Research Report

Resistance Exercise Training Rescues Mitochondrial Dysfunction in Skeletal Muscle of Patients with Myotonic Dystrophy Type 1

Valeria Di Leo[a,b], Conor Lawless[a], Marie-Pier Roussel[c], Tiago B. Gomes[a,d], Gráinne S. Gorman[a,b,d], Oliver M. Russell[a,b], Helen A.L. Tuppen[a], Elise Duchesne[e,f,1,*] and Amy E. Vincent[a,b,1,*]

[a]*Wellcome Centre for Mitochondrial Research, Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, UK*
[b]*NIHR Newcastle Biomedical Research Centre, Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University, Newcastle upon Tyne, England*
[c]*Department of Fundamental Sciences, Université du Québec à Chicoutimi, Quebec, Canada*
[d]*NHS Highly Specialised Service for Rare Mitochondrial Disorders, Royal Victoria Infirmary, Newcastle Upon Tyne, UK*
[e]*Department of Health Sciences, Université du Québec à Chicoutimi, Québec, Canada*
[f]*Neuromuscular Diseases Interdisciplinary Research Group (GRIMN), Saguenay-Lac-St-Jean Integrated University Health and Social Services Center, Saguenay, QC, Canada*

Abstract.
Background: Myotonic dystrophy type 1 (DM1) is a dominant autosomal neuromuscular disorder caused by the inheritance of a CTG triplet repeat expansion in the Dystrophia Myotonica Protein Kinase (*DMPK*) gene. At present, no cure currently exists for DM1 disease.
Objective: This study investigates the effects of 12-week resistance exercise training on mitochondrial oxidative phosphorylation in skeletal muscle in a cohort of DM1 patients ($n = 11$, men) in comparison to control muscle with normal oxidative phosphorylation.
Methods: Immunofluorescence was used to assess protein levels of key respiratory chain subunits of complex I (CI) and complex IV (CIV), and markers of mitochondrial mass and cell membrane in individual myofibres sampled from muscle biopsies. Using control's skeletal muscle fibers population, we classified each patient's fibers as having normal, low or high levels of CI and CIV and compared the proportions of fibers before and after exercise training. The significance of changes observed between pre- and post-exercise within patients was estimated using a permutation test.

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[1]These authors contributed equally to the manuscript.

*Correspondence to: Prof Elise Duchesne, PT, PhD, Department of Health Sciences, Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada. E-mail: Elise1_duchesne@uqac.ca and Dr. Amy E. Vincent, PhD, Wellcome Centre for Mitochon-

drial Research, 4th Floor Cookson Building, Framlington Place, Newcastle University, Newcastle upon Tyne, Tyne and Wear, UK. E-mail: amy.vincent@newcastle.ac.uk.

Results: At baseline, DM1 patients present with significantly decreased mitochondrial mass, and isolated or combined CI and CIV deficiency. After resistance exercise training, in most patients a significant increase in mitochondrial mass was observed, and all patients showed a significant increase in CI and/or CIV protein levels. Moreover, improvements in mitochondrial mass were correlated with the one-repetition maximum strength evaluation.

Conclusions: Remarkably, 12-week resistance exercise training is sufficient to partially rescue mitochondrial dysfunction in DM1 patients, suggesting that the response to exercise is in part be due to changes in mitochondria.

INTRODUCTION

Myotonic dystrophy type 1 (DM1) is a dominant autosomal disorder that affects 1 in 20,000 worldwide [1]. Population incidences vary in different parts of the world, reaching a higher frequency of 1 in 475 in the Québec region of Saguenay—Lac-Saint-Jean (Canada) [2]. DM1 etiology is explained by the toxic gain of function of the Dystrophia Myotonica Protein Kinase (DMPK), which originates from a CTG triplet repeat expansion in the 3' untranslated region of the *DMPK* gene [3, 4]. The CTG triplet repeat microsatellite region contains between 5 and 37 repeats in non-DM1 individuals [5, 6]. In DM1 patients however, the expansion may range from 50 to thousands of repeats but may further expand in post-mitotic cells, such as skeletal muscle (SKM), presenting as somatic mosaicism [7, 8]. Depending on the number of inherited CTG triplet repeats, the age of onset can vary (congenital, childhood, juvenile, adult, or late) [9]. Although presenting as a systemic disease, the most prominent symptoms affect the SKM apparatus with muscle weakness, muscle wasting and myotonia [10].

The DMPK gain of function triggers RNA toxicity, characterized by nuclear sequestration of transcription and splicing factors that eventually dysregulate the downstream alternative splicing machinery [11]. Traditionally, research has focused on understanding RNA toxicity and reversing the mechanisms of the aberrant splicing program. However, a myriad of proteins and mechanisms are affected in DM1 pathology, including nuclear and cytosolic alterations, but also organelle-specific perturbations, such as mitochondria [12]. DMPK has been found to specifically bind to the outer mitochondrial membrane [13], and its overexpression in myoblasts induces fragmentation and perinuclear clustering of mitochondria, resulting in the increase of both autophagy and apoptosis [14]. Moreover, DMPK interacts with tyrosine kinase Src and Hexokinase-II in the formation of a multimeric complex on the outer mitochondrial membrane, which orchestrates a fine-tuned regulation of intracellular oxidative stress and pro-survival processes via glucose starvation stimuli [15–17]. Strikingly, there is evidence that SKM from DM1 patients presents with different mitochondrial morphology, dynamics and function affecting the structure of the sarcoplasmic reticulum with subsequent mitochondrial aggregation [18], metabolic impairment [19] and neuromuscular junction alterations [20, 21]. Recent *in vivo* studies demonstrated that DM1 patients present with oxidative metabolism impairment in both SKM and brain [22]. Like mitochondrial myopathy patients, DM1 patients present with elevated FGF21 serum level, which is thought to be correlated to insulin resistance and mitochondrial dysfunction [23, 24]. Furthermore, it was recently demonstrated that metformin treatment could reverse the impaired metabolism and mitochondrial dysfunction in DM1-derived fibroblasts [25, 26].

