STRATEGIES TO FIGHT EXERCISE INTOLERANCE IN NEUROMUSCULAR DISORDERS

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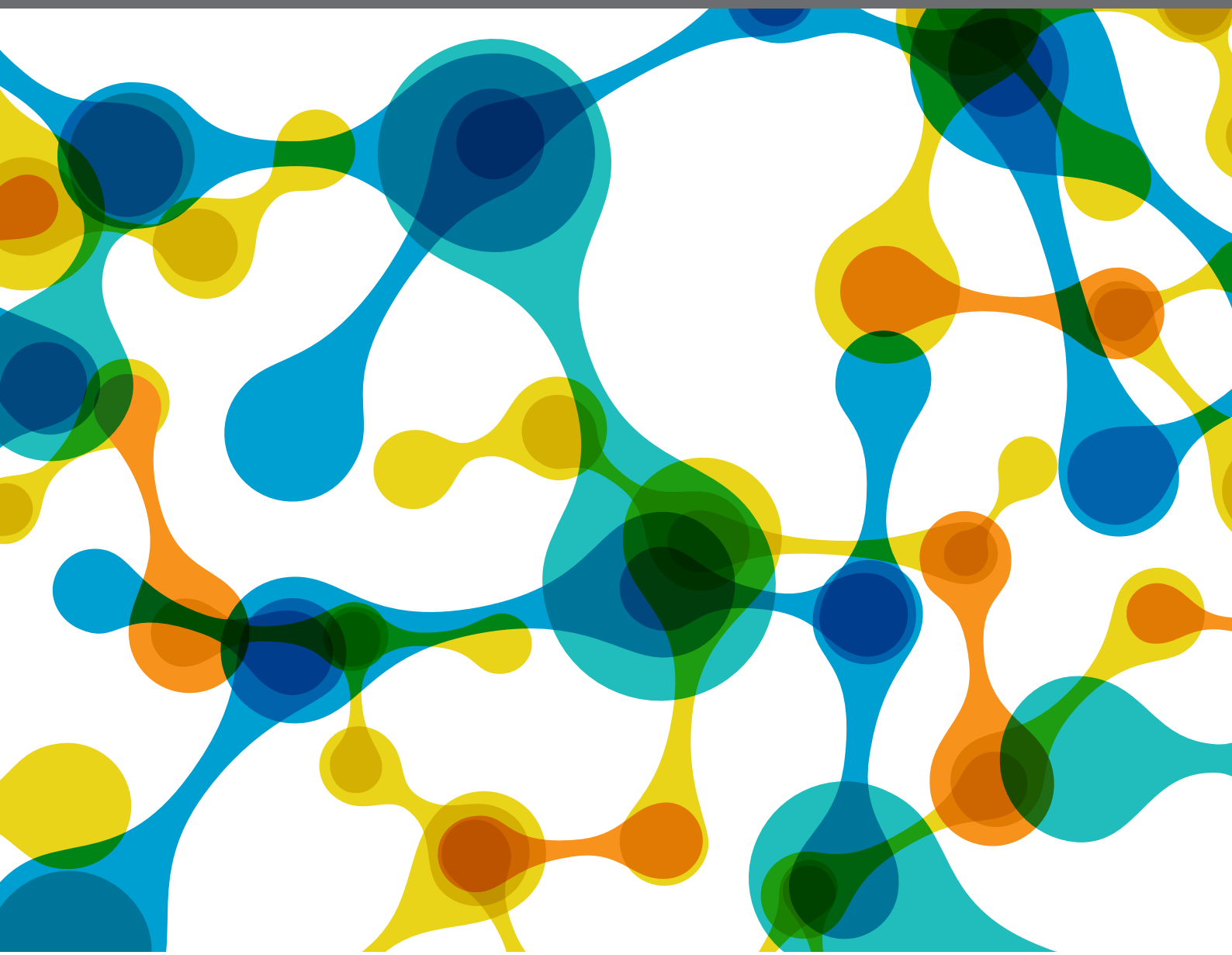
PUBLISHED IN: Frontiers in Physiology and Frontiers in Neurology



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ISSN 1664-8714
ISBN 978-2-88966-077-3
DOI 10.3389/978-2-88966-077-3

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STRATEGIES TO FIGHT EXERCISE INTOLERANCE IN NEUROMUSCULAR DISORDERS

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Citation: Lanfranconi, F., Tremolizzo, L., Marzorati, M., eds. (2020). Strategies to Fight Exercise Intolerance in Neuromuscular Disorders. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-077-3

Table of Contents

04 ***Editorial: Strategies to Fight Exercise Intolerance in Neuromuscular Disorders***
Francesca Lanfranconi, Mauro Marzorati and Lucio Tremolizzo

06 ***Combined HIIT and Resistance Training in Very Long-Chain Acyl-CoA Dehydrogenase Deficiency: A Case Report***
Alba M. Herrera-Olivares, Jose A. Fernández-Luque, Carmen Paradas, Alejandro Lucia and Alfredo Santalla

13 ***The Role of Exercise as a Non-pharmacological Therapeutic Approach for Amyotrophic Lateral Sclerosis: Beneficial or Detrimental?***
Stavroula Tsitkanou, Paul Della Gatta, Victoria Foletta and Aaron Russell

27 ***Physical Exercise and Mitochondrial Disease: Insights From a Mouse Model***
Carmen Fiuza-Luces, Pedro L. Valenzuela, Sara Laine-Menéndez, Miguel Fernández-de la Torre, Verónica Bermejo-Gómez, Laura Rufián-Vázquez, Joaquín Arenas, Miguel A. Martín, Alejandro Lucia and María Morán

37 ***Low-Intensity Running and High-Intensity Swimming Exercises Differentially Improve Energy Metabolism in Mice With Mild Spinal Muscular Atrophy***
Léo Houdebine, Domenico D'Amico, Jean Bastin, Farah Chali, Céline Desseille, Valentin Rumeau, Judy Soukkari, Carole Oudot, Thaïs Rouquet, Bruno Bariohay, Julien Roux, Delphine Sapaly, Laure Weill, Philippe Lopes, Fatima Djouadi, Cynthia Bezier, Frédéric Charbonnier and Olivier Biondi

58 ***Can Proprioceptive Training Reduce Muscle Fatigue in Patients With Motor Neuron Diseases? A New Direction of Treatment***
Ayman A. Mohamed

69 ***Tailored Exercise Training Counteracts Muscle Disuse and Attenuates Reductions in Physical Function in Individuals With Amyotrophic Lateral Sclerosis***
Alessandra Ferri, Francesca Lanfranconi, Giovanni Corna, Riccardo Bonazzi, Samuele Marchese, Andrea Magnoni and Lucio Tremolizzo for the ME&SLA Study Group

82 ***Beneficial Effects of Ketogenic Diet on Phosphofructokinase Deficiency (Glycogen Storage Disease Type VII)***
Minna E. Similä, Mari Auranen and Päivi Liisa Piirilä

89 ***Metabolic Myopathies: "Human Knockout" Models and Translational Medicine***
Bruno Grassi, Simone Porcelli and Mauro Marzorati

92 ***Exercise-Related Oxidative Stress as Mechanism to Fight Physical Dysfunction in Neuromuscular Disorders***
Gabriele Siciliano, Lucia Chico, Annalisa Lo Gerfo, Costanza Simoncini, Erika Schirinzi and Giulia Ricci

106 ***Aerobic Exercise Training in Patients With mtDNA-Related Mitochondrial Myopathy***
Tina Dysgaard Jeppesen

frontiers in Physiology

EDITORIAL
published: 19 August 2020
doi: 10.3389/fphys.2020.00968

Editorial: Strategies to Fight Exercise Intolerance in Neuromuscular Disorders

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Keywords: metabolic myopathies, motor neuron diseases (MNDs), skeletal muscle oxidative metabolism, mitochondrial myopathies, functional evaluation

Editorial on the Research Topic

Strategies to Fight Exercise Intolerance in Neuromuscular Disorders

We are pleased to have the opportunity to edit this Research Topic. We and the other scientists involved in this Research Topic have established a consistent direction toward the possibility of applying the tools of exercise physiology functional evaluation to improve the quality of life (QoL) of frail patients. The goal of this research is the exploration of the complex multifactorial pathogenesis of neuromuscular disorders. The extent of the research ranges from cellular mechanisms to systems derangements and involves pragmatic clinical approaches. We believe that improving the resilience of such patients by addressing exercise intolerance can be a formidable challenge for clinical human physiologists.

Metabolic myopathies (MM) and motor neuron diseases (MND) comprise a heterogeneous group of disorders characterized by impaired oxidative metabolism and reduced muscle strength. Patients affected by these disorders show progressive signs of reduced exercise tolerance and increased fatigability, with devastating effects on their QoL. Taking care of these patients requires clear understanding of those mechanisms which are known to jeopardize oxidative metabolism and skeletal muscle strength during exercise. Especially in MM and MND, exercise triggers inherent flaws in skeletal muscle cellular energy metabolism and motor unit recruitment resulting in severe impairments. Accordingly, the assessment of the impact of precision training and/or diet, "as medicine," can lead to new therapies aimed at counteracting the effects of these conditions.

It is appropriate that this Research Topic opens with the opinion of Grassi et al.. These authors highlighted the usefulness of widely used, non-invasive physiological approaches to identify and quantify impaired skeletal muscle oxidative metabolism, factors limiting exercise tolerance, and ultimately the patient's QoL.

Questions related to the possible dose-response effect of a precision-based exercise program include:

1) What is the best approach: moderate or high intensity exercise protocol?
2) What is the appropriate duration of rest between exercise sessions?
3) Should aerobic training be performed alone or in combination with strength/balance/flexibility exercises?

If exercise is medicine, these questions are not trivial and change is needed in clinical culture, because the assumption that "exercise is good, exercise is for everybody, so go out and do exercise" is not well-suited for patients with MM and MND. Based on animal model studies, it is observed by Houdebine et al., that in mice with Spinal Muscular Atrophy (SMA), active physical exercise,

OPEN ACCESS

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *20 May 2020*
Accepted: *16 July 2020*
Published: *19 August 2020*

Citation:
Lanfranconi F, Marzorati M and Tremolizzo L (2020) Editorial: Strategies to Fight Exercise Intolerance in Neuromuscular Disorders. Front. Physiol. 11:968. doi: 10.3389/fphys.2020.00968

including high-intensity protocols, induced metabolic adaptations. This finding provides further evidence that exercise in association with gene therapies increases the "survival rate of motor neuron" proteins. Analogous to this, Fiuza-Luces et al., reported the effects of an 8 week training intervention program combining aerobic and resistance exercise in a mouse model with respiratory chain complex I deficiency. It was observed that exercise training increased aerobic fitness and muscle strength, activated signaling pathways involved in muscle mitochondrial biogenesis and anabolism, and improved respiratory chain complexes activity and redox status in muscle tissue.

Physical exercise has been considered harmful for patients with MND and MM for a long time. However, a growing body of evidence now suggests that supervised exercise training is safe and may be effective in improving oxidative capacity and muscle function in patients. MND is a classical paradigm where this issue is fiercely debated. As reported by Tsitkanou et al., the fear of exercise induced damage in motor units and excessive ROS production, leads to the conclusion that accurate dosing of exercise should be considered on an individual basis (see Siciliano et al.). This is a critical issue, since exercise intolerance in MND and functionally similar patients (see Jeppesen et al. for mitochondrial myopathies) will lead to a sedentary life-style (disuse) that, as a vicious cycle, further interferes with QoL. It is noteworthy to mention that Ferri et al. reported that no damage is observed in MND patients doing a precisely-tailored 12-week exercise program. Such evidence is contributing to the body of knowledge that eventually will see the "overwork damage dogma" shattered.

Mohamed et al. shifts the perspective by focusing on training aimed at the sensory portion of muscle control in older patients with MND. The physiological effects of proprioceptive training can possibly decrease the occurrence of muscle fatigue. To our knowledge, a training program aimed at the sensory portion of motor control is a new avenue for further investigation.

The impact of diet on patients with MM and MND have not been deeply explored in this Research Topic. However, Similä et al. reported on the long-term effects of a ketogenic diet in a patient affected by GSD type VII. In the course of a 5-year follow up, an alleviation of muscle symptoms, beneficial effects on breathing, and improvement in exercise performance and oxygen uptake were observed. Similarly, Herrera-Olivares et al. reported on an anecdotal case of a very long-chain acyl-CoA dehydrogenase deficiency, a rare disorder of mitochondrial fatty acid β-oxidation. The patient completed a 6-month supervised aerobic + resistance training program, with the addition of a medium chain triglyceride + carbohydrate supplement ingested 60 min. before each session. Diet and exercise helped to increase the oxidative metabolism safely without muscle contractures or rhabdomyolysis.

One of the challenges in studying exercise in MM and MND is that these diseases are rare and heterogeneous, so it is difficult to recruit a sufficient number of participants for randomized controlled trials, and one type of exercise may not be suitable for everyone.

Therefore, despite the valuable contributions of this research, several points still remain open.

1) Determination of the time, intensity and duration of exercise for different diseases and phenotypes to create a guideline that clinicians and patients can use;
2) Determination of whether continued compliance with nutrition and exercise slows disease progression and minimize muscle deterioration;
3) Most of the data have been obtained in ambulatory patients. The effects of exercise using assistive devices should be further explored in very weak and wheelchair-bound patients;
4) Sometimes patients did not feel any benefit from the exercise nor did the intervention increase their functional capacities. Thus, strategies aimed at empowering these patients and increasing physical function in their habitual tasks should be investigated.

We strongly believe that the future of clinical exercise physiology will be closely linked to the establishment of a new cultural approach among clinicians and researchers about the use of exercise (and diet) as a safe and appropriate way to fight neuromuscular diseases so as to provide suitable care for children and adults.

AUTHOR CONTRIBUTIONS

FL wrote the first draft of the present manuscript. LT and MM contributed to its critical revision. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We sincerely thank the authors who have contributed to the success of this Research Topic. Their articles demonstrate the wide-ranging and multidisciplinary interest on this topic. We are grateful to Mr. Bejitual Berhanu for his careful revision of our manuscript as native speaker.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

CASE REPORT
published: 28 May 2019
doi: 10.3389/fphys.2019.00650

Combined HIIT and Resistance Training in Very Long-Chain Acyl-CoA Dehydrogenase Deficiency: A Case Report

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OPEN ACCESS

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *23 February 2019*
Accepted: *08 May 2019*
Published: *28 May 2019*

Citation:
Herrera-Olivares AM, Fernández-Luque JA, Paradas C, Lucia A and Santalla A (2019) Combined HIIT and Resistance Training in Very Long-Chain Acyl-CoA Dehydrogenase Deficiency: A Case Report. Front. Physiol. 10:650. doi: 10.3389/fphys.2019.00650

Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is a rare disorder of mitochondrial fatty acid β-oxidation characterized by a spectrum of clinical manifestations. Patients with the adult-onset form can present with muscle pain, rhabdomyolysis and myoglobinuria after physiological stress, such as fasting and exercise. We report on a 23-year-old female patient with a history of recurrent rhabdomyolysis. The patient completed a 6-month supervised combined (high-intensity interval training [HIIT] + resistance training) program, with the addition of a medium chain triglyceride + carbohydrate supplement provided 60 min before each session. The HIIT consisted of 6 sets of 70–80 s performed at maximum intensity with a minimum cadence of 100 rpm. Resistance training consisted of a circuit of basic exercises with dumbbells and elastic bands, with sets of 4–7 repetitions. The patient was evaluated at months 0, 3 and 6 using an incremental discontinuous step protocol, with steps of 1 min of exercise/1 min of passive recovery, at a high pedal cadence. The test started at 10 W, with a load increase of 10 W/step. Blood creatine kinase (CK) concentration was measured before each evaluation. There was a training-induced increment of 90.2% in peak oxygen uptake (VO_{2peak}), 71.4% in peak power output and 24.7% in peak heart rate. The patient reported no muscle pain, contractures, rhabdomyolysis (basal CK concentration was always <200 U/L) or hospital admissions during the training period. After completion of 6-month program, the patient remained active, doing similar but non-supervised training for 1.5 years (to date). During this period, the patient has not reported myalgias, contractures, rhabdomyolysis or hospital admissions. Our preliminary data suggest that it is possible to carry out a combined (HIIT + strength) training program in patients with VLCADD, safely (without muscle contractures or rhabdomyolysis) and obtaining high values of VO_{2peak} and cycling power output.

Keywords: VLCADD, training, exercise, neuromuscular disorders, FATmax

INTRODUCTION

Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency (VLCADD, OMIM 201475) is an autosomal recessive disorder (Andresen et al., 1999; Wanders et al., 1999; Lindner et al., 2010) with an estimated incidence of 1 in 30,000–100,000 newborns (Tucci et al., 2014). VLCAD is a mitochondrial membrane enzyme that catalyzes the dehydrogenation of very long chain fatty acids (14–20 carbons) (Andresen et al., 1999), which is the first step in the β-oxidation of fatty acids (Leslie et al., 1993). VLCADD is a clinically heterogeneous disease with three major phenotypes: (i) a severe infantile form with early onset and high mortality, which manifests with cardiac arrhythmia, cardiomyopathy and liver disease; (ii) a milder childhood form with late onset, which typically presents with hypoketotic hypoglycemia but with rare cardiomyopathy and low mortality; and (iii) an adult form with isolated involvement of the skeletal muscle that is commonly accompanied by rhabdomyolysis and myoglobinuria (Andresen et al., 1999; Arnold et al., 2009).

The most common form of VLCADD is the adult manifestation, and treatment consists mainly of dietary advice aimed at preventing catabolism, including following a diet rich in carbohydrates (CHO), low in very long chain fatty acids and supplemented with medium-chain triglycerides (MCT) (Arnold et al., 2009; Spiekerkoetter et al., 2009). It is also recommended that patients with VLCADD avoid prolonged exercise and fasting (Voermans et al., 2005; Diekman et al., 2016; Bleeker et al., 2018) to prevent rhabdomyolysis and myoglobinuria (Merinero et al., 2018). However, even following these recommendations, patients can still experience exercise intolerance, myalgia and, occasionally, episodes of rhabdomyolysis (Diekman et al., 2016), which is a serious complication (Hisahara et al., 2015) and can cause kidney damage and even kidney failure (Laforêt et al., 2009).

Perhaps for these reasons, there has been only one study examining the bioenergetic responses to aerobic exercise in patients with VLCADD (Diekman et al., 2016). Patients performed 45 min of exercise at a constant load corresponding to the maximal fat oxidation (FATMAX) (38 ± 4% of peak oxygen uptake [VO_{2peak}]), of which the last 5 min were performed inside a magnetic resonance scanner. Results showed an impairment in energetic muscle balance, including a decrease in phosphocreatine and an increase in inorganic phosphate concentrations, without changes in plasma acyl-carnitine levels. Based on these results, the authors hypothesized that this energy crisis during exercise (insufficient ATP turnover) was responsible for the observed rhabdomyolysis (Diekman et al., 2016).

Accordingly, increasing ATP synthesis capacity during exercise through a training program could be an effective strategy to reduce the symptomatology of VLCADD (exercise intolerance, myalgia and risk of rhabdomyolysis). However, to our knowledge, no study has analyzed the effects of exercise training in VLCADD patients. Here we tested the applicability of combined (high-intensity interval training [HIIT] + strength training) exercise program in a young VLCADD patient.

CASE REPORT

The patient was a 23-year-old woman (height: 167 cm, weight: 72.3 kg) from a non-consanguineous family, with asymptomatic parents and brothers. From 14 years of age she had 19 episodes of rhabdomyolysis, all of them requiring hospitalization (two of them after the genetic diagnosis), with a median duration of 4 days (range 1–14), and a maximum creatine kinase (CK) concentration of 39,994 ± 66,148 U/L (range 2,121–276,000 U/L). Three episodes of rhabdomyolysis were accompanied by renal failure (**Figure 1**). Physical examination and CK levels were normal between the episodes. At 21 years of age, the patient was assessed using a targeted next-generation sequencing-based panel containing 256 neuromuscular disease genes, and found to have a compound heterozygous mutation c.589G > A (p.Val197Met)/c.1742T > C (p.lle581Thr) in the gene (*ACADVL*, MIM 609565) encoding VLCAD. The patient gave her written consent to participate in the study and for the data to be published, after a thorough explanation about VLCADD and the purpose of the study, which was approved by the local institutional ethics committee.

Laboratory Evaluation

At the first visit to our laboratory (March 2017), the patient underwent ergospirometry. It was our intention to use a stepped incremental ergospirometry protocol with an initial power of 0 watts and a power increase of 30 watts/3 min, at 60 rpm of pedaling rate (Diekman et al., 2016), but the patient developed muscle pain and was unable to complete the 1st step (0 watts). Thus, she was asked to maintain a high pedaling rate (∼100 rpm) with the aim of recruiting type IIA and IIX fibers (not dependent on fatty acids) (Vollestad and Blom, 1985). As the patient reported no pain at these pedaling cadences, a test was performed on an electromagnetic cycle ergometer (Ergometrics 900, Ergoline, Barcelona, Spain) using an incremental discontinuous step protocol, with steps of 1 min of exercise interspersed with periods of 1 min of passive recovery, always maintaining a high cadence. The test was started at 10 watts, with a load increase of 10 watts/step. At the end of each step, a rating of perceived effort (RPE) was assessed according to the OMNI scale (Utter et al., 2004), and lower limb pain perception was scored using a numeric rating scale (NRS) (0–10) (Hawker et al., 2011). She was also asked at the end of the test whether she had a perception of muscle crises. The test was performed in the exercise physiology laboratory of Pablo de Olavide University under medical supervision, and the intensive care unit of Hospital Virgen del Rocío (Seville, Spain) was advised to be prepared.

Heart rate (HR, in bpm) was continuously monitored during the test from a 12-lead ECG. A breath-by-breath automatic system (CPX ultima, Medical Graphics Corporation, St Paul, MN, United States) was used to measure gas-exchange parameters: oxygen uptake (VO_2, in $mL \cdot min^{-1}$ and $mL \cdot kg^{-1} \cdot min^{-1}$),

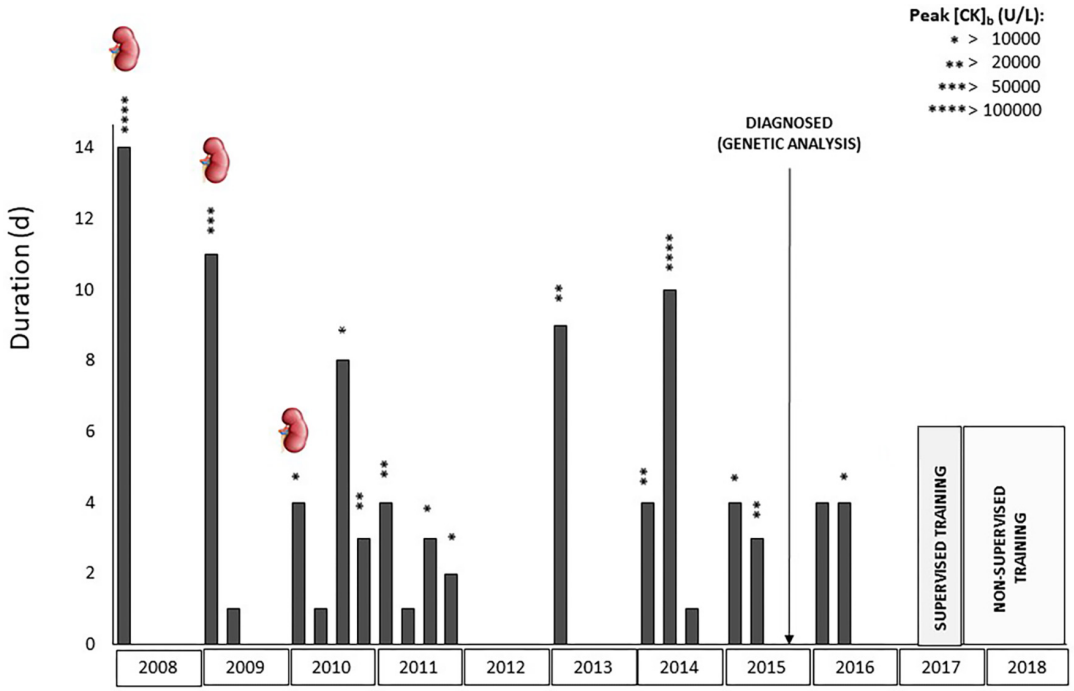

FIGURE 1 | Clinical history of the patient from her first hospital admission to date. Each bar represents a hospitalization, detailing its duration (days) and highest concentration of creatine kinase (CK) reached. Kidney Symbol: renal failure.

carbon dioxide production (VCO_2, mL·min^{-1}), ventilation (VE, in L·min^{-1}), and ventilatory equivalent for O_2 ($VE{\cdot}VO_2{}^{-1}$). Average values of the last 10 s of each step were obtained.

