); BMI: 25.7±6.8; (16.2-41.7)	564±324 (80-1130)	MIRS: 3.0±1.2 MIRS I (n): 6 MIRS II (n): 16 MIRS III (n): 9 MIRS IV (n): 16 MIRS V (n): 4	QMT: Ankle dorsiflexors	19.3±15.8 lb	Good
				QMT: Grip strength	14±9.3 lb	
				QMT: Hip flexors	29±16.8 lb	
				QMT: Knee extensors	47.8±20.8 lb	
				QMT: Wrist extensors	14.4±9.7 lb	

Esposito et al. (2017), Italy [58]	**DM1**=14; Sex: 14M; Age: 31±12 **Controls**=14; Sex: 14M; Age: 35±7	NR	NR	QMT (EMG): MVIC *Tibialis anterior* – Ankle dorsiflexors	**DM1 Pre fatiguing exercise:** 217±26 N vs **Controls Pre fatiguing exercise:** 475±25 N; p<0.05 **DM1 After fatiguing exercise:** 162±21 N vs **Controls After fatiguing exercise:** 358±23 N; p<0.05 **DM1 At minute 2 of recovery:** 166±19 N vs **Controls At minute 2 of recovery:** 402±28 N; p<0.05 **DM1 At minute 5 of recovery:** 183±20 N vs **Controls At minute 5 of recovery:** 450±27 N; p<0.05 **DM1 At minute 10 of recovery:** 194±21 N vs **Controls At minute 10 of recovery:** 473±26 N; p<0.05	Good
				QMT (EMG): MVIC *Vastus Lateralis* – Knee extensors	**DM1 Pre fatiguing exercise:** 303±23 N vs **Controls Pre fatiguing exercise:** 612±29 N; p<0.05 **DM1 After fatiguing exercise:** 230±27 N vs **Controls After fatiguing exercise:** 482±29 N; p<0.05 **DM1 At minute 2 of recovery:** 250±31 N vs **Controls At minute 2 of recovery:** 528±27 N; p<0.05 **DM1 At minute 5 of recovery:** 266±35 N vs **Controls At minute 5 of recovery:** 589±21 N; p<0.05 **DM1 At minute 10 of recovery:** 270±34 N vs **Controls At minute 10 of recovery:** 605±20 N; p<0.05	
Gagnon, et al. (2018), Sweden [59]	**DM1 Baseline**=100; Sex: 65F;35M; Age: 43.6±10.6 (20-77); BMI: 24.9±5.4 (14.3-43.11) **DM1 Follow-up (9y)**=100; Sex: 65F;35M; Age: 41.9±7.1; BMI: 25.1±5 **Late-onset (Group A)**=22 **Adult-onset (Group B)**=78	**Baseline** 50-199 (n): 17 200-1000 (n): 55 >1000 (n): 28 **Follow-up** 50-199 (n): 12 200-1000 (n): 34 >1000 (n):	Baseline MIRS I (n):4 MIRS II (n):14 MIRS III (n):26 MIRS IV (n):51 MIRS V (n):5 Follow up MIRS I (n):1 MIRS II (n):3 MIRS III (n):23 MIRS IV (n):63 MIRS V (n): 10	QMT (handheld dynamometry): MIT-Ankle dorsiflexors	**DM1 Baseline:** 16.1±7 Nm vs **DM1 Follow-up:** 7.5±4 Nm; p<0.001; Change:-8.5±5.2 Nm; %change: 52.8% **Group A Baseline:** 21±6.2 Nm vs **Group A Follow-up:** 10.3±3.9 Nm; p<0.05; Change: -10.7±4.7 Nm; % change: 51% **Group B Baseline:** 14.7±6.6 Nm vs **Group B Follow-up:** 6.8±3.7 Nm; p<0.05; Change: -7.9±5.2 Nm; % change: 53.7% Phenotype comparison of subgroups effects: Within participants p=0.06; Between participants: p=0.00 **DM1 (F) Baseline:** 15.3±5.8 Nm vs **DM1 (F) Follow-up:** 7.5±3.7 Nm; p<0.05; Change: -7.9±4.8 Nm; % change: 51.6% **DM1 (M) Baseline:** 17.4±8.8 Nm vs **DM1 (M) Follow-up:** 7.6±4.7 Nm; p<0.05; Change: -9.8±5.7 Nm; % change: 56.3% Sex comparison of subgroups effects: Within participants: p=0.3; Between participants: p=0.14	Good

		54		QMT (handheld dynamometry): MIT - Elbow extensors	**DM1 Baseline:** 21.2±8.8 Nm vs **DM1 Follow-up:** 16.0±7.9 Nm; p<0.001; Change: -5.2±5.1 Nm; % change: 24.5% **Group A Baseline:** 26.9±9.2 Nm; **Group A Follow-up:** 22.7±7.8 Nm; p<0.05; Change: -4.2±3.6 Nm; % change: 15.6% **Group B Baseline:** 19.6±8 Nm vs **Group B Follow-up:** 14.1±6.8 Nm, p<0.05; Change: -5.5±5.4 Nm; % change: 28.1% Phenotype comparison of subgroups effects: Within participants: p=0.29; Between participants: p=0.00 **DM1 (F) Baseline:** 17.7±5.6 Nm vs **DM1 (F) Follow-up:** 14.3±5.6 Nm; p<0.05; Change: -3.4±4.3 Nm; % change: 19.2% **DM1 (M) Baseline:** 27.7±9.9 Nm vs **DM1 (M) Follow-up:** 19.3±10.2 Nm; p<0.05; Change: -8.4±4.9 Nm; % change: 30.3% Sex comparison of subgroups effects: Within participants: p=0.001; Between participants: p= 0.00	
				QMT (handheld dynamometry): MIT- Elbow Flexors	**DM1 Baseline:** 33.9±12.6 Nm vs **DM1 Follow-up:** 24.6±10.3 Nm; p<0.001; change: -9.3±8.9 Nm; % change: 27.4% **Group A Baseline:** 37.3±11 Nm vs **Group A Follow-up:** 30.6±10.1 Nm; p<0.05; Change: -6.6±8 Nm; % change: 17.7% **Group B Baseline:** 33.0±12.9 Nm vs **Group B Follow-up:** 23.0±9.8 Nm; p<0.05; Change: -10±9 Nm; % change: 30.3% Phenotype comparison of subgroups effects: Within participants: p=0.06; Between participants: p=0.001 **DM1 (F) Baseline:** 28.4±7.4 Nm vs **DM1 (F) Follow-up:** 22.8±7.3 Nm; p<0.05; Change: -5.6±5.9 Nm; % change 19.7% **DM1 (M) Baseline:** 44.2±13.9 Nm vs **DM1 (M) Follow-up:** 28.1±13.8 Nm; p<0.05; Change: -16.1±9.3 Nm; % change: 36.4% Sex comparison of subgroups effects: Within participants: p=0.00; Between participants: p= 0.00	
				QMT (handheld dynamometry): MIT - Hip flexors	**DM1 Baseline:** 51.2±16.8 Nm vs **DM1 Follow-up:** 53.7±19.2 Nm; p<0.001; Change: 2.5±15.5 Nm; % change: 4.9% **Group A Baseline:** 54±16.2 Nm vs **Group A Follow-up:** 60±18.4 Nm; p<0.05; Change: 6±3 Nm; % change: 11.1% **Group B Baseline:** 50.4±16.9 Nm vs **Group B Follow-up:** 52±19.2 Nm; p<0.05; Change: 1.6±16.1 Nm; % change: 3.2% Phenotype comparison of subgroups effects Within participants: p=0.3; Between participants: p=0.18 **DM1 (F) Baseline:** 44.3±12.2 Nm vs **DM1 (M) Follow-up:** 50.4±17.2 Nm p<0.05; Change: 6.1±14.2 Nm; % change: 13.8% **DM1 (M) Baseline:** 64.1±16.6 Nm vs **DM1 (M) Follow-up:** 60.0±21.4 Nm; p<0.05; Change: -4.10±15.86 Nm; % change: 6.4%	

					Sex comparison of subgroups effects: Within participants: p=0.03; Between participants: p=0.002	
				QMT (handheld dynamometry): MIT- Knee extensors	**DM1 Baseline:** 81.6±32.5 Nm vs **DM1 Follow-up:** 56.9±27 Nm; p<0.001; Change: -24.7±20.5 Nm; % change: 30.3% **Group A Baseline:** 82.4±27.1 Nm vs **Group A Follow-up:** 69.6±27.8 Nm; p<0.001; Change: -12.8±14.4 Nm; % change: 15.5% **Group B Baseline:** 81.3±34 Nm vs **Group B Follow-up:** 53.3±25.9 Nm; p<0.05; Change: -28.1±20.8 Nm; % change: 34.6% Phenotype comparison of subgroups effects: Within participants: p=0.00; Between participants: p=0.11 **DM1 (F) Baseline:** 69.1±21 Nm vs **DM1 (F) Follow-up:** 50.3±20.7 Nm; Change: -18.9±16.5 Nm; % change: 27.4% **DM1 (M) Baseline:** 104.7±37.4 Nm vs **DM1 (M) Follow-up:** 69.1±32.9 Nm; Change: -35.6±22.9 Nm; % change: -35.6±22.9% Sex comparison of subgroups effects: Within participants: p=0.04;Between participants: p=0.00	
				QMT (handheld dynamometry): MIT- Knee flexors	**DM1 Baseline:** 50.3±16 Nm vs **DM1 Follow-up:** 33.4±13.2 Nm; p<0.001; Change: -17.0±13.1 Nm; % change: 33.8% **Group A Baseline:** 51.8±13.9 Nm vs **Group A Follow-up:** 36.5±12.3 Nm; p<0.05; Change: -15.4±12.7 Nm; % change: 29.7% **Group B Baseline:** 49.9±16.6 Nm vs **Group B Follow-up**: 32.5±13.4 Nm; p<0.05; Change: -17.4±13.2 Nm; % change: 34.9% Phenotype comparison of subgroups effects: Within participants: p=0.35; Between participants: p=0.24 **DM1 (F) Baseline:** 44.2±11.1 Nm vs **DM1 (F) Follow-up:** 30±11.6 Nm; p<0.05; Change: -14.2±10.7 Nm; % change: 32.1% **DM1 (M) Baseline:** 61.8±17.6 Nm vs **DM1 (M) Follow-up:** 39.7±13.8 Nm; p<0.05; Change: -22.1±15.5 Nm; % change: 35.8% Sex comparison of subgroups effects: Within participants: p=0.14; Between participants: p=0.00	
				QMT (handheld dynamometry): MIT -	**DM1 Baseline:** 41.9±14.9 Nm vs **DM1 Follow-up:** 27.7±11.1 Nm; p<0.001, Change: -14.1±8.4 Nm; % change: 33.7%	

				Shoulder abductors	**Group A Baseline:** 44±12.1 Nm vs **Group A Follow-up:** 30.6±10.5 Nm; p<0.05; Change: -13.4±6.2 Nm; % change: 30.5% **Group B Baseline**: 41.2±15.6 Nm vs **Group B Follow-up**: 26.9±11.2 Nm; p<0.05; Change: -14.4±9 Nm; % change: 35% Phenotype comparison of subgroups effects: Within participants: p=0.47; Between participants: p=0.05 **DM1 (F) Baseline**: 33.9±9.2 Nm vs **DM1 (F) Follow-up**: 23.3±7.1 Nm; p<0.05; Change: -10.6±6 Nm; % change: 31.3% **DM1 (M) Baseline**: 56.6±11.9 Nm vs **DM1 (M) Follow-up:** 35.9±12.5 Nm; Change: -20.7±8.4 Nm; % change: 36.6% Sex comparison of subgroups effects: Within participants: p=0.00; Between participants: p=0.00	
				QMT (handheld dynamometry): MIT - Wrist extensors	**DM1 Baseline:** 5.1±2.8 Nm vs **DM1 Follow-up:** 2.4±1.4 Nm; p<0.001; Change: -2.6±1.7 Nm; % change:51% **Group A Baseline:** 7.3±4.4 Nm vs **Group A Follow-up:** 3.7±2.1 Nm; p<0.05; Change: -3.6±2.7 Nm; % change: 49.3% **Group B Baseline:** 4.4±1.7 Nm vs **Group B Follow-up:** 2.1±0.89 Nm; p<0.05; Change: -2.4±1.2 Nm; % change: 54.5% Phenotype comparison of subgroups effects: Within participants: p=0.001; Between participants: p=0.00 **DM1 (F) Baseline:** 4.5±2.9 Nm vs **DM1 (F) Follow-up:** 2.3±1.4 Nm; p<0.05; Change: -2.3±1.7 Nm; % change: 51.1% **DM1 (M) Baseline:** 6±2.3 Nm vs **DM1 (M) Follow-up:** 2.7±1.3 Nm; p<0.05; Change: -3.3±1.4 Nm; % change: 55% Sex comparison of subgroups effects: Within participants: p=0.002; Between participants: p=0.005	
Hammarén et al. (2014), Sweden [60]	**DM1**=51; Sex: 31F; 20M; Age: 41.3±9.7; BMI: 25±5.4	Median: 460 [212;700]	MIRS I-III (n): 19 MIRS IV-V (n): 32	QMT (handheld dynamometry): MIF-Ankle dorsiflexors	**DM1:** Median: 110 N [58;192]; Median: 52% of pred.; **DM1 (F):** Median: 174 N [74;201]; Median: 76% of pred. vs **DM1 (M):** Median: 174 N [44;125]; Median: 27% of pred.; p= 0.033 **MIRS I-III:** 216 N; Median: 200 N [184;225]; **MIRS IV-V:** 54.0 N; Median: 61.6 N [43;96]; p<0.001	Fair
				QMT (handheld dynamometry): MIF- Hip flexors	**DM1:** 169±40 N; 85% of pred.; **DM1 (F):** 155±31 N; 89% of pred. vs **DM1 (M):** 191±44 N; 78% of pred.; p=0.001	
				QMT (handheld dynamometry): MIF-Knee extensors	**DM1:** 277±97 N; 86% of pred.; **DM1 (F):** 252±61 N; 92% of pred. vs **DM1 (M):** 317±126 N; 77% of pred.; p=0.016	
				QMT (handheld dynamometry):MIF-Knee flexors	**DM1:** 100±34 N; 53% of pred.; **DM1 (F):** 97±26 N; 58% of pred. vs **DM1 (M):** 104±44 N; 46% of pred.; p=0.474	

