Arch Rheumatol 2023;38(x):i-xi
doi: 10.46497/ArchRheumatol.2023.9257

ORIGINAL ARTICLE

Exercise training attenuates skeletal muscle fat infiltration and improves insulin pathway of patients with immune-mediated necrotizing myopathies and dermatomyositis

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ABSTRACT

Objectives: This study aims to evaluate the effects of exercise training on intramuscular lipid content and genes related to insulin pathway in patients with systemic autoimmune myopathies (SAMs).

Patients and methods: Between January 2016 and May 2019, a total of seven patients with dermatomyositis (DM; 3 males, 4 females; mean age: 49.8±2.3 years; range, 43 to 54 years), six with immune mediated necrotizing myopathy (IMNM; 3 males, 3 females; mean age: 58.5±10.6 years; range, 46 to 74 years), and 10 control individuals (CTRL group; 4 males, 6 females; mean age: 48.7±3.9 years; range, 41 to 56 years) were included. The muscle biopsy before and after the intervention was performed to evaluate the intramuscular lipid content. Patients underwent a combined exercise training program for 12 weeks. Skeletal muscle gene expression was analyzed and the DM versus CTRL group, DM pre- and post-, and IMNM pre- and post-intervention were compared.

Results: The DM group had a higher intramuscular lipid content in type II muscle fibers compared to the CTRL group. After the intervention, there was a reduction of lipid content in type I and II fibers in DM and IMNM group. The CTRL group showed a significantly higher expression of genes related to insulin and lipid oxidation pathways *(AMPKβ2, AS160, INSR, PGC1-α, PI3K, and RAB14)* compared to the DM group. After exercise training, there was an increase gene expression related to insulin pathway and lipid oxidation in DM group *(AMPKβ2, AS160, INSR, PGC1-α, PI3K,* and *RAB14)* and in IMNM group *(AKT2, AMPKβ2, RAB10, RAB14,* and *PGC1-α)*.

Conclusion: Exercise training attenuated the amount of fat in type I and II muscle fibers in patients with DM and IMNM and increased gene expression related to insulin pathways and lipid oxidation in DM and IMNM. These results suggest that exercise training can improve the quality and metabolic functions of skeletal muscle in these diseases.

Keywords: Exercise training, fat infiltration, insulin resistance, myopathies, myositis.

Systemic autoimmune myopathies (SAMs) are rare heterogeneous chronic and systemic autoimmune disorders that share some clinical manifestations such as progressive proximal skeletal muscle weakness, inflammatory cell infiltrates in muscle and myositis-specific or associated autoantibodies.[1] Patients with SAMs can also experience extra-muscular manifestations in the joints, skin, or lungs. Systemic autoimmune myopathies can be subdivided into dermatomyositis

Received: October 23, 2021 ***Accepted:*** July 21, 2022 ***Published online:*** October 21, 2022

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Citation:
de Oliveira DS, Borges IBP, Marie SKN, Lerario AM, Oba-Shinjo SM, Shinjo SK. Exercise training attenuates skeletal muscle fat infiltration and improves insulin pathway of patients with immune-mediated necrotizing myopathies and dermatomyositis. Arch Rheumatol 2023;38(x):i-xi.

(DM), immune-mediated necrotizing myopathy (IMNM), inclusion body myositis, and polymyositis.[1]

The treatment of SAMs is based on immunosuppressive and immunomodulation therapies, with glucocorticoids being the main treatment.[2] However, patients with SAMs are commonly resistant to treatment, and recent evidence has shown that not only adaptive immune, but also non-immune actions play a key role in decreasing skeletal muscle strength and endurance in these disorders.[1,2] One non-immune mechanism that may lead to impaired muscle function in these diseases is the increasing of fat infiltration in the skeletal muscle.[3] Disturbances in adenosine triphosphate (ATP) production, followed by few skeletal muscle type I fibers, mitochondrial dysfunction and, consequently, low fatty-acid oxidation, culminates with an increase in the fat infiltration in the skeletal muscle, a condition highly present in patients with SAMs,[3,4] responsible for playing a major role in the progression of these diseases.

Skeletal muscle fat infiltration is associated with an important decrease in skeletal muscle strength and function in healthy older adults,[5] chronic diseases[6] and autoimmune rheumatic diseases.[7] Furthermore, during conditions of high lipid infiltrates, the lipid intermediates (e.g., ceramides and diacylglycerol) can activate genes that impair insulin signaling, leading to metabolic disturbances in these conditions.[8] Confirming this assumption, a high prevalence of metabolic disorders (e.g., insulin resistance (IR) and metabolic syndrome) has been described in patients with SAMs.[9,10] Therefore, strategies that can attenuate the fat infiltration in the skeletal muscle and, consequently, improve physical function and metabolic impairments, is essential to be implemented in patients with SAMs.