One hour before evaluation the patient ingested 500 mL of an isotonic drink (30 g CHO) Gatorade, PepsiCo, Purchase, NY, United States) with 15 g of MCT (Myprotein, Cheshire, United Kingdom), proportions that have been recommended for intestinal absorption (Jeukendrup et al., 1998; Jeukendrup, 2014). It is known that MCT can circumvent the block in the β-oxidation of long-chain fatty acids in VLCADD and can provide an alternative energy substrate to long-chain triglycerides (LCT) (Shiraev and Barclay, 2012), in addition to decreasing the oxidation of CHO, and reducing the risk of lactic acidosis induced by exercise (Behrend et al., 2012). Moreover, supplementation with CHO increases blood glucose levels and improves performance (Tsintzas et al., 2000). Accordingly, the role of this supplement was to (i) increase the work capacity during the test, (ii) maintain the glycemia level to reduce fat oxidation during recovery, and (iii) provide MCT for oxidation during the hours after assessment, thereby reducing the risk of post-exercise rhabdomyolysis (Diekman et al., 2016). Blood samples were collected 2 days before each evaluation to measure CK concentration. Laboratory evaluations were repeated at the end of the 3rd and 6th month.

Training Program

The patient followed a 6-month combined (HIIT + strength training) exercise program supervised by a fitness specialist. The first month consisted of only HIIT (2 days·week^{-1}), while in the following 5 months combined training was carried out 4 days·week^{-1} (HIIT 2 days·week^{-1} and strength training 2 days·week^{-1}). HIIT was done in cycle ergometer and consisted of 6 sets of 70–80 s performed at maximum intensity with a minimum cadence of 100 rpm, with 1 min passive recovery periods between sets. We chose this type of training to maintain a high demand for glycolysis during exercise and, therefore, avoid the dependence of lipolysis on energy production (at the highest possible intensity, well above FATMAX) (**Figure 2**). HR

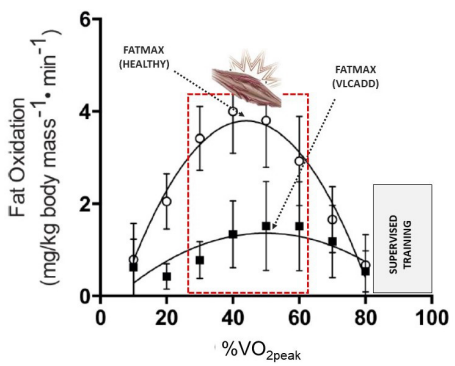

FIGURE 2 | Fat oxidation capacity in exercise in a healthy person vs. a patient with VLCADD. Muscle discomfort during exercise appears at intensities close to FATMAX (marked in red), modified from (10). High-intensity interval training was always at high intensity (in which the demand for fat oxidation is practically null). Completed only 40 s of the last step.

and RPE were registered at the end of each set, while pain perception was registered at the end of the training session (Hawker et al., 2011). Three hours later, the patient was asked if she had a perception of (i) muscle swelling similar to that experienced before her hospital admissions (in order to anticipate a possible muscle crisis) and (ii) risk of actual muscle "crisis" (i.e., rhabdomyolysis).

The following strength training exercises were performed with dumbbells and elastic bands: bench press, biceps curl, bent-over dumbbell row, lying triceps extension and dumbbell lateral rise, and also ½ squat using body weight. The exercise routine was designed as a circuit, with 2 laps of 4–7 repetitions, with 2 min rest between sets and 4 min rest between laps. The OMNI Resistance Exercise Scale (Robertson et al., 2003) was used to measure RPE. The initial load was fixed at 5–7 of this 0–10 scale. Three hours after each strength session the patient was asked about muscle swelling. The morning after each session, she was asked if she had delayed-onset muscle soreness (using a scale of 0–10 in case of presentation).

Given the high intensity of HIIT, and the excess post-exercise oxygen consumption (EPOC) elicited by intense exercise bouts (McGarvey et al., 2005; Knab et al., 2011; Shiraev and Barclay, 2012; Buitrago et al., 2014), all training sessions were performed 60 min after ingestion of the pre-exercise supplement (30 g CHO + 15 g MCT). Warm-up sessions were also designed specifically to avoid fat oxidation and comprised 30 s bouts of intense exercise (at 80% of VO_{2peak} achieved in the incremental step test) before HIIT and sets of low repetitions at high speed (without weights) before strength training. With the same objective, we decided not to include a cool-down phase after HIIT sessions.

RESULTS

The effects of the training program are shown in **Table 1**. VO_{2peak} showed a high increase (90.2%), of which most (70.7%) occurred in the first 3 months. Also, peak values of HR and power output (W) increased 20.6 and 71.4%, respectively, in the first 3 months, without changes in the last 3 months.

In HIIT training sessions, HR reached at the end of sets ranged between 70 to 85% of age-predicted maximum HR (220 minus age). Pain levels reported during the HIIT sessions were low and decreased during the months of training (**Table 2**). In addition, the patient did not perceive muscular swelling or risk of a crisis in the hours following each training session. The RPE in strength training increased during the training period, reaching the desired values in the 5th month. Delayed onset muscle swelling and soreness scores were low and none of the sessions caused her sense of muscular swelling (**Table 2**).

During the 6 months of supervised training the patient had no myalgia or contractures and did not report the presence of dark urine. In addition, and as opposed to the 2 years previous to the program (with one episode of rhabdomyolysis [CK = 24,860 U/L] requiring 3-day hospitalization in 2015 and two episodes in 2016, each requiring 4–day hospitalization [CK = 6,277 and 16,759, respectively]– **Figure 1**), blood parameters were in normal range during the training program, with CK values of 52, 55, and 169 U/L (months 0, 3, and 6, respectively), without any episode of rhabdomyolysis. The patient was not admitted to hospital during this period. The patient has remained active, performing similar but non-supervised training (including also pre-exercise MCT+CHO supplementation) for 1.5 years (to date). During this period, she has not experienced myalgia, contractures or rhabdomyolysis, and has not been admitted to hospital; she only visited the hospital once for a routine medical visit (in September 2018), showing a low CK value (=61 U/L). Similar to during the period of supervised training, she described less limitation in daily life activities and a higher quality of life.

DISCUSSION

The main finding of our study was that the combined (HIIT + strength training) exercise regimen with pre-exercise CHO + MCT supplementation yielded high benefits (i.e., ↑20.6% peak HR, ↑90.2% VO_{2peak}, and ↑71.4% peak power output) for the patient. Our results also indicated that this regimen was safe, with no muscle pain, contractures, rhabdomyolysis (baseline CK < 200 U/L) or hospital admissions reported in the entire training period.

VO_{2peak} has been described as an indicator of cardiorespiratory health (Jones and Carter, 2000), morbidity (Levine, 2008) and mortality (Jones and Carter, 2000) in the general population. The improvement in VO_{2peak} can be attributed to multiple factors that increase aerobic fitness, including improvement of respiratory muscles, and both cardiovascular and peripheral factors (Vendrusculo et al., 2019). In this case, the improvement of VO_{2peak} could be explained by both peripheral (i.e., increased mitochondrial content of skeletal muscle and capillary density) and central cardiovascular adaptations (i.e., increased cardiac contractility, blood volume and cardiac output), widely described after HIIT (MacInnis and Gibala, 2017).

The training program and supplement were designed to prevent muscular discomfort during training, post-exercise muscle crises and increases in basal CK. Post-exercise rhabdomyolysis has been described in patients with VLCADD as a consequence of the impossibility in obtaining energy during exercise when it is performed close to FATMAX (Diekman et al., 2016). Although MCT supplementation can bypass the blockade in the oxidation of long chain triglycerides (LCT) and can provide a useful source of fatty acids for both the heart and the skeletal muscle in exercise (Behrend et al., 2012), it has been shown to have no benefits in patients with VLCADD when they exercise at FATMAX, when the energy demand of fats is very high (Ørngreen et al., 2007). However, the energy demand of LCT in the HIIT protocol used in our patient was much lower since skeletal muscle depends almost entirely on CHO as a source of fuel as exercise intensity increases (Van Loon et al., 2001), greatly reducing the energy demand of fats. Under these conditions, the MCT + CHO supplement seems to have been effective both during and after exercise. Indeed, CHO can be used as fuel during exercise (Behrend et al., 2012), increasing

TABLE 1 | Ergospirometry data (peak values) during the laboratory assessments, before and after the combined (high-interval training HIIT + resistance training) exercise program.

Variables	Pretraining	Posttraining (3 months)	% Change (from pretraining to 3rd month of training)	Posttraining (6 months)	% Change (from pretraining to 6th month of training)
Test duration	13 min 45 s	23 min 40 s	+72.1	23 min 38 s	+71.9
VO_{2peak} (ml·kg^{-1}·min^{-1})	11.6	19.9	+70.7	22.3	+90.2
VO_{2peak} (ml·min^{-1})	846	1441	+70.3	1610	+90.3
VCO_2 (ml·min^{-1})	932	2,235	+139.3	1,714	+83.9
VE (ml·min^{-1})	27.3	73.5	+169.2	81	+196.7
VE/VO_2	32	51	+59.4	50	+56.3
RER	1.10	1.55	+40.9	1.06	−3.6
HR (beats·min^{-1})	144	182	+26.4	180	+25
% HRmax	73	92	+26.0	91	+24.7
Power output (watts)	70	120*	+71.4	120*	+71.4

**Completed only 40 s of the last step. HR, heart rate; HR_{max}, age-predicted maximum heart rate (220 minus age, in years); RER respiratory exchange ratio; VCO_2 carbon dioxide production; VE, ventilation; VE/VO_2 ventilatory equivalent for oxygen; VO_{2peak}, peak oxygen uptake.*

TABLE 2 | Aerobic and resistance training sessions averaged data during the combined training program.

	HIIT						Resistance training			
Month	HR (beats·min^{-1})	% of HR_{max}	RPE	Pain (0 to 10 score)	Risk* (yes/no)	Swelling* (yes/no)	RPE_{Legs} (0 to 10 score)	RPE_{Arms} (0 to 10 score)	DOMS (0 to 10 score)	Swelling (yes/ no)
1	166 ± 13	85	2 ± 0	1 ± 1	No	No	–	–	–	–
2	154 ± 6	79	5 ± 1	0	No	No	2 ± 1	2 ± 1	2 ± 1	No
3	144 ± 12	73	4 ± 1	2 ± 0	No	No	1 ± 0	2 ± 0	2 ± 1	No
4	150 ± 8	77	3 ± 1	0	No	No	3 ± 0	4 ± 1	1 ± 1	No
5	138 ± 4	70	5 ± 0	0	No	No	5 ± 1	5 ± 1	3 ± 2	No
6	140 ± 4	70	5 ± 0	0	No	No	6 ± 0	5 ± 1	1 ± 1	No

*Data are presented as mean ± SD. DOMS, delayed onset muscle soreness (0–10 scale, one day after training sessions); HIIT, high-intensity interval training; HR, heart rate; HRmax, age-predicted maximum heart rate (220 minus age, in years); RPE, rating of perceived exertion (0–10 scale). *perception of swelling and of risk of actual muscle "crisis" (rhabdomyolysis) (yes/no) was reported 3 h after the end of training sessions.*

blood glucose levels and their use during and after exertion (Tsintzas et al., 2000). The sparing effects on the utilization of muscle glycogen stores, and the higher glycemia after exercise, seem to be responsible for the fact that the energy demand of fat oxidation during EPOC is moderate enough for MCT to be sufficient.

After the supervised training period, the patient maintained a similar training regimen until today, without experiencing muscular contractures or hospital admissions. The patient also reported improvement in her quality of life due to the relief from muscular discomfort. This could be due to the training adaptations, a better control of diet and a greater understanding of the disease. It is known that high pedaling cadences, high contraction speeds and low number of sets (such as those used in our HIIT sessions) produce muscle fiber type shifts characterized by an increase of type IIA fibers and a decrease of type I fibers (Wilson et al., 2012). Since type IIA fibers are more dependent on CHO metabolism and less on LCT metabolism (Egan and Zierath, 2013; Bourdeau Julien et al., 2018), a higher proportion of type IIA would allow an increase in the use of muscle glycogen and a decrease in the demand for LCT. This would reduce the limitations and the risk of muscle crises in the patient. Indeed, this metabolic adaptation (although without fiber type shift) has already been described in $VLCAD^{-/-}$ mice as a strategy to compensate for VLCAD deficiency (Tucci et al., 2012). It is also known that strength training, similar to that used here, has proven to reduce rhabdomyolysis in other myopathies (Santalla et al., 2014; Pietrusz et al., 2018) as a consequence of an improvement in the resistance of muscle fibers to damage during physical activity. In addition, less muscle damage could allow for the maintenance of higher exercise intensities without experiencing symptoms (Pietrusz et al., 2018) and would imply a lower energy expenditure, from CHO and LCT, for the synthesis of muscle tissue during EPOC.

The patient followed a diet high in protein and low in fat during and subsequent to the intervention period. A diet regimen low in LCT is recommended for patients with VLCADD, but the necessary intake of essential fatty acids should be provided (Spiekerkoetter et al., 2009). Although this diet was prescribed after diagnosis (before the training period), in the 1st visit to our laboratory we explained to the patient the importance of diet and of avoiding fasting. She was also informed about the influence of exercise intensity and CHO availability on muscle

fuel utilization during exercise and recovery. Relating to her own medical history, the patient was informed about how prolonged low intensity exercise performed during fasting caused rhabdomyolysis and hospital admissions. In the same way, the patient was instructed to carry out daily life activities at a higher speed and explosiveness, to avoid the energetic demand from oxidation of fatty acids (Egan and Zierath, 2013; Bourdeau Julien et al., 2018).

There are several limitations of this study that merit attention. As a single case report, the results must be interpreted with caution and cannot be generalized to other patients with VLCADD. In addition, gains in strength, quantitative changes (i.e., dual-energy X-ray absorptiometry assessments) and qualitative changes in muscle mass (i.e., muscle biopsy) were not quantified. We believe that the training program is the main reason that the patient stopped experiencing rhabdomyolysis and hospital admissions. However, we cannot establish the precise degree of responsibility, since the dietary habits were also changed and the way of carrying out activities of daily life was modified. Finally, we did not assess the patient's quality of life or ability to cope with daily life activities, which should be done in future research.

In summary, the results of this study show that it is possible to carry out a combined (HIIT + strength training) exercise program in patients with VLCADD safely (without muscle contractures or rhabdomyolysis) and to achieve high values of VO_{2peak} and cycling power output. In our opinion, this must be supervised by a fitness specialist given the need to control the loads of HIIT, the strength training load and the monitoring of muscle perception during and after each training session.

ETHICS STATEMENT

Ethics committee of Hospital 12 de Octubre, Madrid. Written informed consent was obtained.

AUTHOR CONTRIBUTIONS

AH-O: writing the work. JF-L: acquisition, analysis or interpretation of data (training). CP: medical care, testing and medical tracking of the patient, and also contributing to critical revision of the work. AL: contributing to the analysis and interpretation of the data and critical revision of the work. AS: conception and design the word, writing contribution and critical review of it.

FUNDING

Research by AL is funded by the Spanish Ministry of Economy and Competitiveness (Fondo de Investigaciones Sanitarias and Fondos FEDER, Grant Nos. PI15/00558 and PI18/00139).

ACKNOWLEDGMENTS

We would like to thank Macarena Cabrera and Nigel Laing at PathWest Laboratory Medicine WA, Section of Neuropathology, Royal Perth Hospital (Perth, Australia) for the genetic study.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

REVIEW
published: 17 July 2019
doi: 10.3389/fneur.2019.00783

The Role of Exercise as a Non-pharmacological Therapeutic Approach for Amyotrophic Lateral Sclerosis: Beneficial or Detrimental?

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Amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease, involves the rapid deterioration of motor neurons resulting in severe muscle atrophy and respiratory insufficiency. It is considered a "multisystemic" disease with many potential mechanisms responsible for its pathology. Currently, there is no cure for ALS. Exercise training is suggested as a potential approach to reduce ALS pathology, but its beneficial role remains controversial. This review provides an overview of the effects of exercise training in ALS-affected mice and patients. It will compare the intensity, duration, and type of exercise on the health of $SOD1^{G93A}$ mice, a mouse model of familial ALS, and review clinical studies involving ALS patients undergoing both endurance and resistance training. In summary, mild-to-moderate swimming-based endurance training appears the most advantageous mode of exercise in $SOD1^{G93A}$ mice, improving animal survival, and delaying the onset and progression of disease. Furthermore, clinical studies show that both endurance and resistance training have an advantageous impact on the quality of life of ALS patients without extending life expectancy. However, small sample sizes, non-representative control populations, heterogeneous disease stage of patients, and the presence of confounders often exist in the exercise studies conducted with ALS patients. This raises concerns about the interpretation of these findings and, therefore, these results should be considered with caution. While promising, more pre-clinical and clinical studies with improved experimental design and fewer limitations are still necessary to confirm the impact of exercise training on the health of ALS patients.

Keywords: amyotrophic lateral sclerosis/ALS, motor neuron disease, exercise, endurance training, resistance training, $SOD1^{G93A}$ mice, ALS patients

OPEN ACCESS

Edited by:
Lucio Tremolizzo, University of Milano-Bicocca, Italy

Reviewed by:
Christian Lunetta, Centro Clinico Nemo, Italy
Mamede De Carvalho, University of Lisbon, Portugal

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Specialty section:
This article was submitted to Neuromuscular Diseases, a section of the journal Frontiers in Neurology

Received: *23 May 2019*
Accepted: *04 July 2019*
Published: *17 July 2019*

Citation:
Tsitkanou S, Della Gatta P, Foletta V and Russell A (2019) The Role of Exercise as a Non-pharmacological Therapeutic Approach for Amyotrophic Lateral Sclerosis: Beneficial or Detrimental? Front. Neurol. 10:783. doi: 10.3389/fneur.2019.00783

INTRODUCTION

Amyotrophic lateral sclerosis (ALS), a subtype of fatal motor neuron disease (MND), is a progressive and degenerative neuromuscular disorder. It is characterized by the death of the upper and lower motor neurons at the spinal or bulbar level, leading to paralysis and eventual death usually from severe muscle atrophy and respiratory insufficiency. Median survival is between 2 and 4 years from the onset of symptoms (1). The disease usually appears between 40 and 70 years of age, and affects about five in 100,000 people worldwide (2). There are two types of

ALS: a familial ALS (FALS), heritable form (∼5–10% of ALS), and the sporadic ALS (SALS) form, which occurs randomly (∼90–95% of ALS). Mutations in the human copper/zinc (CuZn) superoxide dismutase (SOD1) gene account for ∼20% of FALS cases, and the mutant $SOD1^{G93A}$ rodent model has been used extensively to help understand ALS pathology and to test potential therapeutic treatments for this disease. Several factors are suggested to contribute to motor neuron death in ALS. Excitotoxicity (3, 4), deficits in axonal transport (5, 6) and neurofilament aggregation (7, 8) are neuron-related mechanisms potentially involved in pathophysiology of ALS. Non-neuronal defects, including oxidative stress (9, 10), protein misfolding/aggregation (11), neuroinflammation caused by non-neuronal cells (e.g., glial cells, astrocytes) (12, 13), and skeletal muscle dysfunction (14–16) resulting from disequilibrium in mitochondria function (17, 18), satellite cell (SC) activity (19, 20) and microRNA expression (16, 21), are also considered potential contributors to ALS pathophysiology. Therefore, ALS is considered a "multisystemic" disease, where changes in different cell types may act mutually and synergistically to contribute to disease onset and severity (22). Given the complexity of the disease, it is difficult to find a specific treatment for ALS patients. Currently, there is no effective pharmacological therapy for ALS. Interestingly, non-pharmacological approaches, such as non-invasive ventilation, have been suggested to prolong the survival in ALS patients (23, 24).

Physical activity is considered as a non-pharmacological therapeutic intervention for many diseases. In the general population, exercise training induces beneficial adaptations in both cardiovascular and neuromuscular systems, including improvements in respiratory and heart function (25), capillary density increase (26), muscle hypertrophy and muscle strength/power increase (27). In addition, it promotes psychological well-being by decreasing depression and anxiety levels, and positively influencing mood state, self-esteem and self-image (28, 29). However, exercise as an approach to reduce ALS pathology is controversial. Previous epidemiological studies suggest that a lifestyle involving vigorous exercise (30–32) and reduced body fat (32) are associated with an increased incidence of ALS. Also, there are studies which suggest an increased risk for ALS among professional soccer and American football players (33, 34). This association is physiologically plausible as strenuous and prolonged exercise may induce oxidative stress, glutamate excitotoxicity and increased calcium loads, which promote selective degeneration of vulnerable motor neurons (35, 36). On the other hand, several neurotrophic factors are up-regulated by exercise in neural and muscle tissue including brain-derived neurotrophic factor (37), which promotes survival and growth of spinal cord cells (38, 39) and glial cell-derived neurotrophic factor (40), which causes synaptic remodeling and hyperinnervation of neuromuscular junctions (41). Based on these findings, the hypothesis that physical activity is a risk factor for developing ALS seems unlikely. Instead, it has been suggested that genetic profiles modified by exogenous factors, which promote physical fitness and raise ALS susceptibility, might be a more credible explanation for the association between physical activity and ALS (17). The discordant results among the epidemiological studies of ALS can be explained by the methodological limitations inherent in studying a relatively low incidence disease. Specifically, most of these studies did not entirely account for potential confounders, such as trauma, possible use of drugs, drugs of abuse, dietary supplements, exposure to environmental toxins such as pesticides or characteristics of physical activity (mode, frequency, duration, intensity) (42, 43).

Multiple studies in ALS mice and patients have been performed to assess the effect of exercise training on disease progression. In preclinical studies, data are conflicting as exercise has been suggested to be beneficial (44), null (45) or even harmful (46) in transgenic $SOD1^{G93A}$ mice. Similarly, in humans, there are studies supporting the benefit of exercise training on the quality of life for ALS patients (47, 48), while other studies concluded that exercise had negative outcomes on neuromuscular function of ALS patients (49). Irrespective of these findings, physical activity is still considered a potential therapeutic approach for ALS improving body function, slowing disease progression and lessening caregiver burden (50). Apart from the biological and mental improvements observed after exercise, physical activity also activates specific mechanisms disrupted during the progression of ALS. For example, exercise evokes signaling pathways modifying skeletal muscle metabolism and triggers structural myofiber remodeling through SC activation (51). Regardless of mode (endurance, resistance or concurrent training), regular exercise lowers basal oxidative stress and increases anti-oxidative capacity (52–54). Moreover, endurance exercise potently increases skeletal muscle mitochondrial content (55, 56), neurogenesis (57, 58) and has a neuroprotective effect on the brain protecting against ischemic neuronal damage in the hippocampal formation (59).