Hammarén et al. (2015), Sweden [61]	**DM1 Baseline**=43, Sex:25F; 18M; Age: 41±9.1; Median: 39 (23-60); BMI: 24.4 (17.2-37.5) **DM1 Follow-up (5y)**=41; BMI 25.6 (17.5-39.4); **DM1 (F)**=25; Age; 41.1±8.7; Median: 39 (25-58) **DM1 (M)**=18; Age: 40.8±9.8; Median: 40.5 (23-60)	**DM1:** 558±385; Median: 475 (64-2000) **DM1 (F):** 543±461; Median: 450 (64-2000) **DM1 (M)**: 580±253; Median: 590 (105-1100)	DM1: MIRS III (n):16 MIRS IV (n):24 MIRS V (n): 3 DM1 (F): MIRS III (n):14 MIRS IV (n):11 DM1 (M): MIRS III (n): 2 MIRS IV (n):13 MIRS V (n): 3	QMT (handheld dynamometry)**:** MIF-Ankle dorsiflexors	**DM1 Baseline:** 130±86 N; Median: 111 N (5-450) vs **DM1 Follow-up:** 15±91 N; Median: 74 N (3-395); Change: -15±30 N; Median: -10 N (-93 to 55); p<0.01 **DM1 (F):** 151±66 N; Median: 176 N (30-246) vs **DM1 (M):** 101±101 N; Median: 71.5 N (5-450) **DM1 (F):** Change: -9.8±33 N; Median: -9.2 N (-88 to 55) vs **DM1 (M):** Change: -22±26 N; Median: -12.4 N (-93 to 20)	Good
				QMT (handheld dynamometry)**:** MIF-Hip flexors	**DM1 Baseline:** 171±39 N; Median: 172 (114-283) vs **DM1 Follow-up:** 159±36 N; Median: 155 N (86-232); Change: -12±18 N; Median: -12 N (-57 to 55); p<0.001 **DM1 (F):** Change: -10.7±13 N; Median: -12 N (-38 to 19) vs **DM1 (M):** Change: -14±25 N; Median: -14.8 N (-57 to 26)	
				QMT (handheld dynamometry): MIF-Knee extensors	**DM1 Baseline:** 274±95 N; Median: 274 [92;474] vs **DM1 Follow-up:** 258±10.3 N; Median: 269 [47;473]; Change: -15±43 N; Median: -17 N [-98;105]; p<0.05 **DM1 (F):** Change: -1.7±45 N; Median: -2.7 N (-98 to 105) vs **DM1 (M):** Change: -35±33 N; Median: -37 N [-95;30]	
				QMT (handheld dynamometry)**:** MIF-Knee flexors	**DM1 Baseline:** 101±33 N; Median: 106 N (3.5-171) vs **DM1 Follow-up:** 93±35 N; Median: 101 N (0-154); Change: -8±14 N; Median: -8.2 N (-38 to 23); p<0.01 **DM1 (F):** Change: -4.2±12 N; Median: -0.5 N (-28 to 19) vs **DM1 (M):** Change: -13.2±14.3 N; Median: -16.3 N (-38 to 23); p=0.021	
Hébert et al. (2018), Canada [62]	**DM1 Quebec (Group A) baseline**=23 **Group A Follow-up (5y)**=16; Sex:7F; 9M; Age: Median:34.5 (22-57) **DM1 Lyon (Group B) baseline**=26 **Group B Follow-up (5y)**=10; Sex: 2F; 8M; Age: Median: 42 (31-62)	**Group A** Median: 750 (130-1400) **Group B:** Median: 500 (100-867)	NR	QMT (handheld dynamometry): MIT - Ankle dorsiflexors	**Group A:** Change (%Change): -3.1 Nm (36%) **Group B:** Change (%Change): -2.3 Nm (27.7%) Baseline muscle strength vs %Change: r=0.58; R2=0.33;p<0.05 Baseline muscle strength vs Change: r=0.67; R2=0.45; p<0.01	Fair
				QMT (handheld dynamometry): MIT - Ankle evertors	**Group A: Change (%Change)**: -2.6 Nm (31.3%) **Group B: Change (%Change):** -3.7 Nm (35.5%) Baseline muscle strength vs %Change: r=0.40; R2=0.16;p<0.01 Baseline muscle strength vs Change:r=0.69; R2=0.07; p<0.01	
Hébert et al (2010), Canada [63]	**DM1 Quebec Total**=22; 11F; 11M; Age: 41.1±13.8 **DM1 Quebec (Group A)**=11 **DM1 Quebec (Group B)**=6 **DM1 Quebec (Group C)**=5 **Controls for DM1 Quebec (Controls 1)**=7; Sex: 2F; 5M; Age: 43.1±9 **DM1 Lyon Total** =24; Sex:	NR	NR	QMT (handheld dynamometry): MIT - Ankle dorsiflexors	**Group A right**: 12.8±3.7 Nm vs **Group B right**: 6.9±2.4 Nm vs **Group C right**: 3.2±1.1 Nm vs **Controls 1**: 25.8±6 Nm **Group D right:** 9.4±3.8 Nm vs **Group E right**: 5.3±2.2 Nm vs **Group F right**: 2.7±1.5 Nm vs **Controls 2**: 26.8±5.8 Nm **Group A left**: 12.7±4.9 Nm vs **Group B left**: 6.9±2.4 Nm vs **Group C left**: 3.2±1.5 Nm vs **Controls 1**: 24.6±4.6 Nm **Group D left:** 9.2±2.8 Nm vs **Group E left**: 6.2±3.7 Nm vs **Group F left**: 3.8±1.4 Nm vs **Controls 2**: 26.3±6.2 Nm	Fair

	13F; 11M; Age: 41.6±10.2 **DM1 Lyon (Group D)**=11 **DM1 Lyon (Group E)**=6 **DM1 Lyon (Group F)**=7 **Controls for DM1 Lyon (Controls 2)**=9; Sex: 2F; 7M; Age: 45.7±9			QMT (handheld dynamometry): MIT - ankle evertors	**Group A right:** 12.3±3.8 Nm vs **Group B right:** 6±2.2 Nm vs **Group C right:** 5±2.5 Nm vs **Controls 1:** 19.6±5.1Nm **Group D right:** 8.9±3.2 Nm vs **Group E right:** 5.2±1.8 Nm vs **Group F right**: 2.1±2.2 Nm vs **Controls 2:** 18.1±5 Nm **Group A left**: 12.5±5.3 Nm vs **Group B left**: 6±1.7 Nm vs **Group C left**: 3.8±2.9Nm vs **Controls 1**: 18.5±3.9 Nm **Group D left:** 8±3.2 Nm vs **Group E left**: 4.7±1.5 Nm vs **Group F left**: 1.8±1.5 Nm vs **Controls 2**: 17.9±4.2 Nm	
Hermans et al. (2011), the Netherlands [64]	**DM1**=93; Sex:40F; 53M; Age: 45±11 **DM1 with peripheral neuropathy (Group A)**=16; Sex: 4F; 12M; Age: 43.4±1.9; BMI: Median:23.3 (15.6-33.6) **DM1 without peripheral neuropathy (Group B)**=77; Sex: 36F; 41M; Age: 45.4±1.3; BMI: Median: 23.9 (15.4-41.5)	NR	NR	MMT: MRC scale (0-5 points) compound score - Proximal Limb (bilateral shoulder abductors, elbow flexors and extensors, hip flexors, knee extensors, and flexors)	**Group A:** Median: 3.4 (2.4-5) vs **Group B:** Median: 4.3 (2.8-5); p=0.001	Good
				MMT: MRC scale (0-5 points) compound score - Distal Limb (bilateral wrist extensors, digits flexors, ankle dorsiflexors, and ankle plantar flexors)	**Group A:** Median: 4.7 (4-5) vs **Group B:** Median: 5 (3.3-5); p=0.02	
				MMT: MRC scale Sum Score (0-110 points)	**Group A:** Median: 92 (75-107) vs **Group B:** Median: 100 (70-110); p=0.001	
Hiba et al (2011), France [65]	**DM1**=19; Sex: 9F; 10M; Age: 39.5±13 (20-62); **DM1 ≥4 and <5 MRC (Group A)**=9 **DM1≥3 and <4(Group B)**= 4 **DM1<3 MRC(Group C)**=6 **Controls**=6; Age: 42.6±9.5 (31-58)	NR	NR	QMT (handheld dynamometry): MIT - Ankle dorsiflexors	**Group A:** 11.21±2.62 Nm vs **Group B**: 6.58±1.91 Nm vs **Group C**: 2.42±1.48 Nm vs **Controls**= 23.12±5.04 Nm	Fair
Hogrel et al. (2009), France [66]	**DM1**=16; Age: 45.8±8.2 **Control=**16; Age: 40.5±13.3	(133-1066)	MIRS III (n): 8 MIRS IV (n): 8	QMT (handgrip dynamometry): MVIC - grip strength	**DM1 (F):** 12.1±4.7 daN vs **Controls**: 33.8±4.8 daN; p<0.0001 **DM1 (F):** 0.19±0.1 daN/Kg vs **Controls:** 0.58±0.1 daN/Kg; p<0.0001 **DM1 (M):** 8.5±4.6 daN vs **Controls:** 47.4±3.5 daN; p<0.0001 **DM1 (M):** 0.1±0.1 daN/Kg vs **Controls:** 0.63±0.1 daN/Kg; p<0.0001	Fair
Hogrel et al. (2017), France [67]	**DM1**=144 Sex:76F; 68M Age: 42.2±11 **Controls** = 44; Sex:24F; 20M; Age: 40.2±11.5	679±414	MIRS: 3.2±0.9 MIRS I (n):3 MIRS II (n):17 MIRS III (n):62 MIRS IV (n):36 MIRS V (n): 8	QMT: MVIC - Grip strength	**DM1:** 125.2±80.7 N; 35.4±19.4% of pred. vs **Controls:** 371.5±103.8 N; 101±13.8% of pred.; p<0.001 and p<0.001, respectively Grip strength vs CTG repeat length: ρ=-0.29; p<0.001 Grip strength (% of pred.) vs CTG repeat length: ρ= -0.269; p=0.002 Muscle strength vs MIRS: ρ= -0.326; p<0.0001	Fair

					DM1 (F) vs DM1 (M) % of pred.: p=0.02	
Jimenez-Moreno et al. (2019), U.K. (cross-sectional) [68]	**DM1**=213; Sex: 109F; 104M; Age: 45.2±14.5; BMI: 22±5.9 **Adult-onset (Group A)**= 172 **Late-onset (Group B)**=41	500±357	MIRS I (n): 22 MIRS II (n): 59 MIRS III (n):46 MIRS IV (n):70 MIRS V (n): 16	QMT: Ankle dorsiflexors	**DM1:** 26±13 lb **MIRS I:** 42±14 lb vs **MIRS II:** 31±12 lb vs **MIRS III:** 21±11 lb vs **MIRS IV:** 19±9 lb vs **MIRS V:** 14±10 lb **Group A:** 23±14 lb vs **Group B:** 36±15 lb; p≤0.001 **DM1 (M):** 29±15 lb **MIRS I:** 47±12 lb vs **MIRS II:** 35±12 lb vs **MIRS III:** 20±11 lb vs **MIRS IV:** 21±9 lb vs **MIRS V:** 18±16 lb **Group A:** 25±13 lb vs **Group B:** 40±16 lb; p≤0.001 **DM1 (F):** 23±11 lb **MIRS I:** 30±10 lb vs **MIRS II:** 28±11 lb vs **MIRS III:** 23±11 lb vs **MIRS IV:** 21±9 lb vs **MIRS V:** 12±6 lb **Group A:** 22±11 lb vs **Group B:** 30±13 lb; p≤0.001	Good
				QMT: Hip flexors	**DM1:** 33±13 lb **MIRS I:** 44±11 lb vs **MIRS II:** 38±13 lb vs **MIRS III:** 35±11 lb vs **MIRS IV:** 27±11 lb vs **MIRS V:** 25±12 lb **Group A:** 32±13 lb vs **Group B:** 39±13 lb; p≤0.001 **DM1 (M):** 39±13 lb **MIRS I:** 48±10 lb vs **MIRS II:** 45±12 lb vs **MIRS III:** 38±11 lb vs **MIRS IV:** 33±13 lb vs **MIRS V:** 29±13 lb **Group A:** 39±15 lb vs **Group B:** 42±12 lb **DM1 (F):** 28±10 lb **MIRS I:** 32±7 lb vs **MIRS II:** 33±12 lb vs **MIRS III:** 30±9 lb vs **MIRS IV:** 23±7 lb vs **MIRS V:** 18±3 lb **Group A:** 28±10 lb vs **Group B:** 34±114 lb; p≤0.001	
				QMT: Knee extensors	**DM1:** 46±20 lb **MIRS I:** 61±19 lb vs **MIRS II:** 54±17 lb vs **MIRS III:** 47±17 lb vs **MIRS IV:** 37±19 lb vs **MIRS V:** 30±16 lb **Group A:** 44±20 lb vs **Group B:** 56±17 lb **DM1 (M):** 54±20 lb **MIRS I:** 67±17 lb vs **MIRS II:** 65±16 lb vs **MIRS III:** 52±15 lb vs **MIRS IV:** 47±20 lb vs **MIRS V:**35±18 lb **Group A:** 55±20 lb vs **Group B:** 47±15 lb **DM1 (F):** 38±17 lb **MIRS I:** 48±15 lb vs **MIRS II:** 47±15 lb vs **MIRS III:** 41±18 lb vs **MIRS IV:** 29±15 lb vs **MIRS V:** 22±8 lb	

					Group A: 37±17 lb vs **Group B:** 47±15 lb	
Jimenez-Moreno et al.(2019), U.K. (cohort) [68]	**DM1**=98; Sex: 43F, 55M; Age: 46±14; BMI 26±6	500±357	MIRS I (n): 22 MIRS II (n): 59 MIRS III (n):46 MIRS IV (n):70 MIRS V (n):16	QMT: Ankle dorsiflexors	**DM1:** 25.6±1.9 lb; Change: 1.9 lb vs % change: 7%; 95% CI: 4.5 to -0.7; p=Ns **Group A:** 21.6±12 lb; Change: 3.5 lb vs % change: 16%; 95% CI: 6.3 to 0.6; p=0.02 **Group B:** 33.3±15.4 l; Change: -2.2 lb vs % change: -7%; 95% CI: 3.3 to -7.7; p=Ns	Good
				QMT: Hip flexors	**DM1:** 33.7±13.1lb; Change: 2.2 lb vs % change: 6%; 95% CI: 4.8 to -0.5; p=Ns **(Group A):** 31.6±14 lb; Change: 3.3 lb vs % change: 10%; 95%CI: 6.3 to 0.3; p=0.03 **Group B:** 37.3±12 lb; Change: -1.9 lb vs % change: -5 %; 95% CI: 4 to -7.7; p=Ns	
				QMT: Knee extensors	**DM1:** 46.4±19.4 lb; Change: 2.1 lb vs % change: 5%; 95% CI: 5.4 to -1.1; p=Ns **Group A:** 44.2±19 lb; Change: 1.1 lb vs % change: 2%; 95% CI: 4.4 to -2.2; p=Ns **Group B:** 54.3±19.1 lb; Change: 5.8 lb vs % change: 11%; 95% CI: 15.6 to -4; p=Ns	
Kierkegaard et al. (2017), Sweden [69]	**DM1**=70; Sex: 41F; 29M; Age: 45±13; BMI: 24±5	NR	MIRS I (n):3 MIRS II (n):13 MIRS III (n):21 MIRS IV (n):13 MIRS V (n):20	QMT (hand dynamometry): Grip strength	**DM1 Right hand:** Avg. of trials: 11.4±10.1 Kg; Median: 8 Kg (4.3-15.4) vs Best of trials: 12.3±10.3 Kg; Median: 9 Kg (5-16); ICC: 1; 95% CI: 0.8-1 **DM1 Left hand:** Avg. trial 11.1±10.3 Kg; Median: 7.8 Kg (4.3-14.4) vs Best of trials: 11.8±10.7 Kg; Median: 8 Kg (5-14), ICC: 1; 95% CI:0.9-1	Good
Kierkegaard et al. (2018), Sweden [70]	**DM1 Baseline**=113; Sex:71F; 42M; Age: 52 (29-85) **DM1 Follow-up (9y)** =113	NR	DM1 Baseline MIRS I-III (n): 47 MIRS IV-V (n): 66 DM1 Follow-up MIRS I-III (n): 29 MIRS IV-V (n): 84	QMT (handheld dynamometry): Grip strength	**DM1 Baseline (n=105):** Median: 11.2 Kg (0.8-51); [5.2;17.5] vs **DM1 Follow-up (n=105):** Median: 8.9 Kg (0.8-51.5); [5;15.6]; Change: Median: -1 Kg (-24.2 to -6.8); [-2.7; -0.7]; % change: Median: -13.3 (-81.8 to 220); [-30.4;11.1]	Fair
				QMT (pinch gauge): Pinch strength	**DM1 Baseline (n=109):** Median: 5.7 Kg (2.2-13.7); [4.5;7.2] vs **DM1 Follow-up (n=109):** Median: 4 Kg (0.8-12.5); [3;5]; Change: Median: -1.4 Kg (-5.2 to 0); [-2;-1]; % change: Median: -27.8 (–79.1 to -0.4); [–37.1;20.6] Pinch strength vs grip strength (% change): 0.4	
				QMT (handheld dynamometry): MIT - Ankle dorsiflexors	**DM1 Baseline (n=105):** Median: 15.1 Nm (2.2-38.7); [11;19.6] vs **DM1 Follow-up (n=105):** Median: 6.8 Nm (0.5-29.5); [4.5;10]; Change: Median: -8.1 Nm (-25.3 to 2.6); [-11.4;-4.9]; % change: Median: -56.4 (-90.9 to -20.8) [-67.4;-40.1] Ankle dorsiflexors vs lower-limb score % change: 0.5	
				QMT (handheld dynamometer): MIT - Hip flexors	**DM1 Baseline (n=105):** Median: 50.8 Nm (19.7-137.5), [39.8;62.2] vs **DM1 Follow-up (n=105):** Median: 52.2 Nm (41-71.2); [14-121.6]; Change: Median: 1.8 Nm (-47 to – 44.9); [-5.5;12.3]; % change: Median: 3.4; (-53.5- 143.9); [-12.6; 23] Hip flexors vs lower limb (% change): 0.6 Hip flexors vs ankle dorsiflexors (% change): 0.2 Hip flexors vs knee extensors (% change): 0.4 Hip flexors vs knee flexors (% change): 0.3	