Exercise training programs have been considered an adjuvant tool capable of reestablishing muscle strength, function, and endurance, as well as attenuating the inflammation and disease-related symptoms in patients with SAMs.[11] Moreover, exercise training is known to attenuate skeletal muscle fat infiltration in several conditions.[12,13] Nevertheless, until now, no study has investigated the effects of exercise training on skeletal muscle fat infiltration of patients with SAMs. In the present study, we hypothesized that exercise training could lead to an increase in skeletal muscle fat oxidation, attenuating the fat infiltration, which could, in turn, improve both physical and metabolic impairments in these patients. We, therefore, aimed to evaluate the effects of exercise training on intramuscular lipid content and genes related to insulin pathway in patients with SAMs.

PATIENTS AND METHODS

This single-center, quasi-experimental, prospective cohort study was conducted at University of Sao Paulo, Department of Rheumatology between January 2016 and May 2019. Except for one case, all patients were selected from our previous studies.[14,15] Exclusion criteria were overlapped myositis, infection, established diabetes mellitus, uncontrolled systemic arterial hypertension, use of statins or fibrates (or both), any disorder that could preclude participation in the exercise training, use of more than 10 mg/day of prednisone within the past three months, engagement in exercise programs within the past six months, and reactivation of disease.

Seven patients with DM (3 males, 4 females; mean age: 49.8±2.3 years; range, 43 to 54 years) diagnosed according to the classification criteria of European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) 2017[16] and six with IMNM (3 males, 3 females; mean age: 58.5±10.6 years; range, 46 to 74 years) diagnosed according to the Myositis Study Group (MSG) and the 119th European Neuromuscular Centre workshop criteria[17] were selected, as well as a healthy control group (CTRL group, n=10; 4 males, 6 females; mean age: 48.7±3.9 years; range, 41 to 56 years) matched for age, sex and body mass index with the DM group. All cases (before and after physical training) and controls individuals (only at the beginning of the study) were submitted to a cardiopulmonary exercise test, maximal graduation, and an exercise test to determine the peak of oxygen consumption (VO_2 peak), anaerobic ventilator threshold (AVT), respiratory compensation point (RCP), and time to exhaustion (min).

The International Myositis Assessment & Clinical Studies Group (IMACS) set scores were used, including the following: manual muscular

testing (MMT)-8, a Visual Analog Scale (VAS) for global disease activity from the point of view of the patient and the physician, Myositis Disease Activity Assessment VAS (MYOACT), and Health Assessment Questionnaire (HAQ) to assess disease status.[18] Creatine phosphokinase levels (reference value ≤167 U/L) were also assessed.

Autoantibodies: The identification of specific and myositis-associated autoantibodies (anti-Mi-2, Jo-1, PL-7, PL-12, OJ, EJ, Ku, PM/Scl, SRP, Ro-52) was performed using the commercial kit (Myositis Profile 3, EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) according to the manufacturer's instructions. The HMGCR antibody was tested by enzyme-linked immunosorbent assay (ELISA) using recombinant HMGCR protein and polyclonal anti-HMGCR antibodies (MyBioSource, San Diego, CA, USA). In this study, patients with anti-HMGCR values of more than three standard deviations from the mean of eight healthy subjects were considered positive.

Patients with DM and IMNM underwent a combined physical training program for 12 weeks twice a week. These exercises consisted of resistance, aerobic and stretching exercises and were described elsewhere.[14,15]

A biopsy of the vastus lateralis skeletal muscle was performed at pre- and post-12-weeks of intervention. These biopsies were performed in fasting (at least 8 h) and stored in liquid nitrogen immediately after collection. They were free of blood and visible adipose tissue.

We stained lipids with the Oil Red O (ORO) to evaluate the skeletal muscle fat content. A light microscope was used to evaluate stained muscles using a 200× oil immersion objective and bright field settings. Fields within the biopsy section (free from artifact) were analyzed for lipid content. Quantitative image analysis was then carried out on at least 100 fibers, or approximately 10 contiguous fibers per field.