Although evidence supports the advantageous impact of exercise in ALS, it is still not clear whether physical activity should be recommended as a treatment option for ALS patients. Even if exercise training *per se* is not a sufficient intervention to decelerate disease progression or improve patients' survival, it could be an adjunct therapy to other pharmacological treatments of ALS. Considering the lack of treatments at this time in ALS, non-pharma therapeutics, such as physical activity, is likely to be more important than previously appreciated. This review highlights our knowledge of the role of exercise as a non-pharmacological therapeutic approach for ALS. More specifically, we have critically reviewed both pre-clinical animal and clinical studies to understand whether physical activity could improve the quality of life and life expectancy in ALS, as well as which type of exercise elicits greater compliance and efficacy in ALS patients. While we acknowledge the importance of alternative training methods, such as respiratory muscle training and range-of-motion exercises/stretching, used mainly by respiratory therapists, physiotherapists and occupational therapists to improve physical performance (e.g., respiratory function and flexibility), this review is focused on specific physical activity modes (endurance, resistance, and concurrent training) prescribed by exercise physiologists.

EFFECTS OF EXERCISE IN SOD1^{G93A} ALS MICE

Pre-clinical studies in rodents have assessed the effect of exercise on ALS pathophysiology. Most of them suggest mild-to-moderate endurance exercise as a positive treatment (44, 60–63); whereas, vigorous endurance training seems to have null (45) or harmful (46, 61) effects in SOD1^{G93A} ALS mice (**Tables 1**, **2**). Compared to running-based training, swimming seems to be more beneficial in SOD1^{G93A} mice (64, 65). Therefore, the exercise mode, intensity and duration are likely critical factors in eliciting potential positive effects from endurance training in ALS mice. Interestingly, none of the exercise studies in ALS mice is resistance-based, although resistance training is suggested as a major type of exercise in many disorders, including neuromuscular diseases (66).

Gender Differences in SOD1^{G93A} ALS Mice in Response to Exercise

Kirkinezos et al. (60) was the first to investigate the effect of exercise in ALS mice and concluded that 10 weeks of endurance treadmill training can significantly extend life expectancy in high-copy number SOD1^{G93A} mice. However, when male and female mice were separately analyzed, the increase in life expectancy was only statistically significant for males, implying that testosterone levels and increased muscle mass may play a protective role in ALS (60). Sex differences in response to endurance training were also reported in ALS mice by Veldink et al. (44). However, in this case, endurance treadmill training delayed the onset of disease only in the SOD1^{G93A} female mice with a low-copy number of transgenes, but not in male mice with a low-copy number (44), suggesting a possible neuroprotective effect of female sex hormones (e.g., estrogens) in ALS. Indeed, the neuroprotective role of estrogens (67, 68) has been suggested as the potential reason why the incidence in ALS is lower in women than in men (69, 70) and why this gender difference declines gradually among older ALS patients (70) when estrogen levels decrease. Considering that testosterone levels also decrease with age (71, 72), a postmenopausal drop in the male:female ratio in ALS may not be fully explained by sex hormones, but inherent limitations that characterize epidemiologic investigations of rare and very rapidly fatal diseases, such as ALS studies.

In any case, the inconsistency of two aforementioned pre-clinical studies (44, 60) could be explained by the different training regime (see **Table 1**) and transgene copy number [high (60) vs. low (44)] of the SOD1^{G93A} transgene in the mice. Our experience in SOD1^{G93A} mice has shown that treatment efficacy alters to a great degree when the transgene copy number is different. When Veldink et al. (44) repeated the study using only female SOD1^{G93A} mice with a high-copy number of the transgene, they observed a significant delay in total survival time in the exercised group, compared to the sedentary group. This could be associated with the fact that the sedentary SOD1^{G93A} mice (low-copy number) presented a period of anestrus (i.e., lack of a normal estrus cycle) related with low levels of estrogens (73) contrary to the counterpart exercised mice that all of them appear a normal estrus cycle (mice with anestrus: 3/10 vs. 0/10, $p = 0.05$) (44). Although estrogen levels were not presented in this study (44), as the plasma levels of estrogens were below the detection threshold of their radioimmunoassay, there are numerous studies reporting that the expression of estrogen receptors (74, 75), and serum (74) and cerebellar (76) estrogen levels increase following endurance training. High-dose treatment of 17β-estradiol, which is an estrogen steroid hormone, is suggested to delay ALS disease progression (77, 78) and rescue the lifespan (78) of ovariectomized female SOD1^{G93A}. Surprisingly, histological analysis in the lumbar ventral horns of low-copy SOD1^{G93A} mice showed no significant differences in motor neuron counts and muscle fiber size between exercising and sedentary mice in both male and female animals (44). More extensive histological analysis is needed to investigate whether these results could be explained by the low transgene copy number of SOD1^{G93A} mice.

Effects of Exercise Intensity in SOD1^{G93A} ALS Mice

The intensity of endurance training is a parameter that potentially influences the health outcome of SOD1^{G93A} mice. Carreras et al. found that moderate intensity endurance exercise significantly delayed the onset of motor performance deficit in male SOD1^{G93A} mice (copy number is not reported) (61). This delay correlated with a 2-fold higher motor neuron density in the ventral horn of the lumbar spinal cord. This observation is contrary to results of Veldink et al. (44), who found no significant differences in motor neuron counts between exercising and sedentary low-copy SOD1^{G93A} mice in both male and female after moderate-intensity endurance exercise. Although copy number is not reported by Carreras et al. (61), any mention of SOD1^{G93A} usually refers to the more common high-copy number unless it is not specified. Therefore, the potential different copy number and the different age and, as such, disease stage that mice started exercising [30 days old (61) vs. 56 days old (44)] may explain the discrepancies observed between these two studies. Given that SOD1^{G93A} mice become symptomatic approximately at 80–90 days old (79), experimental designs including exercise training, as a therapeutic approach, which starts before disease onset are not clinically relevant. Such pre-symptomatic studies can provide important information about the effects of exercise on ALS development rather than exercise as an ALS therapeutic on which this review focuses.

Although the study of Carreras et al. (61) was not designed to monitor the longevity of the mice, a trend for higher survival rate was detected among moderate-exercised SOD1^{G93A} mice compared to sedentary or high intensity-exercised SOD1^{G93A} mice (premature deaths: 5/22 moderate-exercised vs. 7/22 sedentary vs. 10/23 high intensity-exercised). In contrast, high intensity endurance exercise significantly hastened the onset of motor performance deficits in SOD1^{G93A} mice (61). In parallel with these results, Mahoney et al. (46) concluded

TABLE 1 | Summary of the effects of running-based endurance training in SOD1^{G93A} ALS mice.

Study	Sex	Age (days)	Duration	Exercise protocol	Various outcomes
Kirkinezos et al. (60)	M ($n = 15$)	49	70 days	Treadmill running at 13 m/min for 30 min, 5 days/week	↑ Survival
	F ($n = 15$)	49	70 days	Treadmill running at 13 m/min for 30 min, 5 days/week	↔ Survival
Veldink et al. (44)	M ($n = 13$) Low-copy SOD1 ^{G93A}	56	Scores >15 points (3 points for each electrical shock, 1 point after resting for 5 s)	Treadmill running at 16 m/min for 45 min, 5 days/week	↔ Onset of disease ↔ Survival
	F ($n = 11$) Low-copy SOD1 ^{G93A}	56	Scores >15 points (3 points for each electrical shock, 1 point after resting for 5 s)	Treadmill running at 16 m/min for 45 min, 5 days/week	↑ Onset of disease ↔ Survival
	F ($n = 9$) High-copy SOD1 ^{G93A}	56	Scores >15 points (3 points for each electrical shock, 1 point after resting for 5 s)	Treadmill running at 16 m/min for 45 min, 5 days/week	↔ Onset of disease ↑ Survival
Kassa et al. (63)	M ($n = 5$)	50	Until symptom onset	Treadmill running at 20 m/min for 45 min, 5 days/week	↔ Onset of disease ↔ Motor neuron counts
Carreras et al. (61)	M ($n_{total} = 30$, $n = 10$ for each time point)	30	40, 65, and 90 days	Moderate intensity: treadmill running at 10 m/min, for 30 min, 3 days/week	↑ Motor performance ↑ Motor neuron density
	M ($n_{total} = 30$, $n = 10$ for each time point)	30	40, 65, and 90 days	High intensity: treadmill running at 20 m/min, for 60 min, 5 days/week	↓ Motor performance ↔ Motor neuron density
Mahoney et al. (46)	M ($n = 7$)	40	A minimum of 9 m/min for 45 min	Treadmill running at 9–22 m/min for 20, 25, and 30 min for 3 days/week, the first 3 weeks, and 45 min for 5 days/week thereafter	↔ Onset of disease ↓ Survival ↓ Motor performance
	F ($n = 7$)	40	A minimum of 9 m/min for 45 min	Treadmill running at 9–22 m/min for 20, 25, and 30 min for 3 days/week, the first 3 weeks, and 45 min for 5 days/week thereafter	↔ Onset of disease ↔ Survival ↔ Motor performance
Kaspar et al. (62)	M ($n = 9$) + F ($n = 9$)	40	To right themselves within 30 s	2 h daily exposure to running wheels (3,162 revolutions/day)	↔ Motor performance ↑ Survival
	M ($n = 9$) + F ($n = 9$)	40	To right themselves within 30 s	6 h daily exposure to running wheels (5,551 revolutions/day)	↑ Motor performance ↑ Survival
	M ($n = 9$) + F ($n = 9$)	40	To right themselves within 30 s	12 h daily exposure to running wheels (10,482 revolutions/day)	↑ Motor performance ↑ Survival
Liebetanz et al. (45)	M ($n = 7$) + F ($n = 5$)	35	Speed exceeding preset wheel speed	10 h daily exposure to running wheels: 40 × 10 min of running, 5 min rest (1,400–5,000 m/day)	↔ Onset of disease ↔ Motor performance ↔ Survival
Deforges et al. (64)	M ($n = 20$)	70	45 days or until mice death	Running in a speed-regulated treadmill (max. 13 m/min), for 30 min, 5 days/week	↔ Onset of disease ↔ Survival ↔ Motor performance
Desseille et al. (65)	M ($n = 18$)	70	45 days	Running in a speed-regulated treadmill (max. 13 m/min), for 30 min, 5 days/week	↔ Glucose tolerance ↔ Plasma lactate ↔ GLUT4 expression ↔ Lipid synthesis

M, male; F, female; GLUT4, glucose transporter type 4; statistically significant increase (↑), decrease (↓) and no statistically significant changes (↔) compared to sedentary/no-exercising SOD1^{G93A} ALS mice.

TABLE 2 | Summary of the effects of swimming-based endurance training in SOD1^{G93A} ALS mice.

Study	Sex	Age (days)	Duration	Exercise protocol	Various outcomes
Deforges et al. (64)	M ($n = 25$)	70	45 days or until mice death	Swimming in an adjustable-flow swimming pool (max. 5 l/min), for 30 min, 5 days/week	↑ Onset of disease ↑ Survival ↑ Motor performance
Desseille et al. (65)	M ($n = 18$)	70	45 days	Swimming in an adjustable-flow swimming pool (max. 5 l/min), for 30 min, 5 days/week	↑ Glucose tolerance ↑ Plasma lactate ↑ GLUT4 expression ↑ Lipid synthesis
Flis et al. (85)	M ($n = 8$) [M ($n = 6$)— survival study]	70	45 days	Swimming in an adjustable-flow swimming pool (max. 5 l/min), for 30 min, 5 days/week for the first 35 days, and then 3 days/week in the last 10 days.	↑ Survival ↓ Mitochondrial dysfunction ↓ Oxidative stress

M, male; F, female; GLUT4, glucose transporter type 4; statistically significant increase (↑) and decrease (↓) compared to sedentary/no-exercising SOD1^{G93A} ALS mice.

that regular, high intensity endurance training accelerated the deficit in motor performance and reduced survival in male, but not female, SOD1^{G93A} mice. High intensity endurance training did not increase survival in female SOD1^{G93A} mice (46). These sex differences could be explained by the potential neuroprotective role of estrogens, but further investigation is needed for confirming this postulation. Surprisingly, this type of exercise did not affect the onset of clinical symptoms in either male or female SOD1^{G93A} mice (46). Based on the authors' assumptions (46), the detrimental effects of high intensity endurance training on SOD1^{G93A} can be explained either from the intense activation of antioxidant system leading to increased oxidative stress in skeletal muscle and motor neurons or from the increased demand of adenosine triphosphate leading to excessive mitochondrial activation, which disrupts mitochondrial capacity (46). Although these physiological assumptions sound reasonable considering the potential decreased capacity of SOD1^{G93A} mice to restore the exercise-induced neuromuscular damage, more studies are needed for supporting this notion. Similarly, Kassa et al. did not find any change in disease onset after performing high intensity, endurance exercise in SOD1^{G93A} mice (63). Surprisingly, they suggested that this type of exercise may exert a neuroprotective effect on motoneurons acting on glial environment, reducing astrocytic activation and activating a protective microglial phenotype in SOD1^{G93A} mice. This finding seems inconsistent, as exercise did not improve the ALS phenotype. Considering that the sample size was only 5 SOD1^{G93A} mice, these results do not provide scientifically meaningful information.

Generally, high intensity endurance exercise is considered as a more potent stimulus for antioxidant enzyme adaptation than low intensity exercise (80). For this reason, it would be expected that higher exercise intensity may further improve the ALS phenotype and physiological responses of SOD1^{G93A} mice. However, the existing studies suggest that SOD1^{G93A} mice seem more vulnerable to high intensity stress-related stimuli (46, 61). Given that this type of mouse model has mutation in a major antioxidant enzyme (i.e., SOD1), the ability of these mice to deal with increased exercise-induced reactive oxygen species (ROS) levels may be restricted. Therefore, any extrapolation of the results should be interpreted with caution.

Effects of Exercise Duration in SOD1^{G93A} ALS Mice

The duration of physical activity may also affect the therapeutic benefit associated with endurance exercise in SOD1^{G93A} mice. Six and 12 h, but not 2 h, daily exposure to running wheels improves motor function in SOD1^{G93A} mice with a high-copy number of the transgene, with the 6 h exercise exhibiting the most beneficial effect on lifespan (62). These exercise benefits were observed in both male and female SOD1^{G93A} mice (62). In contrast, Liebetanz et al. (45) concluded that extensive endurance exercise including 10 h daily exposure to running wheels in either gender had no significant effect on the clinical onset and progression of disease or in the lifespan of SOD1^{G93A} mice (copy number is not reported) (45). However, exercised SOD1^{G93A} mice showed an increase in their survival by 1 week compared to the sedentary counterparts, although it was not statistically significant (45). Considering that 1 week in the age of mice corresponds to ∼1 year of life for humans when correlating their entire lifespan (81) and that the median survival of ALS patients is between 2 and 4 years from the onset of disease (1), the 1 week improvement in SOD1^{G93A} mice lifespan could be biologically significant, even if it did not reach the statistical significance ($p < 0.05$). In any case, the sample size ($n = 12$, 5 females/7 males) used in this study (45) is unlikely underpowered given previous evidence shown through power calculations that $n = 8$ SOD1^{G93A} mice per group are sufficient to give a significant difference in lifespan means ($p = 0.05$) with 80% power (82).

Table 1 provides an overview of the effects of exercise intensity and duration, as well as the effects of gender in response to exercise in SOD1^{G93A} mice.

Effects of Exercise Mode (Running vs. Swimming) in SOD1^{G93A} ALS Mice

Skeletal Muscle Improvements in Response to Swimming

Another type of endurance training investigated in SOD1^{G93A} mice is swimming. Swimming-based training using an adjustable-flow swimming pool can significantly delay the disease onset and extend the mean survival in male SOD1^{G93A} mice compared to sedentary male SOD1^{G93A} mice (64). Surprisingly, no improvement was found with running-based

training in the same study (64). Kirkinezos et al. who followed exactly the same training program, found a significant increase in the lifespan of male SOD1^{G93A} mice after running-based training (60). Considering that both studies used a similar sample size of male SOD1^{G93A} mice, the inconsistency of these results may be explained by the different time point that mice started exercising [49 day (60) vs. 70 days (64)]. In any case, findings of pre-clinical studies could have practical applications in clinical conditions only if exercise starts after disease onset. Moreover, only swimming-based training efficiently preserved the muscle phenotype including the number of myofibers, their cross-section area and distribution, in the ALS mice to the extent that it was similar in morphology to the muscles of the control group. Specifically, swimming limited the ALS-induced hypotrophy in both slow- and fast- twitch muscles, as well as maintaining the fast phenotype in fast-twitch muscles (64).

Metabolic Improvements in Response to Swimming

Improvements in glucose metabolism in the SOD1^{G93A} mice after swim training have also been reported (65). Specifically, swimming-based training restored impaired glucose tolerance in late symptomatic SOD1^{G93A} mice (65). On the other hand, running-based training had more modest effects (65). The benefits of swimming-based training were related to changes in skeletal muscle energetic metabolism of SOD1^{G93A} mice, shifting energetic fuels to the anaerobic glycolytic pathway (65). This metabolic shift was associated with an enhanced triglyceride storage in skeletal muscle and reduced the dysregulated expression of autophagic molecules such as Bcl2, Becn1, LC3b, and Sqstm1 (P62) (65). In case of huge energetic demands, autophagy can play a crucial role in skeletal muscle metabolic balance involving the degradation of cellular intrinsic components to produce the three main energetic macromolecules, glucose, lipids and amino acids (83). Given that ALS mice are characterized by an excessive lipid catabolism (84), glucose re-use and fat deposition induced by the swimming-based training are considered beneficial adaptations in SOD1^{G93A} mice. The beneficial role of swimming in ALS energetic metabolism is confirmed by Flis et al. who found improvements in skeletal muscle energy metabolism, oxidative stress, and mitochondrial and endoplasmic reticulum membrane modeling and function in SOD1^{G93A} mice after training in a swimming pool with an adjustable flow (85). The swim training prolonged the lifespan of SOD1^{G93A} mice with accompanying changes including increased caveolin-1, a key regulator of cholesterol efflux, decreased cholesterol accumulation in the crude mitochondrial function, improved bioenergetics (cytochrome c oxidase activity, respiratory capacity ratio, lactate dehydrogenase activity) and decreased oxidative stress.

Neuroprotective Improvements in Response to Swimming

Swimming also exhibited neuroprotective effects and improved motor function with delayed spinal motoneuron death and preservation of motoneurons with large soma area (64). In contrast, a dramatic motoneuron loss and an increased proportion of motoneurons with small soma area were observed after running-based training. Additionally, reduced astrogliosis and apoptosis of oligodendrocytes were evident in the spinal cord of SOD1^{G93A} mice after swimming-based training (64). This may be explained by the fact that swimming is associated with high hindlimb movement amplitude (cm) and frequency (cycles min^{-1}) exercise, preferentially activating a sub-population of large motoneurons innervating fast motor units (86). In contrast, running is related with low hindlimb movement amplitude and frequency exercise resulting in the activation of a sub-population of small motoneurons (86). Therefore, the combined beneficial effects of swimming on muscle, neuronal tissue and glucose metabolism suggests swimming, at least in ALS SOD1^{G93A} mice, as a very encouraging exercise intervention. **Table 2** provides an overview of the physiological adaptations to swimming in SOD1^{G93A} mice.

Overall, mild-to-moderate endurance training seems to have positive effects in SOD1^{G93A} mice, increasing the survival and motor performance as well as delaying the onset and progression of the disease with swimming-based endurance training perhaps the most beneficial type of exercise in ALS SOD1^{G93A} mice. Given that resistance training is suggested as a major type of exercise in many clinical cases, future studies using resistance-based exercise intervention in ALS mice are needed. Furthermore, since ALS is a disease with complex and multiple pathologic abnormalities, successful combinatorial therapeutic approaches with different but complementary mechanism of action may have a beneficial effect in ALS treatment. Scientific evidence suggests running with *ad libitum* exposure in running wheels, combined with virally-induced Insulin Growth Factor-1 overexpression, has synergistic and maximal effects on survival and motor function of SOD1^{G93A} mice, compared with either monotherapy-treated or no-treated SOD1^{G93A} mice (62). As a result, exercise training could be an adjunct therapy to other pharmacological treatments of ALS, but further investigation is needed to confirm this notion. Finally, an additional limitation is that the SOD1^{G93A} transgenic mouse, the most extensively used mouse model, is not representative of all ALS cases as mutations in the *SOD1* gene account for only 2% of all ALS, primarily familial, cases (87). In addition, *SOD1* gene is a major antioxidant enzyme and its mutation may make SOD1 mice unable to deal with any exercise-induced oxidative-stress. For these reasons, pre-clinical studies involving additional animal models based on both sporadic and familial ALS phenotype (88–90), are needed to advance our understanding of the effects of exercise in ALS.

EFFECTS OF EXERCISE IN ALS PATIENTS

Several clinical studies have been performed to determine whether endurance and/or resistance exercise ameliorates symptoms and improves the health of ALS patients. Regardless of its modality, exercise seems to have beneficial effects on the quality of life of ALS patients, but its impact on survival has not been confirmed. In any case, no negative outcomes have been found in ALS progression after an exercise intervention.

Endurance Exercise

Even though endurance exercise promotes cardiorespiratory fitness, cellular metabolism increasing mitochondrial biogenesis and intramuscular fuel stores, and psychological well-being, little is known about its effect on ALS pathophysiology. Pinto et al. (91) investigated the possibility of reducing motor decline by exercising ALS patients to the anaerobic threshold, simultaneously ensuring their respiratory insufficiency with the assistance of a non-invasive ventilator, the Bipap STD®. ALS patients performing a ramp treadmill exercise protocol for 1 year decreased the rate of respiratory deterioration and improved their functional independent mobility score, compared to a non-exercised control group (91). Similarly, Sanjak et al. (92) demonstrated that an 8 week walking program on a weight-supported treadmill for 30 min, three times weekly, significantly improved the ALS Functional Rating Scale (ALSFRS) score as well as tolerability, gait speed, distance and stride length during 6 min walk tests for ALS patients (92). The feasibility of performing moderate endurance training with non-invasive ventilation or body weight supporting system in ALS patients was confirmed recently where improvements in functionality and cardiorespiratory factors were found (93). The same research group also suggested that tele-monitored home-based endurance exercise consisting of 15 min walking on a treadmill or outdoors, is feasible, safe, user-friendly and had excellent compliance in ALS patients (94). Despite the absence of a non-exercise ALS control group in this study, there were no differences in cardiorespiratory factors (percentage of saturation of oxygen and force vital capacity) observed over the course of the exercise program (6 months). This implies a beneficial role of a home-based endurance training in ALS patients protecting them from the degenerative effects during disease progression. On the other hand, Clawson et al. recently reported that 24 week endurance training in both lower and upper body using a minicycle was not as well-tolerated as resistance training or stretching/range of motion exercises (95). Although the endurance training was not harmful for ALS patients in this study, it did not increase their muscle strength, functionality or quality of life. The inconsistency of these results could be explained by the different type of endurance training [cycling (95) vs. walking (91–94)] and the different muscle groups [upper and lower limbs (95) vs. only lower limbs (91–94)] activated during training sessions.

While respiratory muscle training is beyond the scope of this review, it is worth highlighting its potential to improve respiratory function, one of the major factors of aerobic/endurance training, in ALS patients. Specifically, scientific evidence shows that a 12 week inspiratory muscle training programme consisted of inhaling and exhaling through a specific device (Respironics®) may slow the decline in respiratory function in ALS patients through strengthening their inspiratory muscles (96). However, more detailed clinical assessment is needed as a later study using this type of training did not find either negative or positive effects on respiratory function of ALS patients (97). Furthermore, a combinatorial training including both respiratory muscle and endurance training could potentially induce positive adaptations for ALS patients, but this requires experimental validation.