Although DM1 is one of the most common adult-onset neuromuscular disorders, there is no curative therapies currently available. Many different approaches, including the genomic correction of the *DMPK* gene, the generation of antisense oligonucleotides for the alternative splicing correction and the use of small molecules for the modulation of signaling pathways, have been extensively explored to treat DM1 pathology with several clinical trials now underway [27, 28]. At present, the only option for DM1 patients is symptomatic therapy. To this end, in the last few decades, exercise therapy has been investigated as a beneficial and effective approach to treat neuromuscular disorders patients [29, 30], as well as in conjunction with cognitive behavioral therapy in DM1 patients [31, 32]. Resistance exercise training has been proved to be an accessible, low-cost way to improve SKM weakness and increase strength by inducing muscle hypertrophy in DM1 patients [33–39]. In mitochondrial myopathy patients, resistance exercise training

was demonstrated to induce amelioration in the oxidative phosphorylation (OXPHOS) defects [40]. Mitochondrial OXPHOS defects can be detected in cells and tissues by using a single cell analysis combined to immunofluorescence, which has previously been shown to correlate with results obtained both from cytochrome c oxidase/succinate dehydrogenase histochemistry and mitochondrial enzymatic activity assays [41, 42]. Recently, mitochondrial OXPHOS deficiency has been found in DM1 patients compared to healthy individuals [43]. However, the effects of resistance exercise training on mitochondrial OXPHOS deficiency have never been assessed before at a single cell level in SKM from DM1 patients. Therefore, the aim of this study is to investigate baseline mitochondrial dysfunction and the impact of 12-week resistance exercise training in a cohort of DM1 patients to better elucidate the role of mitochondria in DM1 disease.

MATERIALS AND METHODS

Experimental design

This study is a secondary analysis of a larger project, in which DM1 patients ($n = 11$, men) were recruited to participate in a 12-week resistance exercise training program [38]. DM1 patients were aged between 31 and 60 years old; were able to walk independently and to give informed consent. Control muscle was obtained from healthy individuals, who voluntarily donated their tissue. Control muscle has previously been shown to have normal OXPHOS in immunofluorescence experiments. *Vastus lateralis* muscle biopsies from controls ($n = 3$, men) and DM1 patients ($n = 11$, men) pre- and post-exercise (Table 1) were collected at the *Centre Intégré Universitaire de Santé et de Services Sociaux du Saguenay–Lac-St-Jean* (Canada) [38]. Biopsy sections (10 μm) were cut on glass slides at the Université du Québec à Chicoutimi (Canada). Samples were stored on dry ice until shipment to Newcastle University (Newcastle upon Tyne, United Kingdom). Upon receipt, samples were stored at –80°C.

Resistance exercise training

All participants completed a supervised 12-week resistance exercise training with two exercise sessions every week [38]. During the first session, the weight for each type of exercise was determined with the one-repetition maximum (1 RM) method, which consists in the execution of full-range movement before failure for each participant, calculated using the Epley formula [44]. During all sessions, participants completed 3 series of 6–8 RM of five lower limb training exercises, which included leg extension, leg press, hip abduction, squat, and plantar extension.

Clinical measurements

All clinical measurements related to the DM1 cohort, such as age, age of onset, CTG triplet repeat expansion in blood and muscular impairment rating scale (MIRS), were collected accordingly to previously published protocols [38]. Muscle strength was assessed with the 1-RM method pre- and post-training. Individual changes after the training program were reported in percentage compared to the pre-exercise value ([post-exercise value – pre-exercise value]/pre-exercise value * 100). An average composite score of lifted weight was then calculated with the following exercise (leg extension, leg press, hip abduction and squat). The phenotypical presentations were classified as juvenile when the age of onset is between 10 and 20 years old; adult when the age of onset is between 20 and 40 years old; late when at least two of these criteria are met: i) age of onset is >40yo, ii) CTG triplet repeat expansion is <200, iii) MIRS is 2 (minimal signs) or 1 (no muscle impairment) (Table 1).

Quadruple immunofluorescence

SKM sections were stained for quadruple immunofluorescence (QIF) to assess OXPHOS. The staining included the immunolabelling of key subunit NADH:Ubiquinone Oxidoreductase Subunit B8 (NDUFB8) for CI and Mitochondrially Encoded Cytochrome C Oxidase I (COX1) for CIV, together with Voltage Dependent Anion Channel 1 (VDAC1) as a mitochondrial mass marker and Laminin subunit alpha-1 (LAMA1) as a cell membrane marker [41].

Fluorescent microscopy

Fluorescent images were taken with Zeiss Cell Discoverer 7 (CD7) and analyzed using Zen 2011 (black edition) software. The CD7 includes the following parts: a Hamamatsu Fusion and a Zeiss Axiocam 506 monochrome camera; 5x/0.35, 20x/0.7 and 50x/1.2NA lenses; a Zeiss LED light source (Colibri 7). Image acquisition was performed at 20x

Table 1
Characteristics of sex matched controls and DM1 participants enrolled in the resistance exercise training program

Controls #	Sex	Ages years	PA hs/wk				
C4	M	33	4				
C10	M	45	10				
C13	M	23	1				
Mean		33.7					
SD		9					

Participant number #	Sex	Age years	Age of onset years	Duration of disease years	CTG n	MIRS	Phenotypical presentation onset
523	M	58	35	23	85	2	Late
907	M	59	34	25	533	3	Adult
1242	M	53	20	33	1200	3	Juvenile
1791	M	47	25	22	349	4	Adult
1806	M	40	13	27	608	4	Juvenile
1955	M	60	50	10	63	3	Late
2002	M	31	25	6	603	2	Adult
2005	M	60	54	6	74	3	Late
2019	M	50	44	6	67	3	Late
2110	M	34	30	4	86	1	Late
2182	M	36	24	12	414	4	Adult
Mean		48	32.2	15.8	371.1		
SD		10.6	12.2	9.9	339.0		

The following details are listed: participant number, sex, age, physical activity reported in hours per week, age of onset of the disease, duration of disease since diagnosis or first symptoms appearance, number of CTG triplet repeats expansion measured in blood, muscle impairment rating scale (MIRS) and phenotypical presentation.

magnification using a motorized stage AxioImager M1 and the tiling function in Zen software. For each section, a.czi file was generated using the stitching function in Zen.

Image analysis

Stitched images of the SKM sections (.czi files) were analyzed using *Quadruple Immuno Analyser*, an in-house software written in MatLab R2015a. The software automatically created a segmentation map of the SKM fibers' boundaries using LAMA1 signal. The mean signal intensities for each channel in each single SKM fiber were exported in tabular format as.csv files [41].

Linear regression and 95% predictive interval model

Statistical analysis of QIF data was conducted using a 95% predictive interval linear regression model based on combined control population of fibers. As previously described, combined controls with normal OXPHOS is needed to quantify relative levels of deficiency in putative samples [45–47]. In patients' samples, all fibers lying within the control predictive interval were classified as normal for NDUFB8 or COX1 (fibers[normal]).

Fibers below the interval were classified as fibers with a low level (fibers[low]), and above the interval as fibers with a high level (fibers[high]). The relationships between VDAC1 and NDUFB8 or COX1, respectively, for each patient and all controls are shown as scatterplots, here referred as 2Dmito plots (https://github.com/VDLNCL/DM1-mitochondria/blob/main/Report.pdf).