For ribonucleic acid (RNA) extraction, the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) was used and these samples were treated with RNase-free DNase (Qiagen, Valencia, CA, USA). The RNA integrity and concentration assessments were performed using RNA ScreenTape at 4200 Tapestation (Agilent Technologies, Santa Clara, CA, USA). Libraries were constructed from 300 ng of total RNA for analysis of the transcriptome using the QuantSeq 3 'mRNA-Seq Library Kit FWD for Illumina (Lexogen GmbH, Vienna, Austria) according to the manufacturer's instructions. Libraries were pooled and quantified by quantitative real-time polymerase chain reaction (PCR) using the Kapa Library Quantification Kit (Kapa Biosystems, Wilmington, MA, USA). Sequencing was performed in a NextSeq 500 (Illumina Inc., San Diego, CA, USA) with a single-read run (75 cycles) in the SELA Faculity Core. The sequencing generated an average of 5 million readings per sample. Quality control analysis was performed using the FASTQC version 0.11.9 software??. The raw reads were aligned to hg38 using STAR version 2.7.1a software.[19] The quantification of gene expression data was performed using the feature Counts version 1.6.4 software[20] and the data (counts per million, CPM) were transformed in a log and normalized to Z-score for heatmap visualization.

Statistical analysis

Statistical analysis was performed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max) or number and frequency, where applicable. The Kolmogorov-Smirnov test was applied to check data distribution. Independent samples were compared using an unpaired t-test and Mann-Whitney U test when data were parametric or non-parametric, respectively. Dependent samples were compared with a paired t-test or Wilcoxon test, when data were parametric or non-parametric, respectively. Categorical variables were analyzed using the Fisher exact test. A p value of <0.05 was considered statistically significant with 95% confidence interval (CI).

RESULTS

All clinical, laboratory, and disease parameters and changes in aerobic capacity, muscle strength, and function of control are described in Table 1. The mean disease durations of the DM and IMNM groups were 9.8±6.2 and 19.1±7.6 years, respectively. Two patients in the DM group had the presence of autoantibody anti-Mi-2, whereas in the IMNM

Table 1. Disease status and physical capacity data from DM, IMNM and CTRL groups before and after exercise training program

	DM Pre (n=7)			DM Post (n=7)			IMNM Pre (n=6)			IMNM Post (n=6)			CTRL (n=10)			DM Pre *vs.* Post	IMNM Pre *vs.* Post	DM Pre *vs.* CTRL
	Mean±SD	Median	Interquartile 25^{th}-75^{th} percentage	Mean±SD	Median	Interquartile 25^{th}-75^{th} percentage	Mean±SD	Median	Interquartile 25^{th}-75^{th} percentage	Mean±SD	Median	Interquartile 25^{th}-75^{th} percentage	Mean±SD	Median	Interquartile 25^{th}-75^{th} percentage	*p*	*p*	*p*
Disease status																		
Patient's VAS (0-10)		1.2	0.0-1.8		0.0	0.0-0.0		0.0	0.0-1.2		0.0	0.0-0.0		-	-	0.236	0.370	-
Physician's VAS (0-10)		0.4	0.2-1.8		0.0	0.0-0.0		1.2	0.9-4.9		0.7	0.4-2.8		-	-	0.459	0.400	-
MMT-8 (0-80)		80	80-80		80	80-80		78	75-80		80	80-80		-	-	1.000	0.960	-
MYOACT (0-60)		0	0-1.0		0	0-0		0	0-2.2		0	0-0		-	-	0.654	0.370	-
HAQ (0.00-3.00)		0.1	0.1-1.2		0.4	0.1-1.4		0.1	0.1-1.2		0.1	0.1-0.6		-	-	0.499	0.320	-
Creatine phosphokinase (U/L)		143	120-312		112	96-210		215	178-1559		195	161-1644		173	79-210	0.645	0.857	-
Physical capacity																		
VO_2 peak (mL/kg/min)	18.9±3.2			20.3±4.5			18.8±4.4			19.6±4.2			26.1±4.0			0.052	0.397	0.011
Time to achieve VAT (min)	4.2±1.0			5.9±1.6			4.5±1.3			5.6±0.8			5.9±0.9			0.049	0.054	0.034
Time to achieve RCP (min)	9.3±1.9			10.9±1.4			7.0±1.5			9.6±1.9			11.5±2.3			0.062	0.000	0.039
Time to exhaustion (min)	11.2±2.0			13.3±1.1			9.2±2.2			11.3±1.8			13.9±2.3			0.001	0.002	0.014
Leg press (kg)	70.3±13.9			81.3±14.2			48.2±25.0			51.6±27.6			87.2±13.5			0.025	0.270	0.046
Bench press (kg)	27.9±8.7			31.3±9.2			26.0±12.2			29.3±13.5			38.0±8.7			0.001	0.042	0.055
TST (reps)	13.5±2.4			15.9±3.8			7.1±0.7			6.4±0.6			16.4±1.9			0.005	0.053	0.044
TUG (s)	7.8±1.3			6.8±1.3			13.8±2.3			16.2±2.2			5.7±0.6			0.035	0.000	0.017