Overall, endurance training with a supplemental support such as ventilation or weight support seems to have positive effects on respiratory capacity, functionality, tolerability and physical performance in ALS patients (**Table 3**), but further studies with a larger number of participants and inclusion of a control group are needed to confirm these findings. Given that each type of endurance training activates particular muscle groups and creates specific mechanical loading, different adaptations are induced by walking, cycling and swimming. Although swimming is suggested as an advantageous type of exercise in many neurological disorders (98–100), its beneficial role has not yet been experimentally validated in ALS patients. For this reason, further investigation is required to optimize an endurance training protocol for ALS patients.

Resistance Exercise

Resistance exercise improves muscle force/power, induces muscle hypertrophy, maintains skeletal muscle function and prevents disability (101). In ALS patients, resistance training imparts protective benefits despite not reducing disease progression (**Table 4**). One of the first published reports of resistance training in ALS patients concluded that resistance exercise to the upper extremities can increase muscle static strength of the upper body (102). A subsequent study observed that moderate regular resistance training may have a mild, temporary positive effect on motor deficit and disability in ALS patients (103). Specifically, modest-intensity resistance exercises involving most muscle groups of the four limbs and trunk, induced significant improvements on functional and spasticity scores, but not on muscle strength, fatigue, pain and quality-of-life scores after 3 months of intervention (103). However, after 6, 9, or 12 months of resistance training, no further change was observed in these parameters. Similarly, Kitano et al. (104) recently suggested a 6 month home-based, whole-body strength and stretching exercise program as a safe mode of exercise training without adverse effects in ALS patients. This exercise program alleviated the functional deterioration in patients with early- stage ALS, but did not improve their upper and lower body muscle strength. In addition to these results, a new study showed that although 24 week resistance training using both lower and upper body is well-tolerated and safe for ALS patients, it is not sufficient to improve their muscle strength, functionality or quality of life (95).

Furthermore, Lunetta et al. found that a 6 month strength training program, based on either active exercises against gravity in upper and lower limbs or passive exercises consisting of flexion-extension movements in the upper and lower limbs, cannot improve the functionality (ALSFRS total score), survival or quality of life in ALS patients, even though they were reporting subjectively an improvement in their sense of well-being at the end of every exercise session (48). On the other hand, a randomized controlled trial showed that a 6 month resistance training program with moderate intensity and moderate load involving both lower- and upper-body muscles, can induce significantly better function, less decline in leg strength, and higher quality of life in ALS patients without any adverse effects (47).

TABLE 3 | Summary of the effects of endurance training in ALS patients.

Study	Sex	Age (years)	Disease duration	Duration	Exercise protocol	Various outcomes
Clawson et al. (95)	M ($n = 15$) + F ($n = 5$)	58	7 months	24 weeks	10 min of upper limb exercise followed by 10 min lower limb exercise using a minicycle. 40–70% of target HR or 13–15 in Borg scale, 3 days/week	↓ Tolerability and compliance to exercise ↔ Functionality (ALSFRS score) ↔ Muscle strength ↔ VO_2 max
Pinto et al. (91)	M ($n = 6$) + F ($n = 2$)	62	13 months	12 months	Ramp treadmill protocol. Exercise performed with the assistance of the ventilator Bipap STD®.	↓ rate of respiratory deterioration ↑Functional independent mobility
Sanjak et al. (92)	M ($n = 3$) + F ($n = 3$)	58	N/A	8 weeks	Walking on a weight-supported treadmill for 30 min (6 sets × 5 min, 5 min rest), 3 days/week	↑ Tolerability (measured by RPE) ↑ Functionality (ALSFRS score) ↑ Exercise performance (distance, speed, stride length)
Braga et al. (93)	M ($n = 18$) + F ($n = 6$)	63	11 months	6 months	Moderate aerobic exercise on a treadmill with non-invasive ventilation and body weight supporting system, 2 days/week	↑ ALSFRS total score ↔ ALSFRS total score slope ↑ VO_2 peak
Braga et al. (94)	M($n = 7$) + F ($n = 3$)	57	8 months	6 months	Walking 15 min with 5 min warm-up and 5 cool-down, at 75% of HR_{max}, at least 1 day/week	↓ Functionality (ALSFRS score) ↓ Level of physical activity

M, male; F, female; N/A, not available; ALSFRS, ALS functional rating scale; VO_2 max, maximum rate of oxygen consumption; HR_{max}, maximum heart rate; statistically significant increase (↑), decrease (↓) and no statistically significant changes (↔) compared to no-exercising/"usual care" treated ALS patients or compared to pre-exercise values. Age represents the average age of patients.

In contrast, negative outcomes in neuromuscular function of ALS patients have been found after 12 weeks of resistance training (49). The whole-body resistance training did not significantly affect functionality and average cross-section area of type I and II muscle fibers, but loss of muscle strength and power in both lower and upper body muscle groups increased following the training period (49). Also, an increase in the percentage of both atrophied and greatly (abnormal) hypertrophied type II fibers combined with a decrease in normal-sized type II fibers were observed after the training period (49). Taking into account of the small number of patients (only 5) recruited in this study (49), their heterogeneous disease progression and the study design (12 week "lead-in" control period without exercising), the findings should be interpreted with caution. In ALS studies, the use of a lead-in control period (i.e., a period with no exercise), before receiving an intervention instead of using a control, non-exercised ALS group may confound the final results. Considering the potential rapid decline in health and the immediacy of death in ALS patients, the muscle fiber atrophy and the deteriorated muscle strength after resistance training may be partly explained by the disease progression occurring during the 12 week lead-in period rather than by any detrimental effects caused by exercise.

In summary, most studies suggest that resistance training can improve the quality of life of ALS patients increasing their functionality and, in some cases, their muscle strength. However, resistance training seems to have no effect on the lifespan of ALS patients. Future attempts to restrict, as much as possible, the inherent limitations of ALS clinical studies such as the small sample size and the heterogeneity of patients are needed for confirming the existing findings. Ideally, further studies with a stronger experimental design including a greater number of participants with homogeneous disease progression would provide more valid information about the effects of resistance training in ALS patients. Unfortunately, the reality is different as there are too few patients and only a small proportion of them eventually participates in exercise clinical trials.

Concurrent Endurance and Resistance Exercise

Concurrent training is a type of exercise that combines endurance and resistance training models and can stimulate both aerobic and anaerobic metabolic pathways, inducing a variety of beneficial adaptations. A recent randomized controlled trial suggested that a 6 month exercise program involving a combination of resistance and endurance training can reduce the motor deterioration of ALS patients (48). Specifically, the exercise training consisting of active exercises against gravity in the upper and lower limbs combined with cycle ergometer activity for 20 min increased the functionality (ALSFRS total score) of ALS patients. Furthermore, all exercised ALS patients reported subjectively an improvement in their sense of well-being at the end of every exercise session, although no improvements in their survival, respiratory function and quality of life were observed (48). However, the limited number of patients ($n = 10$, sex ratio is not reported) (48) raises concerns about the interpretation of these findings and, therefore, these results should be considered with caution. Additionally, a recent study suggested concurrent training as a feasible and beneficial intervention for ALS patients (105). Specifically, Merico et al. found that 5 week moderate, submaximal resistance and endurance training improved the functional independence, oxygen consumption, fatigue and muscle strength in ALS patients (105). The positive effects of combined endurance and resistance training in ALS patients were also confirmed in a retrospective, non-consecutive case

TABLE 4 | Summary of the effects of resistance training in ALS patients.

Study	Sex	Age (years)	Disease duration	Duration	Exercise protocol	Various outcomes
Bohannon (102)	F ($n = 1$)	56	22 months	75 days	Resistance exercises to upper extremities (2 sets × 10 reps, 5 min rest), for 6 days/week	↑ Static force in 14 muscle groups of upper extremities ↓ Static force in 4 muscle groups of upper extremities
Drory et al. (103)	M ($n = 8$) + F ($n = 6$)	58	21 months	12 months	Resistance exercises to whole body, against modest loads, lasted 15 min twice daily	↑ Functionality (ALSFRS score) at 3 months only ↔ Manual muscle strength
Kitano et al. (104)	M ($n = 15$) + F ($n = 6$)	63	2 months	6 months	Resistance training and stretching for the trunk muscles, upper and lower limbs, 6 days/week	↔ ALSFRS total score, but ↑ compared to control group ↔ Muscle strength of upper and lower body
Clawson et al. (95)	M ($n = 9$) + F ($n = 9$)	64	7 months	24 weeks	Resistance exercise in upper and lower body at 40% (week: 0–2), 50% (week: 3–4), and 70% (week: 5–24) 1RM, 3–5 s between reps, 2 min between sets, 4 min between muscle groups, 3 days/week	↔ Tolerability and compliance to exercise ↔ Functionality (ALSFRS score) ↔ Muscle strength
Bello-Haas et al. (47)	N/A ($n = 13$)	N/A	N/A	6 months	Moderate-load and moderate-intensity resistance exercise program to upper and lower extremities, 3 days/week	↑ Functionality (total and combined ALSFRS score) ↑ Quality of life (SF-36 score) ↓ Decline in leg strength
Jensen et al. (49)	M ($n = 5$) + F ($n = 1$)	62	<12 months ($n = 5$) 180 months ($n = 1$) Mean value N/A	12 weeks	Resistance exercises targeting both upper and lower body (2 sets × 5 reps at 6RM), 2–3 days/week	↔ Functionality (ALSFRS score and TUG) ↑ Functionality (measured by 30 s chair rise) ↓ Lower and upper body strength and power
Lunetta et al. (48)	N/A ($n = 10$)	18–75	≤24 months	6 months	Active exercises against gravity in six muscle groups in the upper and lower limbs (3 sets × 3 reps each muscle group) performed daily for 2 weeks each month	↔ Functionality (ALSFRS total score) ↔ Survival ↔ Respiratory function ↑ Subjective sense of well-being
	N/A ($n = 10$)	18–75	≤24 months	6 months	Passive exercises consisting of 20 min of 20 flexion–extension movements per minute in the upper and lower limbs, daily for 2 weeks/month	↔ Functionality (ALSFRS total score) ↔ Survival ↔ Respiratory function

M, male; F, female; N/A, not available; Reps, repetitions; ALSFRS, ALS functional rating scale; SF-36, MOS 36-Item Short Form Survey; TUG, timed up and go; statistically significant increase (↑), decrease (↓) and no statistically significant changes (↔) compared to no-exercising/"usual care" treated ALS patients or compared to pre-exercise values. Age represents either the average age or the age range of patients.

series study (106). In this study, 2 week endurance training on a cycle ergometer combined with lower-body resistance training appeared feasible and beneficial for ALS patients improving their muscle strength at the early stage of disease but not at the late stage of disease (106). Given that initial improvements in muscle strength, especially among untrained people, are explained mainly by the increased voluntary neural activation of the trained muscles and not by muscle hypertrophy which has a gradually increasing role in strength development as the training proceeds (after the first 3 to 5 weeks) (107, 108), a longer exercise intervention (>5 weeks) could induce further and greater skeletal muscle adaptations in ALS patients at either early or late stage of disease. In addition, while encouraging, these findings should be interpreted carefully considering the lack of statistical analysis due to the very small sample size ($n = 2$) used in this study (106). An overview of the effects of concurrent endurance and resistance training in ALS patients is summarized in **Table 5**.

Given the inherent methodological limitations involving clinical studies of rare and rapidly fatal diseases, such as ALS studies, further investigation with sufficient cohort size, limited variability of ALS causality, less heterogeneity in the disease stage of patients and adequate control of confounders is needed for confirming the existing findings of endurance, resistance, and concurrent training clinical studies in ALS patients. In addition, experimental designs including control group and proper statistical analysis are important for concluding to valid results. Also, future studies involving alternative training modes with less risk of muscle damage and injuries, such as swimming, could provide useful information to conclude to an optimal exercise program for ALS patients. Recent pre-clinical studies suggest swimming (64, 65, 85) as the most beneficial type of

TABLE 5 | Summary of the effects of concurrent endurance and resistance training in ALS patients.

Study	Sex	Age (years)	Disease duration	Duration	Exercise protocol	Effects of exercise
Lunetta et al. (48)	N/A ($n = 10$)	18–75	≤24 months	6 months	Active exercises against gravity in the upper and lower limbs (3 sets × 3 reps each muscle group) combined with cycle ergometer activity at 60% maximal power output, for 20 min. Performed daily for 2 weeks/month	↑ Functionality (measured by ALSFRS total score) ↔ Survival ↔ Respiratory function
Merico et al. (105)	M ($n = 17$) + F ($n = 9$)	62	30 months	5 weeks	Resistance training with submaximal isometric contractions, 3 reps with 30 s of rest between for each bilateral muscle segment, daily for 5 weeks. + Endurance training at 65% HR_{max}, 15–20 min on a cycle ergometer, ergometry arm-leg and/or treadmill, based on the weakness pattern of each patient, daily for 5 weeks.	↑ Functionality (FIM) ↓ VO_2 submax ↑ Muscle strength (MRC muscle grading scale) ↔ Muscle strength (dynamometric measures) ↔ 6 min walk test ↑ Fatigue Severity Scale (compared to the baseline, but not to the control group)
Kato et al. (106)	M ($n = 2$)	1st case: 60 2nd case: 52	1st and 2nd case: 1st intervention: 10 and 15 months; 2nd intervention: 20 months for both	1st and 2nd case: 2 weeks for each intervention	Both cases: 1st and 2nd intervention: lower-body resistance training using weights and machines, at 5 score of Borg scale (lower limbs), combined with endurance training on a bicycle ergometer, respiratory exercises and gait exercises	1st and 2nd case: 1st intervention: ↑ knee extension muscle strength ↔ and ↑ Functionality (FAC score) 2nd intervention: ↔ knee extension muscle strength ↔ Functionality (FAC score)

M, male; F, female; N/A, not available; Rep, repetitions; ALSFRS, ALS functional rating scale; VO_2 submax, oxygen uptake at submaximal work; HR_{max}, maximum heart rate; FIM, Functional Independence Measure; MRC, medical research council; FAC, functional ambulation categories; statistically significant increase (↑), decrease (↓) and no statistically significant changes (↔) compared to no-exercising/"usual care" treated ALS patients or compared to pre-exercise values. Age represents either the average age or the age range of patients.

exercise in ALS, but clinical studies are needed to translate these mouse exercise results to humans.

CONCLUSIONS

Although previous evidence suggests a potential relationship between heavily active lifestyles and an increased incidence of ALS (30, 31, 109), recent studies support that physical activity is not necessarily a risk factor for ALS (42, 43, 110). There is now a large body of evidence suggesting physical activity as a potential therapeutic or even holistic approach for ALS.

Pre-clinical mouse studies conclude that the intensity, duration and mode of exercise, as well as gender, can influence health outcomes in ALS progression as identified in $SOD1^{G93A}$ mice in response to regular long-term endurance training (44, 61, 62, 64). Findings from this review suggest that mild-to-moderate endurance training is a potential beneficial exercise intervention for ALS $SOD1^{G93A}$ mice (44, 60, 61), in particular, swimming (64, 65, 85). However, many of these pre-clinical studies are characterized by limitations related to variable sample sizes, heterogeneous transgene copy number or a non-clinically-relevant timing of intervention. An additional limitation is the lack of pre-clinical studies focused on the effect of resistance-based exercise, a major type of exercise, in ALS. A current *in vivo* study shows that repeated sessions of isometric tetanic contractions, which represent a resistance-based exercise protocol for experimental animals, can improve contractile function and ameliorate distinct histopathological features of skeletal muscle in Duchenne muscular dystrophy circumventing the concern of potentially injurious eccentric contractions (111). A similar resistance-based exercise program including non-injurious isometric contractions could also have positive effects in skeletal muscle of ALS mice. Further pre-clinical studies are needed to investigate the effects of exercise not only *per se* but also as an adjunct therapy to other pharmacological treatments of ALS. Finally, except for the $SOD1^{G93A}$ transgenic mouse, which represents only the 2% of all, primarily familial, cases, additional animal models based on both sporadic and familial ALS phenotype (88–90) are needed to advance our understanding of the effects of exercise in ALS.

Despite mild-to-moderate exercise training improving the survival of ALS mice, currently, there is no clinical evidence supporting this notion in ALS patients (48). Regardless, most clinical studies suggest that every type of exercise training including stretching, resistance, endurance or concurrent training, has an advantageous impact on the quality of life of ALS patients increasing mainly their functionality (47, 48, 91–93, 103, 105) and sometimes their muscle strength (105, 106) and/or their cardiorespiratory function (91, 93, 105). The discrepancy between preclinical and clinical findings could be partly explained by the limitations observed in most of clinical studies in ALS, such as a small sample size, non-representative

control populations, inadequate control of confounders and heterogeneous disease stage of patients.

Importantly, the effect of swimming has not yet been experimentally validated in ALS patients, although exercising in water is commonly recommended for patients with other neurological disorders such as Parkinson's disease and multiple sclerosis (98–100). Given that swimming movements mainly incorporate concentric muscle contraction, a swim-based training may protect skeletal muscles of ALS patients from the high muscle stress induced by eccentric muscle contraction experienced with other types of exercise, such as running or resistance training. Furthermore, the water minimizes biomechanical stress on muscles and joints decreasing the risk of muscle damages and injuries. As water itself creates resistance to movement, performing exercises in water has been suggested as an alternative training mode to improve neuromuscular conditioning in healthy population (112, 113). Considering all these aspects, swimming with supplemental support or aquatic exercise training could be a potential exercise treatment for ALS patients.

While the benefits of exercise in ALS patients are still not clear, nor have the effects of swimming yet been determined, there appears promise ahead for further studies investigating the therapeutic benefits of exercise, which should be supported by well-designed and statistically powered, pre-clinical studies in multiple rodent models of ALS. In addition to the physical impact, the holistic value of exercise may also be key to the improved well-being of ALS patients.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

Deakin University (Victoria, Australia) provided funding support for publishing this work.

ACKNOWLEDGMENTS

ST was supported by an International Postgraduate Research Scholarship, awarded by the Deakin University (Victoria, Australia) and a Greek Scholarship for doctoral studies abroad, awarded by the Onassis Foundation (Greece).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 25 July 2019
doi: 10.3389/fneur.2019.00790

Physical Exercise and Mitochondrial Disease: Insights From a Mouse Model

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Purpose: Mitochondrial diseases (MD) are among the most prevalent neuromuscular disorders. Unfortunately, no curative treatment is yet available. This study analyzed the effects of exercise training in an animal model of respiratory chain complex I deficiency, the Harlequin (*Hq*) mouse, which replicates the clinical features of this condition.

Methods: Male heterozygous Harlequin (*Hq*/Y) mice were assigned to an "exercise" ($n = 10$) or a "sedentary" control group ($n = 11$), with the former being submitted to an 8 week combined exercise training intervention (aerobic + resistance training performed five times/week). Aerobic fitness, grip strength, and balance were assessed at the beginning and at the end of the intervention period in all the *Hq* mice. Muscle biochemical analyses (with results expressed as percentage of reference data from age/sex-matched sedentary wild-type mice [$n = 12$]) were performed at the end of the aforementioned period for the assessment of major molecular signaling pathways involved in muscle anabolism (mTOR activation) and mitochondrial biogenesis (proliferator activated receptor gamma co-activator 1α [PGC-1α] levels), and enzyme activity and levels of respiratory chain complexes, and antioxidant enzyme levels.

Results: Exercise training resulted in significant improvements in aerobic fitness (−33 ± 13 m and 83 ± 43 m for the difference post- vs. pre-intervention in total distance covered in the treadmill tests in control and exercise group, respectively, $p = 0.014$) and muscle strength (2 ± 4 g vs. 17 ± 6 g for the difference post vs. pre-intervention, $p = 0.037$) compared to the control group. Higher levels of ribosomal protein S6 kinase beta-1 phosphorylated at threonine 389 (156 ± 30% vs. 249 ± 30%, $p = 0.028$) and PGC-1α (82 ± 7% vs. 126 ± 19% $p = 0.032$) were observed in the exercise-trained mice compared with the control group. A higher activity of respiratory chain complexes I (75 ± 4% vs. 95 ± 6%, $p = 0.019$), III (79 ± 5% vs. 97 ± 4%, $p = 0.031$), and V (77 ± 9% vs. 105 ± 9%, $p = 0.024$) was also found with exercise training. Exercised mice presented with lower catalase levels (204 ± 22% vs. 141 ± 23%, $p = 0.036$).

Conclusion: In a mouse model of MD, a training intervention combining aerobic and resistance exercise increased aerobic fitness and muscle strength, and mild

OPEN ACCESS

Edited by:
Mauro Marzorati, Institute of Biomedical Technologies (CNR), Italy

Reviewed by:
Olimpia Musumeci, University of Messina, Italy
Marshall Riley, Belfast City Hospital, United Kingdom

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Specialty section:
This article was submitted to Neuromuscular Diseases, a section of the journal Frontiers in Neurology

Received: *28 March 2019*
Accepted: *09 July 2019*
Published: *25 July 2019*

Citation:
Fiuza-Luces C, Valenzuela PL, Laine-Menéndez S, Fernández-de la Torre M, Bermejo-Gómez V, Rufián-Vázquez L, Arenas J, Martín MA, Lucia A and Morán M (2019) Physical Exercise and Mitochondrial Disease: Insights From a Mouse Model. Front. Neurol. 10:790. doi: 10.3389/fneur.2019.00790

improvements were found for activated signaling pathways involved in muscle mitochondrial biogenesis and anabolism, OXPHOS complex activity, and redox status in muscle tissue.

Keywords: rare diseases, mitochondrial diseases, OXPHOS, harlequin mutant mouse, resistance training, AIF deficiency, respiratory chain complex I

INTRODUCTION

Mitochondrial diseases (MD) encompass a heterogeneous group of rare diseases caused by defects in oxidative phosphorylation (OXPHOS) (1, 2), and are among the most common neuromuscular disorders, but showing variable estimated prevalence (3–5). Given the ubiquity of mitochondria and their essential role in ATP production, MD can affect several tissues and those with higher metabolic demands, particularly (but not only) the skeletal muscle, are usually the most affected, with mitochondrial myopathy and poor exercise capacity (as reflected by low levels of aerobic fitness) being frequent features among patients with MD (6, 7).

Although no curative treatment is yet available for MD, preclinical evidence from animal and *in vitro* studies suggests that promoting the increase in mitochondrial content or mitochondrial biogenesis, through the activation of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) might be a therapeutic option (8, 9). In this respect, regular endurance exercise is known to be a potent stimulus for muscle mitochondrial biogenesis (10), and in fact previous research has shown that endurance-based exercise interventions can increase the muscle oxidative capacity of patients with MD, as directly reflected by increases in the activity of citrate synthase (CS) and respiratory chain complexes in skeletal muscle biopsies (11–13), or indirectly, by improvements in patients' aerobic fitness (11–18). Another exercise modality, resistance or "strength" training (e.g., weight lifting), is also known to promote muscle mitochondrial biogenesis (19) together with other benefits, notably improvements in muscle mass and strength (20), but scarce data are available in MD patients. One study assessed the effects of a 12 week resistance training program in 8 patients with mitochondrial DNA large scale deletions, showing strength improvements and a decrease in mitochondrial DNA heteroplasmy without side effects (21). Another study found an increase in upper-body muscle strength after 12 weeks of combined resistance and endurance training in 10 mitochondrial patients with biopsy diagnosis of MD but with no genetic confirmation (22). Finally, we recently described significant improvements in upper-, lower-body, and respiratory muscle strength in 12 well-characterized MD patients (all with genetic diagnosis) after 8 weeks of combined resistance, endurance and respiratory training (14).

On the other hand, studying mouse models of MD allows to gain mechanistic insight into the molecular processes underlying exercise benefits in these conditions. In this respect, although previous exercise training studies have been published with mouse models of respiratory chain complex IV deficiency (23) or of a defect in mitochondrial polymerase gamma (i.e., "mutator" mice) (24–26), the effects of exercise-training on a mouse model of the commonest mitochondrial defect, respiratory chain complex I deficiency, have yet not been assessed. Further, the effects of resistance training remain to be determined in mouse models of MD.