				QMT (handheld dynamometry): MIT - Knee extensors	**DM1 Baseline (n=104):** Median: 79.5 Nm (18.9-165.3); [59.6;98.9] vs **DM1 Follow-up (n=104):** Median: 53.4 Nm (4.60-153.85); [38.9;72.1]; Change: Median: -25.2 Nm (-80 to -16); [-37.8 to -10.3] Nm; % change: Median:-33.6 (-86.3 to 22.3) [-43.5;17.7] Knee extensors vs lower-limb (% change): 0.8 Knee extensors vs ankle dorsiflexors (% change): 0.4 Knee extensors vs knee flexors (% change): 0.5	
				QMT (handheld dynamometry): MIT - Knee flexors	**DM1 Baseline (n=106):** Median: 48.9 Nm (20.3-114.4); [40;63.1] vs **DM1 Follow-up (n=106):** Median: 31.9 Nm (4.5-88.4); [23.2;43.1]; Change: Median: -16.5 Nm (-55.4-16.7); [-25.9;-8.1]; % change: Median: -33.9 (-81.5-23.4); [50.2; -19] Knee flexors vs lower-limb score (% change): 0.8 Knee flexors vs ankle dorsiflexors (% change): 0.3	
				QMT (handheld dynamometry): MIT - Lower limb	**DM1 Baseline (n=102):** Median: 390.9 Nm (156.7-723.2); [310.6;474.5] vs **DM1 Follow-up (n=102):** Median: 302.5 Nm; (57.1-743.2); [237.7;393.4]; Change: Median: -100.2 Nm (-323 to -101.5); [-133.3;-48.8]; % change: Median: -24.3 (-63.5 to 29); [-35.7;-12.5]	
Knak et al. (Jul 2020), Denmark [71]	**DM1 Baseline=** 63 Sex: 30F; 33M; Age: 41±10; BMI: Median: 24 [21;27] **DM1 Follow-up (1y)** =63;	NR	DM1 Baseline MIRS II (n): 13 MIRS III (n): 2 MIRS IV (n):42 MIRS V (n): 6	QMT (Handheld dynamometry): MIT - Ankle dorsiflexors	**DM1 Baseline:** Median: 18.2 Nm [5.7;25.7] vs **DM1 Follow-up:** Median: 18.1 Nm [6.6;27.5]; p=0.79; Change: 95%CI: 0.9-1.1 Nm; % change: 1%; 95% CI: -7% to 10%	Good
				QMT (Handheld dynamometry): MIT- Ankle plantar flexors	**DM1 Baseline:** Median: 18 Nm [10.3;25.7] vs **DM1 Follow-up:** Median: 15.9 Nm [10.3;24.6]; p=0.01; Change: -1.3; 95%CI: -2.3 to -0.3%; % change: -7.1; 95%CI: -12.6 to -1.6%	
				QMT (Handheld dynamometry): MIT- Hip extensors	**DM1 Baseline:** 64.2±22.6 Nm vs **DM1 Follow-up:** 59.9±19.9 Nm; p=0.065; Change: -3.5; 95%CI: -7.3 to 0.2 %; % change: -5.5; 95% CI: -11.4 to 0.3%	
				QMT (Handheld dynamometry): MIT - Hip flexors	**DM1 Baseline:** 65.3±20.4 Nm vs **DM1 Follow-up:** 61.3±22.4 Nm; p=0.03; Change: -3.8; 95%CI: -7.2 to -0.51%; % change: -5.8; 95%CI: -10.9 to -0.7%	
				QMT (Handheld dynamometry): MIT - Knee extensors	**DM1 Baseline:** 105.3±38.2 Nm vs **DM1 Follow-up:** 108.5±41.7 Nm, p=0.16; Change: 3.6; 95%CI: -1.3 to 8.5%; % change: 3.4; 95%CI: -1.2 to 8.1%	
				QMT (Handheld dynamometry): MIT - Knee flexors	**DM1 Baseline:** 68.1±26.4 Nm vs **DM1 Follow-up:** 65.7±25.6 Nm; p=0.11; Change: -2.2; 95%CI: -5 to 0.4%; % change: -5.8; 95%CI: -10.9 to -0.7%	
				QMT (stationary dynamometry) MIT – Ankle dorsiflexors	**DM1 Baseline:** Median:16.8 Nm [7.7;27.3] vs **DM1 Follow-up:** Median: 17.1 Nm [8.5;27.8], p=0.5; Change: 95%CI: 0.9-1%; % change: 1; 95%CI: -3 to 6%	
				QMT (stationary dynamometry) MIT – Ankle plantar flexor	**DM1 Baseline:** Median: 27.2 Nm [14.8;39.2] vs **DM1 Follow-up:** Median: 26.9 Nm [14.6;47.1]; p=0.52; Change: 1; 95%CI: 0.9-1.1%; % change: 3; 95%CI: -6 to 12%	

				QMT (stationary dynamometry) MIT – Hip extensors	**DM1 Baseline:** Median: 128.4 Nm [98.2;176.7] vs **DM1 Follow-up:** Median: 106.7 Nm [72;143.6]; p<0.0001; Change: -28.6; 95%CI: -37.8 to -19.4%; % change: -20.1; 95%CI: -26.6 to -13.6%	
				QMT (stationary dynamometry): MIT – Hip flexors	**DM1 Baseline:** 69.4±28.4 Nm vs **DM1 Follow-up:** 85.5±30.4 Nm; p<0.0001; Change: 16.3; 95% CI: 12.4-20.2 Nm; % change: 23.5; 95% CI: 17.8- 29.2%	
				QMT (stationary dynamometry): MIT – Knee extensors	**DM1 Baseline:** 139.7±64.4 Nm vs **DM1 Follow-up:** 133.5±66.7 Nm; p=0.02; Change: -6; 95% CI: -11 to -0.9; % change: -4.1%; 95% CI: -7.9% to -0.7%	
				QMT (stationary dynamometry): MIT – Knee flexors	**DM1 Baseline:** 61.1±27.2 Nm vs **DM1 Follow-up:** 58.2±26.3 Nm; p=0.009; Change: -2.8; 95% CI: -4.9 to -0.7 Nm; % change: -4.6; 95% CI: -8 to -1.3%	
Knak et al. (Jun 2020), Denmark [72]	**DM1 Baseline**=78; Sex: 39F; 39M; Age: 40±10; BMI: Median: 24 (16-37) **DM1 Follow-up (2 weeks)**=76	NR	MIRS II (n): 17 MIRS III (n): 3 MIRS IV (n):51 MIRS V (n): 7	QMT (handheld dynamometry): MIT - Ankle dorsiflexors	**DM1 Baseline (n=64):** 19.4±9.1 Nm vs **DM1 Follow-up (n=64):** 19.8±9.7 Nm; p=0.198; Change: 0.4±3.6 Nm; SEM (SEM%): ±1.7 (±9%); MDD95 (MDD 95% and MDD 95% without outliers): ±4.7 (±24%); ICC (95%CI): 0.9 (0.95-0.98); MCID (MCID %): 4.5 (24%)	Good
				QMT (handheld dynamometry): MIT - Ankle plantar flexors	**DM1 Baseline (n=69):** 19±9.3 Nm vs **DM1 Follow-up (n=69):** 19.3±9.1 Nm; p=0.436; Change: 0.3±3.6 Nm; SEM (SEM%): ±2.5 (±13%); MDD95 (MDD 95% and MDD 95% without outliers): ±7 (±37%) [±31%]; ICC (95%CI): 0.9 (0.88-0.95); MCID (MCID %): 4.6 (25%)	
				QMT (handheld dynamometry): MIT- hip extensors	**DM1 Baseline (n=70):** 62.3±24.3 Nm vs **DM1 Follow-up (n=70):** 62.3±23.8 Nm; p=0.436; Change: -0.02±11.8 Nm; SEM (SEM%): ±8.37 (±13%); MDD95 (MDD 95% and MDD 95% without outliers): ±23.2 (±37%); ICC (95%CI): 0.8 (0.82-0.92); MCID (MCID %): 12.1 (20%)	
				QMT (handheld dynamometry): MIT - hip flexors	**DM1 Baseline (n=70):** 63±22.9 Nm vs **DM1 Follow-up (n=70):** 64.8±24.6 Nm; p=0.173; Change: 1 (0.9, 1); 3 (-1,7); SEM (SEM%): ±1.1 (±11%); MDD95 (MDD 95% and MDD 95% without outliers): ±1.34 (±34%) [±31%]; ICC (95%CI): 0.89 (0.84-0.93); MCID (MCID %): 11.48 (18%)	
				QMT (handheld dynamometry): MIT - knee extensors	**DM1 Baseline (n=71):** 102.8±35.8 Nm vs **DM1 Follow-up (n=71):** 102.7±37.3 Nm; p=0.962; Change: -0.1±13.4; SEM (SEM%): ±9.5 (±9%); MDD95 (MDD 95% and MDD 95% without outliers): ±26.4 (±26%) [±22%]; ICC (95%CI): 0.9 (0.89-0.96); MCID (MCID %): 17.9 (17%)	
				QMT (handheld dynamometry): MIT - knee flexors	**DM1 Baseline (n=72):** 66±24.1 Nm vs **DM1 Follow-up (n=72):** 67.2±25.7 Nm; p=0.165; Change: 1.2±7.2 SEM (SEM%): ±5.1 (±8%); MDD95 (MDD 95% and MDD 95% without outliers): ±14.2 (±21%); ICC (95%CI): 0.9 (0.93-0.97); MCID (MCID %): 12.1 (18%)	
				QMT (stationary dynamometry): MIT - Ankle dorsiflexors	**DM1 Baseline (n=58):** Median: 20.5 Nm; [5.1-49.5] vs **DM1 Follow-up (n=58):** Median: 20.7 Nm; [4.1-47.9]; p=0.17; Change: 0.8±4.8; SEM (SEM%): ±3.4 (±15%); MDD95 (MDD 95% and MDD 95% without outliers): ±9.4 (±41%) [±17%]; ICC (95%CI): 0.9 (0.8-0.9); MCID (MCID %): 1.3 (32%)	

				QMT (stationary dynamometry): MIT - Ankle plantar flexors	**DM1 Baseline (n=68):** Median: 26.3 Nm; [2.5-97.5% Percentiles]: [5.6-90.1] vs **DM1 Follow-up (n=68):** Median: 27.4 Nm; [2.5-97.5% Percentiles]: [3.6-96.8]; p=0.348; Change: Median: 1 [0.9, 1.1]; 4 [-5,14]; SEM (SEM%): ±1.3 (±30%); MDD95 (MDD 95% and MDD 95% without outliers): ±2.1 (±107%) [±71%]; ICC (95%CI): 0.7 (0.6-0.8); MCID (MCID %): 1.4 (43%)	
				QMT (stationary dynamometry): MIT - Hip extensors	**DM1 Baseline (n=71):** 128.5±58.1 Nm vs **DM1 Follow-up (n=71):** 137.3±65.4 Nm; p=0.003; Change: 1 (1-1.1); 5 (1-10); SEM (SEM%): ±1.1(±14%); MDD95 (MDD 95% and MDD 95% without outliers): ±1.4 (±45%) [±38%]; ICC (95%CI): 0.9 (0.8-0.9); MCID (MCID %): 29 (23%)	
				QMT (stationary dynamometry): MIT - Hip flexors	**DM1 Baseline (n=71):** 63.1±25.8 Nm vs **DM1 Follow-up (n=71):** 67.1±28.3 Nm; p=0.013; Change: 4±13.5; SEM (SEM%): ±9.5 (±15%); MDD95 (MDD 95% and MDD 95% without outliers): ±26.5 (±41%) [±25%]; ICC (95%CI): 0.8 (0.7-0.9); MCID (MCID %): 12.9 (20%)	
				QMT (stationary dynamometry): MIT - knee extensors	**DM1 Baseline (n=72):** 136.9±59.9 Nm vs **DM1 Follow-up (n=72):** 135.3±62.6 Nm; p=0.452; Change: 0.9 (0.9, 1); -2 (-6,1); SEM (SEM%): ±1.1 (±10%); MDD95 (MDD 95% and MDD 95% without outliers): ±1.3 (±32%) [24%]; ICC (95%CI): 0.9 (0.93-0.97); MCID (MCID %): 29.9 (22%)	
				QMT (stationary dynamometry): MIT - knee flexors	**DM1 Baseline (n=72):** 57.3±25.9 Nm vs **DM1 Follow-up (n=72):** 59.6±27 Nm; p=0.080; Change: 2.3±11.1 SEM (SEM%): ±7.8 (±13%); MDD95 (MDD 95% and MDD 95% without outliers): ±21.8 (±37%) [±25%]; ICC (95%CI): 0.9 (0.8-0.9); MCID (MCID %): 12.9 (23%)	
Moxley et al. (2007), USA [73]	**DM1**=29 Sex:11F; 18M; Age: 45.9 (23-62) **Control**=17; Sex: 8F; 9M; Age:40.9 (21-62)	563 (169-1731)	NR	MMT: Modified MRC scale (0-5 points)	**DM1:** 3.9 (2.7–4.8); MRC vs CTG repeat length: r=-0.37; p<0.05	Good
				QMT (hand dynamometry): Peak force grip strength	**DM1:** 10.4 kg vs **Controls:** 2.5 kg; p<0.001; Peak force grip strength vs CTG repeat length: r=-0.42; p<0.03	
				QMT (force transducer): MVIC (elbow flexors and extensors, knee flexors and extensors, ankle flexor, and grip muscles)	DM1 vs QMT normative data: z-score: -4.81 (-10.32 to -1.88); MVIC vs CTG repeat length: r=-0.42; p<0.03	
Petitclerc et al. (2017), Canada [74]	**DM1**=107; Sex: 69F; 38M; Age: median: 42 (20-77); BMI: median: 25.7 (14.5-44.2) **Adult-onset (Group A)**=84; Age: median: 41 (20-58);BMI: Median: 25.8 (14.5-44.2) **Late-onset (Group B)**=23; Age: Median: 50 (32-77);	**DM1**: Median: 700 (60-2000) **Group A:** Median: 925 (150-2000) **Group B**: Median: 100	Hip flexors MIRS I (n):6 MIRS II (n):14 MIRS III (n):27 MIRS IV (n):55 MIRS V (n): 4 Knee extensors MIRS I (n):5 MIRS II (n):14 MIRS III (n):27	MMT: MRC scale (0-10) - Ankle dorsiflexors	**Group A (n=83)**: Median:7 (3-10) vs **Group B (n=23)**: Median:10 (6.5-10); p<0.001	Good
				MMT: MRC scale (0-10)- Hip flexors	**Group A (n=83)**: Median:9 (5-10) vs **Group B (n=23)**: Median:10 (7-10); p=0.005	
				MMT:MRC scale (0-10)- Knee extensors	**Group A (n=83):** Median:9.5 (3-10) vs **Group B (n=23):** Median:10 (6-10); p=0.020	
				MMT: MRC scale (0-10) - Knee flexors	**Group A (n=83):** Median:9.5 (5-10) vs **Group B (n=23):** Median:10 (8.5-10); p=0.001	

	BMI: Median 24.4 (17.6-39.7)	(60-700)	MIRS IV (n):55 MIRS V (n): 4 Knee flexors MIRS I (n): 6 MIRS II (n): 14 MIRS III (n):27 MIRS IV (n): 6 MIRS V (n): 4 Ankle dorsiflexors MIRS I (n): 6 MIRS II (n): 14 MIRS III (n):26 MIRS IV (n): 6 MIRS V (n): 4	QMT (handheld dynamometry): MIF - Ankle dorsiflexors	**DM1:** 61.3±29.2% pred.; 99% CI (55.7-67) **Group A (n=83):** 54.2±26.6% pred.; 99% CI (48.4-60) vs **Group B (n=23):** 88.3±23.1% pred.; 99%CI (77.3-97.3); p<0.001 **DM1 MIRS I (n=6):** 75.9±14.5% pred.; 99% CI (60.7-91); **DM1 MIRS II (n=14):** 85.5±17.4% pred.; 99% CI (75.5-95.6); **DM1 MIRS III (n=26):** 76.6±27.3% pred.; 99% CI (65.5-87.6); **DM1 MIRS IV (n=56):** 49.1±25.8% pred.; 99% CI (42.2-56); **DM1 MIRS V (n=4):** 26.9±16% pred.; 99% CI (1.5-52.33); Ankle dorsiflexors vs MIRS: r=- 0.610; p<0.05	
				QMT (handheld dynamometry): MIF - Hip flexors	**DM1:** 69.5±19% pred.; 99% CI (65.8-73.1); **Group A (n=83):** 65.6±17.9% pred.; 99% CI (61.7-69.5) vs **Group B (n=23):** 83.5±16.3% pred.; 99% CI (76.4-90.5); p<0.001; **DM1 MIRS I (n=6):** 87.6±14% pred.; 99% CI (72.9-102.3); **DM1 MIRS II (n=14):** 87.2±17% pred.; 99% CI (77.3-97); **DM1 MIRS III (n=27):** 77.3±12.7% pred.; 99% CI (72.3-82.3); **DM1 MIRS IV (n=55):** 60.2±16.7% pred.; 99% CI (55.7-64.7); **DM1 MIRS V (n=4):** 53.9±8.2% pred.; 99% CI (40.9-67) Hip flexors vs MIRS: r=-0.626; p<0.05	
				QMT (handheld dynamometry): MIF - Knee extensors	**DM1:**67.1±20.9% pred.; 99% CI (63.1-71.1); **Group A (n=83):** 63.8±20.5% pred.; 99% CI (59.3-68.3) vs **Group B (N=22):** 79.6±17.6% pred.; 99% CI (71.8-87.4); p=0.001 **DM1 MIRS I (n=5):** 86.2±13.8% pred.; 99%CI (69.1-103.4); **DM1 MIRS II (n=14):** 88.7±14.6% pred.; 99%CI (80.3-110.1); **DM1 MIRS III (n=27):** 74.7±12.9 % pred.; 99%CI (69.6-79.7); **DM1 MIRS IV (n=55):** 59.5±17% pred.; 99%CI (54.9-64.1) ; **DM1 MIRS V (n=4):** 20.3±6% pred.; 99%CI (10.8-29.9); Knee extensors vs MIRS: r=-0.532; p<0.05	
				QMT (handheld dynamometry): MIF- Knee flexors	**DM1:** 86.1±22.8% pred.; 99%CI (81.7-90.4); **Adult-onset (n=84):** 81.2±21.4% pred.; 99%CI (76.5-85.9) vs **Late-onset (n=23):** 103.9±18.5% pred.; 99%CI (95.9-111.9); p<0.001; **DM1 MIRS I (n=6):** 106.7±11.8% pred.; 99%CI (94.3-119); **DM1 MIRS II (n=14):** 101.9±14.1% pred.; 99%C (93.8-110.1); **DM1 MIRS III (n=27):** 94.5±20.4% pred.; 99%CI (86.4-102.6)**; DM1 MIRS IV (n=56):** 78.2±20.9 % pred.; 99%CI (72.6-83.8); **DM1 MIRS V (n=4):** 52.8±18.4% pred.; 99%CI (23.5-82) Knee flexors vs MIRS: r=-0.580; p<0.05	
Pruna et al. 2011, France [75]	**DM1**=69; Sex: 45F;24M; Age: 42.3±12.5	975±748	NR	MIRS	MIRS vs Age: r=0.37; p=0.002	Fair
				QMT (dynamometry): Grip strength	**DM1 (n=59):** 41.3±20.9% (10.8-101) Grip strength vs MIRS: r=-0.47; p=0.001	