DM: Dermatomyositis; IMNM: Immune-mediated necrotizing myopathy; CTRL: Control individuals; SD: Standard deviation; VAS: Visual Analogue Scale; MMT: Manual muscle testing; MYOACT: Myositis Disease Activity Assessment Visual Analog Scale; HAQ: Health Assessment Questionnaire; VO_2: Maximum oxygen uptake; VAT: Ventilatory anaerobic threshold; RCP: Time elapsed until respiratory compensation point; TST: Timed stands-test; TUG: Timed up-and-go test.

group, three had the presence of anti-signal recognition particle (anti-SRP) and three had the presence of autoantibody anti-hydroxy-methyl-glutaryl coenzyme A reductase (anti-HMGCR). In relation to drugs, only one patient in the DM group was using prednisone (5 mg/day), whereas all patients in both experimental groups were using at least one immunosuppressive drug (azathioprine 2 to 3 mg/kg/day, methotrexate 15 to 25 mg/week, and/or mycophenolate mofetil 2 to 3 g/day). Drug regime remained unchanged during the study. The IMACS set scores showed that patients in both groups had stable diseases (Table 1).

The DM group had an impaired aerobic capacity compared to the CTRL group, as demonstrated by lower VO_2 peak, and lower time to achieve ventilatory anaerobic threshold (VAT), RCP, and exhaustion ($p<0.05$). Similarly, the DM group showed worse performance in leg press, timed stands-test (TST), and timed up-and-go test (TUG) tests compared to the CTRL group ($p<0.05$) (Table 1).

After the exercise training program, no impairments in disease status or muscle enzyme were observed. An increase in aerobic capacity was observed in the DM group, demonstrated by an increase in VO_2 peak, and in time to achieve VAT and exhaustion. The patients with IMNM had an increased aerobic capacity, demonstrated by an increase in time to achieve VAT, RCP, and exhaustion. In relation to muscle strength, the patients in the DM group had improved leg press and bench press, whereas patients in the IMNM group only improved in the bench press test. The muscle function improved in both groups, demonstrated by a better performance in TUG and TST after the exercise training program (Table 1).

At baseline, the DM group showed no significant difference observed in type I

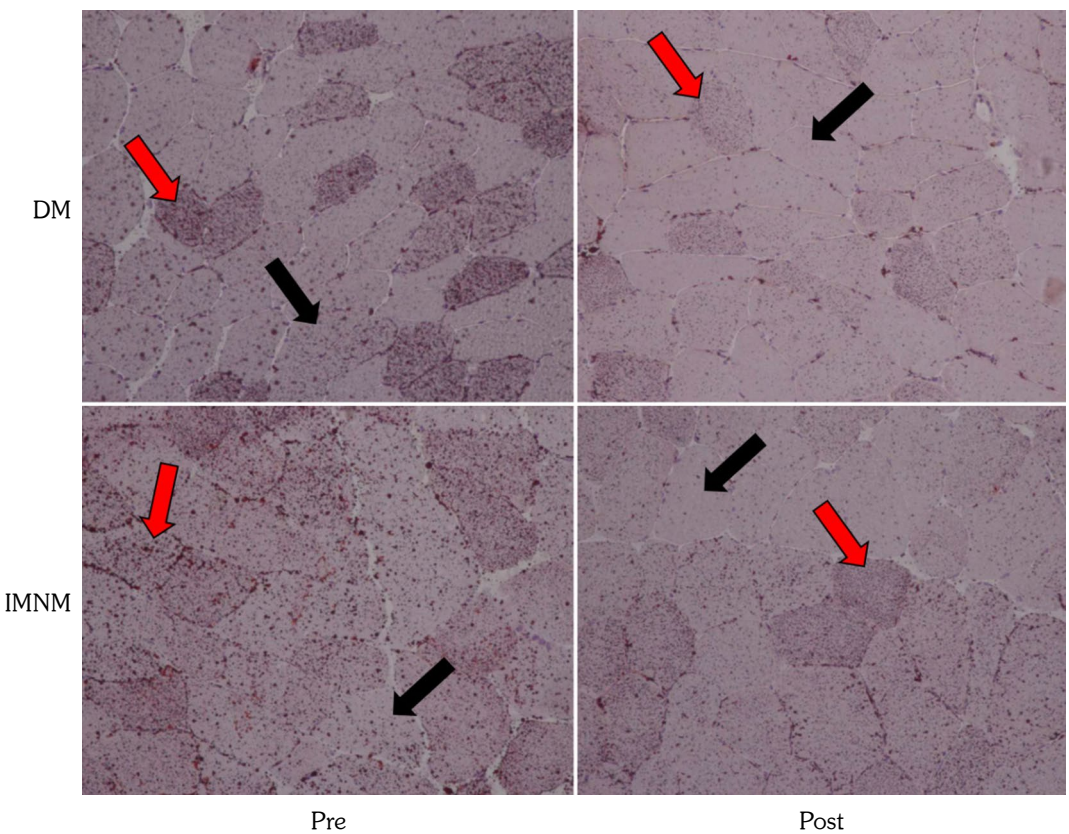

Figure 1. Skeletal muscle lipid content in type I and type II fibers pre- and post-exercise training. Cross-sectional muscle histological section stained with Oil Red O. Magnification of 200×.
Red arrows: Skeletal muscle type I fiber; Black arrows: Skeletal muscle type II fiber.