The aim of the present study was to determine the effects of a combined exercise intervention (aerobic + resistance exercise) in the aerobic fitness, muscle strength and muscle-tissue adaptations at the molecular level of the Harlequin (*Hq*) mutant mouse. In this animal, proviral insertion in the X-linked gene encoding Apoptosis Inducing Factor (AIF) produces a large decrement (∼80%) in the expression of this mitochondrial protein (27), leading to respiratory chain complex I deficiency (28) and to a phenotype that mimics the clinical features of affected patients, including skeletal muscle myopathy (29–31).

MATERIALS AND METHODS

Animals

All experimental protocols were approved by the institutional ethics committee (project number 111/15) and were conducted in accordance with European (European convention ETS 123) and Spanish (32/2007 and R.D. 1201/2005) laws on animal protection in research.

Heterozygous 6–8 week-old, male Harlequin mice ($n = 21$, *Hq*/Y named "*Hq*" hereafter, B6CBACa Aw-J/A-Aifm1Hq/J) and gender and age-matched wild-type (WT) mice ($n = 12$) of the same genetic background (B6CBACa Aw-J/A), were obtained from The Jackson Laboratory (Bar Harbor, ME). Animals were housed at 21°C and 60% humidity with a 12-h light/dark cycle and with free access to food and water in the animal facility of *Hospital Universitario 12 de Octubre* (Madrid, Spain). To ensure that all mice presented with the same degree of disease development at the moment of their incorporation into the study groups, each *Hq* mouse was previously evaluated from 8 weeks of age until the first signals of ataxia appeared following a locomotor skill and balance evaluation (**Figure 1**). The data obtained from the WT animals during the same period were used as a reference for absence of the aforementioned alterations as well as for biochemical analyses (see below).

Locomotion analysis was assessed while the mouse walked at a speed of 10 $cm{\cdot}s^{-1}$ (15 min, 15% inclination) on a treadmill (Harvard Apparatus; Panlab, Barcelona, Spain) during the even weeks of the locomotor skill evaluation phase. All mice were allowed to adapt to the treadmill in three familiarization sessions. An electric grid was placed at the end of the treadmill to deliver electrical shocks of constant intensity to encourage the animals to continue running on the treadmill. These sessions involved a gradual increase in running time and intensity,

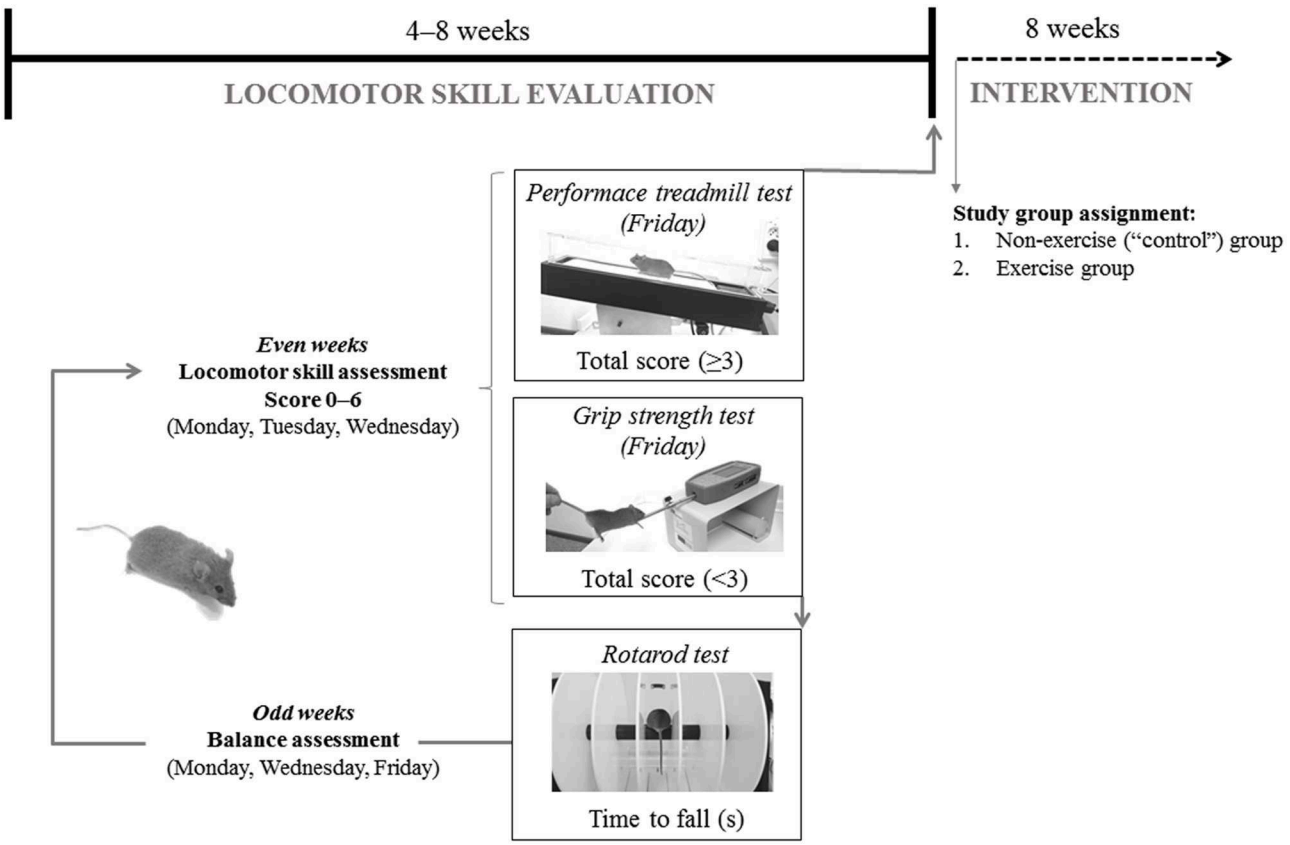

FIGURE 1 | Representation of mice baseline evaluation before group assignment.

starting just placing the mice on the treadmill the first day: 0% inclination and 0 $cm{\cdot}s^{-1}$ speed for 1 min, with no electrical stimulation, and ending with a 15 min running period at low intensity on the third day (15% and 10 $cm{\cdot}s^{-1}$, electrical stimulation 0.1 mA, 1 Hz, 200 ms). Two examiners scored the motor function of each mouse and, if two scores did not match, the lowest score was chosen. Scores ranged from 0 (absence) to 2 (maximal severity grade) in the following manner: (i) walking gait balance, 0, 1, or 2 for smooth gait pattern, mild imbalanced and unstable walking gait, respectively; (ii) 0, 1, or 2 for ability to walk straight, first signs of diagonal walk and gait instability, or inability to walk straight, respectively; (iii) alterations in hind limb gait, 0, 1, or 2 for normal coordination, first signs of alterations in the stride frequency, and lack of hind left-right limb coordination, respectively. A total score (0–6) was generated by summing individual scores. Data from the 3 days was averaged and if the score was <3, the animals continued in the locomotor skill evaluation phase and performed the grip strength test (described below). Conversely, animals with an average score ≥ 3 were included in the training phase and were subjected to a treadmill performance test (described below).

During the locomotor skill evaluation phase, cerebellar ataxia was also tested, using the rotarod device (Letica Scientific Instruments; Panlab, Barcelona, Spain) on alternative days (odd weeks, 3 days in total) to avoid fatigue and following the protocol described elsewhere (27). To familiarize with the apparatus, mice underwent a training session of three trials (60 s each) in which the rod was kept stationary for the first trial and held at 4 rpm for the last two trials (32).

Group Assignment

Hq mice were paired-matched based on their aerobic fitness in the treadmill test (see below for a description of the assessment method) at baseline. Thus, among each pair of *Hq* mice with similar performance in the pre-intervention aerobic treadmill tests, one mouse was randomly assigned to an *exercise* group ($n = 10$), subjected to an 8 week exercise training program, and the other to a *non-exercise* (control) group ($n = 11$), allowed to freely move in the cage, but not performing the program (**Figure 2**). On the other hand, none of the WT mice used as reference underwent exercise training (i.e., same treatment as for the *Hq* control group).

Exercise Training

The exercise intervention included 5 weekly sessions (Monday–Friday; session duration of 40–60 min), each including aerobic and resistance training.

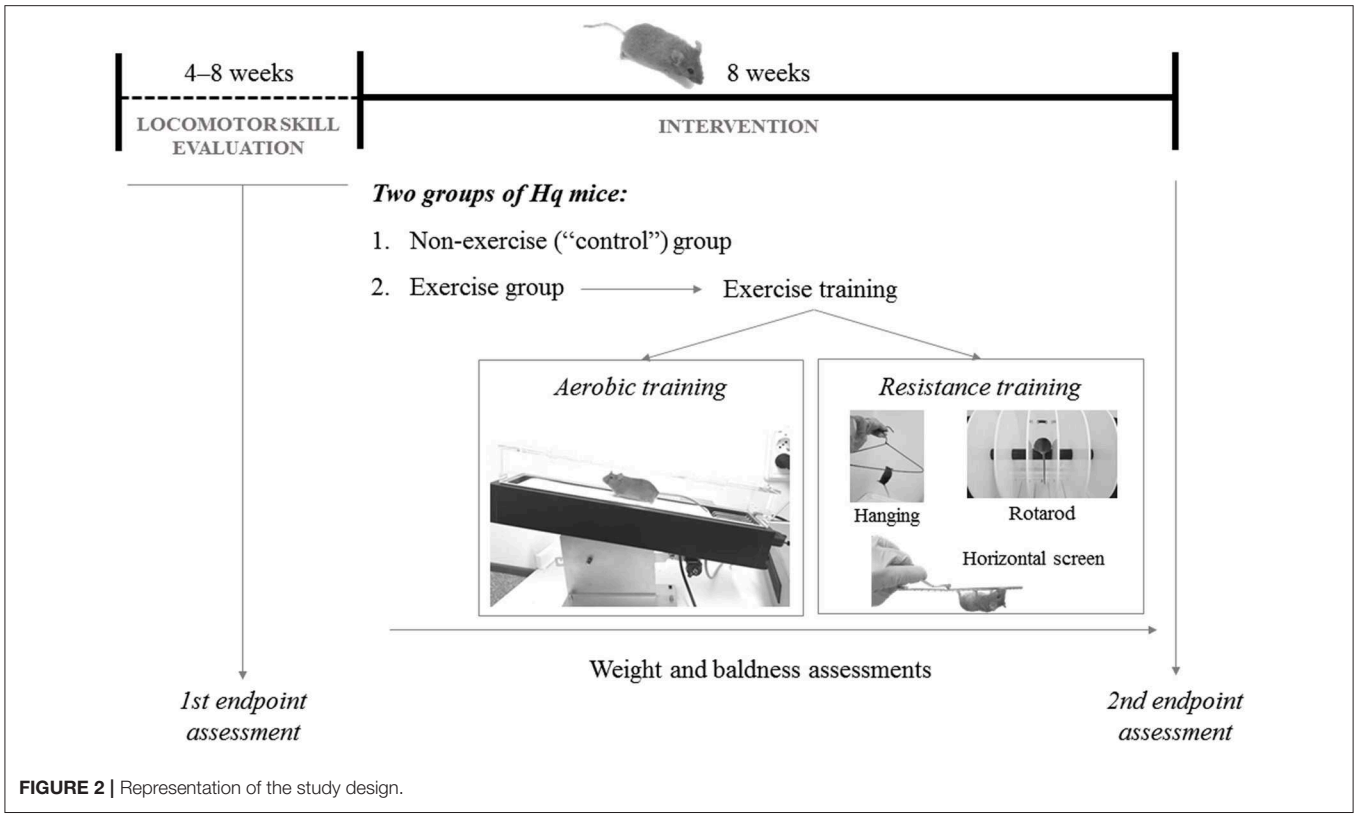

FIGURE 2 | Representation of the study design.

Aerobic Training

Hq mice were allowed to adapt to the treadmill (Harvard Apparatus; Panlab, Barcelona, Spain) before their incorporation into the exercise intervention, as previously described (33). Exercise duration, treadmill speed, and inclination were gradually increased during the program following a defined interval training protocol from our group, with slight modifications (33). Briefly, beginning at very low workloads in the first session (20 min at 35% of the maximal velocity obtained during the aerobic fitness test that is described below) and 0% gradient on the first day, and ending with 40 min at 65–70% of maximal velocity and 15% gradient in the last sessions. Only gentle tail touching was used to prompt the mice to run, and no electrical stimulation was applied during the training sessions.

Resistance Training

This followed the aerobic training and included three exercises: (i) horizontal screen exercise (Monday); (ii) hanging exercise with two limbs (Wednesday and Friday); and (iii) rotarod exercise (Tuesday and Thursday). For (i), the *Hq* mouse was placed inverted on top of a screen and had to climb back over to the top. When this was achieved, the mouse was placed again in the initial position. The number of repetitions was gradually increased (from 1 to 6), each also of increasing duration (from 30 s at the beginning to 90 s at the end of the training phase), with a constant 2 min rest period between repetitions. For (ii), *Hq* mice were picked up by the tail and placed on a metal cloth hanger taped to a shelf and maintained at 40 cm above a layer of bedding to cushion the falls (32). Mice were allowed to grasp the wire only using the two forepaws for as long as they could during one set of six repetitions, with a 10 min rest period between them. For (iii), each *Hq* mouse was placed onto the rod in a rotarod device (Letica Scientific Instruments, Panlab; Barcelona, Spain) and walked the rotating rod at speeds that increased from 4 to 40 rpm over a 300 s period. When the first fall occurred, the number of rpm was recorded, and the mouse exercised at this speed during 5 min (32).

Measurements

Study endpoints were assessed at the start and the end of the exercise intervention except for muscle molecular measurements, which could only be measured at the end. Serum samples were obtained before and after concluding the exercise program. Forty eight hours after the last exercise test, *Hq* mice were killed by cervical dislocation and the *biceps femoris* and *quadriceps femoris* muscles were dissected and immediately snap-frozen in liquid nitrogen before storage at −80°C until molecular analysis. The WT mice were sacrificed with the aforementioned procedure at the same time point.

Aerobic Fitness

An incremental treadmill test was used to determine the maximal aerobic fitness (expressed as the total distance [meters] run by the mice) of *Hq* mice (33). The test was performed after a warm-up period of 10 min at a speed of 10 $cm \cdot s^{-1}$ (with 15% inclination) and followed a previous protocol from our group

with slight modifications in workload increases (33). Thus, the initial velocity was 5 cm·s^{-1} and was increased in 2 cm·s^{-1} every 2 min until exhaustion, with a constant treadmill inclination of 15% during the whole test. Mice were considered exhausted when they spent more than 5 continuous s on the electric grid and were unable to continue running at the next speed (33).

Forelimb Grip Strength

Grip strength of *Hq* mice was measured using an isometric force transducer (Harvard apparatus; Panlab, Barcelona, Spain). Maximum force (grams) exerted by the mouse before losing grip was recorded in three trials (each separated from the next by a 5 min interval). The best score for each animal was recorded as the maximal grip strength.

Hq Phenotype

Cerebellar ataxia in *Hq* mice was tested on the rotarod device. Each mouse walked the rotating rod at speeds that increased from 4 to 40 rpm over a 300 s period (27). The latency to fall from the rotating rod was recorded in each trial. The best of the 3 day latency was considered for analysis. We additionally evaluated two other hallmarks of the *Hq* phenotype (27) weekly throughout the study: (i) hair loss, which was assessed as the percentage of body surface area without hair (30), and (ii) weight.

Serum Determinations

Muscle damage in *Hq* mice was assessed through the measurement of creatine kinase (CK) activity in serum samples (Creatine Kinase Activity Assay Kit; Sigma Aldrich, MO).

Tissue Processing

Muscle tissue of both *Hq* and WT mice was either processed to obtain total homogenates as described previously (33), or processed to obtain mitochondria-enriched fractions with a specific kit (Mitochondria Isolation Kit for Tissue; Abcam, UK) according to manufacturers' instructions.

Western Blotting

Aliquots of total homogenates or mitochondria-enriched fractions containing equal amount of protein were resolved by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) electrophoresis, transferred to polyvinylidene difluoride (PVDF) membranes, blocked, and incubated with primary antibodies for the determination of levels of the following proteins: catalase (CAT), citrate synthase (CS), cytosolic superoxide dismutase (cSOD), gluthathione reductase (GR), mitochondrial superoxide dismutase (mtSOD), mitochondrial transcription factor A (TFAM), NADH-ubiquinone oxidoreductase subunit B8 (NDUFB8) and S1 (NDUFS1), peroxisome proliferator-activated receptor-gamma co-activator 1α (PGC-1α), and ribosomal protein S6 kinase beta-1 total (P70S6K) or phosphorylated at threonine 389 (P70S6K Thr389, or simply "phosphorylated" pP70S6K) (**Supplementary Table 1**). After incubation with peroxidase-conjugated secondary antibodies, immunoreactive bands were detected with the ECL Prime Western Blotting Detection Reagent (Amersham Biosciences, UK) in a ChemiDoc^{TM} MP Imager (Bio-Rad, Hercules, CA). Band densities were evaluated by densitometric scanning [ImageJ software, National Institutes of Health (34)]. Either glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was immunodetected and used as loading control for total homogenates, or total protein load per lane was determined with Coomassie Blue staining of the PVDF membranes (35). When needed, membrane stripping was performed before immunodetection of GAPDH.

Spectrophotometry

Activities of OXPHOS complexes were determined in muscle total homogenates prepared in 225 mM mannitol, 75 mM sucrose, 0.1 mM EDTA, and 10 mM Tris–HCl pH 7.4, according to standardized protocols for spectrophotometric assays (36), and in mitochondria-enriched fractions by a researcher blinded to the mouse group (control *Hq*, exercised *Hq*, or WT) from which the samples were obtained. For complexes II–IV, 20 μg of protein was used in total homogenates, and 6 μg in mitochondria-enriched fractions, whereas 60 μg of protein was added to the assay for complex I in total homogenates, and 10 μg in mitochondria-enriched fractions. Complex V activity was assayed as F1-ATPase using 20 μg of protein according to a previously described method (37). CS activity, was spectrophotometrically determined at 30°C in the presence of 0.1% Triton X-100 following the formation of 5-thio-2-nitrobenzoic acid at 412 nm, as previously described (38), using 60 μg of protein in total homogenates and 6 μg in mitochondria-enriched fractions.

All the aforementioned muscle biochemical variables obtained with western blotting or spectrophotometry in samples of *Hq* mice were expressed relative to the corresponding mean values for the WT mice.

Histochemistry

For muscle histology *tibialis anterior* muscles were frozen in N_2-cooled isopentane, and hematoxylin-eosin staining on transverse sections of muscle was performed on cryostat sections according to standard techniques (39), and cross-sectional area of fibers was determined with Image J software.

Statistical Approach

Data are presented as mean ± standard error of measurement (SEM). The normal distribution (Shapiro-Wilk test) and homoscedasticity (Levene's test) of the data were checked before any statistical treatment. A two-factor (group [exercise, control] by time) ANOVA with repeated measures on time was used for between-group comparisons of outcomes with more than one measurement over time (i.e., aerobic fitness, muscle strength, balance, serum CK, hair loss and weight). To minimize the risk of type I error, *post hoc* comparisons were only performed within-groups (with the Bonferroni test) when a significant interaction group by time was found. The non-parametric Mann–Whitney's *U*-test was used for between-group comparisons of outcomes assessed at one single time point (i.e., muscle biochemical analyses). All statistical analyses were performed with the SPSS 23.0 package (SPSS, Inc., Chicago, IL) setting the significance level at 0.05.

RESULTS

Endpoints With Repeated Measurements in Time

A significant group by time interaction effect was found for aerobic fitness ($p = 0.014$, effect size [η^2] = 0.304, power = 0.728), with *post hoc* analyses indicating an improvement after the training intervention ($p = 0.016$) and no change in control mice after the same time period ($p = 0.277$) (**Table 1**). A significant group by time interaction effect was also observed for muscle strength ($p = 0.037$, $\eta^2 = 0.209$, power = 0.566), which increased in the exercise group ($p = 0.003$) but did not change in control mice ($p = 0.751$). Individual data on aerobic fitness and muscle strength by group are shown in **Supplementary Figure 1**. On the other hand, no significant group by time interaction was found for time to fall ($p = 0.544$), serum CK ($p = 0.290$) (**Table 1**), body weight ($p = 0.058$), or hair loss ($p = 0.406$) (**Supplementary Figure 2**).

Endpoints With Single Measurement in Time

Higher levels of total P70S6K and pP70S6K were observed at the end of the training intervention in the exercise group compared with the control group (**Table 2**; **Supplementary Figure 3A**), which also showed high levels compared to the wild-type animals, as well as a non-significant trend toward a higher pP70S6K/ P70S6K ratio (**Table 2**; **Supplementary Figure 3A**). Exercise resulted in higher levels of PGC-1α and a tendency toward higher levels of TFAM (**Table 2**; **Supplementary Figure 3B**).

The activities of the OXPHOS complexes I, III, and V measured in muscle homogenates were higher in the exercised than in the control group and a trend toward significant differences was also found for CS activity (**Table 2**). However, no significant between-group differences were found for the muscle protein levels of representative subunits of the different OXPHOS complexes or for CS as measured by western blotting in total homogenates (**Table 2**; **Supplementary Figure 3C**), the enzymatic activity of respiratory chain complexes, or the representative OXPHOS proteins measured in mitochondria-enriched fractions (**Table 2**; **Supplementary Figure 3D**).

Exercised mice presented with lower CAT levels at the end of the training intervention compared to control mice, which showed abnormally high levels compared to the wild-type mice with no between-group differences for the remainder of antioxidant enzymes (**Table 2**; **Supplementary Figure 3E**).

To further assess the time course of the myopathy in the *Hq* mice, we analyzed forelimb strength in an independent cohort of *Hq* and WT mice from 1.5 to 3 months of age, with the results showing significantly lower muscle strength in the former since 1.5 months (**Supplementary Figure 4**). Finally, to study the effects of exercise training and P70S6K activation on skeletal muscle mass, we studied the cross-sectional fiber area of *tibialis anterior* muscles from some WT and *Hq* mice. The results indicated lower cross-sectional area in both control and exercise *Hq* animals compared to WT (**Supplementary Figure 5**).

DISCUSSION

The present study shows that an exercise intervention combining aerobic and resistance training induced significant benefits on the aerobic fitness and muscle strength of *Hq* mice, partially counteracting the myopathy progression that characterizes the time-course of the disease. These improvements were accompanied by mild increases in the activity of OXPHOS complexes I, III, and V, and by a trend to higher CS activity in total homogenates. Further, the intervention did not induce major skeletal muscle damage, as reflected by the lack of differences between groups in a well-accepted marker of this phenomenon, serum CK activity. No benefits were noted on phenotypes not directly related to muscle tissue such as cerebellar ataxia (i.e., balance performance) or growth impairment, which, as opposed to the aforementioned fitness/muscle variables, showed a similar evolution over time in the two groups. Of note, the fact that the exercise training program was initiated when the first signs of ataxia and myopathy were already present might explain, at least partly, why we did not find greater improvements in muscle biomarkers. In this respect, however, a strength of our design is that it mimics a frequent situation in patients, that is, the treatment starts when the disease phenotype is already well-established.