Bootstrapping and permutation test

We used statistical bootstrapping to estimate uncertainty about the proportions of fibers classified as fibers[normal], fibers[low] and fibers[high]. We used the permutation test to estimate whether observed differences in proportions after resistance exercise training are significant. The number of resamples was set as N = 5,000. We also generated bootstrapping estimates of Δfibers[low] and Δfibers[high], the differences in proportion of fibers in each class (post-exercise - pre-exercise) to quantify our uncertainty about changes after exercise. A permutation test was performed for each class of fibers in each patient to calculate whether exercise-induced changes were statistically significant using the null hypothesis that the labels "pre-exercise" and "post-exercise" are interchangeable. The permutation test provided *p*-values through

a one-sided *t*-test. Δfibers^{low} for both NDUFB8 and COX1 was classified as significant when there was a decrease after exercise (Δfibers$^{low} < 0$) and $p < 0.05$. Δfibers^{high} for both NDUFB8 and COX1 was classified as significant when there was an increase after exercise (Δfibers$^{high} > 0$) and the *p*-value was <0.05. All *p*-values were corrected for multiple testing by controlling for the False Discovery Rate [48].

Statistical tests

All statistical tests were performed in R (version 3.5.2, https://intro2r.com/citing-r.html). A *t*-test was used to assess the difference between the population means for VDAC1. The function *t*.test was used to perform two-sided *t*-test. An ANOVA test was used to test any significant difference in the mean of independent populations (control and pre-exercise fibers' populations, or pre- and post-exercise fibers' populations). The function *aov* was used to perform two-way ANOVA test. An over-representation test was used to test the statistical over-representation of a subset of observations (COX1^{low} or COX1^{high}) within a subset of collected data (NDUFB8^{low} or NDUFB8^{high}). The function *hypertest* (https://github.com/VDLNCL/DM1-mito chondria/blob/main/Over-representation%20test.R) was used to perform the over-representation test, where *x* was defined as number of NDUFB8^{low} &COX1^{low} or NDUFB8^{high}&COX1^{high}, *m* as number of COX1^{low} or COX1^{high}, *y* as number of NDUFB8^{low} or NDUFB8^{high}, *n* as the total number of fibers per sample.

RESULTS

DM1 cohort genotypic and phenotypical presentations

The cohort of subjects used in this study included DM1 participants ($n = 11$, men) and control muscle with normal OXPHOS ($n = 3$) (Table 1). The DM1 cohort presents with a mean age of 48 ± 10.6 years, and a mean age of onset of 32.2 ± 12.2 years. The CTG triplet repeat expansion varies between a minimum $n = 63$ (patient #1955) and a maximum $n = 1,200$ (patient #1242) of CTG triplet repeats. As expected, the CTG triplet repeat expansion shows a strong negative correlation with the age of onset displaying a slope significantly different from zero (Pearson, R = –0.7; *p*-value = 0.016, Fig. 1). The phe-
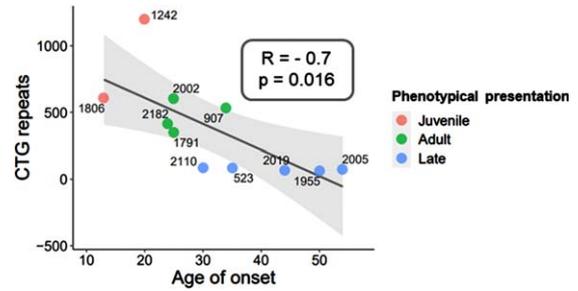

Fig. 1. Genotypic and phenotypical presentation of DM1 cohort. The CTG triplet repeat expansion negatively correlates with the age of onset of DM1 disease, resulting in three phenotypical presentation classes: juvenile, adult, and late onset. Correlation coefficient (R) and *p*-value are indicated.

notypical presentations observed in the DM1 cohort were juvenile ($n = 2$), adult ($n = 4$) and late ($n = 5$) onset (Table 1), in accordance with previous publications [9, 38]. Importantly, duration of disease varies extremely across the DM1 cohort with more than 20 years in some patients (min = 22, max = 33, $n = 5$) or less than 20 years in others (min = 4, max = 12, $n = 6$) (Table 1).