fiber (DM: 43.7±14.2 *vs.* CTRL: 39.3±10.3; p=0.356), while a higher lipid content of skeletal muscle was observed in type II fibers compared to the CTRL group (DM: 12.3±4.3 *vs.* CTRL: 6.1±3.3; p=0.023). After the intervention, a decrease in lipid content of skeletal muscle in type I (43.7±14.2 *vs.* 32.4±8.3; p=0.038) and type II (12.3±4.3 *vs.* 7.6±4.1; p=0.032) muscle fibers were observed in DM group, as well as in the IMNM group (type I fibers: 45.9±16.8 *vs.* 35.9±9.6; p=0.023; type II fibers: 16.8±6.1 *vs.* 7.2±3.8; p=0.013) (Figure 1).

Skeletal muscle gene expression was analyzed through transcriptome of biopsies. The DM cases were compared to the CTRL group, and biopsies collected before and after exercise intervention were compared for both the DM group and the IMNM group. Genes that showed significant differences in expression levels of all comparisons in the insulin pathway and lipid oxidation are shown in Table 2 (bold). The CTRL group showed a higher expression of genes related to insulin pathways and lipid oxidation [*AMPKβ2* (p=0.043), GLUT-4 (p=0.031), *INSR* (p=0.0043), *PGC1-α* (p=0.050), RAC1 (p=0.025), and *RAB14* (p=0.010)], and compared to the DM group (Table 2, Figure 2).

After exercise training, there was an upregulation in the expression of genes related to lipid oxidation and the insulin pathway in both DM [*AMPKβ2* (p≤0.001), *AS160* (p=0.026), *INSR* (p=0.038), *PGC1-α* (p=0.050), *PI3K*

Table 2. Gene expression in dermatomyositis, immune-mediated necrotizing myopathies and control groups before and after exercise training

		DM Pre *vs.* CTRL	DM: Pre *vs.* Post	IMNM: Pre *vs.* Post
Gene symbol	Gene name	*p*	*p*	*p*
Insulin pathway				
INSR	Insulin receptor	**0.043**	**0.038**	0.235
IRS-1	Insulin receptor soluble-1	0.546	0.485	0.385
IRS-2	Insulin receptor soluble-2	0.326	0.235	**0.018**
PI3KCA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	0.126	**0.045**	0.162
AKT2	AKT serine/threonine kinase 2	0.145	0.095	**0.032**
MTOR	Mammalian target of rapamycin	0.263	0.126	0.216
RAC1	Rac family small GTPase 1	**0.025**	0.210	0.129
TBC1D1	TBC1 domain family member 1	0.546	0.362	0.356
AS160	Akt substrate of 160 kda	0.343	**0.026**	0.268
RAB10	Ras-related protein 10	0.235	0.169	**0.015**
RAB14	Ras-related protein 14	**0.010**	**0.041**	**0.032**
GLUT-4	Glucose transporter-4	**0.031**	0.564	0.127
Lipid oxidation				
PPARGC1A	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	**0.050**	**0.050**	**0.042**
PRKAB2	Protein kinase AMP-activated non-catalytic subunit beta 2	**0.043**	**0.000**	**0.032**
TFAM	Mitochondrial transcriptor factor A	0.463	0.256	0.365

DM: Dermatomyositis; IMNM: Immune-mediated necrotizing myopathy; *INSR*: Insulin receptor; IRS1: Insulin receptor substrate 1; IRS2: Insulin receptor substrate 2; *PI3KCA*: Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; AKT2: AKT serine/threonine kinase 2; MTOR: Mammalian target of rapamycin; RAC1: Rac family small GTPase 1; TBC1D1: TBC1 domain family member 1; *AS160*: AKT substrate of 160 kDa; RAB10: Ras-related protein 10; *RAB14*: Ras-related protein 14; GLUT4: Glucose transporter-4; PPARGC1A: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PRKAB2: Protein kinase AMP-activated non-catalytic subunit beta; TFAM: mitochondrial transcription factor A.