Raymond et al (2017), Canada [76]	**DM1 Baseline**= 108; Sex: 70F; 38M; Age:43.6±10.6 (20-77) **DM1 Follow-up (9y)**= 108; Sex: 70F; 38M; Age: 52.2±10.6 (29-85) **Late-onset (Group A)**=25 **Adult-onset (Group B)**=83	**Baseline:** 50 to 199 (n): 20 200 to 1000 (n): 56 >1000 (n): 32 **Follow-up:** 50 to 199 (n): 13 200 to 1000 (n): 35 >1000 (n): 60	Baseline: MIRS I (n): 6 MIRS II (n): 14 MIRS III (n): 27 MIRS IV (n): 56 MIRS V (n): 4 Follow-up: MIRS I (n): 1 MIRS II (n): 5 MIRS III (n): 23 MIRS IV (n): 63 MIRS V (n): 13	QMT (Hand dynamometry): Grip strength	**DM1 Baseline right hand:** 12.8±10.1kg vs **DM1 follow-up right hand:** 11.1±9.1kg, p<0.001 **Group A (F) Baseline right hand:** 20.1±4.5kg vs **Group A (F) follow-up right hand:** 20.5±44.4kg ; Change (%Change): 0.4±4.4kg (2%) **Group A (M) Baseline right hand:** 38.5±9.9kg vs **Group A (M) follow-up right hand:** 31.1±13.9kg; Change (%Change): -7.4±11.8kg (-19.2%) **Group B (F) Baseline right hand:** 8.5±5.7kg vs **Group B (F) follow-up right hand:** 7.7±4.4kg; Change (%Change): -0.8±4.2kg (-9.4%) **Group B (M) Baseline right hand:** 10.1±7.5kg vs **Group B (M) follow-up right hand:** 7.1±5.3kg; Change (%Change):-3.1±3.4kg (-30.7%) Phenotype comparison of subgroup effects: within participants: p=0.21; between participants: p<0.001 Sex comparison of subgroup effects: within participants: p<0.001; between participants: p<0.001 Phenotype comparison vs sex: within participants: p=0.02; between participants: p<0.001 **DM1 Baseline left hand**: 12.3±10.2kg vs **DM1 follow-up left hand**:10.8±9.4kg; p<0.001 **Group A (F) Baseline left hand:** 18.5±4.7kg vs **Group A (F) follow-up left hand:** 19.5±5kg; Change (%Change): 0.8±4.5kg (4.3%) **Group A (M) Baseline left hand:** 37.1±10.3kg vs **Group A (M) follow-up left hand:** 31.7±12.4kg; Change (%Change): -5.4±10.1kg (-14.6%) **Group B (F) Baseline right hand:** 7.9±5.6kg vs **Group B (F) follow-up right hand:** 7.1±4.3kg; Change (%Change): -0.8±4kg (-10.1%) **Group B (M) Baseline right hand:** 9.7±7.7kg vs **Group B (M) follow-up right hand:** 7±5.9kg; Change (%Change): -2.8±3.9kg (-28.9%) Phenotype comparison of subgroup effects: within participants: p=0.66; between participants: p<0.001 Sex comparison of subgroup effects: within participants: p<0.001; between participants: p<0.001 Phenotype comparison vs sex: within participants: p=0.06; between participants: p<0.001	Good
				QMT (pinch gauge): Pinch strength	**DM1 Baseline right hand**: 6.1±2.3kg vs **DM1 follow-up right hand**: 4.5±2.1kg; p<0.001 **Group A (F) Baseline right hand:** 7.8±1.2kg vs **Group A (F) follow-up right hand: 6.1**±1.2kg; Change (%Change): -1.7±1.3kg (-21.8%) **Group A (M) Baseline right hand:** 11±2.3kg vs **Group A (M) follow-up right hand:** 9.1±2.6kg; Change (%Change): -1.9±1.4 (-17.3%) **Group B (F) Baseline right hand:** 4.9±1.3kg vs **Group B (F) follow-up right hand:** 3.6±1.2kg; Change (%Change): -1.3±0.9kg (-26.5%) **Group B (M) Baseline right hand:** 6±1.6kg vs **Group B (M) follow-up right hand:** 4.1±1.5kg; Change (%Change): -1.9±1.1kg (-31.7%) Phenotype comparison of subgroup effects: within participants: p=0.34; between participants: p<0.001 Sex comparison of subgroup effects: within participants: p=0.13; between participants: p<0.001 Phenotype comparison vs sex: within participants: p=0.41; between participants: p<0.01 **DM1 Baseline left hand**: 5.9±2.1kg vs **DM1 follow-up left hand**: 4.2±2kg; p<0.001 **Group A (F) Baseline left hand:** 7.4±1.2kg vs **Group A (F) follow-up left hand:** 5.5±1.3; Change (%Change): -1.9±1.4kg (-25.7%) **Group A (M) Baseline left hand:** 10.2±1.9kg vs **Group A (M) follow-up left hand:** 8.8±2.8kg; Change (%Change): -1.3±1.4kg (-12.7%) **Group B (F) Baseline left hand:** 4.8±1.4kg vs **Group B (F) follow-up left hand:** 3.2±1kg; Change (%Change): -1.6±0.9kg (-33.3%) **Group B (M) Baseline left hand:** 5.7±1.6kg vs **Group B (M) follow-up left hand:** 3.8±1.4kg;	

					Change (%Change): -1.9±0.9kg (-33.3%) Phenotype comparison of subgroup effects: within participants: p=0.71; between participants: p<0.001 Sex comparison of subgroup effects: within participants: p=0.53; between participants: p<0.001 Phenotype comparison vs sex: within participants: p=0.09; between participants: p<0.01	
Raymond et al. (2015), Canada [77]	**DM1**=200; Sex: 121F; 79M; Age: 47±11.8; **Adult-onset (Group A)**=158; Sex: 96F; 62M; Age: 44.3±9.2 **Late-onset (Group B)**=42; Sex: 25F; 17M; Age: 57.4±14.4	NR	DM1 MIRS I (n):10 MIRS II (n):31 MIRS III (n):36 MIRS IV (n):98 MIRS V (n): 25 Group A MIRS II (n):18 MIRS III (n):30 MIRS IV (n): 5 MIRS V (n):25 Group B MIRS I (n):10 MIRS II (n):3 MIRS III (n):6 MIRS IV (n): 3	QMT (hand dynamometry): Grip strength	**Group A (F) Right hand:** 7.1±5.8 Kg vs **Group B (F) Right hand:** 20.1±4.3 Kg **Group A (F) Left hand:** 6.5±5.5 Kg vs **Group B (F) Left hand:** 19.3±4.8 Kg **Group A (F) Both hands:** 6.8±5.6 Kg vs **Group B (F) Both hands:** 19.7±4.3 Kg **Group A (M) Right hand:** 7.8±8 Kg vs **Group B (M) Right hand:** 31±11.8 Kg **Group A (M) Left hand:** 7.3±8.4 Kg vs **Group B (M) Left hand:** 28.9±12 Kg **Group A (M) Both hands:** 7.5±8.1 Kg vs **Group B (M) Both hands:** 30.4±12.2 Kg Group A vs Group B: p<0.01	Good
				QMT (pinch gauge): Lateral pinch strength	**Group A (F) Right hand:** 4.6±1.4 Kg vs **Group B (F) Right hand:** 8.1±1.1 Kg **Group A (F) Left hand:** 4.5±1.5 Kg vs **Group B (F) Left hand:** 7.8±1.2 Kg **Group A (F) Both hands:** 4.5±1.4 Kg vs **Group B (F) Both hands:** 8±1.1 Kg **Group A (M) Right hand:** 5.3±2 Kg vs **Group B (M) Right hand:** 9.2±3 Kg **Group A (M) Left hand:** 5±1.8 Kg vs **Group B (M) Left hand:** 8.7±2.5 Kg **Group A (M) Both hands:** 5.2±1.9 Kg vs **Group B (M) Both hands:** 9±2.7 Kg Group A vs Group B: p<0.01	
Rinninella et al. (2019), Italy [78]	**DM1**=40; Sex: 22F; 18M; Age: Median 42 [30;58]; BMI: Median 25.5 [21.6;27.1]	NR	MIRS I (n):4 MIRS II (n):20 MIRS III (n):11 MIRS IV (n): 5 MIRS V (n): 0	QMT (Hand dynamometry): Grip strength	**DM1 Right hand:** Median: 10.5 Kg [4.7;16.9] vs **DM1 Left hand:** Median: 9.6 Kg [4.7;17.2]	Fair
Roussel et al. (2019), Canada [79]	**DM1 Baseline**=19; Sex: 19M; Age; 46±10; **DM1 Follow-up (1 week)**=19; Sex: 19M	458±372	NR	QMT (handheld dynamometry): MIT - Knee extensors	**DM1 Baseline:** 110.13±49.16 Nm vs **DM1 Follow-up:** 106.76±47.62 Nm **DM1 Follow-up right leg:** 105.47±50.33 DM1 Baseline vs DM1 Follow-up: R2=0.94; ICC: 0.98; 95% CI: 0.96-0.99; SEM: 1.05 Nm; MDC (MDC%): 2.92 Nm (2.69%)	Fair
				QMT (isokinetic dynamometry): MIT- Knee extensors	**DM1 Follow-up right leg:** 129.15±61.50 Nm QMT handheld dynamometry vs QMT isokinetic dynamometry: R2: 0.94,; r=0.98; p<0.0001	
Sansone et al. (2007), Italy [80]	**DM1 Baseline**=56; Sex: 23F;33M; Age: 44.9±14.8; **DM1 Follow-up** (2-10y) =20	(500-700)	NR	MMT: Mega MRC scale Sum Score (0-150 points)	**DM1 Baseline (N =56):** 134.2±13.6 **DM1 Baseline (N =20):** 134.5±9 vs **DM1 Follow-up:** 124.6±14; Z=3.31; p<0.01 Decline per year: -1.23±1.43	Fair

Sedehizadeh et al. (2017), UK [81]	**DM1 Baseline**=38; Sex: 18F; 20M; Age: Median 41.6 (23-67); BMI: 27.3±5.5 **DM1 Follow-up (18 months)**=36	Median: 857 (50-2947)	MIRS Median: 4 (1-5)	MMT: MRC Scale (0-5 points)	**DM1 Follow-up:** No significant decline was observed for right or left ankle dorsiflexion MIF (p=0.13 and p=0.15, respectively)	Good
				QMT (handheld dynamometry): MIF - Ankle dorsiflexors	**DM1 Right Leg:** Median: 37.8% of pred. (2-85.3) vs **DM1 Left Leg:** Median: 41.2% of pred. (2.4-96.5)	
				QMT (Hand dynamometry): Grip strength	**DM1 Baseline dominant hand:** Median: 31.7% of pred. (5-79.1) **DM1 Follow-up:** 36 patients showed significant decline in right grip strength (-1.2kgF, p=0.0016) and a decreasing trend in left grip strength (-0.54 kgF, p=0.06)	
Sjögreen L et al. (2011), Switzerland [82]	**DM1**=12; Sex: 7F; 5M; Age: 45±11 **Controls** = 50; Sex: 29F; 21M; Age: 40±15	NR	NR	QMT (Lip dynamometry): Lip strength	**DM1:**12±5·5 N vs **Controls:** 29±9 N; p<0.05 Lip weakness - Lip Force <12 N (n): 7	Poor
Solbakken et al. (2016), Norway [83]	**DM1**=38; Sex: 17F; 21M; Age: 39±12.4 (20-63); BMI: 27.7±6.9 (17-53) **Disease duration <11 y (Group A)**=10 **Disease duration 11-17 y (Group B)**=10 **Disease duration 18-27 y (Group C)**=9 **Disease duration >27 y (Group D)**=9	1.75±1.39 (0.23-5.4) kb	NR	MMT: modified MRC Score (0-3 points) - Ankle dorsiflexors	**DM1:** 2.4±0.7; **Group A:** 2.5±0.8; **Group B:** 2.3±0.6; **Group C:** 2.4±0.5; **Group D:** 2.1±0.6	Good
				MMT: modified MRC Score (0-3 points): Back extensors	**DM1:** 2.2±0.5; **Group A:** 2.6±0.5; **Group B:** 2.2±0.4; **Group C:** 2.1±0.3; **Group D:** 1.9±0.6	
				MMT: modified MRC Score (0-3 points): Distal extremity muscle group (Dorsal extension wrist, dorsal extension ankle)	Distal extremity muscle group vs disease duration: r=-0.33; p=0.04 Distal extremity muscle group vs CTG Repeat Length: r=-0.4; p=0.015 Distal extremity muscle group vs BMI: r=-0.09; p=0.58	
				MMT: modified MRC Score (0-3 points): Elbow extensors	**DM1:** 2.4±0.5; **Group A:** 2.7±0.5; **Group B:** 2.3±0.5; **Group C:** 2.4±0.5; **Group D:** 2.4±0.5	
				MMT: modified MRC Score (0-3 points): Elbow flexors	**DM1:** 2.7±0.5; **Group A:** 2.8±0.4; **Group B:** 2.8±0.4; **Group C:** 2.8±0.4; **Group D:** 2.4± 0.5	
				MMT: modified MRC Score (0-3 points): Hip flexors	**DM1:** 2.6±0.5; **Group A:** 2.9±0.3; **Group B:** 2.8±0.4; **Group C:** 2.5±0.5; **Group D:** 2.32 ± 0.4	
				MMT: modified MRC Score (0-3 points): Knee extensors	**DM1:** 2.9 ± 0.3; **Group A:** 3±0; **Group B:** 2.9±0.3; **Group C:** 3±0; **Group D:** 2.5 ± 0.5	
				MMT: modified MRC Score (0-3 points): Knee flexors	**DM1:** 2.8±0.4; **Group A:** 2.9±0.3; **Group B:** 3±0; **Group C:** 2.8±0.4; **Group D:** 2.5±0.5	