Mitochondrial mass deficiency and changes after resistance exercise training

QIF labelling of NDUFB8, COX1, VDAC1 and LAMA1, was used to investigate mitochondrial mass and OXPHOS defects in the SKM biopsies of the DM1 cohort. QIF staining was not possible in pre-exercise section of case #2002, so the patient was excluded from further analyses. A representative image of the QIF staining is provided in Fig. 2, including one control and one patient sample (pre- and post-exercise). VDAC1 intensities from single myofibres in each section were assessed to calculate a mean value for each pre- and post-exercise sample across the DM1 cohort [45]. The DM1 pre-exercise group (mean = 5979.1 AU) shows significantly decreased mitochondrial mass compared to healthy controls (mean = 6591.6 AU) (two-way ANOVA test, *p*-value <0.0001, Fig. 3A). Grouped analysis of pre- and post-exercise samples (mean = 5,998 AU) does not show any significant change after exercise (Fig. 3A). However, when comparing individually each pair of pre- and post-exercise samples, six of 10 patients exhibit a significant increase in VDAC1 mean value (*t*-test two-sided, *p*-value <0.05, Fig. 3B). Four patients (cases #2182, #1955, #2005 and # 2019) present with a significant decrease in mitochondrial mass after
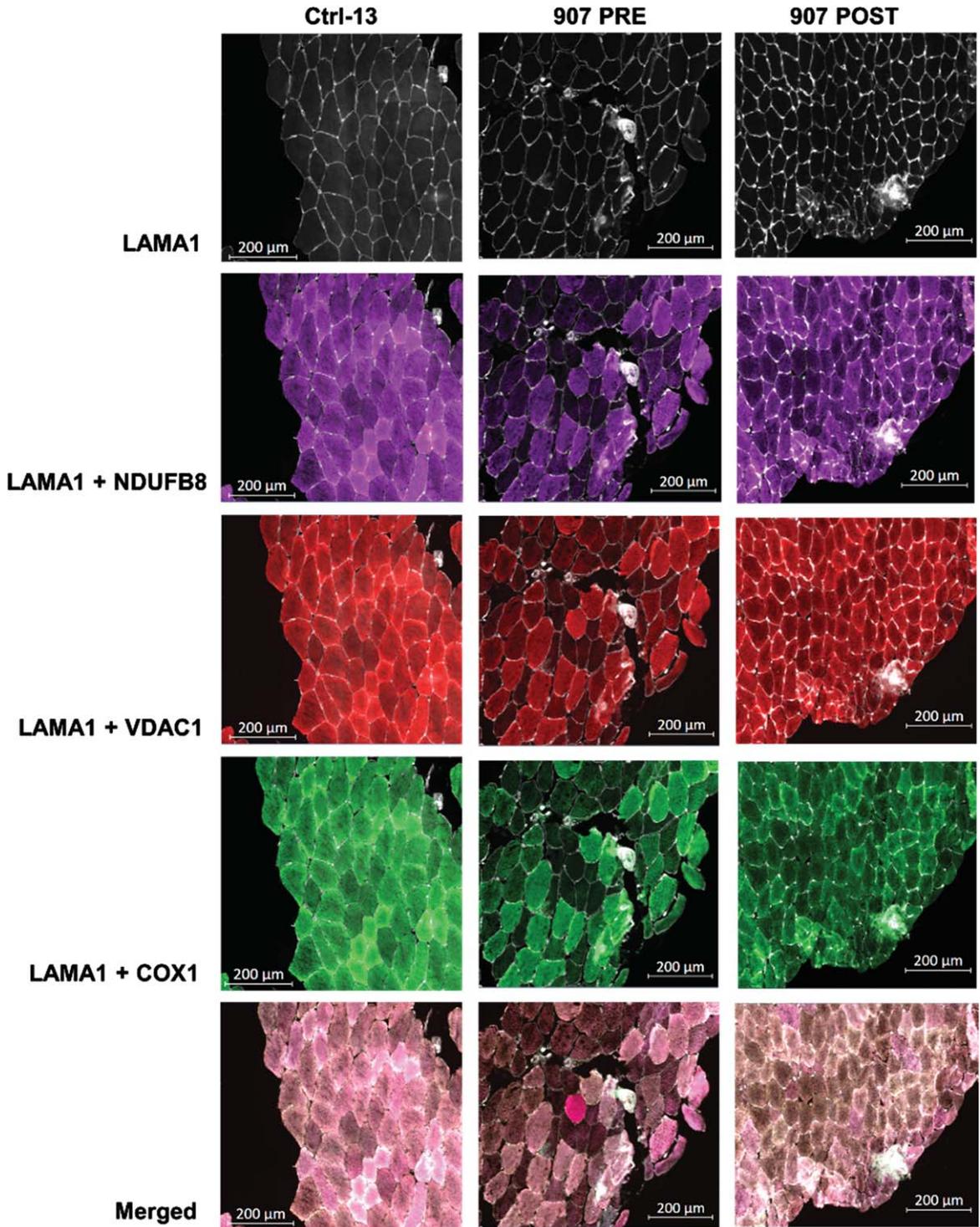
Fig. 2. Representative QIF images of control subject, pre- and post-exercise pair from DM1 cohort after resistance exercise training. Representative images of quadruple immunofluorescence staining for LAMA1 (membrane marker), VDAC1 (mitochondrial mass marker), NDUFB8 (complex I marker) and COX1 (complex IV marker) in SKM sections from healthy Ctrl-13, P907 PRE (pre-exercise) and P907 POST (post-exercise) biopsies. The last row is the merge of all channels.
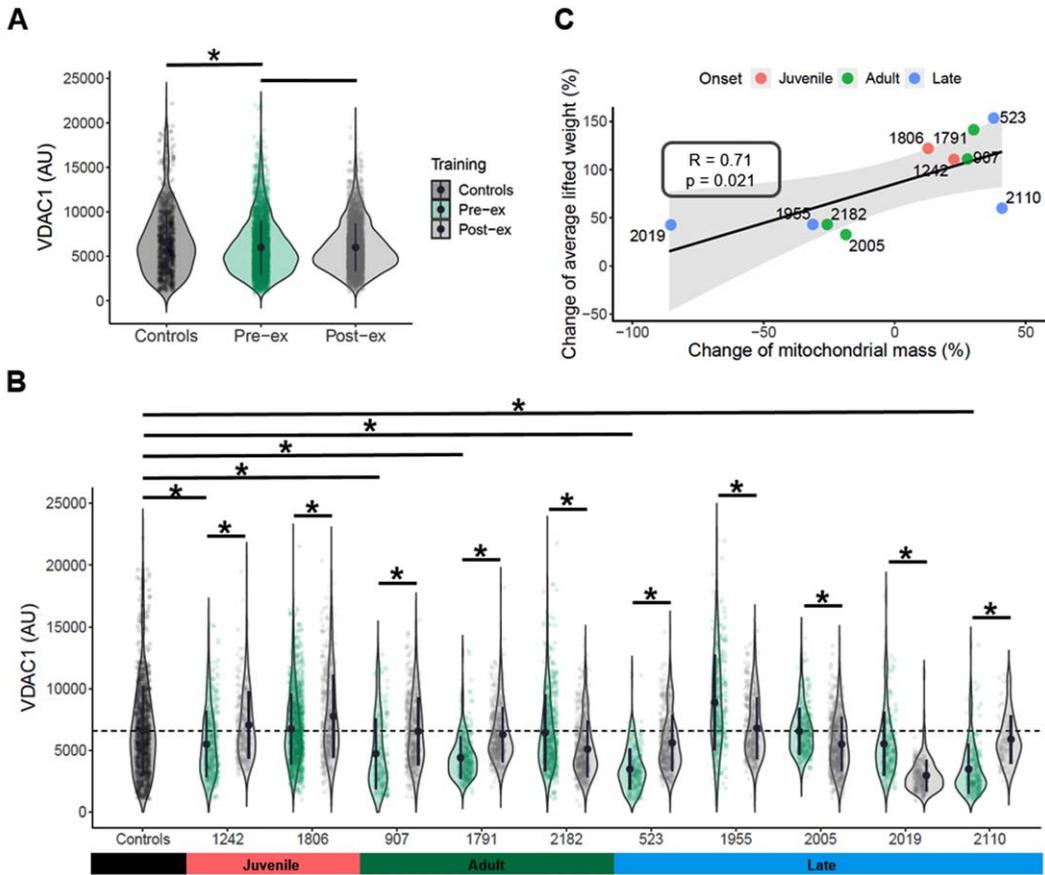
Fig. 3. Mitochondrial mass and exercise-induced effects in DM1 cohort. VDAC1 values were compared between pre-exercise group and controls or post-exercise group. Mean values, two-way ANOVA test, *p*-value <0.05 (*) (A). The mean value of mitochondrial mass in controls fibers is indicated as dashed black line. VDAC1 mean values were compared between each pre-exercise case and grouped controls. VDAC1 mean values were compared between pre- and post-exercise samples for each patient. Whiskers indicate the min and max values. Mean values, two-sided *t*-test, *p*-value <0.05 (*) (B). The change of mitochondrial mass after exercise was correlated to the change of the average composite score of lifted weight. Pearson's correlation test was performed in R core; R factor and p value are reported for each correlation test (C).

exercise (*t*-test two-sided, *p*-value <0.05, Fig. 3B). Interestingly, after exercise these patients present with the smallest change both in mitochondrial mass and in average of lifted weight, which show a significant positive correlation (R = 0.71; *p*-value = 0.021, Fig. 3C).