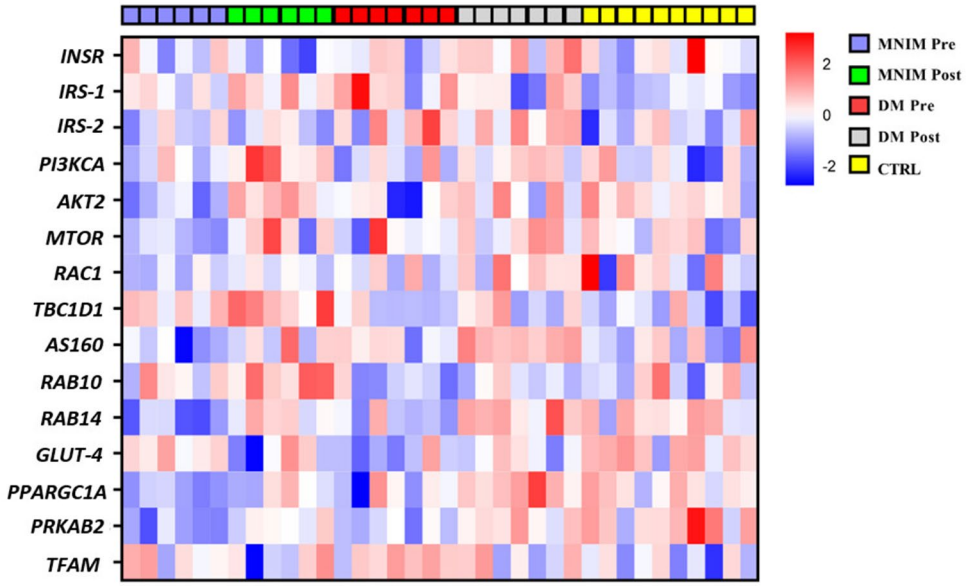

Figure 2. Differential gene expression before and after exercise training in IMNM, DM, and CTRL groups. Heatmap of RNAseq expression z-score representing the expression of genes coding for proteins of insulin pathway and lipid oxidation.

IMNM: Immune-mediated necrotizing myopathy; DM: Dermatomyositis; CTRL: Control individuals; *INSR*: Insulin receptor; *IRS1*: Insulin receptor substrate 1; IRS2: Insulin receptor substrate 2; *PI3K*: Phosphoinositide 3-kinase; *AKT2*: AKT serine/threonine kinase 2; *MTOR*: Mammalian target of rapamycin; *RAC1*: Rac family small GTPase 1; *TBC1D1*: TBC1 domain family member 1; *AS160*: AKT substrate of 160 kDa; *RAB10*: Ras-related protein 10; *RAB14*: Ras-related protein 14; *GLUT-4*: Glucose transporter-4; *PPARGC1A*: Peroxisome proliferator-activated receptor coactivator 1-alpha; *PRKAB2*: Protein kinase AMP-activated non-catalytic subunit beta; *TFAM*: mitochondrial transcription factor A; *PPARGC1A*: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

(p=0.045), and *RAB14* (p=0.041)] and IMNM groups [(AKT2 (p=0.032), *AMPKβ2* (p=0.032) RAB10 (p=0.015), *RAB14* (p=0.032), and *PGC1-α* (p=0.042)] (Table 2, Figure 2).

DISCUSSION

In the present study, we investigated the impact of a combined exercise training program on skeletal muscle fat infiltration and insulin pathway in patients with DM and IMNM. Our study results showed that: *(i)* skeletal muscle lipid content was higher in type II fibers of the DM group compared to the CTRL group; *(ii)* gene expression of *AMPKβ2*, GLUT-4, *INSR*, *PGC1-α*, RAC1, and *RAB14* was higher in the CTRL group than in the DM group; and *(iii)* the combined exercise training program promoted an increase in the skeletal muscle gene expression of insulin pathway and fat oxidation in patients with DM (e.g., *AMPKβ2*, *AS160*, *INSR*, *PGC1-α*, *PI3K*, and *RAB14*) and IMNM (e.g., AKT2, *AMPKβ2*, *PGC1-α*, RAB10, and *RAB14*).

Skeletal muscle responds physiologically to muscle fiber degeneration with a coordinated regenerative mechanism. Despite the regenerative capacity of healthy skeletal muscle, extensive and widespread destruction of muscle fibers is a feature of many diseases in which the trigger cannot be eliminated, such as skeletal muscular dystrophies and SAMs. In these diseases, persistent injury compromises the tissue ability to replace muscle fibers with ectopic tissues such as bones, lipids, and fibrotic tissue.[21] Several studies have demonstrated that patients with SAMs show an important fat infiltration in skeletal muscle.[22-25] This lipid infiltration is a critical measure to assess the degree of disease progression.[23-25] It is well known that patients with more severe disease and/or a long-term disease outcome have a higher lipid infiltration into the skeletal muscle.[23] The use of glucocorticoids, overweight or obesity, disuse, decreased oxidative capacity of the skeletal muscle, and the disease pathophysiology may all contribute to this condition.[22] Once that fat infiltration in skeletal muscle is associated with

muscle dysfunction and metabolic disturbances,[26] attenuating this situation can contribute to improved muscle strength and function and, consequently, attenuate the cardiometabolic risk of these patients.