The *Hq* mouse model replicates the main features of respiratory chain complex I deficiency in humans (30), which is the most common respiratory chain defect (40). *Hq* mutant mice not only mimic clinical features of this condition in important aspects such as tissue specificity, disease onset and course, or inter-individual variability (30), but also show disturbances frequently found in other diseases associated with respiratory chain deficiencies such as ataxia, myopathy and retinal degeneration (30). Therefore, this model has been considered a valuable tool for assessing potential therapeutic approaches in MD, and previously nutritional, genetic, and pharmacological interventions, have been proven effective to attenuate several disease-related markers (41–44). In this regard, muscular weakness, the main feature of myopathy, is an early finding in the *Hq* mouse model, in which we observed weakness since 1.5 month of age. In turn, the latter result correlates well with the finding of complex I deficiency in skeletal muscle previously reported in 1 month-old *Hq* mice, a molecular phenotype trait that remains during the course of the disease (30). The early appearance of myopathy in *Hq* mice also makes this model a valuable tool for the study of exercise training as therapy for MD. Our results showing physical capacity improvements in trained *Hq* are in agreement with previous studies analyzing exercise training as therapy in other mouse models of MD. For example, Wenz et al., reported a lower number of falls during a treadmill test after a 1.5 month training program in a mouse model of cytochrome oxidase deficiency (23). After a 1 month training program, "Mutator" mice were able to reach levels of treadmill running performance similar to those of healthy (WT) animals (25). Our results demonstrating improvements in the physical capacity of *Hq* mice in response to exercise training are novel for this strain, and might allow to gain insight into the molecular underpinnings of exercise-induced adaptations in this model.

TABLE 1 | Effects of the exercise intervention on study endpoints with repeated measurements before and after the intervention.

Endpoint	Group	*N* with data	Pre-training	Post-training	Group (*p*)	Time (*p*)	Group by time interaction (*p*)
Aerobic fitness (distance, in m)	Control	10	191 ± 40	158 ± 37	0.372	0.256	**0.014**
	Exercise	9	176 ± 18	259 ± 43*			
Strength (forelimb test, g)	Control	11	109 ± 9	111 ± 8	0.537	**0.015**	**0.037**
	Exercise	10	110 ± 10	126 ± 10†			
Balance (time to fall, in s)	Control	11	59 ± 12	23 ± 7	0.073	**<0.001**	0.544
	Exercise	10	79 ± 14	52 ± 6			
Serum CK (U/L)	Control	11	93 ± 14	96 ± 8	0.976	0.499	0.290
	Exercise	9	94 ± 33	106 ± 21			

Data are mean ± standard error of the mean. Significant p-values (<0.05) are highlighted in bold. CK, creatinekinase.
**p = 0.016 for pre vs. post; †p = 0.003 for pre vs.post.*

The promotion of mitochondrial biogenesis through either genetically or pharmacologically (bezafibrate administration) stimulation of PGC-1α, has been shown to increase OXPHOS capacity in *cytochrome c oxidase*-deficient mice (9). Accordingly, exercise training-induced promotion of mitochondrial biogenesis through the activation of PGC-1α, has also proven to mediate the enhancement of oxidative capacity in animal (23, 25) and patient studies of MD (11–13). In this regard, in the present study, exercise training slightly improved muscle levels of PGC-1α and TFAM, and complex I, III, and V enzyme activities in muscle homogenates of *Hq* mice. Additionally, CS activity, which is a common marker of mitochondrial content, also showed a trend to increase in this group. Overall, these results indicate that exercise training was able to slightly promote mitochondrial biogenesis in the *Hq* mice, leading to partial normalization of the altered oxidative capacity. Moreover, we observed higher activities of OXPHOS complexes in muscle tissue homogenates but not in mitochondria-enriched fractions, reflecting that the results in the homogenates of the exercised *Hq* were due to a higher content of mitochondria rather than to a higher density of OXPHOS complexes per mitochondria. Therefore, our results support the role of mitochondrial biogenesis as a mediator of the beneficial effects of physical exercise on muscle OXPHOS capacity in the *Hq* mice. Of note, administration of the PGC-1α agonist bezafibrate has failed to improve mitochondrial biogenesis or muscle strength in *Hq* mice, with this treatment in fact inducing liver disease as a major adverse effect (41). Thus, up to date exercise training appears as the safest and most effective strategy to induce mitochondrial biogenesis in the *Hq* mouse model.

One aspect of our study that must be highlighted is the novelty of the exercise intervention, which included both endurance and resistance training. Previous studies in animals and patients with MD have focused on aerobic exercise owing to its potential to promote mitochondrial biogenesis and eventually increase aerobic fitness (11–13, 16–18, 45). Resistance training, however, might not only improve OXPHOS capacity (19) but also provide benefits on muscle mass and strength (20), and indeed we recently observed improvements with this type of intervention not only in the aerobic fitness, but also in the muscle strength and mass of patients with MD (14). Here, we also observed training-induced increases in muscle OXPHOS capacity and a trend to higher CS activity [being a classical marker of muscle aerobic adaptation and upregulated mitochondrial biogenesis (46)], and also in muscle strength. The higher levels of activation of P70S6K (i.e., p70S6K) and the trend toward higher levels of the ratio p70S6K/total P70S6K (a marker of muscle anabolism) observed in the exercise group could also support the role of combined resistance and aerobic exercise in the promotion of muscle anabolism, as P70S6K (a downstream effector of the mammalian target of rapamycin [commonly known as "mTOR"] pathway) is involved in protein synthesis and consequently muscle mass growth (47). Of note, the benefits observed in muscle strength are of particular relevance given that the *Hq* phenotype is characterized by marked muscle atrophy (i.e., lower muscle cross-sectional area and strength, and lower number of fast-twitch fibers compared to wild-type mice) (29, 48), and we recently showed that patients with MD also present with a lower (−18%) lean mass than their healthy peers (14). Nevertheless, preliminary assessment of fiber cross-sectional area in the *tibialis anterior* of control and exercised animals suggested no differences between both groups and thus no major occurrence of exercise-promoted skeletal muscle anabolism. In this respect, studies involving patients with MD have also demonstrated improvements in physical performance and strength without increases in the cross-sectional area of muscle fibers in response to training (21).

Another interesting finding of our study were the lower levels of the antioxidant enzyme CAT found in the exercised mice. *Hq* mice typically present with an 80% decrease in AIF, a protein that might be involved not only in apoptosis and OXPHOS complexes I and IV biogenesis (49, 50), but also in facilitating cellular redox homeostasis (27). Specifically, AIF has been proposed to exert an antioxidant activity as a peroxide scavenger, with reduced AIF levels resulting in increased peroxide sensitivity and consequently in greater levels of oxidative damage and cell death (27). Elevated levels of oxidative stress—as reflected by increased CAT activity in the cerebellum, heart, and skeletal muscle—have been previously reported in *Hq* mice and in other AIF-deficient mouse models (27, 51), which is in agrement with our results (i.e., the CAT levels of control *Hq* mice were significantly higher [by ∼2-fold] than those of wild-type mice). Our results, therefore, suggest that the exercise training intervention might have contributed to improve redox status, with the antioxidant levels of exercised *Hq* mice resembling more those of wild-type mice compared to their non-exercise pairs.

TABLE 2 | Biochemical variables in *Hq* mice.

	Variable	*N* with data (control/ exercise)	Control group	Exercise group	*P*-value Mann-Whitney's *U*-test
Anabolism protein levels in *Biceps femoris* homogenates (% of WT)	P70S6K	10/9	168 ± 20	292 ± 16	**<0.001**
	pP70S6K	10/9	156 ± 30	249 ± 30	**0.028**
	pP70S6K/P70S6K	10/9	91 ± 3	109 ± 6	0.095
Mt biogenesis protein levels in *Biceps femoris* homogenates (% of WT)	PGC-1α	5/4	82 ± 7	126 ± 19	**0.032**
	TFAM	11/10	83 ± 3	94 ± 4	0.060
OXPHOS activities in *Biceps femoris* homogenates (% of WT)	Complex I	10/9	75 ± 4	95 ± 6	**0.019**
	Complex II	10/9	82 ± 5	94 ± 5	0.161
	Complex III	1109	79 ± 5	97 ± 4	**0.031**
	Complex IV	10/9	88 ± 4	102 ± 4	0.136
	Complex V	10/9	77 ± 9	105 ± 9	**0.024**
	Citrate synthase	10/8	79 ± 3	91 ± 4	0.059
OXPHOS protein levels in *Biceps femoris* homogenates (% of WT)	ATP5a	11/9	86 ± 12	98 ± 13	0.387
	UQCRC2	11/9	77 ± 9	91 ± 21	0.705
	MTCO1	11/9	76 ± 13	108 ± 25	0.529
	SDHB	11/9	86 ± 5	85 ± 4	0.468
	NDUFB8	11/9	77 ± 6	81 ± 10	0.809
	NDUFS1	10/8	85 ± 5	93 ± 5	0.095
	Citrate synthase	11/9	84 ± 4	124 ± 14	0.132
OXPHOS activities in *Quadriceps* mitochondrial fraction (% of WT)	Complex I	10/5	87 ± 12	93 ± 19	0.953
	Complex II	5/5	89 ± 12	70 ± 7	0.548
	Complex III	5/5	117 ± 16	86 ± 14	0.310
	Complex IV	4/5	104 ± 7	99 ± 8	0.811
	Citrate synthase	5/5	118 ± 10	117 ± 17	0.905
OXPHOS protein levels in *Quadriceps* mitochondrial fraction (% of WT)	ATP5a	4/5	108 ± 14	125 ± 20	0.343
	UQCRC2	4/5	123 ± 12	146 ± 14	0.486
	MTCO1	4/5	118 ± 4	119 ± 8	0.886
	SDHB	4/5	99 ± 2	94 ± 10	1.00
	NDUFB8	4/5	79 ± 9	74 ± 10	0.886
	NDUFS1	4/5	30 ± 3	21 ± 6	0.343
Antioxidant enzymes protein levels in *Biceps femoris* homogenates (% of WT)	mtSOD	11/10	94 ± 13	94 ± 5	0.607
	cSOD	11/10	120 ± 20	97 ± 10	0.557
	GR	11/10	99 ± 4	109 ± 7	0.512
	CAT	11/10	204 ± 22	141 ± 23	**0.036**

Data (mean ± SEM) are expressed and analyzed as a percentage of mean value of an age- and gender-matched matched non-exercised wild type (WT) group (n = 4–12). Significant p-values are highlighted in bold. ATP5A, ATP synthase alpha subunit; CAT, catalase; cSOD, cytosolic superoxide dismutase; GR, glutathione reductase; Mt, mitochondrial; MTCO1, mitochondrial cytochrome c oxidase subunit I; mtSOD, mitochondrial superoxide dismutase; NDUFB8, ubiquinone oxidoreductase subunit B8; NDUFS1, ubiquinone oxidoreductase subunit S1; P70S6K, total ribosomal protein S6 kinase beta-1; pP70S6K, ribosomal protein S6 kinase beta-1 phosphorylated at threonine 389; PGC-1α, proliferator activated receptor gamma co-activator 1α; SDHB, succinate dehydrogenase complex iron sulfur subunit B; TFAM, transcription factor A mitochondrial; UQCRC2, ubiquinol-cytochrome C reductase core protein 2.

The present study has some limitations. First, randomization, allocation concealment, and blinding outcome assessment can reduce the risk of bias in animal studies. In this respect, although we could not adhere to these recommendations, we followed some measures to limit bias. First, we pair-matched mice in both groups based on their baseline aerobic fitness. Second, researchers in charge of muscle biochemical assessments were blinded to the mouse group from which samples were taken. Furthermore, we limited the risk of statistical error type I by using non-parametric statistical tests unpaired comparisons between exercise and control group, respectively. Another limitation is that we did not assess the effects of exercise training on muscle mass, which would have provided additional information, and we only performed preliminary assessment of muscle fiber cross-sectional area in a few mice. In turn, one of the major novelties of our study was the type of intervention applied, as to our knowledge this is the first study in which a mouse model of MD is subjected to resistance training. Moreover, we assessed both functional (i.e., fitness status) and biological (OXPHOS activity) markers and analyzed potential molecular pathways involved in these adaptations, which we consider as a major strength of our study.

CONCLUSIONS

In summary, an intervention combining aerobic and resistance exercises improved markers of physical capacity (aerobic fitness and muscle strength) in *Hq* mutant mice, a model of respiratory chain complex I deficiency. In turn, this intervention induced mild increases in the activation of signaling pathways involved in mitochondrial biogenesis (i.e., PGC-1α) and muscle anabolism (i.e., pP70S6K), muscle OXPHOS activity, and redox status–as reflected by a decrease in CAT levels. Although more research is needed, these results are encouraging and might provide useful information for the management of MD.

DATA AVAILABILITY

Datasets are available on request to the authors.

ETHICS STATEMENT

All experimental protocols were approved by the institutional ethics committee (project number 111/15) and were conducted in accordance with European (European convention ETS 123) and Spanish (32/2007 and R.D. 1201/2005) laws on animal protection in research.

AUTHOR CONTRIBUTIONS

CF-L: study design, experimental procedures, and drafting of the manuscript. PV: data analysis and drafting of the manuscript. SL-M, MF-T, VB-G, and LR-V: experimental procedures. JA, MAM, and AL: data analysis and manuscript edition. MM: study design, drafting of the manuscript, and direction. All authors revised the manuscript critically for important intellectual content and approved the final version.

FUNDING

This study was funded by FIS, Fondo de Investigaciones Sanitarias (grant numbers PI14/01085, PI15/00558, PI15/00431, and PI17/00093), and FEDER funds from the European Union. MM was supported by Miguel Servet contract (CPII16/00023) by the Institute of Health Carlos III (ISCIII) and FEDER funds. CF-L was supported by Miguel Servet contract (CP18/00034) by ISCIII. PV was supported by a contract granted by University of Alcalá (FPI2016). This study was awarded with the 2nd prize of the Premios Liberbank de Investigación en Medicina del Deporte, año 2017. Escuela de Medicina del Deporte. Universidad de Oviedo.

ACKNOWLEDGMENTS

We acknowledge Belén Fernández Pérez for her kind support with the histochemical analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.00790/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 01 October 2019
doi: 10.3389/fphys.2019.01258

Low-Intensity Running and High-Intensity Swimming Exercises Differentially Improve Energy Metabolism in Mice With Mild Spinal Muscular Atrophy

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: 06 April 2019
Accepted: 17 September 2019
Published: 01 October 2019

Citation:
Houdebine L, D'Amico D, Bastin J, Chali F, Desseille C, Rumeau V, Soukkari J, Oudot C, Rouquet T, Bariohay B, Roux J, Sapaly D, Weill L, Lopes P, Djouadi F, Bezier C, Charbonnier F and Biondi O (2019) Low-Intensity Running and High-Intensity Swimming Exercises Differentially Improve Energy Metabolism in Mice With Mild Spinal Muscular Atrophy. Front. Physiol. 10:1258. doi: 10.3389/fphys.2019.01258

Spinal Muscular Atrophy (SMA), an autosomal recessive neurodegenerative disease characterized by the loss of spinal-cord motor-neurons, is caused by mutations on Survival-of-Motor Neuron (SMN)-1 gene. The expression of *SMN*2, a *SMN1* gene copy, partially compensates for *SMN1* disruption due to exon-7 excision in 90% of transcripts subsequently explaining the strong clinical heterogeneity. Several alterations in energy metabolism, like glucose intolerance and hyperlipidemia, have been reported in SMA at both systemic and cellular level, prompting questions about the potential role of energy homeostasis and/or production involvement in disease progression. In this context, we have recently reported the tolerance of mild SMA-like mice ($Smn^{\Delta 7/\Delta 7}$; $huSMN2^{+/+}$) to 10 months of low-intensity running or high-intensity swimming exercise programs, respectively involving aerobic and a mix aerobic/anaerobic muscular metabolic pathways. Here, we investigated whether those exercise-induced benefits were associated with an improvement in metabolic status in mild SMA-like mice. We showed that untrained SMA-like mice exhibited a dysregulation of lipid metabolism with an enhancement of lipogenesis and adipocyte deposits when compared to control mice. Moreover, they displayed a high oxygen consumption and energy expenditure through β-oxidation increase yet for the same levels of spontaneous activity. Interestingly, both exercises significantly improved lipid metabolism and glucose homeostasis in SMA-like mice, and enhanced oxygen consumption efficiency with the maintenance of a high oxygen consumption for higher levels of spontaneous activity. Surprisingly, more significant effects were obtained with the high-intensity swimming protocol with the maintenance of high lipid oxidation. Finally, when combining electron microscopy, respiratory chain complexes expression and enzymatic activity measurements in muscle mitochondria, we found that (1) a muscle-specific decreased in enzymatic activity

of respiratory chain I, II, and IV complexes for equal amount of mitochondria and complexes expression and (2) a significant decline in mitochondrial maximal oxygen consumption, were reduced by both exercise programs. Most of the beneficial effects were obtained with the high-intensity swimming protocol. Taking together, our data support the hypothesis that active physical exercise, including high-intensity protocols, induces metabolic adaptations at both systemic and cellular levels, providing further evidence for its use in association with SMN-overexpressing therapies, in the long-term care of SMA patients.

Keywords: Spinal Muscular Atrophy, energy metabolism, physical exercise, fat oxidation, oxygen consumption, muscle mitochondria, respiratory chain

INTRODUCTION

Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease characterized by the specific loss of spinal cord motor neurons (MNs) which induce progressive muscular atrophy and could lead to patient death when respiratory muscles are affected (Crawford and Pardo, 1996). SMA is mainly caused by mutations in the telomeric copy of the Survival of Motor Neuron (*SMN*) genes, named *SMN1* (Lefebvre et al., 1995). *SMN2*, the SMN centromeric gene copy, only partially compensates the lack of *SMN1* expression because the exon 7 is excluded in 90% of transcripts, leading to the production of an unstable SMN protein. Consequently, the number of *SMN2* genes and their expression levels are directly linked to SMA clinical severity, classified from the most severe type 1 to the mild form type 3, based on the age of onset and on disease progression (Harding and Thomas, 1980).

Although the molecular origin of neurodegeneration in SMA is established in the vast majority of cases, i.e., a depletion of SMN protein in MNs leading to their degeneration, the physiopathology of the disease is today considered to be much more complex than initially thought. Noteworthy, SMN protein has a largely ubiquitous expression and is involved in mRNA metabolism. Thus, SMN-depletion induced defects have been reported in many different tissues in addition to the central nervous system and, independently of MN death, notably in the heart (Finsterer and Stollberger, 1999; Bevan et al., 2010; Heier et al., 2010; Shababi et al., 2010; Biondi et al., 2012), vasculature (Somers et al., 2016), skeletal muscles (Braun et al., 1995; Cifuentes-Diaz et al., 2001; Nicole et al., 2003; Biondi et al., 2008), pancreas (Bowerman et al., 2012, 2014) and liver (Vitte et al., 2004; Sahashi et al., 2013). Interestingly, pancreas and liver are directly involved in energy metabolism regulation, vasculature in tissue-oxygenation and heart and skeletal muscles are the main energy consumers in the body. These observations prompted to study in patients and mouse models energy metabolism state in SMA and their potential role in the pathophysiology. Altogether, these data pointed out profound alterations in the main catabolic pathways, including glycolysis (Bowerman et al., 2012; Davis et al., 2015) and fatty acid oxidation (Tein et al., 1995; Crawford et al., 1999). Furthermore, these defects could also be associated with severe perturbations in insulinemia (Davis et al., 2015) and glucose tolerance (Bowerman et al., 2012; Davis et al., 2015). At the cellular level, fatty acids and carbohydrates fuel mitochondria, the most important provider of energy in eukaryotic cells, through the functioning of the respiratory chain in the mitochondrial inner membrane that leads to efficient ATP production. In energy voracious tissues such as skeletal muscles, the maintenance of the mitochondrial network, qualitatively and/or quantitatively, is crucial to adapt to the workload requested for setting up moving or breathing. Interestingly, mitochondrial dysfunctions have been reported in SMA muscles, with alterations in the muscular mitochondrial biogenesis (Ripolone et al., 2015) and in the expression levels of respiratory chain components (Sperl et al., 1997; Jongpiputvanich et al., 2005; Miller et al., 2016).

Following the introduction of adequate clinical care and SMN-restoration therapies in MNs, such as Nusinersen, SMA patients are living longer (Chiriboga et al., 2016; Hache et al., 2016; Finkel et al., 2017). However, SMN expression is still not enhanced in all the affected tissues. Therefore, it appears of paramount importance to find efficient ways to induce whole-body adaptations in order to limit the potential impact of metabolic impairments, to improve muscle resistance to fatigue and to personalize the clinical care for the long-term quality of life of patients. In this context, physical exercise is expected to efficiently improve muscular energy metabolism and consequently limit muscle fatigue, with subsequent whole-body glycemic benefits, even in case of insulin sensitivity impairments, glucose resistance (Wojtaszewski et al., 2000; Cunha et al., 2015; Naufahu et al., 2018), and perturbations in lipids metabolism (Pistor et al., 2015; Wang et al., 2017; Mika et al., 2019). However, despite several recent trials (Lewelt et al., 2015; Madsen et al., 2015; Montes et al., 2015; Bora et al., 2018; Bartels et al., 2019), the use of physical exercise in SMA patient care is still under debate and no data concerning the potential impact of exercise on SMA-induced metabolic defects are available to date. Thus, additional studies directly addressing the potential benefits provided by different types of physical exercise on the energetic metabolic state in SMA are highly warranted.

In the present work, we analyzed the metabolic adaptations of mild SMA-like mouse ($Smn^{\Delta 7/\Delta 7}$; $huSMN2^{+/+}$) population exposed to two different types of long-term exercises, i.e., a low-intensity running- or a high-intensity swimming-based training, which we previously showed efficient to improve several SMA hallmarks in an SMN-expression independent manner,

including MN death, muscle atrophy and locomotor behavior (Chali et al., 2016). Here (Deforges et al., 2009), we report that several SMA-induced systemic metabolic defects, notably glucose homeostasis impairments and lipid overload, were significantly improved by physical exercise. These benefits were associated in particular with an improvement in fast-twitch SMA muscle mitochondrial efficiency. Furthermore, each exercise paradigm provided differential effects on SMA muscle metabolism calling for personalized exercise designs when transferring these data to patients more so when knowing the diversity of SMA patient metabolic state.

MATERIALS AND METHODS

Mild SMA-Like Mouse Model

The knockout-transgenic mild SMA-like mice (FVB/NRj-Smn$^{\Delta 7/\Delta 7}$, *huSMN2*$^{+/+}$) derived from mice obtained from the Institute of Molecular Biology (Hsieh-Li et al., 2000) (Academia Sinica, Taipei, Taiwan) have been purified on the FVB/NRj genetic background (Janvier Labs, Le Genest-Saint-Isle, France) by backcross for more than 10 generations and were designated as 'SMA' (n = 53). The control mice (CTRL; n = 53) were heterozygous knock-out for murine *Smn*, expressing homozygous human *SMN2* transgene (FVB/NRj-*Smn*$^{+/\Delta 7}$, *huSMN2*$^{+/+}$). Only males were selected for experimentation to standardize the analyses, and all the experiments were performed in a blind systematic manner to minimize bias. From two to four animals were housed in each cage, with food and water *ad libitum*. Animal handling and experimentation were performed in line with approved Institutional Animal Care and Use Committee protocols at the University of Paris Descartes (CEEA 34, agreement number B75-06-07) and followed the national authority (Ministere de la Recherche et de la Technologie, France) guidelines for the detention, use and the ethical treatment of laboratory animals based on European Union Directive 2010/63/EU.

Exercise Protocols

Two-month-old mice were submitted to 10 consecutive months of training either to running-based exercise on a speed-regulated treadmill at 13 m.min^{-1}, or to swimming-based exercise in an adjustable-flow swimming pool at 5 L.min^{-1} as previously described (Chali et al., 2016). Sedentary control and SMA (35 Sed CTRL and 35 Sed SMA) mice were placed in the pool without flow (18 SMA and 18 CTRL mice) and floated at the water surface or on the treadmill without speed (17 SMA and 17 CTRL mice).We formed four groups of trained mice, one running group of controls and one of SMA (Run CTRL and Run SMA; n = 18 for each) and one swimming group of controls and one of SMA (Swim CTRL and Swim SMA; n = 18 for each).