CI and CIV deficiency in DM1 patients

We classified single myofibres after QIF staining using a linear regression between VDAC1 intensity and either NDUFB8 or COX1, using data from combined healthy control muscle. Patients' fibers were classified by comparison with the 95% predictive interval from the regression. A typical output of the statistical analysis is shown in Supplementary Figure 1. Percent of fibers below and above the control population, NDUFB8^{low} and COX1^{low}, NDUFB8^{high} and COX1^{high} fibers respectively, were calculated for each pair of samples for each patient in the DM1 cohort (Table 2). At baseline, only patient #523 presents with 0% NDUFB8^{low} fibers. The rest of the DM1 cohort present with NDUFB8^{low} fibers, although patients #1242, #1791, #2182 and #2005 display less than 5% fibers classified as NDUFB8^{low} (Fig. 4A). Similarly, five of 10 patients show a high proportion of COX1^{low} fibers (#1242, #907, #523, #1955 and #2019, (Fig. 4C). The proportion of NDUFB8^{high} fibers is high in patient #523 before exercise (Fig. 4B), whereas the proportion of COX1^{high} fibers is close to 0% in most patients before exercise (Fig. 4D).

Table 2
Classification of fibers across the DM1 cohort

#Patient	Training	NDUFB8^{low}	NDUFB8^{high}	COX1^{low}	COX1^{high}
#523	PRE	0%	19.5%	30.2%	0%
	POST	0.2%	2.1%	0%	0%
#907	PRE	32.2%	0%	41%	0%
	POST	1.1%	0%	0.2%	0%
#1242	PRE	3.8%	0.3%	13.4%	0%
	POST	1.4%	0.0%	1%	0%
#1791	PRE	2.9%	3.2%	0%	0.3%
	POST	0.4%	1.1%	0%	10.2%
#1806	PRE	12.4%	1.5%	1.1%	0.3%
	POST	0.5%	8.8%	0%	0.2%
#1955	PRE	30.8%	0%	10.6%	0%
	POST	1.4%	0.5%	0.3%	0%
#2005	PRE	2.9%	0.4%	0.7%	1.1%
	POST	7%	4.1%	1.3%	5.1%
#2019	PRE	47.7%	0%	11.9%	0%
	POST	6.2%	5.8%	10.9%	4.7%
#2110	PRE	5.7%	0%	4.9%	0%
	POST	0%	0%	0%	0%
#2182	PRE	3%	0%	1.8%	0%
	POST	0%	0.4%	1.6%	9%

Fibers with low and high level of NDUFB8 and COX1 are reported in percent (%) for each sample of the DM1 cohort. Abbreviations: POST, post-exercise; PRE, pre-exercise.

Exercise-induced OXPHOS effects

To assess any significant change in the proportion of fibers^{low} and fibers^{high} after resistance exercise training, the changes between post- and pre-exercise cases were estimated by calculating delta (Δ). All patients, except #1242, #523 and #2005, display a significant decrease in the proportion of NDUFB8^{low} fibers after exercise (p-value <0.05, Fig. 5A). Five patients (#1242, #907, #523, #1955, and #2110) exhibit a significant decrease in the proportion of COX1^{low} fibers too (p-value <0.05, Fig. 5C). Furthermore, after exercise a significant increase in both NDUFB8^{high} ($n = 3$) and COX1^{high} ($n = 4$) proportion of fibers was observed (p-value <0.05, Fig. 5B-D). Overall, after resistance exercise training all patients in the DM1 cohort display a significant change (p-value <0.05) in at least one of the four classes of fibers identified (NDUFB8^{low}, NDUFB8^{high}, COX1^{low} and COX1^{high} fibers).

Combined CI/CIV deficiency is improved after resistance exercise

To establish whether DM1 patients present specifically with isolated CI deficiency, isolated CIV deficiency or combined CI/CIV deficiency, an over-representation test was utilized to assess the proportion of COX1^{low} fibers ($n = 323$) among NDUFB8^{low} fibers ($n = 528$). NDUFB8^{low} &COX1^{low} fibers were found to be significantly over-represented across the DM1 cohort including both pre- and post-exercise cases (p-value <0.05, Fig. 6A). However, a significant proportion of NDUFB8^{low} &COX1^{low} fibers is over-represented in seven pre-exercise cases (#1242, #1806, #907, #2182, #1955, #2019 and #2110), and in only three post-exercise cases (#1242, #907 and #2019), where the proportion of all deficient classes of fibers decrease dramatically after exercise intervention (Fig. 6B). Similarly, the proportion of fibers^{high} was investigated and an over-representation test was used to assess the proportion of COX1^{high} fibers ($n = 145$) among NDUFB8^{high} fibers ($n = 168$). Only eight NDUFB8^{high} &COX1^{high} fibers were identified in patient #2005 post-exercise sample, suggesting no overall significant over-representation of this class of fibers across the DM1 cohort (Fig. 6C).