Impaired aerobic capacity and decreased muscle strength and function have been demonstrated in SAMs.[27] Alterations in skeletal muscle, such as inflammation, capillary depletion, decreasing of type I fibers, and mitochondrial dysfunction, leading to an increasing fat infiltration in skeletal muscle, may contribute to these impairments.[28,29] Confirming this assumption, histological analysis of the present study showed that patients in the DM group had a higher lipid content in type II muscle fibers in relation to the CTRL group, while no significant difference in lipid content was found for type I muscle fibers. Of note, type II muscle fibers are more glycolytic fibers and more prone to IR, a condition highly prevalent in patients with SAMs.[8,9] Therefore, fat infiltration in these fibers may further contribute to IR in this tissue. In addition, fat infiltration in skeletal muscle also impairs muscle contraction, leading to skeletal muscle dysfunction.[5-7] In patients with rheumatoid arthritis, fat infiltration in skeletal muscle is associated with the decreased muscle mass and lower muscle strength and function, suggesting that this outcome can impair the muscle function.[7] Thus, strategies that can attenuate skeletal muscle fat infiltration in SAMs may contribute to attenuation of the progression of these diseases.

After completing the exercise training program, patients with DM and IMNM had a reduction in the lipid content of type I and II fibers. This decrease was followed by an increase in *AMPKβ2* and *PGC1-α* gene expression, two important transcript factors related to mitochondrial biogenesis and fat oxidation.[30] In our previous studies,[14,15] we showed that patients with DM and IMNM had a significant outcome in aerobic capacity, muscle function and strength after completing the exercise training program. Decreased muscle strength and function is the main factor affecting the quality of life of patients.[31] Thus, improving these variables could corroborate to the improvement in quality of life of these patients. In elderly and individuals with obesity, intramuscular lipid infiltration plays a mechanistic role in the progress of anabolic resistance and in the progression of muscle atrophy.[32] In these individuals, decreased muscle mass and strength are associated with the increased accumulation of bioactive lipid metabolites and appear to be sovereign of increased inflammation.[32] Thus, we believe that the improvement of strength and functional capacity through exercise training in the previous studies was due to the improvement of muscle quality following the reduction of skeletal muscle fat infiltration.

In our previous study, we also demonstrated that exercise training was capable of attenuating IR in patients with DM.[15] The cause of IR is complex, but it is known that factors such as physical inactivity, overweight, obesity, chronic inflammation, oxidative stress and mitochondrial dysfunction play significant roles in its development.[33] In addition to these factors, several studies have demonstrated the importance of skeletal muscle fat infiltration in the etiology of IR.[33-35] The accumulation of toxic lipid species in skeletal muscle may occur as a result of increased fatty acid absorption or decreased oxidation of these substances,[36] and has been considered the main factor responsible for the appearance of IR in skeletal muscle.[34-36] Intramuscular lipid content can be defined as any form of lipid that is localized within muscle cells and stored as lipid droplets, most often in the form of triglycerides, but in some cases, as lipid intermediates such as diacylglycerol and ceramides.[36] It has been shown that under conditions of excess intramuscular lipids, these lipid intermediates are increased in skeletal muscle, which can activate genes that result in serine phosphorylation of IRS-1, hampering its ability to associate with the insulin receptor and interfering with *PI3K* activation, leading to IR.[35] This phenomenon is known as lipotoxicity.[36]

Besides the decreased skeletal muscle fat infiltration, we also observed, in the present study, an increase in several genes associated with insulin signaling in the DM group (e.g., *AMPKβ*, *AS160*, *INSR*, *PI3K*, and *RAB14*) and IMNM group (e.g., *AMPKβ2*, AKT2, RAB10, and *RAB14*) after completion of the exercise training program. Furthermore, as commented earlier, genes related to skeletal muscle fat oxidation (*AMPKβ2* and *PGC1-α*) were increased in both DM and IMNM group. This data corroborated to the improvement in

insulin sensitivity and lipid oxidation found in the present and previous[15] study.