Glucose Homeostasis Evaluation

An Oral Glucose Tolerance Test (OGTT) and an Insulin Tolerance Test (ITT) were performed on sedentary groups of mice at 3, 6, and 12 months of age and on trained groups of mice at 6 and 12 months. The two tests were performed on each mice with a delay of 1 week between each. Briefly, overnight fasted mice received an oral load of glucose (2 g.kg^{-1}) or received an intraperitoneal injection of insulin (0.75IU.kg^{-1}; Actrapid,^{TM} Novonordisk). A drop of blood was collected from tail tip just prior the administration (T0) and 5, 20, 60, 90, and 120 min following glucose ingestion or insulin injection and glycemia was determined via a glucometer (Accu-Chek® performa glucometer, France).

Measurement of Body Weight, Food Intake, Respiratory Exchanges and Related Parameters

Body weight was measured daily, before the onset of the light phase. At 12 months of age, and 48 h after the last training session, food intake, total spontaneous activity, oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured for each mouse, individually, during 2 consecutive days (D1 and D2) by using the Oxylet Physiocage System (Panlab/Harvard apparatus, Cornella, Spain), the software suite METABOLISM (V2.2.01, Panlab) and the obtained data were represented as a mean of the 2 days. Mice were habituated to the metabolic chambers for 24 h before data collection.

The respiratory exchange ratio (RER) was calculated as VCO_2/VO_2, Energy Expenditure (EE) was calculated according to the formula: EE (kcal.day^{-1}.kg$^{-0.75}$) = $VO_2 \times 1.44 \times [3.815 + (1.232 \times RER)]$, the fat oxidation (Fat ox.) was calculated according to the formula: Fat ox. (g.day^{-1}.kg$^{-0.75}$) = (1.72 × VO2) – (1.72 × VCO2) – (1.96 × 0.2) and the carbohydrate oxidation (CH ox.) was calculated according to the formula: CH ox. (g.day^{-1}.kg$^{-0.75}$) = $(-2.97 \times VO_2) + (4.17 \times VCO_2) - (2.44 \times 0.2)$.

The analyses of total spontaneous activity, respiratory exchanges and related parameters were also analyzed by dividing the day in 4 periods of 6 h: two periods (0–6 h and 6–12 h) during the diurnal phase and two periods (12–18 h and 18–24 h) during the nocturnal phase.

Finally, the mice EE was evaluated during minimal (Min) and maximal (Max) activity in diurnal and nocturnal phases. Briefly, the analyses of the total spontaneous activity, expressed in arbitrary unit (a.u), were performed by plotting data every 7 min 30. We defined a minimal activity as an activity below 10 a.u over a period of 7 min 30 and a maximal activity as an activity up to 100 a.u. over a period of 7 min 30. A Min EE was represented as a mean of each EE related to a minimal activity during diurnal or nocturnal phases. In contrast, a Max EE was represented as a mean of each EE related to a maximal activity during diurnal or nocturnal phases.

Insulin ELISA

Blood samples were collected from right ventricle on anesthetized mice, with 1% pentobarbital solution (6 μL.g^{-1}) diluted in 0.9% saline buffer, using a 1 mL syringe mounted with a 22-gauge needle and coated with heparin (5000 UI/mL, Panpharma Luitré, France) in resting condition for all group of mice (24 h after training). Blood samples were centrifuged

(1000 *g*, 10 min, + 4°C) and serum was frozen at −80°C. The serum insulin level was measured using a rat/mouse ELISA assay kit (EZRMI 13K, Millipore) following manufacturer's instructions. For each mouse, 10 μL of serum were incubated with biotinylated anti-insulin antibody at room temperature for 2 h in agitation at moderate speed, about 400–500 rpm followed by 30 min incubation with streptavidin-horseradish peroxidase conjugate buffer. Insulin concentration was determined reading on spectrophotometer at 590 nm wavelength and 450 nm wavelength. Insulin levels were given in nmol/mL.

Thermoregulation Assessment

The thermoregulation of mice was assessed under two thermal stress tests: the cold air test (+12°C) and the cold water test (+10°C). For the cold air test, mice were individually housed in an air-conditioned room at +12°C and rectal temperature was recorded every 20 min until 2 h using a rodent rectal temperature probe (BIO-9882 Dual Input Thermometer, Bioseb, Vitrolles, France). For the cold water test, mice were individually put in plastic cage with 4 cm of 10°C water in order to recover the entire body of mice avoiding swimming. Rectal temperature was recorded every 10 min for 1 h.

Muscles Transmission Electron Microscopy

Tibialis and *soleus* muscles of 12-month-old mice were dissected immediately after cervical dislocation, divided in pieces with blade then were fixed and stored with a 4% paraformaldehyde solution supplemented with 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4. Pieces of muscles were then supported by the electron microscopy platform of Cochin institute for all processes and imaging, as follows. Pieces of muscle were washed in phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in graded ethanol series, and embedded in epoxy resin. Ultrathin muscles sections (80–90 nm) were cut longitudinally on an ultramicrotome (UC-7, Leica) and collected on 200-mesh nickel grids. Staining was performed on drops of 4% aqueous uranyl acetate, followed by Reynolds's lead citrate (Reynolds, 1963). Mitochondrial ultrastructural analyses were performed in a JEOL jem-1011 electron microscope and digitalized with DigitalMicrograph software on submembranar and intramyofibrillar mitochondria.

Muscles Proteins and Western Blot Analysis

Twelve-month-old mice were anesthetized with intraperitoneal injection of pentobarbital 1% (6 $\mu L.g^{-1}$). *Tibilias*, *plantaris* and *soleus* muscles dissected, immediately frozen in liquid nitrogen and homogenized in 100 μL per 5 mg tissues in the presence of ice-cold RIPA buffer [50 mM Tris, 150 mM NaCl, 0.1% SDS, 1% NP40, 10 mM NaF, 1X protease inhibitor (Roche, Basel, Switzerland), 1% phosphatase inhibitor (Sigma-Aldrich, St. Louis, MO, United States)] using metal beads in 2 mL tubes and mechanically stressed with a TissueLyser II apparatus (Qiagen ID85300, United States). Protein concentration of the clarified homogenates (+4°C, 20 min, 17,000 g) was determined on all samples using the Lowry protein assay (Lowry et al., 1951). Whole cell extracts [15 μg VDAC1/Porin, Cyclophilin D or 10 μg (Oxphos)] were fractionated by 12.5% SDS-PAGE(1.5 M Tris pH 8.3, 12.5% acrylamide, 0.07% Bis, 0.1% SDS, 0.05% ammonium persulfate, 0.06% tetramethylethylenediamine). The separated proteins were transferred on PVDF membranes (Bio-Rad Laboratories) according to the Towbin protocol (Towbin et al., 1984). Equal loading of samples was checked by Ponceau dye staining of the transferred gels. Western blot analysis was performed on membranes overnight at +4°C in 4% BSA, 0.05% Tween 20, TBS pH 7.4. Each of the following primary antibodies, including, mouse anti-total OXPHOS rodent cocktail (1:1000; Abcam, ab110413), mouse anti-VDAC1/Porin (1:2000, 36 kDa, Abcam, ab14734), mouse anti-alpha Tubulin (1:10000, 50 kDa, Abcam, ab7291), anti-cyclophilin D (1:1000, 18 kDa, MitoSciences, MSA04), was incubated overnight at +4°C in the above blocking medium. Membranes were rinsed in 0.1% Tween 20 in TBS three times for 10 min each time at room temperature and then incubated in horseradish peroxidase-conjugated goat secondary antibody directed against mouse Igs (1:5000; Bio-Rad Laboratories) and in horseradish peroxidase-conjugated goat secondary antibody directed against rabbit Igs (1:10,000; Jackson ImmunoResearch) in 0.1% Tween 20 in TBS for 1 h at room temperature. Bound antibody complexes were developed with AmershamTM ECL^{TM} Western Blotting Analysis System (GE Healthcare, Bio-Science, Upsala, Sweden). In some instances, membranes were stripped after immunoblotting by incubation in stripping buffer (100 mM-mercaptoethanol, 2% SDS, and 62.5 mM Tris-HCl, pH 6.7) for 30 min at +55°C with agitation, and membranes were then blocked and reprobed with monoclonal mouse anti-glyceraldehyde-3-phosphate dehydrogenase antibody (GAPDH) (1:1000, 37 kDa, Millipore, MAB374). Images were done using ImageQuant LS4000 (GE Healthcare Bio-Science, Upsala, Sweden) and quantification performed using Image J v1.52i software.

Isolated Mitochondrial Oxygen Consumption

Tibialis and *plantaris* muscles were dissected after cervical dislocation of 12-month-old mice and transferred in a glass cupule containing ice-cold extraction buffer pH 7.2 (20 mM Tris-HCl, 250 mM sucrose, 2 mM EDTA, and 40 mM KCl). The muscles were immediately weighed, finely minced all together with scissors in 1 mL of extraction buffer supplemented with 1 $mg.mL^{-1}$ BSA (medium A), and homogenized immediately by 10–15 strokes at 500 rpm with a motor-driven Teflon/glass homogenizer. The homogenate was filtered through a 90 μm nylon gauze and centrifuged at 1000 *g* for 5 min at +4°C. The supernatant was centrifuged at 14000 g, +4°C, for 10 min, and the pellet containing mitochondria was resuspended in 1 mL of medium A supplemented with 5% Percoll, and centrifuged at 14000 *g* for 10 min. The pellet of washed mitochondria was carefully resuspended in medium A with 5% Percoll on ice, by adjusting the resuspension volume with a ratio of 1.33 $\mu L.mg^{-1}$ of muscle wet weight. Measurements of mitochondrial oxygen consumption rates were performed right after isolation using

the Oxoplate technology, based on 96-well microplates with integrated optical oxygen sensors (PreSens, Germany). Each well was filled with 100 μL of incubation medium containing 20 mM KH_2PO_4/K_2HPO_4 pH 7.4, 300 mM Mannitol, 10 mM KCl, 5 mM $MgCl_2$, and either 1 mM pyruvate + 2 mM malate or 37 μM palmitoy-CoA + 2 mM malate + 1 mM carnitine, pre-warmed at +37°C. Then, 5 μL of purified mitochondria were added in each well, which were covered with 200 μL of mineral oil to isolate from ambient oxygen. Oxoplates® were then read out from the bottom every 30 s for 20 min by a fluorescence intensity microplate reader (infinite®M200, Tecan). The kinetics of fluorescence intensities were analyzed according to the manufacturer's instruction manual, and the oxygen consumption assessed by determining the maximal slope of decrease of oxygen partial pressure relative to 100%, at +37°C. The results were finally expressed as percent of O_2 per minute per mg of mitochondrial proteins. Total mitochondrial protein content was determined by the Lowry method (Lowry et al., 1951).

Respiratory Chain Activity

Twelve-month-old mice were anesthetized by intraperitoneal injection of pentobarbital 1% (6 $\mu L.g^{-1}$ body weight). *Tibialis*, *plantaris* and *soleus* muscles were dissected, immediately frozen in liquid nitrogen and stored at −80°C. Frozen muscles were weighed and homogenized in cold extraction buffer (1/25 weight/volume) using motor-driven Teflon/glass homogenizer, and the homogenates were centrifuged at 1000 *g* for 10 min at +4°C. The supernatants were then frozen at −80°C and used within 2 months for enzyme determinations. Total protein content of homogenates was determined by the Lowry method (Lowry et al., 1951).

The methods used for measurements of respiratory chain (RC) complex I and II activities, based on following the decrease in absorbance of dichloroindophenol (DCIP), were adapted from the method developed by Janssen et al. (2007). RC complex I activity was measured at the spectrophotometer at 614 nanometer (nm) in 1 mL of 25 mM potassium phosphate buffer pH 7.8 at 37°C, containing 3.5 mg/ml BSA, 70 μM DCIP, 90 μM decylubiquinone, and 0,2 mM NADH. The reaction was started by addition of 10–15 μL of muscle homogenate and the slopes were recorded continuously for 1–2 min before and after addition of 10 μM rotenone. CI activity was calculated as the rotenone sensitive fraction, and expressed as nanomol (nmol) DCIP reduced.min^{-1}.mg^{-1} protein. RC complex II activity was immediately measured in the same cuvettes by addition of 12 mM succinate, followed by slope recording for 2 min. The reaction was terminated by addition of 15 mM malonate and CII activity was calculated as the malonate sensitive fraction, and expressed as nmol DCIP reduced.min^{-1}.mg^{-1} protein.

The RC complex IV, cytochrome C oxidase, activity was measured by following the decrease in absorbance of reduced cytochrome C at 550 nm by spectrophotometry, as described by Barrientos et al. (2002). The assay was performed at +37°C using 15–20 μL of muscle homogenate in 1 mL of 10 mM potassium phosphate buffer pH 7.8 containing 1 $mg.mL^{-1}$ BSA, 13.6 μM reduced cytochrome C, and 2.4 mM lauryl maltoside. The slopes were recorded for 1–2 min, stopped by addition of 100 μM KCN, and the results were expressed as nmol cytochrome C oxidized.min^{-1}.mg^{-1} protein.

The activity of citrate synthase was determined by following the increase in absorbance of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) at 405 nm by spectrophotometry in 96-well microplates (Kamemura). The assay mixture (100 μL per well) contained 50 mM potassium phosphate buffer pH 7.8, 1 mM EDTA, 1 $mg.ml^{-1}$ BSA, 100 μM DTNB and 200 μM acetylCoA. The reaction was initiated by addition of 5 μL diluted (1/2) muscle homogenate and reading for 2 min, after which 5 μL of 20 mM oxaloacetate were added for measurements of maximal activity. The results were expressed as nmol DTNB oxidized.min^{-1}.mg^{-1} protein.

mRNA Quantification by RT-qPCR Analysis

Twelve-month-old mice were anesthetized with intraperitoneal injection of pentobarbital 1% (6 $\mu L.g^{-1}$). Perigonadic adipocytes were dissected and immediately frozen in liquid nitrogen. RNA was extracted using TRizol reagent (Invitrogen, Life Technologies, Saint-Aubin, France) with metal beads in 2 ml tubes and mechanically stressed with a TissueLyser apparatus (Qiagen). Each RNA preparation was treated with RQ1 RNase-Free DNase (Promega). One μg of mouse RNA was reverse transcribed with oligodT (20 mer) using reverse transcriptase Improm II (Promega France, Charbonnières, France). Quantitative real time PCR was performed with standard protocols using SYBR Green ROX as a fluorescent detection dye in ABI PRISM 7000 (ABgene, Courtaboeuf, France) in a final volume of 7 μL. Specific primers were used at 300 nM (**Table 1**). The cDNAs for the real time PCR were used at 5 ng/μL. The amounts of cDNA in each sample were determined on the basis of the threshold cycle (Ct) for each PCR product and normalized to proteasome 26 subunit [Rps 26 (26s)] Ct for all the tissues used. This housekeeping gene has been determined as best internal controls in our conditions, using Bestkeeper (Pfaffl et al., 2004) and Normfinder (Andersen et al., 2004) algorithms (data not shown). The calculated relative amount of mRNA was done respective to control samples and given as fold change after $2^{-\Delta\Delta CT}$ calculation (Livak method).

Statistical Analysis

All data are presented as mean and standard deviation (SD). For glucose and insulin tolerance test, as repeated measures experiments, a Holm–Sidak method was performed in order

TABLE 1 | RT-qPCR primers sequences.

Gene name	GenBank ID	Primer sequences
ACC1	NM_133360	Fw 5′-GCCTCTTCCTGACAAACGAG-3′ Rv 5′-TGACTGCCGAAACATCTCTG-3′
FASN	NM_007988	Fw 5′-AGAGATCCCGAGACGCTTCT-3′ Rv 5′-GCCTGGTAGGCATTCTGTAGT-3′
ACC2	NM_133904	Fw 5′-GAGCTGCTGTGTAAACACGAGATTGCT-3′ Rv 5′-CTGGTGCCGGCTGTCCTC-3′

to compare two group of mice, while a two-way ANOVA test followed by *post hoc* Dunnet's test was performed in order to compare multiple group of mice. For other multiple group comparisons, a Kruskal–Wallis test was performed followed by a *post hoc* Mann-Whitney test to verify significant differences between groups (GraphPad Prism v7.05, Chicago, IL, United States). For other two group comparisons, a non-parametric Mann–Whitney test was performed to verify significant differences (GraphPad Prism). All the data presented in this study were considered as statically different when the statistical power exceeds 95% (AnaStats.fr, France). All graphics were done with GraphPad Prism v7.05 and Adobe Illustrator CS6 v16.0.3.

RESULTS

Low-Intensity Running and High-Intensity Swimming Enhanced Whole-Body Metabolism in SMA Mice

The mild SMA model mouse, initially defined as a type III model mouse, has been well phenotypically characterized, with distal necrosis at 2 months of age (tail, ears and toes), quantifiable motor neurons degeneration at 6 months of age, a decrease in motor function and an increase in hind limb muscles atrophy from 9 months of age (Tsai et al., 2006; Chali et al., 2016). However, its metabolic status has never been investigated. Interestingly, despite the hind limb muscle atrophy, no significant modulation of the C57/B6 SMA body weight has been reported at any age when compared to age-matched controls (Tsai et al., 2006). We confirmed these data at 12 months of age in an FVB/NRj genetic background (**Figure 1A**). Interestingly, and for the first time, when we subjected SMA mice to 10 months low-intensity running or high-intensity swimming, we observed a significant reduction in body weight with both protocols when compared to sedentary SMA mice (**Figure 1A**). However, in control mice, unlike running, only swimming-based training significantly decreased the body weight at 12 months of age (**Figure 1A**), suggesting that the high-intensity swimming protocol induced higher effects than low-intensity running on body weight.

In order to determine whether this impact on body weight could be related to a modulation in food intake or to a metabolic change, we focused on the eating behavior of 12-month-old sedentary and trained mice during the whole day, i.e., the diurnal and nocturnal periods. In line with their lack of changes in body weight, the sedentary SMA and CTRL mice exhibited similar food (**Figures 1B,D**) and water (**Supplementary Figures S1A,C**) intakes, whatever the analyzed period of the day. Interestingly, in trained SMA mice, while both types of exercise significantly reduced the body weight, we observed an increase in food (**Figures 1C,D**) and water (**Supplementary Figures S1B,C**) intakes during the nocturnal phase when compared to sedentary SMA mice, without any significant modification during the diurnal phase. Moreover, in trained CTRL mice, a tendency to increase food (**Figure 1D**) and water (**Supplementary Figure S1C**) intakes had been observed for both protocols compared to sedentary CTRL mice, despite a swimming-induced significant increase in food intake during diurnal phase (**Figure 1D**). Taken as a whole, our data suggest that despite no modification in body weight and food intake in sedentary mice, both exercises induced whole-body metabolic change in CTRL and SMA mice by increasing nutrients intake and decreasing body weight.

Since the decrease in body weight observed in trained SMA mice was not linked to a decrease in food intake, we next investigated whether the modulation of body weight was related to a change in the adipose mass. We observed that the perigonadic white adipocytes mass reported on body weight was significantly greater in sedentary SMA mice, when compared to sedentary CTRL mice (**Figure 1E**). However, in trained SMA mice, both types of exercise totally restored this ratio, suggesting that running and swimming-based trainings in SMA mice could reduce the body weight by reducing adipose mass (**Figure 1E**). This ratio was not affected by any type of exercise in trained CTRL mice.

In order to determine whether the sedentary SMA-specific increase in adipose mass was a consequence of an impaired fatty acid metabolism, we focused on the gene expression of lipogenesis enzymes, *Fatty acid synthase* (*Fasn*), *Acetyl-CoA carboxylase 1* (*Acc1*) and *Acetyl-CoA carboxylase 2* (*Acc2*) in perigonadic white adipocytes of 12-month-old mice. Interestingly, the gene expression of *Fasn*, *Acc1* and *Acc2* was significantly increased in sedentary SMA mice, when compared to sedentary CTRL mice (**Figure 1F**). After exposing to the running and swimming protocols, we observed a significant reduction in the gene expression of all three enzymes in trained CTRL and SMA when compared to sedentary mice (**Figure 1F**). Thus, we showed that SMA induced lipogenesis which was counteracted by both exercise protocols.

Taken together, these results strongly suggest that the SMA mice exhibited an impaired fatty acid metabolism, which promotes lipogenesis without any body weight or food intake perturbations. Interestingly, both types of exercises reduce lipogenesis and decrease body weight with no change in food intake, whilst the swimming protocol provided more significant effects.

Low-Intensity Running and High-Intensity Swimming Mitigate Glucose Homeostasis Alteration in SMA Mice

It is now well reported that alterations in body composition, lipogenesis and body weight can be related to glucose homeostasis defects, as observed in type 2 diabetes (Iizuka and Horikawa, 2008; Samuel, 2011; Samuel and Shulman, 2016). In addition, metabolism abnormalities such as metabolic acidosis, altered fatty acid metabolism, hyperlipidemia and hyperglycemia have been reported in SMA patients and mouse models (Dahl and Peters, 1975; Tein et al., 1995; Bowerman et al., 2012). However, no data are available in our SMA mouse model or on the metabolic effects of exercise in the SMA condition. To this

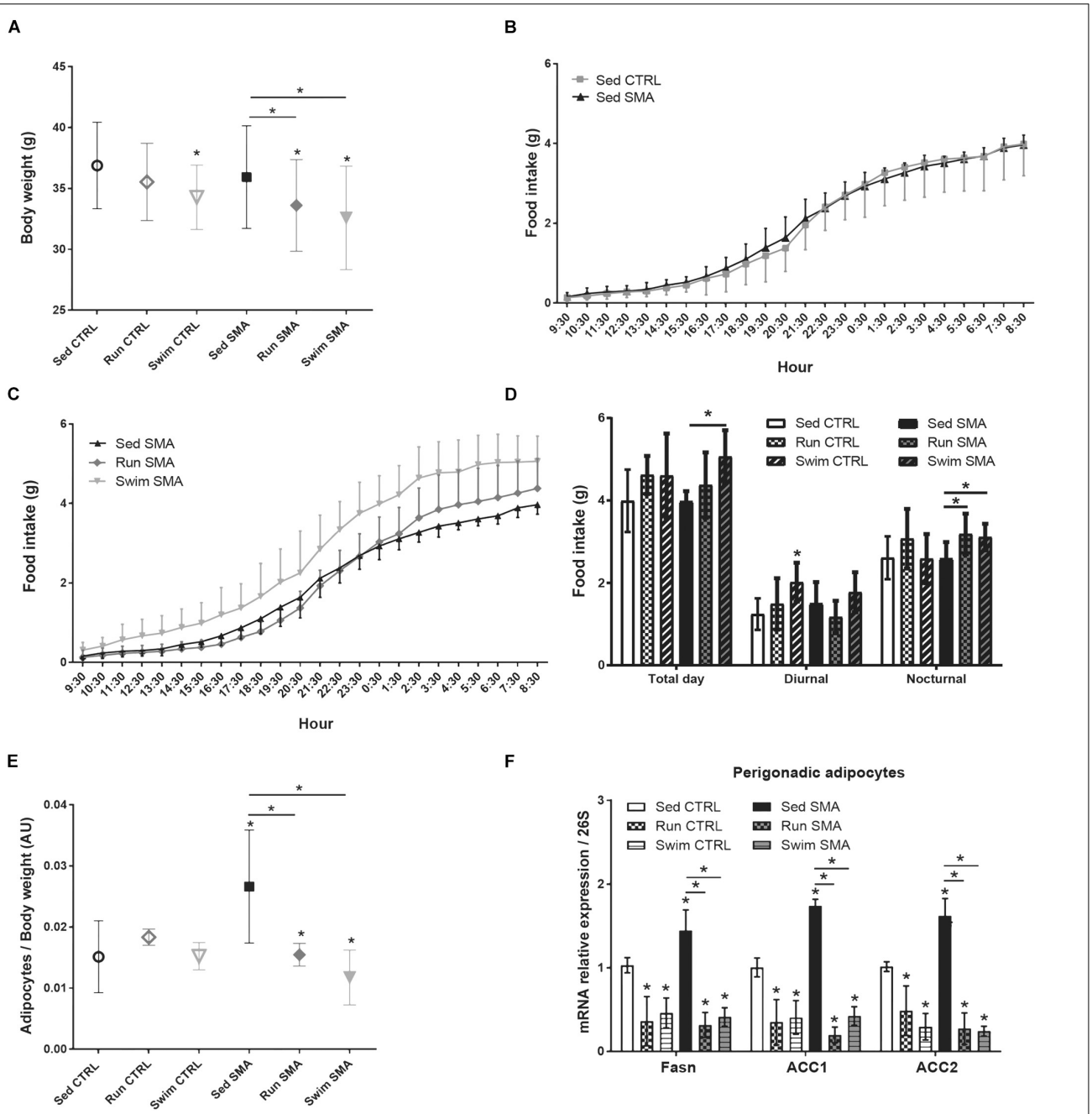

FIGURE 1 | Whole body metabolism in sedentary and trained SMA and control mice. **(A)** Body weight of sedentary control (Sed CTRL $n = 35$), running-trained control (Run CTRL $n = 18$), swimming-trained control (Swim CTRL $n = 18$), sedentary SMA (Sed SMA $n = 35$), running-trained SMA (Run SMA $n = 18$) and swimming-trained SMA (Swim SMA $n = 18$) mice at 12 months of age. **(B,C)** 24 h food intake of **(B)** Sed CTRL ($n = 12$) compared to Sed SMA ($n = 12$) and **(C)** Sed SMA mice compared to Run ($n = 8$) and Swim ($n = 6$) SMA mice. **(D)** Quantification of food intake during all day (Total day), diurnal (9 h 30–21 h 30) and nocturnal periods (21 h 30–9 h 30) of sedentary and trained control and SMA mice at 12 months of age. **(E)** Ratio of white perigonadic adipocytes mass over body weight of sedentary and trained control and SMA mice at 12 months of age ($n = 8$ for each group). **(F)** Quantification of mRNA expression levels of *Fasn*, *Acc1* and *Acc2* by RT-qPCR in adipocytes of 12 months old sedentary and trained control and SMA mice ($n = 4$ for each group). * and -*- indicated significance relative to sedentary control and SMA mice, respectively (with $P < 0.05$).

purpose, we decided to evaluate glucose homeostasis in sedentary and trained CTRL and SMA mice from 3 to 12 months of age.