				MMT: modified MRC Score (0-3 points): Neck flexors	**DM1:** 1.6±0.5; **Group A:** 1.9±0.5; **Group B:** 1.4±0.5; **Group C:** 1.8±0.4; **Group D:** 1.3±0.4	
				MMT: modified MRC Score (0-3 points): Proximal extremity muscle group (Hip flexors, knee flexors and extensors, shoulder abductors, elbow flexors and extensors)	Proximal extremity muscle group vs disease duration: r=-0.5; p=0.001 Proximal extremity muscle group vs CTG Repeat Length: r=-0.57; p<0.001	
				MMT: modified MRC Score (0-3 points): Shoulder abductors	**DM1:** 2.5±0.5; **Group A:** 2.6±0.5; **Group B:** 2.4±0.5; **Group C:** 2.6±0.5; **Group D:** 2.3±0.4	
				MMT: modified MRC Score (0-3 points): Trunk flexors	**DM1:** 1.5±0.6; **Group A:** 2.2 ± 0.4; **Group B:** 1.2±0.4; **Group C:** 1.4±0.5; **Group D:**1.3±0.4	
				MMT: modified MRC Score (0-3 points) - Wrist extensors	**DM1:** 2.3±0.5; **Group A:** 2.6±0.5; **Group B:** 2.3±0.6; **Group C:** 2.2±0.4; **Group D:** 2.1±0.3	
Tieleman et al. (2012), Netherlands [84]	**DM1**=31; Sex: 24F; 7M; Age: 52.8±12	NR	3.8±0.6 MIRS II (n):2 MIRS III (n):3 MIRS IV (n):26	MMT: MRC sum score (0-40 points)	**DM1:**35±2.4 Normal muscle strength (MRC = 40) (n): 1; Mild muscle weakness (MRC 36-39) (n): 10; Moderate muscle weakness (MRC 32-35) (n): 19; Severe muscle weakness (MRC <32)(n): 1	Fair
				QMT (handheld dynamometry): MIF-Ankle dorsiflexors	**DM1:** 56±26 N; **DM1 (F)**: 54±27 N vs **DM1 (M):** 62±23 N	
				QMT (handheld dynamometry): MIF-Elbow flexors	**DM1:** 105±31 N; **DM1 (F):** 100±27 N vs **DM1 (M):** 122±40 N	
				QMT (handheld dynamometry): MIF-Three-point pinch	**DM1:** 49±27N; **DM1 (F):** 46±24N vs **DM1 (M):** 60±35N	
				QMT (handheld dynamometry): MIF- Knee extensors	**DM1:** 113±27N; **DM1 (F):** 109±26N vs **DM1 (M):** 130±27N	
Umemoto et al. (2016), Japan [85]	**DM1**=30; Sex: 13F; 17M; Age: 49	NR	NR	QMT: Tongue strength - Maximum tongue pressure	13.2±6 kPa; Bodyweight vs Maximum tongue pressure: R=0.185; p=0.327	Fair
Umemoto et al. (2009), Japan [86]	**DM1 Baseline**=8; Sex: 4F; 4M; Age: 52.1±5.8 **DM1 Follow-up (5y)**=8; Sex: 4F; 4M	1338 (400-2200)	NR	QMT (Dental Prescale System): Maximum bit strength	**DM1 Baseline:** 82.6±47.9 N vs **DM1 Follow-up:** 40.2±24.3 N; p<0.05	Fair

Wiles et al. (2005), UK [87]	**DM1**=13; Sex:7F; 6M; Age: 46.5±1.68; BMI: 27.1±6.7 **Controls**=12; Sex:7F; 5M; Age: 34.4±1.73; BMI: 25.7±5.2	NR	NR	QMT (wall mounted dynamometry): MVIC - Ankle dorsiflexors	**DM1:** 54.3±28.5 N vs **Controls:** 180.6±67.6 N; p<0.001	Fair
				QMT (wall mounted dynamometry): MVIC - Ankle plantar flexors	**DM1:** 109.4±65.8 N vs **Controls:** 313.1±126.3 N; p<0.001	
				QMT (wall mounted dynamometry): MVIC - Hip abductors	**DM1:** 96.5±53.8 N vs **Controls:** 194.8±91.6 N; p=0.006	
				QMT (wall mounted dynamometry): MVIC - Hip extensors	**DM1:** 141±57.8 N vs **Controls:** 262±145 N; p=0.02	
				QMT (wall mounted dynamometry): MVIC - Knee extensors	**DM1:** 169.5±72.9 N vs **Controls:** 274.4±137 N; p=0.03	
				QMT (wall mounted dynamometry): MVIC - Knee flexors	**DM1:** 95.6±50.7 N vs **Controls:** 143.7±63.6 N; p=0.06	
Respiratory Studies						
Boussaïd et al. (2018), France [88]	**DM1=**283; Sex: 122F; 161M; Age: 43.7±12.5; **BMI (≤18.5):** 34; **BMI (18.5-25):** 112; **BMI (25-30):** 76; **BMI (30-35):** 42; **BMI (35-40):** 19 **DM1 Follow-up (5y)**=231	Median: 600 50-149 (n): 15 150-450 (n): 53 451-1000 (n): 97 >1000 (n): 50	NR	Manometry: PEmax	**DM1 Baseline:** 36±19 % of pred.; Change (per y): -1.1 ± 11.1% of pred. Follow-up vs Change: β=-1.32±0.2; p<0.0001 PEmax vs CTG repeat Length: β=-1.6; p=0.01 Change PEmax vs CTG repeat length (150-450): β=-5.9±4.1; Change PEmax vs CTG repeat length (451-1000): β=-9.72±3.7; Change PEmax vs CTG repeat length (> 1000): β=-13.39±10.8; p=0.01 Change PEmax vs BMI: β=-1.13±0.7; p=0.12 Change PEmax vs Age: β=-0.1±0.1; p=0.19	Fair
				Manometry: PImax	**DM1 Baseline:** 53±23% of pred.; Change (per y): -2.9 ± 12.3% of pred.; Follow-up vs Change: β=-1.34±0.3; p<0.0001 PImax vs CTG repeat length: β=-5.4±1.4;<0.0001 Change in PImax decreased vs Age (baseline): β=-0.23±0.1; p=0.04 Change in PImax vs CTG repeat length (150-450): β=-5.91±3.9 Change in PImax vs CTG repeat length (451-1000): β=-16.71±5.5 Change in PImax vs CTG repeat length (>1000): β=-36.3±14.2; p=0.003 Change in PImax vs BMI (baseline): β=--3.03±0.9; p=0.001	

				Mechanics of breathing (Spirometry): VC	**DM1 Baseline Supine VC:** 59±21% of pred.; **DM1 Baseline Upright VC**:65±20% pred.; Change (per y): -1.8±7% of pred. VC <50 % pred. (n): 72; Upright VC vs supine VC >20% (n): 7 Upright VC vs CTG repeat length: β=-3.8±1.8 p=0.04 Supine fall VC vs CTG repeat length: β= 659.8±482.1; p=Ns Change in VC vs Age: β= -0.43±0.1; p<0.0001 Change in VC vs CTG repeat length (150-450): β= -8.43±5 Change in VC vs CTG repeat length (451-1000): β= -21.48±4.7 Change in VC vs CTG repeat length (>1000): β= -22.56±10.7; p< 0.0001 DM1 Follow-up (per y) coefficient of significance: -1.57±0.2; p<0.0001 Patients with CTG repeats >1000 had a faster VC decrease over time (13%-36%) compared to the patients with CTG between 50-150	
				PCF	DM1 Baseline PCF > 270 L/min (n): 40; DM1 Baseline PCF 180-270 L/min (n): 25; DM1 Baseline PCF <180 L/min (n): 15 PCF vs PEmax: β= 0.15±0.07; p=0.02 PCF vs PImax: β= 0.17±0.06; p=0.005 PCF vs BMI: β= 0.24±0.21; p=Ns PCF vs Age: β= -0.1±0.09, p=Ns PCF (180-270) vs CTG repeat length: β=376±125.7 PCF (<180) vs CTG repeat length: β= 181.4±279.8; p=0.004	
Calabrese et al. 2000, France [89]	**DM1= 9;** Sex:4F; 5F; Age: 48±12	NR	NR	Manometry: PEmax	**DM1 Sitting:** 46.6% of pred. vs **DM1 Supine:** 48.2% of pred.	Fair
				Manometry: PImax	**DM1 Sitting:** 62.2% of pred. vs **DM1 Supine:** 54.8% of pred.	
				Mechanics of breathing (plethysmography): FEV1	**DM1 Sitting:** 76.7% of pred.vs **DM1 Supine:** 73.3% of pred.	
				Mechanics of breathing (spirometry): FEV1/VC	**DM1 Sitting:** 94.3% of pred. vs **DM1 Supine:** 92% of pred.	
				Mechanics of breathing (spirometry):VC	**DM1 Sitting:** 82.6% of pred. vs **DM1 Supine:** 82.3% of pred.	
De Mattia E et al. (2020), Italy [90]	**DM1**=58; Sex: 31F; 27M; Age: Median: 42 [37;52]; BMI: Median: 23.14 [19.97;25.67] **DM1 without respiratory impairment (Group A)**=17; Age: Median: 38.5 [36;42.5]; BMI: Median:21.9 **DM1 with tests suggestive of respiratory impairment (Group B)**=13;Age: Median:	50-200 (n): 9 201-1000 (n): 30 >1000 (n): 3	DM1 MIRS I-III (n): 23 MIRS IV-V (n): 35 Group A MIRS I-III (n): 11 MIRS IV-V (n): 6 Group B MIRS I-III (n): 7 MIRS IV-V (n): 6 Group C	Manometry: PEmax	**Group A:** Median: 94.5 cm H_2O [64;105] vs **Group B:** Median: 77 cm H_2O [48;83] vs **Group C:** Median 42 cm H_2O [34;51]	Good
				Manometry: PImax	**Group A:** Median: 74 cm H_2O [64;105] vs **Group B:** Median: 56 cm H_2O [45;70] vs **Group C:** Median 33 cm H_2O [26;45]	
				Mechanics of breathing: (Spirometry) FEV1	**Group A:** Median: 2.52 L [2.25;2.76] vs **Group B:** Median: 2.41 L [1.89;2.69] vs **Group C:** Median: 1.78 L [1.52;2.26]; p=0.0006 **Group A:** Median: 78.6% of pred. [69.5;86] vs **Group B:** Median: 73.3% of pred. [67;81.1] vs **Group C:** Median: 63% of pred. [49;72.5]; p=0.005	
				Mechanics of breathing: (Spirometry): FVC	**Seated** **Group A:** Median: 3.33 L [3.07;4.18] vs **Group B:** Median: 3.17 L [2.70;4] vs **Group C:** Median: 2.27 L [1.97;2.7]; p<0.0001	

	48 [40;53]; BMI: Median: 21.9 [20.19;24.56] **DM1 with respiratory impairment (Group C)**=28; Age: Median: 42 [38;53]; BMI: Median: 23.86 [19.9;28.9]		MIRS I-III (n): 5 MIRS IV-V (n): 23		**Group A:** Median: 92 % of pred. [82.2;97.6] vs **Group B:** Median: 77.8% of pred. [75;89] vs **Group C:** Median: 63% of pred. [53;73]; p<0.0001 **Supine** **Group A:** Median: 3.1 L [2.85;4.11] vs **Group B:** Median: 2.91 L [5.52;3.7] vs **Group C:** Median: 2 L [1.79;2.47]; p<0.0001 **Group A:** Median: 85.5% of pred. [80.5;95.2] vs **Group B:** Median: 78% of pred. [70;81] vs **Group C:** Median: 54% of pred. [45;65]; p<0.0001 **Seated/supine** **Group A:** Median: 3.68 [2.15;6.52] vs **Group B:** Median: 5.41 [0,8.99] vs **Group C:** Median: 11.27 [7.46;19.03]; p=0.0196	
				Mechanics of breathing: (Spirometry): PCEF	**Group A:** Median: 5.09 L/s [3.66;6.6] vs **Group B:** Median: 3.56 L/s [3.04;4.88] vs **Group C:** Median: 3.39 L/s; [2.29;4.65]; p=0.0428	
				Mechanics of breathing: (Spirometry): PEF	**Group A:** Median: 5.09 L/s [3.66;6.6] vs **Group B:** Median: 3.56 L/s [3.04;4.88] vs **Group C:** Median: 3.39 L/s; [2.29;4.65; p=0.0428	
Evangelista et al. (2017), Brasil [91]	**DM1**=18; Sex: 11F; 7M; Age: 42.3±11.3; BMI: 24.9±3.6 **Controls**=11; Sex: 4F; 7M; Age: 37.4±10.7; BMI: 25.5±4.2	NR	MIRS I (n): 2 MIRS II (n): 10 MIRS III (n): 4 MIRS IV (n): 2	Manometry: PEmax	**DM1:** Median: 43.5 cm H_2O [36.2;62.7] vs **Controls:** Median: 114 cm H_2O [101;145]; p=0.0002 **DM1:** Median: 39.7% of pred. [28.5;60.5] vs **Controls:** Median: 102% of pred. [89;132.6]; p<0.001; ROC: Cut-off= 92.5 cm H_2O; sensitivity = 73.7 %; specificity= 87.5%; AUC=0.92; p= 0.0002	Fair
				Manometry: PImax	**DM1:** 62.6±26.1 cm H_2O vs **Controls:** 105±29.1 cm H_2O; p=0.0006 **DM1:** Median: 48% of pred. [37;57] vs **Controls:** Median: 99% of pred. [75.3;115]; p=0.0016; ROC: Cut-off= 73 cm H_2O; sensitivity= 81.8%; specificity= 75%; AUC: 0.86; p= 0.0077	
				Manometry: SNIP	**DM1:** 46±16.26% vs **Controls:** 70.64±19.6%; p=0.0015 **DM1:** Media: 46 cm H20 [36.2;60.5] vs **Control:** Median: 72 cm H20 [63;89]; p=0.0014 ROC: Cut-off= 51.4 cm H_2O; sensitivity= 90.4%; specificity= 68.7%; AUC=0.84; p=0.0026	
				Mechanics of breathing (Spirometry): FEV1	**DM1:** 70.2±16.5% of pred. vs **Controls:** 93.1±10.9% of pred.; p=0.0004	
				Mechanics of breathing (Spirometry): FEV1/FVC	**DM1:** 97.4±5.6% of pred. vs **Controls:** 101±4.1% of pred.; p=0.781	
				Mechanics of breathing (Spirometry): FVC	**DM1:** 72.1±16.6% of pred. vs **Controls:** 92.6±11.9% of pred.; p=0.0018	
Henke C et al. (2020), Germany [92]	**DM1**=21; Sex: 10F; 11M; Age: 42.1±12.6; BMI: 24.9±6 **Controls**=21; Sex: 10F; 11M; Age: 42.1±12.8; BMI: 24.1±2	NR	3.6±0.8	Manometry: Cough Pgas	**DM1:** 71.9±43.2 cm H_2O vs **Controls:** 102.4±35.5 cm H_2O; p=0.06 CoughPgas vs MIRS: r=-0.68; p=0.02	Good
				Manometry: PEmax	**DM1:** 41.3±13.4 cm H_2O vs **Controls:** 133.8±28 cm H_2O; p<0.01 PEmax vs MIRS: r=-0.86, p=0.03	
				Manometry: PImax	**DM1:** 40.3±19.2 cm H_2O vs **Controls:** 95.8±28.5 cm H_2O; p<0.01 PImax vs MIRS: r=-0.76; p<0.01	
				Mechanics of breathing (Spirometry): FEV1/FVC	**DM1:** 84±6.4% vs **Controls:** 76.4±7.8%; p<0.01	