DISCUSSION

The aim of this study was to investigate any potential OXPHOS dysfunction and OXPHOS protein changes after 12-week resistance exercise training in
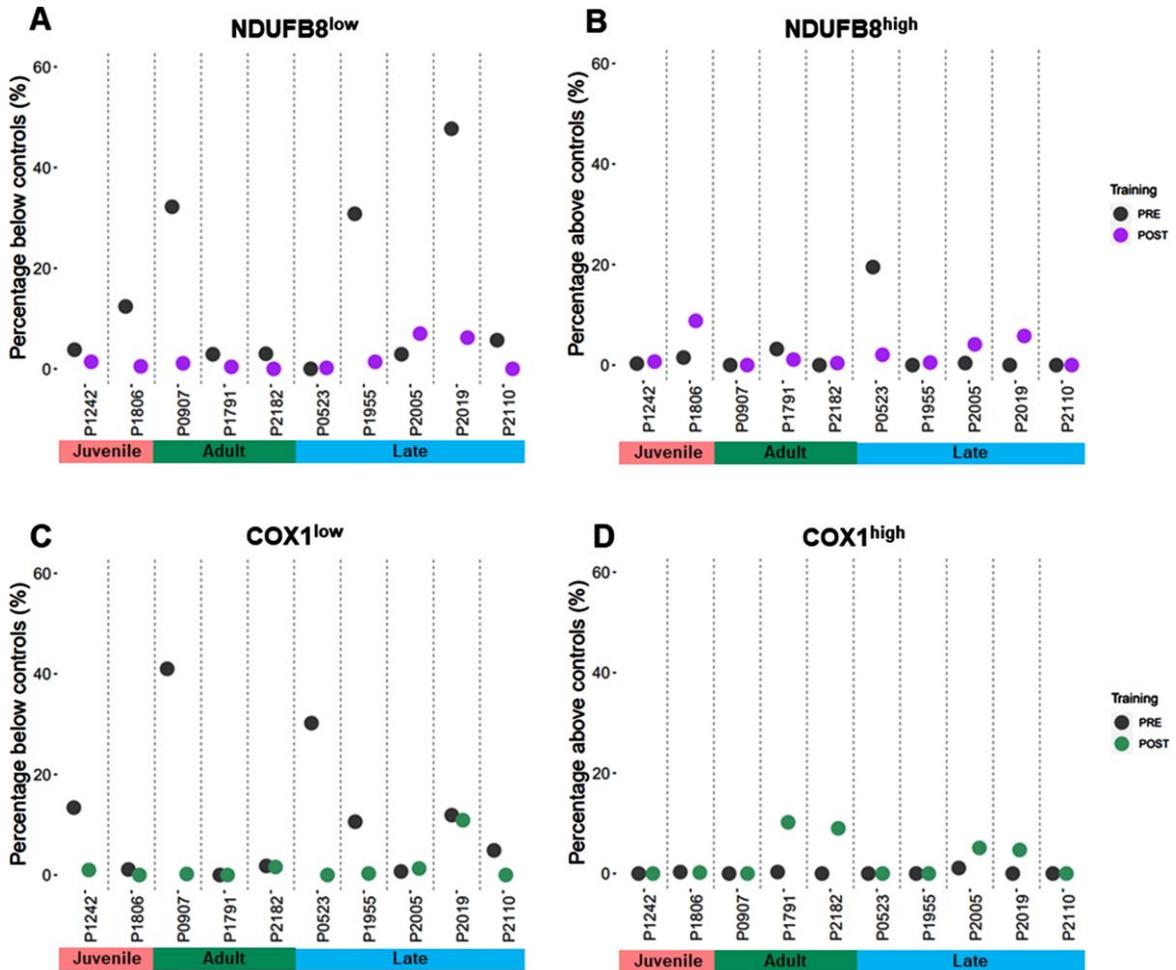
Fig. 4. Overview of proportion of fibers with NDUFB8 and COX1 low or high in DM1 cohort. QIF data were analyzed by the 95% predictive interval model. The proportion of fibers are reported as proportion below controls with low level fibers for NDUFB8 (A) and COX1 (C), or as proportion above the controls with high level fibers for NDUFB8 (B) and COX1 (D).

a cohort of DM1 patients [38]. Aerobic exercise training has been demonstrated to ameliorate OXPHOS deficiency in DM1 patients [43]. However, for the first time in the context of resistance exercise training, we investigated OXPHOS at a single-fiber level in SKM from DM1 patients. Our method using QIF has been extensively applied to study both complex I and complex IV, which before was not possible by using standard COX/SDH histochemistry, since only complex IV and complex II activities are measured, respectively [41]. QIF allows single cell quantification of the level of expression of the putative complexes normalized to the level of mitochondrial mass, which it has been demonstrated to correlate to the enzymatic complexes' activity too [41]. Importantly, the identification of the proteins with western blot and immunofluorescence have been previously

correlated to its genetic loss determined by activity assays and by spectrophotometer [42, 49, 50]. In this way, mitochondrial dysfunction has been investigated in both primary and secondary mitochondrial diseases, gathering information at a single cell level in muscle [47, 51], neurons and brain [52–55], pancreas [56] and bone [57].

It is important to mention that the healthy control cohort used in this study was not aged-matched to the DM1 cohort. We were able to recruit a limited number of healthy volunteers. We chose to focus on control biopsies from *vastus lateralis*, the same muscle biopsied in the DM1 patients, to limit the inter-muscle variability. Indeed, mitochondrial content depends on factors such as age and physical activity [58–60]. It could be speculated that the level of physical activity of the healthy controls would therefore be higher than
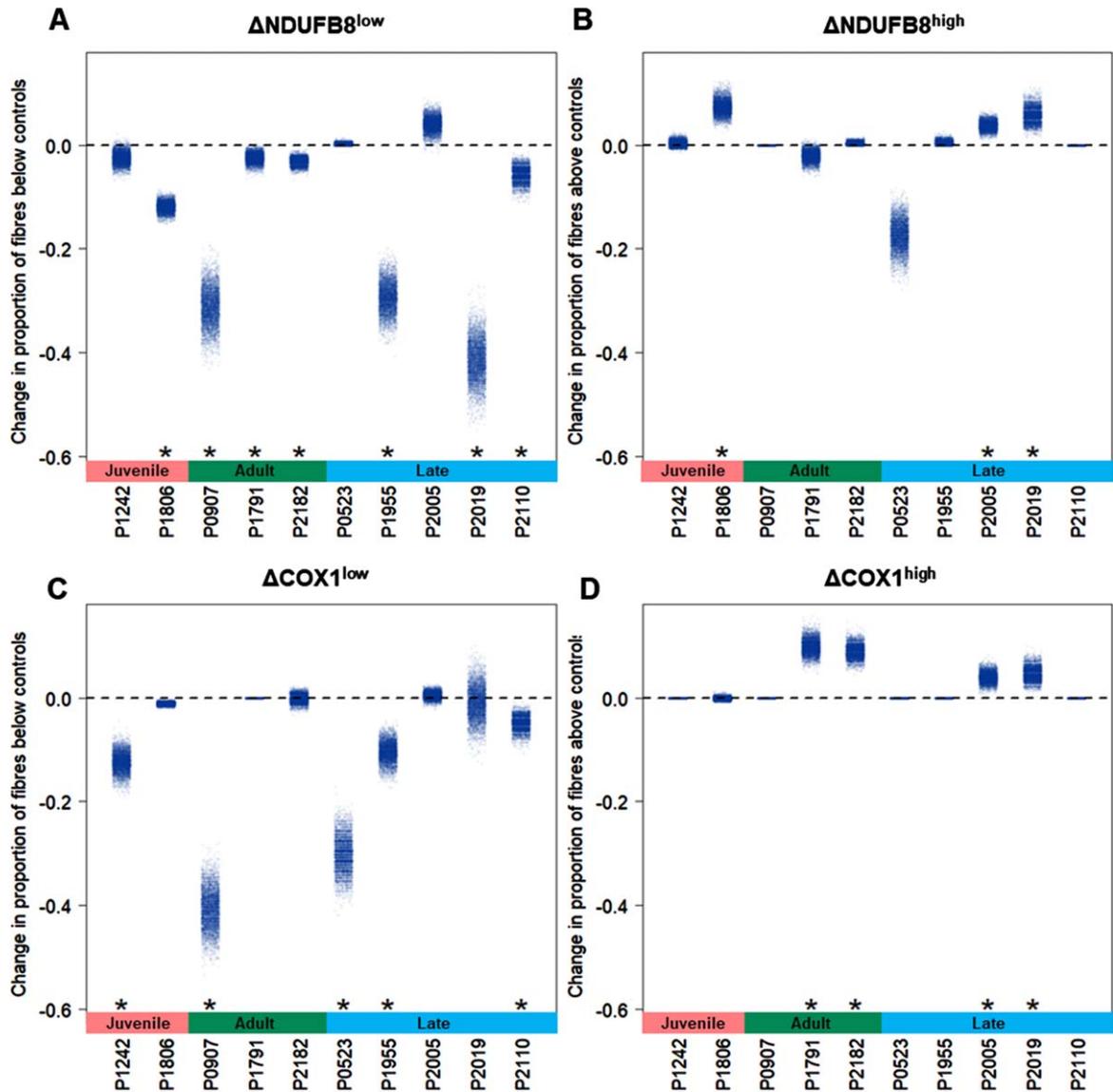
Fig. 5. Exercise-induced effects in OXPHOS activity in DM1 cohort. Changes in the proportion of fibers (Δ) between post- and pre-exercise cases were calculated and bootstrapped to give estimates of uncertainty. Significance of changes were tested using permutation test. Changes in the proportion of fibers were analyzed in fibers below the controls with low level for NDUFB8 (A) and COX1 (C), and in fibers above with high level for NDUFB8 (B) and COX1 (D). One-sided t-test between mean values of the proportion of fibers is indicated for each patient; p-value <0.05 (*).