For the signaling of intracellular insulin to occur in tissues sensitive to its action, insulin binding with a specific membrane receptor (*INSR*) is required. The increased expression of the *INSR* gene suggests a greater activation of the insulin pathway. Insulin binding to its receptor induces the activation of *PI3K*, which also had an increase in the expression of its gene after completion of the exercise training program. In turn, the activation of *PI3K* increases serine phosphorylation of the AKT, allowing glucose transports to skeletal muscle, through the translocation of GLUT-4 to the cell membrane. Although we did not observe any increase in AKT gene in DM, there was an increase in the AKT2 gene in the IMNM group. Furthermore, genes that are activated by AKT in insulin cascade and promote GLUT-4 translocation (*AS160* [DM] and *RAB14* [DM and IMNM]) had their expression increased with exercise training.

As previously described, muscle contraction leads to GLUT-4 translocation and consequent glucose uptake by independent insulin pathways. One of the main pathways involved in this action is dependent of AMPK activation. Exercise training program is considered an adjuvant tool in IR conditions, once it activates AMPK through muscle contraction.[37] The AMPK activation leads to an increase in *AS160* phosphorylation, which consequently leads to GLUT-4 translocation.[38] In patients with systemic lupus erythematosus, a 12-week moderate-intensity aerobic exercise-training program can lead to an increase in skeletal muscle AMPK phosphorylation,[39] culminating in an improvement in insulin sensitivity. Therefore, we believe that the increase in the expression of AMPK by skeletal muscle in patients with DM and IMNM may have led to a greater signaling of the insulin pathway, corroborating the improvement of the insulin sensitivity found in the present study.

The AMPK is also able to increase skeletal muscle lipid oxidation and induce mitochondrial biogenesis.[38] In response to exercise training, mitochondrial biogenesis occurs by an increase in both number and size of mitochondria. Several factors activated by the AMPK contribute to the regulation of mitochondrial biogenesis, with the PGC-1-α transcription factor being mainly responsible for mediating this response.[37,38] The transition of the skeletal muscle fiber phenotype transition supported by PGC-1-α is characterized by enlarged mitochondrial function and density, increased oxidative metabolism, high expression of myofibrillar proteins and change in the use of substrate energy. Therefore, the activation of PGC-1-α by AMPK, can increase skeletal muscle lipid oxidation, and, consequently attenuate skeletal muscle fat infiltration. In addition, studies have indicated that PGC-1-α prevents protein catabolism and loss of muscle mass in several different diseases.[40] When PGC-1-α levels are constantly increased in conditions such as Duchenne muscular dystrophy, denervation-induced muscle atrophy, and statin-associated muscle damage, these conditions are alleviated,[39] suggesting a potential therapeutic effect of this gene in these diseases. The PGC-1-α has also been shown to increase the expression and activity of antioxidant genes,[41] in addition to being able to reduce the production of inflammatory cytokines.[41,42] Therefore, the increase in PGC-1-α expression in the present study may also have contributed to mitigate the negative effects of these diseases on skeletal muscle.

This study has some limitations. First, due to the heterogeneity of these diseases, we were unable to include a control group. Further efforts should be done to replicate these data in randomized controlled trials. Second, the study included only patients with stable diseases. Caution should be exercised in extrapolating these data to patients with more severe disease. Finally, our study had a relatively low sample size. Efforts should be made to tackle these limitations in the future, possibly through multicentre collaborative studies.

In conclusion, the combined exercise-training program was capable of attenuating skeletal muscle fat infiltration in type I and II skeletal muscle fibers of patients with DM and IMNM. This decrease was followed by an increase in skeletal muscle gene expression of AMPKβ2 and PGC-1-α, which could have led to an increase in skeletal muscle lipid oxidation. In turn, the increase in lipid oxidation may have caused a decrease in toxic lipid species which impair insulin signaling in skeletal muscle, contributing to a greater signaling of this pathway, as demonstrated by the increase in several genes of insulin

cascade. Taken together, these results suggest that exercise-training can improve both quality and metabolic functions of skeletal muscle in these diseases.

Ethics Committee Approval: The study protocol was approved by the Registered at ClinicalTrials.gov and approved by the local ethics committee (CAAE number: 61474416.5.0000.0068). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Conception and design, drafting the article; final approval of the article: D.S.O., I.B.P.B., S.K.S.; Analysis and interpretation of data, revising and final approval of the article: S.K.N.M., A.M.L., S.M.O.S.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: This study was funded by the Fundação de Amparo à Pesquisa do Estado de São Paulo, São Paulo, Brazil (2016/19771-5 to D.S.O., 2019/11367-9 to I.B.P.B., and 2017/13109-1 to S.K.S.); Conselho Nacional de Desenvolvimento Científico e Tecnológico, São Paulo, Brazil (303379/2018-9 to S.K.S.); and Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil (to S.M.O.-S., S.K.N.M., and S.K.S.).

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