				Mechanics of breathing (Spirometry): FVC	**DM1:** 2.8±1 L vs **Controls:** 4.8±1.4 L; p<0.01 **DM1:** 69.2±21.4% of pred. vs **Controls:** 111±13.7% of pred.; p<0.01 FVC vs MIRS r=-0.42; p=0.06	
				Mechanics of breathing: (Spirometry): PCF	**DM1:** 284.7±92.5 L/min vs **Controls:** 547.4±156.6 L/min, p<0.01	
				Mechanics of breathing: (Spirometry): PEF	**DM1:** 5.8±2.3 L/s vs **Controls:** 8.8±2.3 L/s; p<0.01 **DM1:** 69.5±23.4% pred. vs **Controls:** 105.7±15.1% pred.; p<0.01	
				Manometry: SniffPdi	**DM1:** 50.4±30.5 cm H_2O vs **Controls:** 92.9±28.9 cm H_2O; p<0.01 SniffPdi vs MIRS: r=-0.72; p=0.02	
				Manometry: twPgas	**DM1:** 19.7±15.5 cm H_2O vs **Controls:** 22.5±13.3 cm H_2O; p=Ns	
				Manometry: TwPdi	**DM1:** 10.8±8.3 cm H_2O vs **Controls:** 21.4±10.1 cm H_2O; p=0.03	
Kaminska et al. 2015, Canada [93]	**DM1 Baseline**=6; Sex: 4F; 2M; Age: 40.3±12.5; BMI: 27±6.7 **DM1 Follow-up (3 months)**=5	NR	NR	Manometry: PEmax	**DM1 Baseline:** 35.5±11.9cmH2O	Fair
				Manometry: PImax	**DM1 Baseline:** 44.3±36.5cmH2O	
				Mechanics of breathing (spirometry): FVC	**DM1 Baseline:** 1.95±1.04L; **DM1 Follow-up:** Change: -0.014L, 95% CI: -0.103-0.075, p=0.46 **DM1 Baseline:** 42.5±26.1% of pred	
				Mechanics of breathing (spirometry): PCF	**DM1 Baseline:** 280±98L/min; **DM1 Follow-up:** Change:5L/min, 95% CI: -36.6-46.6, p=0.89	
Laberge et al. (2009), Canada [94]	**DM1**=43; Sex:29F; 14M; Age: 49.7±10; BMI: 28.7±6.4 **DM1 with ES≥11 (Group A)=**21; Age: 46.7±10.1; BMI: 29.4±8.3 **DM1 with ES <11 (Group B)**=22; Age: 52.7±9.4; BMI: 27.8±3.8 **MSL≤8 min (Group C)**=19; Age: 48.8±6.9; BMI: 28.3±6.7 **MSL > 8 min (Group D)=**24; Age: 50.3±12.1; BMI: 29±6.3	**DM1:** 868±552 **Group A:** 677±494 **Group B:** 1010±532 **Group C:** 1000±545 **Group D:** 764±546	DM1: 3.3±1.1 Group A: 3.3±1 Group B: 3.3±1.2 Group C: 3.9±0.7 Group D: 2.9±1.2	Maximal respiratory pressure	**DM1:** 121.5±49.5 cm H_2O; **Group A:** 136.6±57.6 cm H_2O vs **Group B:** 109.7±34.6 cm H_2O; **Group C:** 104.4±35.5 cm H_2O vs **Group D:** 134.9±55.4 cm H_2O; p<0.05	Fair
				Mechanics of breathing (Spirometry): TLC	**DM1:** 77.6±23.7% of pred.; **Group A:** 82±21.5% of pred. vs **Group B:** 74.7±27.3% of pred.; **Group C:** 68.9±20.6% of pred. vs **Group D:** 84.5±24.2% of pred.; p<0.01	
				Mechanics of breathing (Spirometry): VC	**DM1:** 75.7±27.1% of pred.; **Group A:** 77.9±22.4% of pred. vs **Group B:** 75.3±31.1% of pred.; **Group C:** 63.6±20% of pred. vs **Group D:** 85.2±28.6% of pred.; p<0.01	
Leonardis et al. (2017), Slovenia [95]	**DM1**=30; Sex: 17F;13M; Age: 47±12 (21-69)	NR	NR	Manometry: PImax	**DM1:** 34±16 cm H_2O (2-63)	Fair
				Manometry: SNP	**DM1:** 47±23 cm H_2O (7-112)	
				Mechanics of breathing (Spirometry): VC	**DM1 Sitting:** 62±20% of pred. (15-96) vs **DM1 Supine:** 58±20% of pred. (17-96)	
Lucena Araújo et al. 2010, Brazil [96]	**DM1**=23; Sex: 10F;13M; Age: 40±16; BMI: 23.1±5.3	NR	MIRS I (n): 3 MIRS II(n): 11 MIRS II (n): 4 MIRS IV (n): 5	Manometry: PEmax	**DM1 Sitting:** 71±20 cmH_2O; 64% of pred. MIRS I: 80% of pred.; MIRS II: 71% of pred.; MIRS III: 60% of pred.; MIRS IV: 45%of pred.	Fair
				Manometry: PImax	**DM1 Sitting:** 76±32 cmH_2O; 70% of pred.	
				Manometry: SNIP	**DM1:** 79±28 cmH_2O; 80% of pred	
				Mechanics of breathing (spirometry): FEV1	**DM1:** 77±13% of pred.	

				Mechanics of breathing (spirometry): FEV1/FVC	**DM1:** 83.5±7.4% of pred.	
				Mechanics of breathing (spirometry): FVC	**DM1:** 77.4±13% of pred.	
Poussel et al. (2014), France [97]	**DM1**=58; Sex: 35F;23M; Age: 42.6±12.9 **DM1 (MIRS I-II)**=26; Sex: 17F;9M; Age: 39.5±14.8; BMI: 24.5±5.3 **DM1 (MIRS-III)**=15 Sex: 7F;8M; Age: 41.7±10.5; BMI: 25.3±7 **DM1 (MIRS IV-V)**=17 Sex: 11F;6M; Age: 47.9±10.3; BMI: 27.5±6.3 **DM1 without ventilatory restriction (Group A)**=37; Sex: 27F;10M; Age: 41.3±12.8; BMI: 24.2±6.1 **DM1 with ventilatory restriction (Group B)**=21; Sex: 8F;13M; Age: 44.7±12.1; BMI: 27.9±5.4 **DM1 with fall in supine <20% (Group C)**=41; Sex: 25F;16M; Age: 42.3±13.2; BMI: 24.6±5.7 **DM1 with fall in supine >20% (Group D)**=17; Sex: 10F;7M; Age: 43.2±12.4; BMI: 28±6.4	**MIRS I-II:** 562.7±304 **MIRS III**: 779.2±470 **MIRS IV-V**: 942.9±448.4 **Group A**: 627.8±435.8 **Group B**: 918.8±322.1 **Group C:** 719.4±473.3 **Group D**: 750±278.4	MIRS I-II (n): 26 MIRS III (n): 15 MIRS IV-V (n): 17	Manometry: PEmax	**MIRS I-II:** 24.5±14% of pred. vs **MIRS III:** 19.2±7.4% of pred. vs **MIRS IV-V:** 21.5±8.8% of pred. **Group A:** 23.9±12.1% of pred. vs **Group B:** 19.1±9.2% of pred.; **Group C:** 23.1±11.6% of pred. vs **Group D:** 20.5±10.9% of pred.	Good
				Manometry: PImax	**MIRS I-II:** 44.1±24.1% of pred. vs **MIRS III:** 35.1±9.8% of pred. vs **MIRS IV-V:** 35.3±9.5% of pred. **Group A:** 40.6±20.7% of pred. vs **Group B:** 37.1± 12 % of pred.; **Group C:** 39.8±20.2% of pred. vs **Group D:** 38.4±13.1 % of pred.	
				Mechanics of breathing (Spirometry and plethysmography): FEV1	**Sitting** **MIRS I-II:** 85.8±11.5% of pred. vs **MIRS III:** 66.9±13.1% of pred. vs **MIRS IV-V:** 58.8±17.9% of pred.; p<0.0001 **Group A:** 82.9±12.8% of pred. vs **Group B:** 55.6±12.5% of pred.; p<0.0001 **Group C:** 77.7±17.4% of pred. vs **Group D:** 61.9±15.9% of pred.; p=0.0021 **MIRS I-II:** 77.2±14.5% of pred. vs **DM1 MIRS III:** 56.5±15.8% of pred. vs **MIRS IV-V:** 50.7±18.5; p<0.0001 **Supine** **Group A:** 74.8±14.8% of pred. vs **Group B:** 45.2±12.2% of pred.; p<0.0001 **Group C:** 71.5±17.1% of pred. vs **Group D:** 46.3±14.5% of pred.; p<0.0001 Supine FEV1 vs TLC: p<0.0001	
				Mechanics of breathing (Spirometry and plethysmography): FVC	**Sitting** **MIRS I-II:** 86.3±12.5% of pred. vs **MIRS III:** 69.9±15.9% of pred. vs **MIRS IV-V:** 57±18.8% of pred.; p<0.0001 **Group A:** 84.9±12.8% of pred. vs **Group B:** 53.3±12% of pred.; p<0.0001 **Group C:** 77.9±17.4% of pred. vs **Group D:** 62.8±15.9% of pred.; p=0.0070 **Supine** **MIRS I-II:** 81.4±15.2% of pred. vs **MIRS III:** 63.2±19.2% of pred. vs **MIRS IV-V:** 49.3±20% of pred.; p<0.0001 **Group A:** 80±15.1% of pred. vs **Group B:** 44.8±12.9% of pred.; p<0.0001; **Group C:** 74.8±19.4% of pred. vs **Group D:** 49.2±18.2% of pred.; p<0.0001 Supine FVC vs TLC: p<0.0001	
				Mechanics of breathing (Spirometry and plethysmography): TLC	**Sitting** **MIRS I-II:** 91.7±12.2% of pred. vs **MIRS III:** 77.9±14.4% of pred. vs **MIRS IV-V:** 70.6±21.2% of pred.; p=0.0003 **Group A:** 93±10.4% of pred. vs **Group B:** 62.1±9.8% of pred. p<0.0001; **Group C:** 87±16.7% of pred. vs **Group D:** 70.8±15.9% of pred.; p=0.0013	

				Mechanics of breathing (Spirometry and plethysmography): VC	**Sitting** **MIRS I-II:** 89±12.6% of pred. vs **MIRS III:** 71.4±16.4% of pred. vs **MIRS IV-V:** 59.9±18.2% of pred.; p<0.0001 **Group A:** 87.8±12.1% of pred. vs **Group B:** 54.8±10.6% of pred.; p<0.0001 **Group C:** 81.3±17.8% of pred. vs **Group D:** 64.2±19% of pred.; p=0.0019	
Poussel et al. (2015), France [98]	**DM1**=69; Sex: 43F; 26M; Age: 43.5±12.7 **MIRS I-II**=29; Age: 42±12.8; BMI 24.8±6 **MIRS III**=18; Age: 45±10.6; BMI: 25.1±5.5 **MIRS IV-V**=22; Age: 46.2±13.5; BMI: 29.2±8.1 **DM1 without ventilatory restriction (Group A)**=38; Age: 43.7±13.4; BMI: 25.4±6.7 **DM1 with ventilatory restriction (Group B)**=31; Age: 43.1±11.9; BMI: 29±7.4	**MIRS I-II:** 627±378 **MIRS III**: 718±462 **MIRS IV-V:** 748±329 **Group A**: 680±336 **Group B:** 820±380	MIRS I-II (n): 29 MIRS III (n): 18 MIRS IV-V (n): 22	Manometry: PEmax	**MIRS I-II:** 26.5±15.2% of pred. vs **MIRS III:**19.7±7.8% of pred. vs **MIRS IV-V:** 24.3±13.1% of pred. **Group A:** 26.4±14% of pred. vs **Group B:** 21±11% of pred.	Good
				Manometry: PImax	**MIRS I-II:** 42.2±24% of pred. vs **MIRS III:** 37.3±15.7% of pred. **MIRS IV-V:** 34.5±11.7% of pred. **Group A:** 44±21.7% of pred. vs **Group B:** 34.7±15.9% of pred.	
				Mechanics of breathing (Spirometry and plethysmography): FEV1	**MIRS I-II:** 83.6±20.8% of pred. vs **MIRS III:** 67.9±13.2% of pred. vs **MIRS IV-V:** 53.8±20.3% of pred.; p<0.0001; **Group A:** 84.9±16.9% of pred.; p<0.0001 vs **Group B:** 55.4±15.8% of pred.	
				Mechanics of breathing (Spirometry and plethysmography): FVC	**MIRS I-II:** 87.3±19.2% of pred. vs **MIRS III:** 69.8±15.8% of pred. vs **MIRS IV-V:** 54.7±21.6% of pred.; p<0.0001; **Group A:** 87.4±17.5 % of pred. vs **Group B:** 57.1±17% of pred.; p<0.0001 FVC vs MIP: p=0.0002	
				Mechanics of breathing (Spirometry and plethysmography): TLC	**MIRS I-II:** 88±17% of pred. vs **MIRS III:** 79.1±13% of pred. vs **MIRS IV-V:** 65.3±17.3% pred.; p=0.0015 **Group A:** 93.5±12.5% of pred. vs **Group B:** 63.4±11.4% of pred.; p<0.0001	
				Mechanics of breathing (Spirometry and plethysmography): VC	**MIRS I-II:** 88.8±21 % of pred. vs **MIRS III:** 76±15.3 % of pred. vs **MIRS IV-V:** 56.4±19.7 % of pred.; p<0.0001 **Group A:** 90.8±16.8% of pred. vs **Group B:** 58.6±15.2 % of pred.; p<0.0001	
Rossi et al. (2019), Italy [99]	**DM1**=268; Sex: 117F; 151M; Age: 46.2±12.9; BMI: 26.4±4.9 **DM1 FVC<80% (Group A)**=139; Sex: 61F; 78M Age: 46.7±11.8; BMI: 27±5 **DM1 FVC >80% (Group B)**=129; Sex: 56F;73M; Age: 45.6±14.1; BMI: 25.7±4.7	**Group A:** 686±614.9 **Group B:** 530±623.7	NR	Manometry: PEmax	**DM1 (n=159):** 39.9±22.7% of pred.; **Group A (n=75):** 34.6±15.5% of pred. vs **Group B (n=84):** 44.6±26.8% of pred.; p=0.03	Good
				Manometry: PImax	**DM1 (n=159):** 59.1±32% of pred.; **Group A (n=75):** 52.4±26.8% of pred. vs **Group B (n=84):** 65±35.2% of pred.; p=0.014	
				Mechanics of breathing (Spirometry and plethysmography): FEV1	**DM1 (n=262):** 79±22.2% of pred.; **Group A (n= 134):** 63±16.5 vs **Group B (n= 128):** 95.7±13.5 % of pred.; p<0.0001 **DM1 (n=260):** 2.5±0.9 L; **Group A (n=133):** 2±0.7 L vs **Group B (n=127):** 3±0.8 L; p<0.001	
				Mechanics of breathing (Spirometry and plethysmography): FEV1/FVC	**DM1 (n=245):** 85.7±12.5; **FVC<80% (n=125):** 86.2±13.3 vs **FVC>80% (n=120):** 85.2±11.7; p=Ns	
				Mechanics of breathing (Spirometry and plethysmography): FVC	**DM1:** 77.6±22.1% of pred.; **Group A (n=136):** 60.8±14.6 % of pred.; **Group B (n=128):** 95.5±12.5 % of pred. **DM1 (n= 264):** 2.9±1.1 L; **Group A (n=136):** 2.3±0.8 L; **Group B (n=128):** 3.6±0.9 L;	

				Mechanics of breathing (Spirometry and plethysmography): TLC	**DM1 (n=142):** 76.9±17.6% of pred.; **Group A (n= 78):** 65.5±12.1% of pred. vs **Group B (n=64):** 90.8±12.6% of pred.; p<0.001 **DM1 (n= 140):** 2.5±0.9L; **Group A (n= 76):** 3.8±1.1L vs **Group B (n=64):** 5.2±1.2L; p<0.001	
				Mechanics of breathing (Spirometry and plethysmography): VC	**DM1 (n=234):** 77.2±21.1% of pred.; **Group A (n=119):** 61.3±14.3% of pred. vs **Group B (n=115):** 93.5±13.1 % of pred.; p<0.001 **DM1 (n=260):** 4.5±1.3L; **Group A (n=118):** 2.4±0.8L vs **Group B (n=114):** 3.6±0.9L; p<0.001	
Seijger et al. (2016), Netherlands and UK [100]	**DM1**=105; Sex: 42F; 63M; Age: 46.2±12; BMI: 26.4±5.7 **DM1 with BMI<25kg/m² (Group A)=**43 Sex: 16F; 27M; Age: 45.5±12.4; BMI: 21.7±2.7 **DM1 with BMI ≥25kg/m² (Group B)**=62; Sex: 27F; 35M; Age: 46.7±11.8; BMI: 29.6±5	NR	Median: 4 MIRS I (n): 1 MIRS II (n): 5 MIRS III (n): 24 MIRS IV (n): 46 MIRS V (n): 19	Manometry: PEmax	**DM1:** 55.7±23.5% of pred.; **Group A (n=40):** 50.5±21.3% of pred. vs **Group B (n=53):** 59.6±24.5% of pred.; p=5.98x10^{-1}	Good
				Manometry: PImax	**DM1:** 65±23.4% of pred.; **Group A (n=40):** 63.5±23.5% of pred. vs **Group B (n=51):** 66.1±23.5% of pred.; p=5.73x10^{-2}	
				Mechanics of breathing (spirometry): ERV	**DM1:** 73.1±36.9% of pred.; **Group A (n=39):** 98.5±32.5% of pred. vs **Group B (n=59):** 60.3±31.7% of pred.; p< 0.001; BMI vs ERV: r=-0.59; p=1.33x10^{-10}	
				Mechanics of breathing (spirometry): FEV1	**DM1:** 82.9±18.6% of pred.; **Group A (n=42):** 87.3±16.6% of pred. vs **Group B (n=61):** 79.8±19.3% of pred.; p<0.005	
				Mechanics of breathing (spirometry): FEV1/VC	**DM1:** 101.1±7.9% of pred.; **Group A:** 100.9±8.6% of pred. vs **Group B (n=61):** 101.4±7.5% of pred.; p=7.55x10^{-1}	
				Mechanics of breathing (spirometry): FIV1	**DM1:** 79.6±21.4% of pred.; **Group A (n=38):** 82.4±20% of pred. vs **Group B (n=60):** 78.6±22.1% of pred.; p=3.81x10^{-1}	
				Mechanics of breathing (spirometry): FRC	**DM1:** 72.3±20% of pred.; **Group A (n=39):** 86.6±17.5% of pred. vs **Group B (n=59):** 62.8±15.4% of pred.; p<0.001; BMI vs FRC: r=-0.61; p=3.53x10^{-11}	
				Mechanics of breathing (spirometry): RV	**DM1:** 71.1±16.9% of pred.; **Group A (n=40):** 79.8±19.2% of pred. vs **Group B (n=59):** 65.3±12.1% of pred.; p<0.001	
				Mechanics of breathing (spirometry): TLC	**DM1:** 79±14.9% of pred.; **Group A (n=41):** 84.1±12.6% of pred. vs **Group B (n=59):** 75.4±15.4% of pred.; p<0.001; TLC vs BMI: r=-0.27; p=7.00x10^{-3}; TLC vs PImax: r= 0.38; p= 2.04x10^{-4}; TLC vs FIV1: r= 0.87; p= 1.97x10^{-30}	
				Mechanics of breathing (spirometry): VC	**DM1:** 83.9±18.9% of pred.; **Group A (n=43):** 87.7±17.1% of pred. vs **Group B (n=62):** 81.2±19.8% of pred.; p= 8.58x10^{-1}	
Thil et al. (2017), France [101]	**DM1**=80; Sex: 47F;33M; Age: 37.7±12.5; BMI: 25.3±6.2 **DM1 Restricted at inclusion (Group A)=**22 **DM1 Becoming restricted (Group B)**=30 **DM1 Unrestricted (Group C)**=28	777±401 Group A: 895 ± 279 Group B: 758 ± 401 Group C: 706 ± 469	NR	Mechanics of breathing (Spirometry and plethysmography): FEV1	**Decline DM1:** -1.07±1.75% of pred./y; **Decline Group A:** -0.97±1.39% of pred./y vs **Decline Group B:** -1.585±1.91% of pred./y vs **Decline Group C:** -0.592±1.74% of pred./y; p=Ns **Decline DM1:** -0.043±0.05 L/y; **Decline Group A:** -0.039±0.05 L/y vs **Decline Group B:** -0.056±0.05 L/y vs **Decline Group C:** -0.033±0.05 L/y; p=Ns Annual decline vs BMI: β=-1.06, p<0.04	Fair
				Mechanics of breathing (Spirometry and plethysmography): FVC	**Decline DM1:** -0.72±1.70% of pred./y; **Decline Group A:** -0.901±1.44% of pred./y vs **Decline Group B:** -1.451±1.56% of pred./y vs **Decline Group C:** -0.221±1.61% pred./y; p=0.0004 **Decline DM1:** -0.034±0.06 L/y; **Decline: Group A:** -0.041±0.05 L/y vs **Decline Group B:** 0.054±0.05 L/y vs **Decline Group C:** -0.007±0.06 L/y; p=0.0060 Annual decline vs BMI: β=-30.27, p<0.01	