the DM1 patients since the level of physical activity of this population is known to be reduced. However, due to strict exclusion criteria, participants who have been selected had the physical ability to be enrolled in the resistance exercise study. Overall, the interpretation of our results relies mainly on the comparison of pre- and post- biopsies from each DM1 patient; as such, the controls do not alter our interpretation of these paired comparisons. Whilst each fiber from DM1 patients' samples were clustered in comparison to the control muscle population of fibers, here the importance is that the control samples have no mitochondrial OXPHOS deficiency.

By studying the involvement of mitochondrial dysfunction in DM1 SKM on a single cell level, we showed that DM1 SKM biopsies ($n = 10$) present with an overall decrease in mitochondrial mass when compared to healthy muscle with normal OXPHOS activity. Seven of 10 patients showed isolated or combined NDUFB8 and/or COX1 defi-
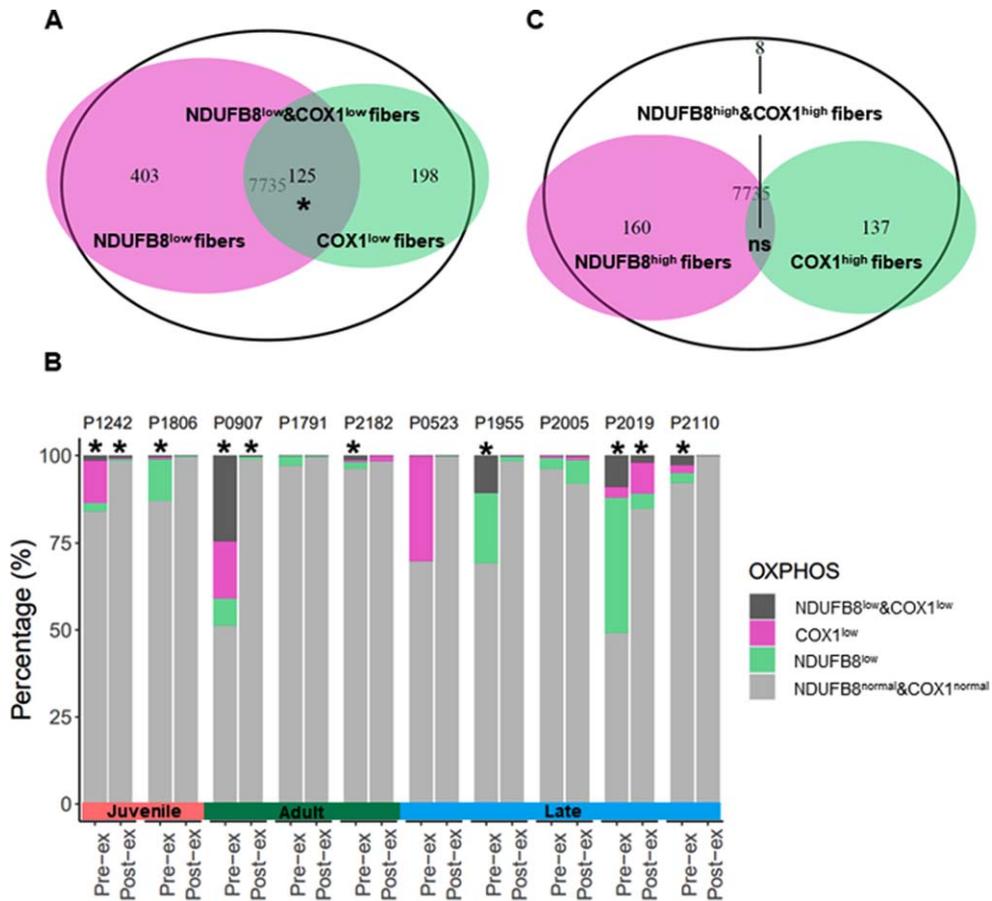
Fig. 6. OXPHOS deficiency is a secondary trait of DM1 pathology. The total number of SKM fibers from the DM1 cohort includes 7,735 fibers, excluding the healthy control subjects. Some of the OXPHOS deficient fibers present with isolated NDUFB8 deficiency (NDUFB8^{low}, $n = 528$), isolated COX1 deficiency (COX1^{low}, $n = 323$) or both NDUFB8 and COX1 deficiency (NDUFB8^{low} &COX1^{low}, $n = 125$) (A). Pre- and post-exercise pairs of samples are shown displaying the percent of fibers NDUFB8^{low}, COX1^{low} and NDUFB8^{low} &COX1^{low} (B). Some fibers present with high level of NDUFB8 (NDUFB8^{high}, $n = 168$), high level of COX1 (COX1^{high}, $n = 145$), and with high level of NDUFB8 and COX1 (NDUFB8^{high} &COX1^{high}, $n = 8$) (C). Over-representation test was performed, p-value <0.05 (*).