				Mechanics of breathing (Spirometry and plethysmography): MEF	**Decline DM1:** -1.86±2.5% of pred./y; **Decline Group A:** -1.2±2.04% of pred./y vs **Decline Group B:** -2.233±2.74% of pred./y vs **Decline Group C:** -2.14±2.53% of pred.; p=Ns **Decline DM1:** -0.093±0.09L/y; **Decline Group A:** -0.06±0.09L/y vs **Decline Group B:** -0.108±0.09L/y vs **Decline Group C:** -0.103±0.09 L/y; p=Ns	
				Mechanics of breathing (Spirometry and plethysmography): RV	**Decline DM1:** -1.49±4.05% of pred./y; **Decline Group A:** 0.466±3.6% of pred./y vs **Decline Group B:** -1.706±4.3% of pred./y vs **Decline Group C:** -3.317±4.74% of pred./y; p=0.0098 **Decline DM1:**-0.014±0.08L/y; **Decline Group A:**0.014±0.69L/y vs **Decline Group B:**-0.037±0.08L/y vs **Decline Group C:** -0.012±0.08L/y; p=Ns	
				Mechanics of breathing (Spirometry and plethysmography): TLC	**Decline DM1:** -1.49±4.05% of pred./y; **Decline Group A:** 0.466±3.6% of pred./y vs **Decline Group B:** -3.317±4.47% of pred./y vs **Decline Group C:** -1.706±4.30% of pred./y; p<0.0001 **Decline DM1:** -1.5±1.68; **Decline Group A:** -0.359±1.19L/y vs **Decline Group B:** -2.369±1.46L/y vs **Decline Group C:** -0.52±1.52L/y; p=0.0005 Annual decline vs BMI: β=-15.26, p<0.03	
Wenninger et al. (2020), Germany [102]	**DM1**=29; Sex: 10F; 19M; Age: 43±12 (21-62); BMI: 22.1±4.5 (16.6-36.2)	NR	NR	Manometry: PEmax	42±15 cm H_2O; Median: 42 cm H_2O (18-71)	Fair
				Manometry: PImax	44±17 cm H_20; Median: 48 cm H_2O (23-87)	
				Mechanics of breathing (Spirometry): FEV1	**DM1:** 2.66±0.61% of pred.; Median: 2.69% of pred. (1.7-4.39) **DM1 PEmax<80 (n= 29):** 79.9±17.1% of pred.**; DM1 PEmax < pred. (n= 26):** 76.6±16.9% of pred.; **DM1 PEmax<LLN (n= 26):** 77.7±16.7% of pred.; **DM1 PImax<40 cm H2O (n= 11):** 75.8±17.1% of pred.; **DM1 PImax < pred. (n=22):** 74.5±16.7% of pred.; **PImax <LLN (11):** 79.1±16.5% of pred.	
				Mechanics of breathing (Spirometry): FVC	**DM1 upright:** 3.19±0.7% of pred.; Median: 3.24% of pred.; (1.89-5.43); **DM1 supine:** 2.98±0.85% of pred.; Median: 3.01% of pred.; (1.59-5.5); **DM1 Drop of FVC:** -7.23±8.79% of pred.; Median: -8.03% of pred. (-28.52 to 9.87) **DM1 PEmax<80 (n= 29):** 88.2±18.4% of pred.; **DM1 PEmax < pred. (n= 26):** 87.9±17.6% of pred.; **DM1 PEmax<LLN (n= 26):** 87.3±17.8% of pred.; **DM1 PImax<40 cm H2O (n= 11):** 81.8±14.1% of pred.; **DM1 PImax < pred. (n=22):** 86.5±15.9% of pred.; **DM1 PImax <LLN (11):** 77.7±12.8 % of pred.	
				Mechanics of breathing (Spirometry): PEF	**DM1:** 413.5±27.32 L/min; Median: 412.8 L/min (358.36-472.45); **DM1 PEmax<80 (n= 29):** 59.8±12.8% of pred.; **DM1 PEmax < pred. (n= 26):** 61±13.4% of pred.; **DM1 PEmax<LLN (n= 26):** 69±15.5% of pred.; **DM1 PImax<40 cm H20 (n= 11):** 59.6±9.3% of pred.; **DM1 PImax < pred. (n=22):** 61±14.57% of pred.; **DM1 PImax <LLN (11):** 62.9±14.2% of pred.	
West et al. (2016), UK [103]	**DM1**=120; Age: 46.9±13.2; BMI: 27.9±7.2	NR	NR	Manometry: PEmax	42.8±28.9% of pred. (10-157)	Fair
				Manometry: PImax	45.1±28.7% pf pred. (2-152)	
				Manometry: SNIP	50±25.9% (9-123)	
				Mechanics of breathing (Spirometry): FEV1	70.4±21.6% of pred. (25-122)	
				Mechanics of breathing (Spirometry): FEV1/FVC	85.4±7.4% (53-100)	
				Mechanics of breathing (Spirometry): FVC	67.6±23.2% of pred. (5.3-129) 12.3±16.3% of fall on lying (0-97)	

Cardiac and Skeletal Studies						
Hermans et al. (2012), Netherlands [104]	**DM1**=80; Sex: 35F; 45M; Age: Median: 48 (24-70) **Mild phenotype (Group A)**=9; Sex: 4F; 5M; Age: Median: 60 (46-70) **Classical phenotype (Group B)**=63; Sex: 30F; 33M; Age: Median: 47 (24-64) **Congenital phenotype (Group C)**=8; Sex: 1F; 7M; Age: Median: 32 (24-51)	NR	NR	CMR: LVEDV	**DM1:** Median: 72 mL/m^2 (38-117); **DM1 (F):** Median: 67 mL/m^2 (38-104) vs **DM1 (M):** Median: 77 mL/m^2 (41-117)	Good
				CMR: LVEF	**DM1:** Median: 58% (38-73); **DM1 (F):** Median: 61% (38-71) vs **DM1 (M):** Median: 57% (45-73)	
				CMR: LVESV	**DM1:** Median: 31 mL/m^2 (11-63); **DM1 (F):** Median: 28 mL/m^2 (11-56) vs **DM1 (M):** Median: 35 mL/m^2 (14-63)	
				CMR: RVEDV	**DM1:** Median: 66 mL/m^2 (40-117); **DM1 (F):** Median: 61mL/m^2 (40-102) vs **DM1 (M):** Median: 71mL/m^2 (40-117)	
				CMR: RVEF	**DM1:** Median: 64% (38-77); **DM1 (F):** Median: 67% (50-76) vs **DM1 (M):** Median: 60% (38-77)	
				CMR: RVESV	**DM1:** Median: 23 mL/m^2 (10-66); **DM1 (F):** Median: 20 mL/m^2 (10-46) vs **DM1 (M):** Median: 28 mL/m^2 (10-66)	
				MMT: modified MRC Score (0-110 points)	**DM1:** Median: 98 (73-110); **Group A:** Median: 110 (107-110) vs **Group B:** Median: 96 (73-109) vs **Group C:** Median: 100; (78-109) DM1 MRC Score vs CTG Repeat Length: p<0.001	
Meola et al. 2002, Italy [105]	**DM1 Baseline**=33; Sex:12F; 21M; Age: 40.6±14, Median: 39 **DM1 Follow-up (2-14y)**	E1 (<500) (n): 4 E2 (500-1000) (n): 26 E3 (1000-1500) (n): 3	MDRS: DM1 Baseline: 3.1±0.7 DM1 Follow-up: 3.6±0.9	ECHO: LVEDD	**DM1 Baseline:** 47±4.4 mm, Median: 47 vs **DM1 Follow-up**: 48±4.7 mm; Median: 45; p<0.05	Fair
				ECHO: LVEF	**DM1 Baseline:** 61±7.8%, Median: 60 vs **DM1 Follow-up**: 58.1±7.7%, Median: 59; p=ns	
				ECHO: LVESD	**DM1 Baseline:** 31±5.8 mm, Median: 28 vs **DM1 Follow-up**: 32±6.2 mm, Median: 30; p<0.05	
				MRC scale (0-150 points)	**DM1 Baseline:** 123±18, Median:128 vs **DM1 Follow-up**: 118±20, Median: 124; t-value: -6.581; p<0.001)	
Petri et al. (2014), Denmark [106]	**DM1**=30; Sex: 13F; 17M; Age: 47±14 **DM1 without abnormal ECG/Holter (Group A)**=12; Sex: 5F; 7M; Age: 41±10 **DM1 with abnormal ECG/Holter (Group B)**=18; Sex:10F; 8M; Age: 51±15 **DM1 with fibrosis (Group C)**=2; Age: 51±18 **DM1 without fibrosis (Group D)**=8; Age: 54±10	NR	NR	CMR: LVEDV	**Group C:** Median: 3 mL/m^2 (53-130) vs **Group D:** Median: 71 mL/m^2 (57-108)	Fair
				CMR: LVEF	**Group C:** Median: 63 % (38-71) vs **Group D:** Median: 66% (60-80);	
				CMR: LVESV	**Group C:** Median: 31 mL/m^2 (15-73) vs **Group D:** Median: 25 mL/m^2 (13-43)	
				ECHO: LVGLS avg.	**Group A:** Median: -19%; (-14 to -23) vs **Group B:** Median: -18 %; (-14 to -25); **Group C:** Median: -19%; (-14 to -23) vs **Group D:** Median: -18%; (-14 to -25)	
				ECHO: LA vol.	**Group A:** Median: 22 mL/m^2 (14-36) vs **Group B:** Median: 25 mL/m^2 (17-38) vs **Group C:** Median: 26 mL/m^2 (17-38) vs **Group D:** Median: 24 mL/m^2 (14-29); LA vol. vs myocardiac fibrosis: r=0.40; p=0.03	
				ECHO: LVEDD	**Group A:** Median: 46 mm (40-60) vs **Group B:** Median: 47 mm (41-56); **Group C:** Median: 50 mm (40-60) vs **Group D:** Median: 46 mm (40-60)	
				ECHO: LVEF	**Group A:** Median: 57% (48-67) vs **Group B:** Median: 58% (45-67) vs **Group C:** Median: 57% (45-53) vs **Group D:** Median: 58% (48-67)	
				MMT: MRC scale (0-5 points) - Ankle dorsiflexion	**Group A:** Median: 5 (2-5) vs **Group B:** Median: 4.5 (1-5); **Group C:** Median: 5 (1-5) vs **Group D:** Median: 4.5 (3-5)	
				MMT: MRC scale (0-5 points) - Grip strength	**Group A:** Median: 4.3 (2-5); vs **Group B:** Median: 4 (1-5) vs **Group C:** Median: 3.8 (1-5) vs **Group D:** Median: 4 (2-5)	

Skeletal and Respiratory Studies						
Kierkegaard et al. (2011), Sweden [107]	**DM1**=70; Sex: 41F; 29M; Age: 45±13; BMI:24.4±5.3 **MIRS I-III**=37; Sex: 20F; 17M; Age: 39±12; BMI: 23.7±4.4 **MIRS IV-V**=33; Sex: 21F; 12M; Age: 52±11; BMI: 25.1±6.1	NR	MIRS I-III (n):37 MIRS IV-V (n): 33	Mechanics of breathing (Spirometry): VC	**DM1:** 75±21% of pred.; **MIRS I-III:** 81±19% of pred. vs **MIRS IV-V:** 67±19% of pred.; p=0.011	Fair
				QMT (hydraulic hand dynamometry): Grip strength	**DM1 Right hand:** Median: 8 Kg [4;15] vs **DM1 Left hand:** Median: 8 Kg [4;14] **MIRS I-III Right hand**: Median: 15 Kg [8;20] vs **MIRS IV-V Right hand**: Median: 5 Kg [3;7]; p<0.001 **MIRS I-III Left hand**: Median: 14 Kg [7;20] vs **MIRS IV-V Left hand**: Median: 4 Kg [3;8]; p<0.001	
Légaré et al. (2019), Canada [108]	**DM1**=90; Sex: 55F; 35M; Age: 41±7.7; BMI: 25.21±5.61	603.4±267.9	MIRS II (n): 7 MIRS III (n): 22 MIRS IV (n): 56 MIRS V (n): 5	Manometry: PEmax	**DM1:** 58.5±22.4 cm H_2O (23-123) PEmax vs sex: β= 0.417; p<0.001	Good
				Manometry: PImax	**DM1:** 66.4±23.5 cm H_2O (26-138) PImax vs CTG repeat length: β=-0.332; p=0.008	
				Mechanics of breathing (Spirometry): FVC	**DM1:** 82.9±18.7 % of pred. (35.8-130.9) FVC vs CTG repeat length: β=-0.283; p=0.012	
				Mechanics of breathing (Spirometry): PEF	**DM1:** 73.1±16.2% of pred. (33.4-113.8)	
				QMT (Hand dynamometry): Grip strength	**DM1:** 8.6±6.4 Kg (0-28.5) Grip strength vs CTG repeat length: β=-0.387; p<0.001	
				QMT (Handheld dynamometry): MIT-Ankle dorsiflexors	**DM1:** 14.2±6.7 Nm (2.2-35.6) vs CTG repeat length: β=-0.442; p<0.001	
				QMT (Handheld dynamometry): MIT - Knee extensors	**DM1:** 80.9±33.1 Nm (18.9-165.3) Knee extensors vs age: β=-0.223; p=0.022 Knee extensors vs sex: β=0.476; p<0.001	
				QMT: Pinch strength	**DM1:** 5.2±1.5 Kg (2.2-9.2) Pinch strength vs sex: β=0.339; p=0.001 Pinch strength vs CTG repeat length: β=-0.387; p<0.001	
				QMT (Handheld dynamometry): MIT-Shoulder abductors	**DM1:** 40.8±15.7 Nm (14.3-94.2) Shoulder abductors vs sex: β=0.703; p<0.001	
Koc et al. (2020), Turkey [109]	**DM1**=33; Sex: 9F;24M; Age: 41±2 **Control**=32; Sex: 9F;23M; Age: 34±2	NR	NR	Manometry: PImax	**DM1 Post-PImax:** 49.8±4.99 cm H_2O vs **Controls Post-PImax:** 77.8±7.44cm H_2O; p<0.05	Fair
				Manometry: SNIP	**DM1:** 42±3.44 cm H_2O vs **Controls:** 66.8±8.90 cm H_2O; p<0.05	
				Mechanics of breathing (Spirometry): FEV1	**DM1 Sitting:** 2.74±0.19*; **DM1 Lying:** 2.58±0.17* vs **Controls Sitting:** 3.89±0.21*; **Controls Lying:** 3.63±0.20*; p<0.05	