ciency compared to healthy controls; findings that strongly resemble mitochondrial defects observed in mitochondrial myopathy patients with primary mitochondrial dysfunction [40, 45–47]. Both DM1 and primary mitochondrial disease patients demonstrate a heterogeneous clinical phenotype; yet both diseases present with overlapping symptoms, including but not limited to neuromuscular symptoms [61, 62]. Interestingly, SKM-specific mitochondrial dysfunction has also been described in other neuromuscular disorders, such as inclusion body myositis [63, 64], dysferlin-related myopathy [65] and myofibrillar myopathy [66]. A 12-week resistance exercise training program was sufficient to induce significant changes in OXPHOS defects in SKM of DM1 patients. A significant increase in mitochondrial mass

was observed in six of 10 patients independent of age and phenotypical presentation. Strikingly, these patients, excluding case #2110, present with duration of disease greater than 20 years and reported considerable improvements in the ability to lift weights after resistance exercise training compared to other participants (Fig. 3B). Patients #2182, #1955, #2005 and #2019 did not exhibit an increase in mitochondrial mass, but rather a significant decrease after exercise intervention. It is important to note that VDAC1 mean value of pre-exercise cases in these patients was similar or even higher than the healthy controls mean, suggesting that the mitochondrial mass was not affected at baseline. Similarly, some of the smallest changes in lifting weights after resistance exercise training were reported in patients who were some

of the strongest at baseline (Fig. 3B). These patients also had a duration of disease less than 20 years compared to the rest of the DM1 cohort. Altogether, these observations suggest that irrespective of which phenotypical presentation patients are classified as, there is potential for improvement in both terms of augmenting muscle strength [38] and increasing SKM mitochondrial mass. Moreover, due to the high heterogeneity observed in the DM1 cohort, significant improvements in OXPHOS function were mainly uncovered when analyzing each patient individually, suggesting that further investigation of DM1 individuals may benefit individual therapy/medicine.

After resistance exercise training, a significant improvement in mitochondrial OXPHOS capacity of complex I and/or complex IV was observed in all DM1 patients by either the decrease of deficient fibers (fibers^{low}) or the increase of fibers with higher protein level (fibers^{high}) compared to baseline measures. Indeed, significant changes were observed in at least one of the four classes of fibers (NDUFB8^{low}, NDUFB8^{high}, COX1^{low} and COX1^{high}) for each patient, indicating that the 12-week resistance exercise training is sufficient to induce increase of OXPHOS protein level in SKM of DM1 patients. Similarly, resistance exercise training has been reported to induce a significant increase in COX1 level in healthy individuals [67, 68], and in mitochondrial myopathy patients [40]. Furthermore, significant improvements have been observed both at the transcriptomic level by inducing changes in splicing events and differential gene expression [69], which strongly correlates to strength improvements, and at the proteomic level by increasing the level of mitochondrial proteins involved in a variety of different functions and pathways [70]. Similarly, mitochondrial functions have been found to play a prominent role in the amelioration of both cognitive and physical capabilities in DM1 disease [43, 71].

In DM1 patients, the CTG triplet repeat expansion is used as a marker of disease severity. The rescue of NDUFB8 and COX1 however is achieved in an independent manner to CTG triplet repeat expansion, meaning that even patients with the highest CTG triplet repeat expansions (#1242 and #1806) showed improvements in NDUFB8 and COX1 deficiency after resistance exercise training intervention. Indeed, the proportion of fibers with combined NDUFB8 and COX1 deficiency (NDUFB8^{low} &COX1^{low}) is significantly higher in pre-exercise cases compared to post-exercise case. Moreover, #1242, #907 and #2019 post-exercise display a decrease of NDUFB8^{low} &COX1^{low} fibers compared to the proportion observed before exercise. The changes in CI and CIV deficiency are independent of the phenotypical presentation classification, implying that overall OXPHOS defects might be a secondary trait in DM1 disease pathology, hence a DM1 hallmark that may be useful to determine the effects of exercise training intervention and disease progression [38, 43]. Herein, we highlight that the SKM adaptations triggered by resistance exercise training in DM1 are driven by the activation of mitochondrial molecular pathways that directly modify the pathogenicity of DM1. A multi-level mitochondrial characterization will be therefore crucial for the understanding of DM1 disease.

Limitations

There are several limitations of this study. Firstly, while this study included the largest cohort of DM1 patients enrolled in a resistance exercise training program, the number of DM1 patients included in the resistance exercise training trial was statistically low [29, 37, 38]. However, the 12-week resistance exercise trial provided effective relief in DM1 patients, and improvements in the clinical outcome showed a significant correlation both with increased mitochondria mass and transcriptomic changes [69]. Secondly, although samples were collected from the same individual and from the same leg muscle, it is unfeasible to conduct the analysis on the same myofibres due to repeated biopsies. This implies a certain degree of natural intra-muscle variability, which is a topic of particular relevance in relation to mitochondrial dysfunction in primary mitochondrial diseases [72]. Finally, only DM1 men were enrolled in the resistance exercise training program [38], although this is not unusual for exercise trials designed for neuromuscular patients' cohort [29]. Hence, findings cannot be necessarily extrapolated to women, since sex is a modifying factor influencing DM1 disease [73, 74]. While this narrowed the inter-individual variability linked to sex, it will be important to assess the effects of resistance exercise training on a cohort of DM1 women, for which an ongoing trial has been carried over (NCT05400629).

CONCLUSIONS

In conclusion, for the first time we demonstrate that a supervised 12-week resistance exercise training program is sufficient to rescue SKM mito-

chondrial deficiency in a cohort of DM1 patients presenting with defects in both mitochondrial mass and OXPHOS. We show that the observed mitochondrial OXPHOS changes are independent of CTG triplet repeat expansion length and display both a decrease of isolated and combined deficient fibers for CI and CIV (NDUFB8^{low}, COX1^{low}, NDUFB8^{low} &COX1^{low}) and an increase of fibers with higher level of NDUFB8 and COX1 (NDUFB8^{high}, COX1^{high}). This implies not only that mitochondrial dysfunction might be classified as a secondary trait of DM1 pathology, but also that physical improvement is independent from the CTG triplet repeat expansion carried by DM1 patients. Resistance exercise training triggers important mitochondrial molecular pathways that lead to the rescue of mitochondrial function and to amelioration of the clinical phenotype observed in DM1 patients.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

AUTHOR CONTRIBUTIONS

E.D. and M-P.R. were responsible for collection of the skeletal muscle samples and training of the DM1 patient cohort. V.D.L. was responsible for the study conception and design. V.D.L., C.L. and T.B.G. performed the statistical analysis of the data generated during the study. M-P.R. and E.D. ensured consistency between the previous study [38] and the study here presented. V.D.L., C.L., M-P.R., E.D. and A.E.V. were involved in the interpretation of the data. A.E.V., G.S.G., O.M.R. and H.A.L.T. were responsible for the supervision of the project as part of a Ph.D. program. V.D.L. was responsible for drafting the manuscript. V.D.L. and all authors evaluated the manuscript and reviewed the final publication.

DATASETS/DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are openly available in VDLNCL/DM1-mitochondria at https://github.com/VDLNCL/DM1-mitochondria.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JND-230099

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