				Mechanics of breathing (Spirometry): FVC	**DM1 Pre-FVC sitting:** 3.30±0.22*; **DM1 lying:** 3.12±0.20 vs **Controls Pre-FVC Sitting:** 4.78±0.22; **Controls lying:** 4.50±0.23*; p<0.05	
				MMT: MRC Sum Score (0-60 points)	**DM1:** 49.8±1.68 vs **Controls:** 60±0.01; p<0.05	
Pincherle et al. (2012), Italy [110]	**DM1**=40; Sex: 16F; 24M; Age: 38.6±9; BMI: 23±3.2 **DM1 without SBD (Group A)**= 8; Age: 37.3±8.9; BMI: 22.3±2 **DM1 with mild SBD (Group B)**=13; Age: 40.1±10.3; BMI: 22.6±3.3 **DM1 with moderate SBD (Group C)**=6; Age: 41.5±10.9; BMI: 25.4±3.2 **DM1 with severe SBD (Group D)**=6; Age: 33.6±8.5; BMI: 24.3±7 **DM1 without PB (Group E)**=35; Age: 39.1±10; BMI: 22.2±2.9 **DM1 with PB (Group F)**= 5; Age: 35.2±3.9; BMI: 22.2±2.9	NR	MIRS Group A: 2.6±0.7 Group B: 3±0.6 Group C: 3.3±0.5 Group D: 2.6±0.5 Group E: 2.8±0.6 Group F: 3.6±0.5	Manometry: PEmax	**Group A (n=18):** 32.4±10.5 cm H_2O vs **Group B (n=13):** 32.1±21.9 cm H_2O vs **Group C (n=6):** 33.4±18.6 cm H_2O vs **Group D (n=3):** 24.3±6.8 cm H_2O;p=Ns ; **Group E (n=35):** 30.5±11.7 cm H_2O vs **Group F (n=5):** 40.9±33.7 cm H_2O; p=Ns	Fair
				Manometry: PImax	**Group A (n=18):** 31.1±14.4 cm H_2O vs **Group B (n=13):** 32.5±21.5 cm H_2O vs **Group C (n=6):** 24.2±9 cm H_2O vs **Group D (n=3):** 23.7±12.1 cm H_2O; p=Ns; **Group E (n=35):** 28.2±12.8 cm H_2O vs **Group F (n=5):** 45.6±28.8 cm H_2O; p=0.024	
				Mechanics of breathing (Spirometry): FEV1	**Group A (n=18):** 91.9±15.7% vs **Group B (n=13):** 83.6±18.7% vs **Group C (n=6):** 93.6±9.5% vs **Group D (n=3):** 81.1±10.3%; p=N ; **Group E (n=35):** 89.8±15.3% vs **Group F (n=5):** 80.7±19.9%; p=Ns	
				Mechanics of breathing (Spirometry):VC	**Group A (n=18):** 86.9±14.5% vs **Group B (n=13):** 77.2±18.4% vs **Group C (n=6):** 84±12.9% vs **Group D (n=3):** 73.7±9.1% vs **Group E (n=35):** 83.1±15.2% vs **Group F (n=5):** 76.6±20%; p=Ns	
				QMT (handheld myometry): Grip strength	**Group A (n=18):** 74.6±48.6* vs **Group B (n=13):** 54±29.7* vs **Group C (n=6):** 35.7±18.7* vs **Group D (n=3):** 63.6±39.3* vs **Group E (n=35):** 61.4±40* vs **Group F (n=5):** 50±38.2*	
Solbakken et al. (2019), Norway [111]	**DM1**=20; Sex:12F; 8M; Age: 39±12.8; BMI: 25±4.7 **Controls**=20; Sex: 12F; 8M; Age: 39±12.8; BMI: 25±4.5	1.4±0.8 kb	NR	Mechanics of breathing (Spirometry): FVC	**DM1:** 74.2±18.9% of pred. (32-103); FVC vs CTG repeat: r=-0.67; p=0.003	Fair
				MMT: MRC scale (0-3 points) - Trunk extensors	**DM1:** Median: 2 (1-3) vs **Controls:** Median: 3 (2-3); p<0.0001	
				MMT: MRC scale (0-3 points) - Trunk flexors	**DM1:** Median: 1.5 (1-3) vs **Controls:** Median: 3 (3-3); p<0.0001	
Cardiac, Skeletal and Respiratory Studies						
Park et al. (2017), Korea [112]	**DM1**=19; Sex: 10F; 9M; Age: 43.7±10.2 (22-62)	409±300 (150-1300)	NR	ECHO: LVEF	**DM1:** 59.4±3.9% (53-67)	Good
				Mechanics of breathing (spirometry): FEV1	**DM1:** 68.5±13.3% of pred. (41-98)	
				Mechanics of breathing (spirometry): FVC	**DM1:** 67.4±12.8% of pred. (37-88)	
				MMT: MRC Sum score (0-60 points)	**DM1:** 49.8±9.8 (28-60)	
Park et al. (2018),	**DM1**=21; Sex: 11F; 10M; Age: 45.2±11.3 (22-68)	**DM1**: 386.67±293.	NR	ECHO: LVEDD	**DM1:** 4.49±0.37 cm (3.15-5.55) LVEDD vs CTG repeat length: r= -0.498; p=0.022	Good

Korea [113]	**DM1 without conduction abnormalities (Group A)**=10**;** Age: 40 ±10.4 **DM1 with conduction abnormalities (Group B)**=11; Age: 48.3 ± 11	92 (150-1300) **Group A**: 400 ± 219.98 **Group B**: 392.5 ± 183.85		ECHO: LVEF	**DM1:** 59±4 % (53-67)	
				ECHO: LVESD	**DM1:** 2.87±0.3 cm (2.35-3.38) LVESD vs CTG repeat length: r=-0.496; p=0.022	
				Mechanics of breathing (Spirometry): FEV1	**DM1:** 68.4±13% of pred. (41-98) vs **Group A:** 66.25±11.55% of pred. vs **Group B:** 71.63±15.22% of pred. FEV1 vs CTG repeat length: r=-0.286; p=0.221	
				Mechanics of breathing (Spirometry): FVC	**DM1:** 67.3±12.5 (37-88) % of pred. vs **Group A:** 70.13±11.55% of pred. vs **Group B:** 65.3±13.2% of pred. FVC vs CTG repeat length: r=-0.444; p=0.05	
				MMT: MRC scale (0-60)	**DM1:** 50.2±9.7 (28-60) vs **Group A:** 49.1±9.7 vs **Group B:** 50.9±10.1 MRC sum score vs CTG repeat length: r=-0.761; p=0.00 MRC sum score vs LVEDD: r=0.600; p=0.004 MRC sum score vs LVESD: r=0.592; p<0.05	

Supplementary table 4.1 footnote: Abbreviations: **ADM**- Adductor Digiti Minimi Muscle; **AUC**- Area Under ROC Curve; **Avg**- Average; **BMI**- Body Mass Index; **BSA**- Body Surface Area; **CI**- Confidence Interval; **CO**- Cardiac Output; **CoughPgas**- Gastric Pressure During a Maximum Cough; **CTG**- Cytosine-Thymine-Guanine; **daN/Kg**- Dalton Newton for Kilogram; **DM1**- Myotonic Dystrophy type 1; **ECHO**- Echocardiography; **EF**- Ejection Fraction; **EMG**- Electromyography; **ERV**- Expiratory Reserve Volume; **ESF**- Endocardial shortening fraction; **F**- Female; **FEV1**- Forced Expiratory Volume in 1 Second; **FIV1**-Forced Inspiratory Volume in 1 Second; **FRC**- Functional Residual Capacity; **FVC**- Forced Vital Capacity; **LV GLS**- Left Ventricular Global Longitudinal Strain; **IQR**- Interquartile Range; **LAEDV**- Left Atrial End-Diastolic Volume; **LAEF**- Left Atrial Emptying Fraction; **LAESV**– Left Atrial End-Systolic Volume; **LA LS**- Left Atrial Longitudinal Strain; **LAD**- Left atrial diameter (mm); **LVDD**- Left Ventricular Diastolic Diameter; **LVEDD**- Left Ventricle End-Diastolic Diameter; **LVEDV**- Left Ventricular End-Diastolic Volume; **LVEF**- Left Ventricular Ejection Fraction; **LVESD**- Left Ventricular End-Systolic Diameter; **LVESV**- Left Ventricular End-Systolic Volume; **LV LS**- Left Ventricular Longitudinal Strain; **LVSD**- Left Ventricular Systole Diameter; **LVSF**- Left Ventricular Shortening Fraction; **M**- Male; **MCID**- Minimal Clinically Important Difference; **MDD**- Minimal Detectable Difference; **MDRS**- Muscle Disability Rating Scale; **MEF**- Maximal Expiratory Flow; **MIF**- Maximum Isometric Force; **MIRS**- Muscle Impairment Rating Scale; **MIT**- Maximum Isometric Torque; **mm**- millimeters; **MMT**- Manual Muscle Test; **MRC**- Medical Research Council; **MVIC**- Maximal Voluntary Isometric Contraction; **Nm**- Newton-meters; **NR**- Not Reported; **PCEF**-Peak Cough Expiratory Flow; **PCF**- Peak Cough Flow; **PEF**- Peak Expiratory Glow; **PEmax**- Maximal Expiratory Pressure; **PImax**- Maximal Inspiratory Pressure; **QMT**- Quantitative Muscle Testing; **RR**- Relative Risk; **RV**- Residual Volume; **RVEDV**- Right Ventricle and End-Diastolic Volume; **RVEF**- Right Ventricle Ejection Fraction; **RVESV**- Right Ventricle End-Systolic Volume; **SEM**- Standard Error of the Mean; **SF**- Shortening Fraction; **sniffPdi**- Transdiaphragmatic Pressure Following a Sniff Manoeuvre; **SNIP** – Sniff Nasal Inspiratory Pressure; **SNP**- Sniff Negative Pressure; **SV**- Stroke Volume; **TLC**- Total Lung Capacity; **twPdi**- Transdiaphragmatic Pressure Following Supramaximal Cervical Stimulation of the Phrenic Nerves; **twPgas**- Gastric Pressure Following Stimulation of the Abdominal Muscle Nerve Roots at the Tenth Vertebra; **VC**- Vital Capacity; **% of pred.**(percentage of the predicted values according to sex, age and

weight);

Note: Data are presented as mean or mean±standard deviation, (range) or [IQR], unless otherwise stated; BMI expressed as kg/m^2; Change and % of change are related to the difference between Follow-up and baseline; ICC = intraclass correlation; MCID = minimal clinically important difference; β- coefficient of significance; Maximal respiratory pressure (Sum of PImax at FRC and PEmax at TLC); *Units of measure not reported

Chapter V. Concluding remarks and future perspectives

5.1 Concluding remarks

Myotonic dystrophy type 1 (DM1) is a autosomal dominant hereditary disease mainly characterized by progressive distal muscle weakness and myotonia. DM1 is caused by the expansion of unstable tri-nucleotide (CTG) in the 3' untranslated region of the *DMPK* gene. To date the identification of the molecular mechanisms underlying this pathology are supported by three hypotheses, namely the rearrangement of DM1 locus, DMPK haploinsufficiency and gain of toxic RNA function. However, the mechanisms underlying these pathologies are not yet fully understood, meaning that more studies are needed, not only to understand the underlying mechanisms but also to understand how these mechanisms are associated for instance with the metabolic alterations observed in patients with DM1, namely Lipin.

Several metabolic alterations are present in patients with DM1, namely, insulin resistance, hypertriglyceridemia, increased fat mass, high levels of low-density lipoprotein (LDL), low levels of high-density lipoproteins (HDL), hypertension, increased levels of glucose and abdominal obesity. Given these metabolic dysfunctions, particularly in the skeletal-muscle tissue, there is a high propensity to develop metabolic syndrome, which is frequently significantly higher in these patients than in the general population. These metabolic alterations could potentially be due to Lipin deficiency, since Lipin is a key enzyme that regulates lipid metabolism and signaling, adipocyte differentiation and transcriptional co-activator of fatty acid β-oxidation.

Therefore, the main goal of this dissertation was to better understand the metabolic alterations in patients with DM1 and the Lipin role in those alterations, as well as, to evaluate if FTIR spectroscopy is a valuable tool for DM1 samples and control group discrimination and characterization, in which, with the aid of multivariate analysis. The latter allowed to identify spectral differences within DM1-derived fibroblasts when compared with control fibroblast. We also, synthetized information regarding the most used outcomes and measurements to evaluate muscle strength in patients with DM1. Our results indicate that:

- Metabolic alterations observed in patients with DM1 are mainly increased: total cholesterol, low-density lipoprotein, triacylglycerol, insulin and HOMA-Insulin resistance levels and glucose levels;
- Patients with DM1 rarely became glucose intolerant, presenting low incidence of diabetes. Additionally, the blood pressure is not elevated as in patients with metabolic syndrome;
- Lipin have a critical metabolic role in adipose tissue, skeletal-muscle and liver;
- Alterations in Lipin levels lead to common features observed in patients with DM1 which are: impaired skeletal-muscles functions (severe myopathies), mithocondrial dysfunction, severe sarcoplasmic reticulum stress, neutral lipid accumulation, insulin

resistance and dyslipidemia, inflammatory state (macrophage) in adipose tissue, endoplasmic reticulum stress and sterol-regulatory element-binding protein (SREBP) activity reduction.

- Clear discrimination was observed particularly in the region between 1800-1500 cm^{-1} in DM1-derived fibroblasts (DM1_2000 and DM1_1000) obtained from Coriell Institute as well as, between cDM1, iDM1, jDM1 and aDM1-derived fibroblasts and Control fibroblast (established at iBiMED);

- Clear separation between the control and DM1-derived fibroblasts (DM1_2000) and also with the ones established at iBiMED (cDM1, iDM1 and jDM1) at 3000-2800 cm^{-1} region;

- The more consensual and important measures to evaluate muscle strength (cardiac, skeletal and respiratory) was successfully gathered.

- There was a consistent use of Echocardiography, Quantitative muscle test, Manual muscle test and Manometry to assess cardiac, skeletal and respiratory muscle strength;

- The measures of choice to assess muscle strength were: (1) ejection fraction fro cardiac muscle strength; (2) muscle isometric torque, grip strength and medical research council (0-5 points and 0-60 points) for skeletal-muscle strength; (3) maximal inspiratory pressure and maximal expiratory pressure in respiratory muscles strength;

In conclusion, our results indicate that it may be a relationship between Lipin and metabolic alterations observed in patients with DM1. FTIR spectroscopy was able to detect biochemical differences within DM1-derived fibroblasts and between DM1-derived fibroblasts and control from Coriell Institute and between DM1-derived fibroblasts and control established at our iBiMED laboratory. Thus, this technique could be a useful screening tool for patients with DM1 characterization of disease severity. The information gathered in the systematic review were important, since it gives valuable information for muscle strength, in which will improve the evaluation of future clinical trials and observational studies, particularly to see if a drug is improving muscle strength in patients with DM1.

5.2 Future perspectives

Despite the promising results regarding Lipin and metabolic alterations in patients with DM1, there is a need to better understand and study the role of Lipin in patients with DM1, since to our current knowledge there are few studies related to this subject. Also, despite FTIR spectroscopy potential to characterize patients with DM1 severity through metabolome analysis as a screening tool, FTIR spectroscopy is not able to identify specific metabolites that might be important in patients with DM1 metabolome. Thus, Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS) may be considered for more detailed analysis of abnormalities in a variety of biological samples of patients

with DM1. Although, the results obtained in the systematic review were important and useful given that they will be valuable for muscle strength evaluation in future clinical trials and observational studies, particularly to test if a drug is improving muscle strength in patients with DM1, it is also, necessary to develop a core outcome set (COMET initiative) for consensus and improvement of standardized methodology regarding muscle strength in clinical practice and research of patients with DM1. In addition, characterization of the different forms of severities in patients with DM1 needs to be included in the studies with robust methodologies to understand the differences of muscle strength and other components of the disease (e.g. lipid abnormalities) across the 5 DM1 phenotypes (congenital-onset, infantile-onset, juvenile-onset, adult-onset and late-onset).