We found that sedentary SMA mice exhibited an early onset significant increase in fasting blood glucose, from 3 months of age, which increased to 6 months and then made a plateau to 12 months of age, when compared to sedentary CTRL mice (**Figures 2A–C**). Fasting hyperglycemia was maintained in trained SMA mice aged 6 and 12 months compared to

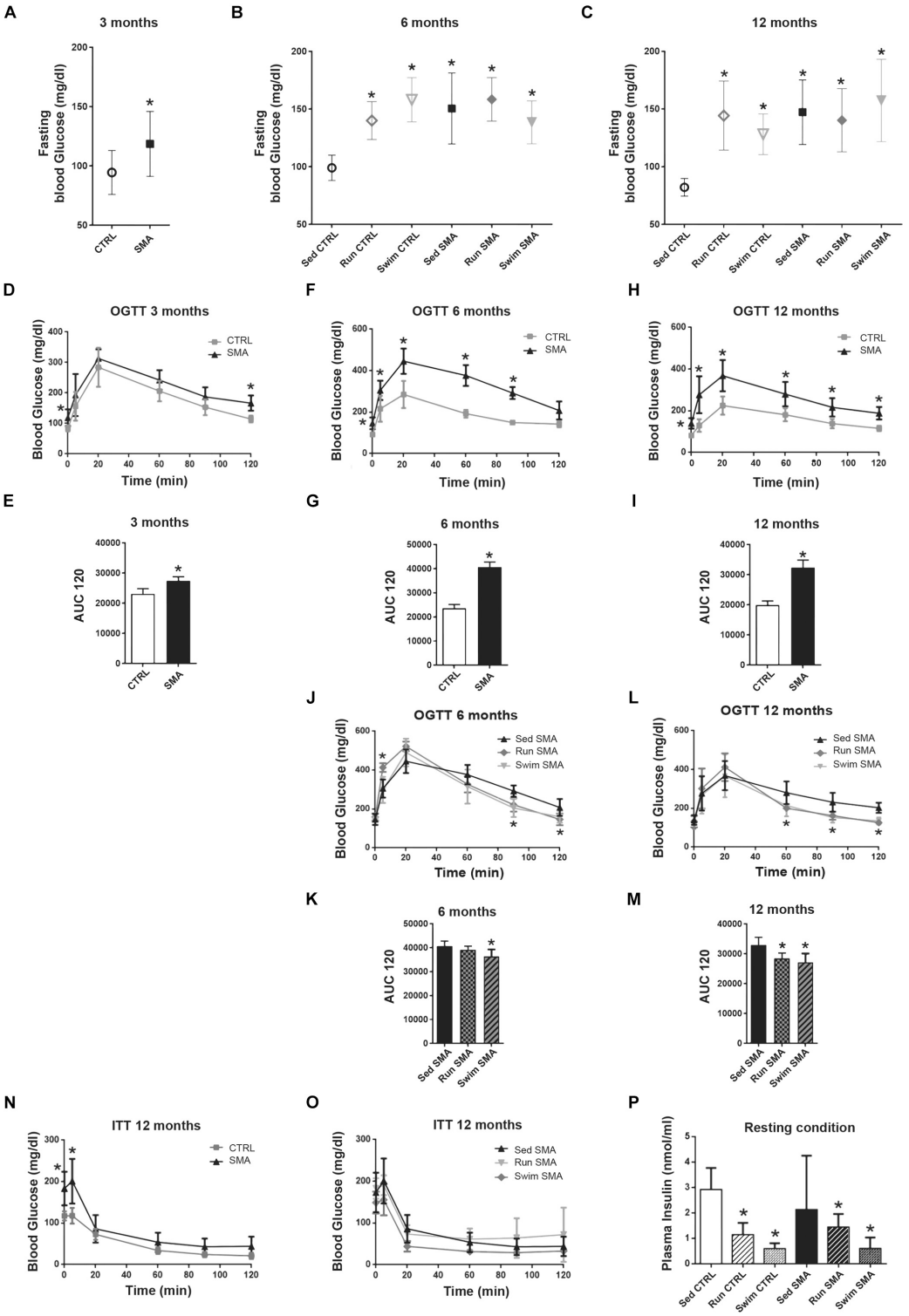

FIGURE 2 | Glucose homeostasis in sedentary and trained SMA and control mice. **(A–C)** Fasting blood glucose measurement of **(A)** 3 months old sedentary control (CTRL) and SMA (SMA) mice, **(B)** 6 months old and **(C)** 12 months old sedentary and trained control and SMA mice. **(D–I)** Oral Glucose Tolerance Test (OGTT) and area under the curve (AUC) calculated over 120 min of **(D,E)** 3 months old, **(F,G)** 6 months old and **(H,I)** 12 months old Sed CTRL and Sed SMA mice (n = 8 for each group). **(J–M)** OGTT and area under the curve (AUC) calculated over 120 min of **(J,K)** 6 months old or **(L,M)** 12 months old Sed SMA compared to Run SMA and Swim SMA mice. **(N,O)** Insuline Tolerence Test (ITT) of **(N)** sedentary control and SMA mice and **(O)** Sed SMA compared to Run SMA and Swim SMA mice at 12 months of age (n = 8 for sedentary mice; n = 6 for trained mice). **(P)** ELISA quantification of plasma insulin levels of fed sedentary and trained controls and SMA mice at 12 months of age (n = 6 for each group). *Indicated significance relative to sedentary mice (with $P < 0.05$).

sedentary SMA mice (**Figures 2B,C**). This hyperglycemia was also observed in trained CTRL mice when compared to sedentary CTRL mice at the same ages (**Figures 2B,C**), suggesting long-term exercise-specific adaptation. Moreover, sedentary SMA mice also exhibited glucose intolerance from the age of 3 months (**Figures 2D,E**), which increased to 6 months and reached a plateau to 12 months (**Figures 2F–I**) with significantly higher glucose levels and *Area under the curve* (AUC) following the oral glucose load, when compared to sedentary CTRL mice. However, both exercise protocols improved glucose tolerance by decreasing glucose levels following the oral glucose load at 90 and 120 min in 6-month-old SMA mice (**Figures 2J,K**), and at 60, 90, and 120 min in 12-month-old SMA mice (**Figures 2L,M**). Our results confirm that, also in our model mouse, glucose homeostasis is altered in SMA and suggest that both exercises modified glucose homeostasis by increasing glucose tolerance, despite maintenance of high glucose level in fasting condition.

We next questioned whether the hyperglycemia and glucose intolerance observed in sedentary SMA mice could be attributed to a reduced insulin sensitivity. To address this question, we performed an insulin tolerance test (ITT) in CTRL and SMA mice at 3 (**Supplementary Figure S2A**), 6 (**Supplementary Figure S2B**) and 12-month-old mice (**Figure 2N**). Interestingly, whatever the age, 20 min after insulin injection, the sedentary SMA mice did not displayed any blood glucose differences compared to sedentary CTRL mice, effects which were maintained until 120 min (**Supplementary Figures S2A,B** and **Figure 2N**). After both exercise protocols, we did not observed a modification of the insulin tolerance compared to sedentary SMA mice at 6 (**Supplementary Figure S2C**) and 12 months of age (**Figure 2O**).

In absence of impaired insulin sensitivity in sedentary SMA mice, we asked whether hyperglycemia and glucose intolerance could be due to an impaired insulin secretion. As already observed in other SMA mouse models (Bowerman et al., 2012, 2014), no significant difference in plasma insulin level was found between sedentary SMA and CTRL fed mice (**Figure 2P**) at 12 months of age, despite high variability in SMA. Interestingly, if both types of exercise did not significantly modify plasma insulin levels in the fed SMA mice, we observed a tendency to decrease plasma level with a decrease in the variability. We also observed a significant decrease in plasma insulin levels in trained CTRL mice (**Figure 2P**), suggesting that both protocols induced a decrease in insulin production in the fed mice.

Taken together, our results suggested that the sedentary SMA mice exhibit an abnormal glucose homeostasis, which seems largely insulin independent, and whose glucose intolerance was limited by both exercise protocols through a potentiation of insulin response.

Low-Intensity Running and High-Intensity Swimming Attenuated SMA-Induced Oxygen Consumption Defects

As previously shown, SMA mice are characterized by impaired lipid metabolism and glucose homeostasis, and by chronic elevation of blood lactate in resting condition (Chali et al., 2016), which can be ameliorated by both exercise protocols (Chali et al., 2016). These data suggest a major abnormality in carbohydrate oxidation and energy expenditure in sedentary mice, which could be prevented by both exercise protocols. To address these issues, we investigated the systemic metabolism in 12-month-old sedentary and trained CTRL and SMA mice by indirect calorimetry with simultaneous measurements of total spontaneous activity.

In sedentary CTRL and SMA mice, the total spontaneous activity appeared identical and followed the same circadian pattern, a low activity during the diurnal phase (9:30 am to 9:30 pm) and a nocturnal phase divided in two sub phases, with a high activity during the first one (9:30 pm – 3:30 am) and a low activity during the second one (3:30 am – 9:30 am) (**Figure 3A**). Surprisingly, the O_2 consumption of sedentary SMA mice appeared higher than that of sedentary CTRL mice over 24 h, with a much greater difference during the second half of the nocturnal phase (**Figure 3C**). Thus, we decided to refine the analyses of each parameters by dividing the day in 4 periods of 6 h: two periods (0–6 h and 6–12 h) during the diurnal phase and two periods (12–18 h and 18–24 h) during the nocturnal phase.

We confirmed that sedentary SMA mice exhibited the same levels of spontaneous activity compared to the sedentary CTRL mice, whatever the analyzed period, with an important increase in activity for both SMA and CTRL mice during the 12–18 h period compared to the other periods (**Figure 3E**). We also confirmed that sedentary SMA mice exhibited significant higher O_2 consumption compared to the sedentary CTRL mice, whatever the analyzed period (**Figure 3F**). However, during the nocturnal 18-24h period, the post-active period, we noted a twofold increase in O_2 consumption difference between sedentary SMA and CTRL mice, suggesting defect in post-active recovery in SMA mice at 12 months of age (**Figure 3F**).

In trained SMA mice, both running and swimming exercises increased total spontaneous activity during the nocturnal phase, when compared to sedentary SMA mice (**Figures 3B,D**), without any modification of the O_2 consumption (**Figure 3F**). While this spontaneous activity increase was significant for running SMA mice at both nocturnal periods, swimming exercise increased significantly this activity for the 12–18h period, but we observed just a tendency during 18–24 h period, when compared to sedentary SMA mice (**Figure 3E**). This suggest a restoration of the ratio activity/O_2 consumption for trained mice and a decrease in the post-active overconsumption of O_2. Interestingly, for control mice, both training protocols induced an elevation of oxygen consumption with the spontaneous activity increasing too, maintaining an energetic adequacy on all analyzed periods (**Figures 3E,F**).

Taken as a whole, our data suggest a drastic imbalance between O_2 consumption and spontaneous activity in sedentary SMA mice compared to controls, which was worsened during the low activity period of the nocturnal phase, just after the high active nocturnal phase. This supports the hypothesis of a longer period of Excess Post-Exercise Oxygen Consumption (EPOC) (Borsheim and Bahr, 2003) in sedentary SMA mice. Importantly,

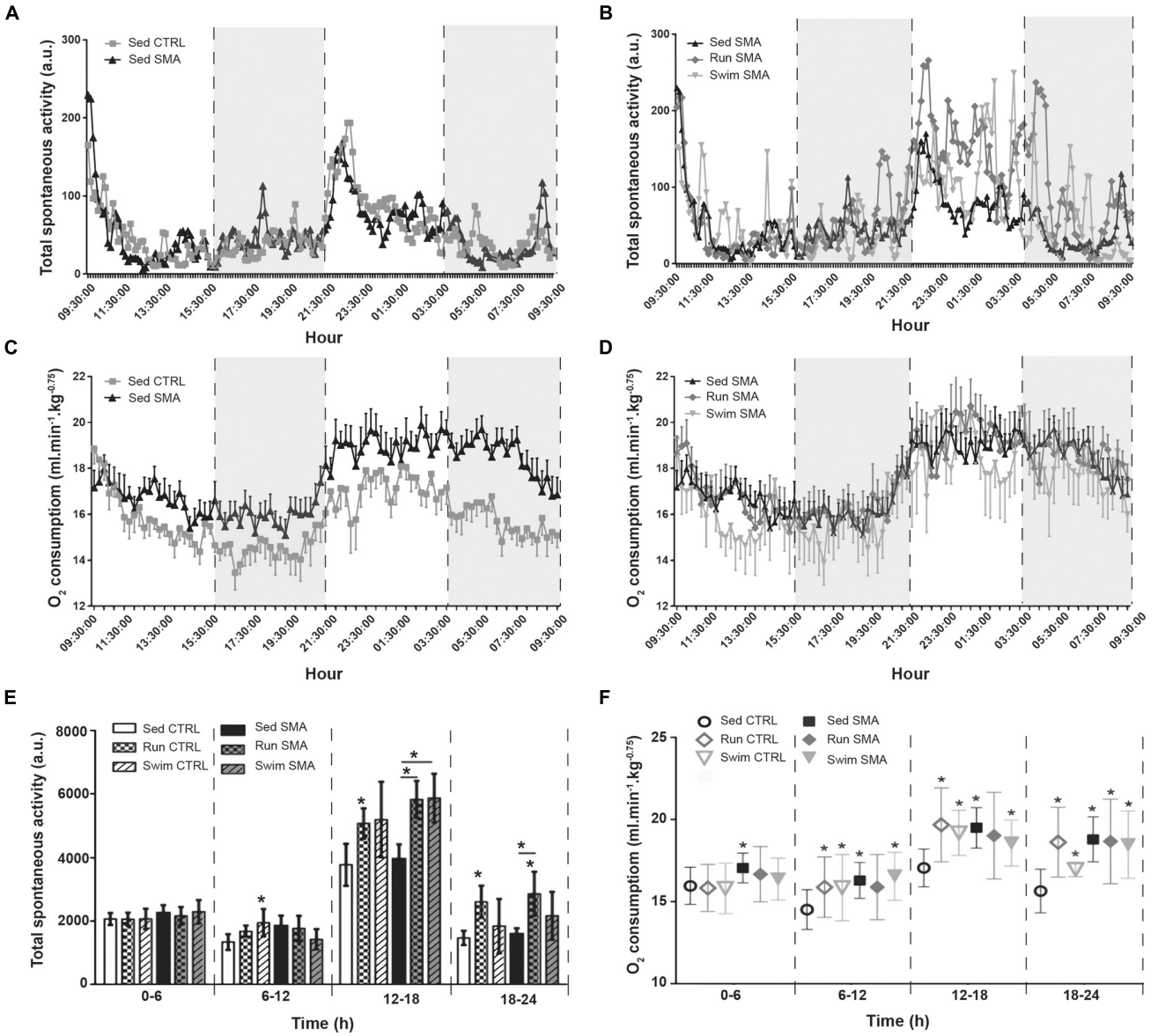

FIGURE 3 | Oxymetry and spontaneous activity analysis in sedentary and trained SMA and control mice: **(A,B)** 24 h spontaneous activity of **(A)** sedentary control and SMA mice and **(B)** Sed SMA compared to Run SMA and Swim SMA mice at 12 months of age. Black dotted lines and gray background delimit periods of 6 h (9 h 30–15 h 30, 15 h 30–21 h 30, 21 h 30–3 h 30 and 3 h 30–9 h 30). **(C,D)** 24 h dioxygen consumption of **(C)** sedentary control and SMA mice, and **(D)** Sed SMA compared to Run SMA and Swim SMA mice at 12 months of age. **(E,F)** Quantification of spontaneous activity **(E)** and oxygen consumption **(F)** according to periods of 6 h from 12 months old sedentary and trained control and SMA mice (n = 12 for sedentary mice, n = 12 for Run CTRL, n = 8 for Run SMA and n = 6 for Swim mice). * and -*- indicated significance relative to sedentary control and SMA mice, respectively (with $P < 0.05$).

both exercise protocols seemed to limit this discrepancy by restoring a normal ratio between O_2 consumption and activity.

Exercise-Specific Modulation of Nutrients Oxidation in SMA Mice

In order to evaluate if the observed systemic lipid and glucose homeostasis defects could parallel or inflict the discrepancy between O_2 consumption and muscle activity, we focused on CO_2 production to determine the RER and then the Fat and Carbohydrate (CH) oxidation levels in sedentary and trained control and SMA mice.

In sedentary SMA mice, and in contrast with O_2 consumption, the CO_2 production was not significantly increased, except during the second half of the nocturnal phase (**Figures 4A,E**) and with lower amplitude when compared to O_2 overconsumption (+8% of CO_2 production and +24% of O_2 consumption for sedentary SMA compared to CTRL mice). This led to a global shift toward a lower RER, indicating an increase in β-oxidation in sedentary SMA mice compared to CTRL mice at 12 months

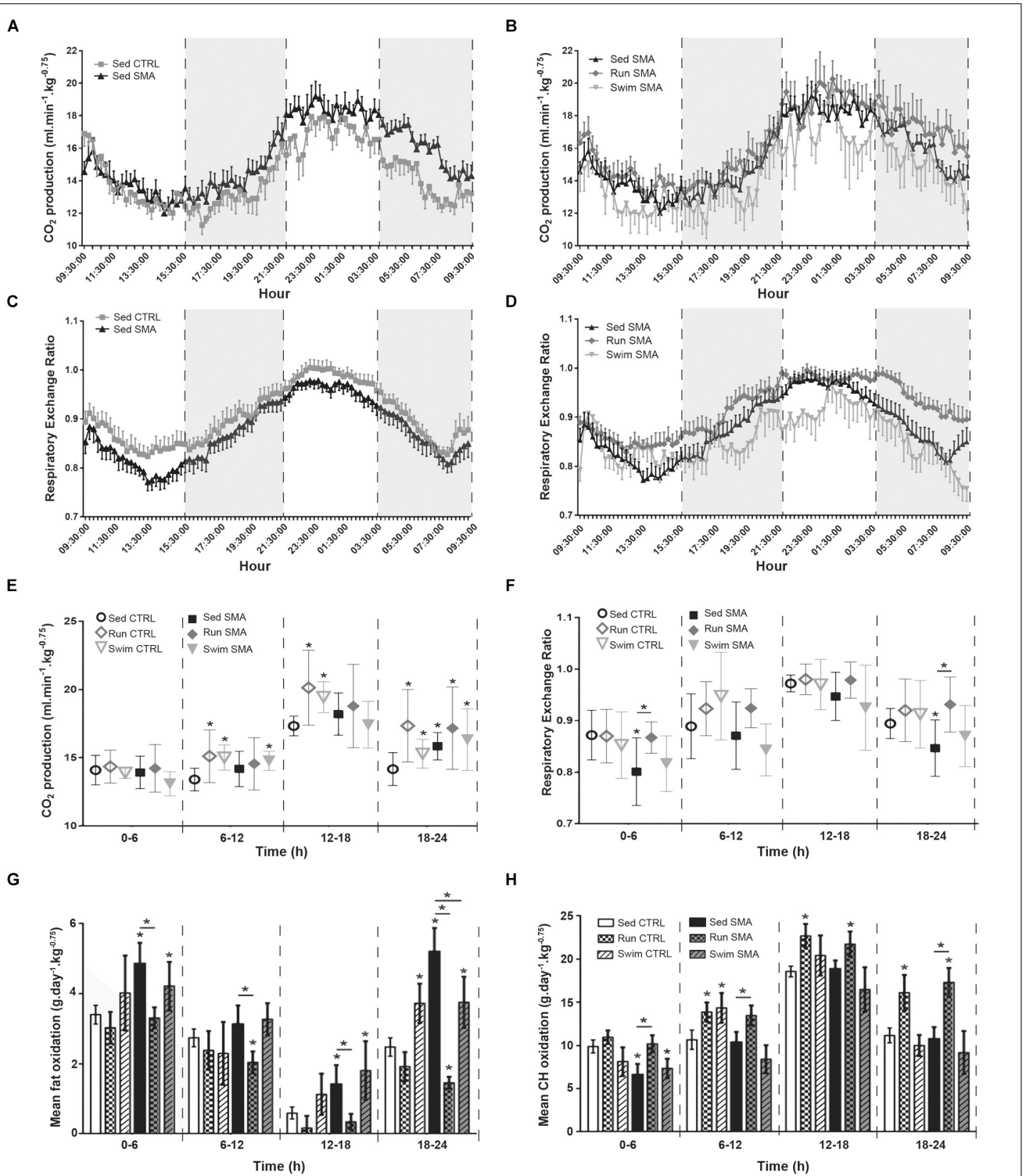

FIGURE 4 | Nutrients oxidation measurements in sedentary and trained SMA and control mice. **(A,B)** 24 h carbon dioxide production of **(A)** sedentary control and SMA mice and **(B)** Sed SMA compared to Run SMA and Swim SMA mice at 12 months of age. Black dotted lines and gray background delimit periods of 6 h (9 h 30–15 h 30, 15 h 30–21 h 30, 21 h 30–3 h 30 and 3 h 30–9 h30). **(C,D)** 24 h respiratory exchange ratio of **(C)** sedentary control and SMA mice, and **(D)** Sed SMA compared to Run SMA and Swim SMA mice at 12 months of age. **(E,F)** Quantification of carbon dioxide production **(E)** and respiratory exchange ratio **(F)** according to periods of 6 h from 12 months old sedentary and trained control and SMA mice. **(G,H)** Quantification of mean fat oxidation **(G)** or carbohydrate oxidation **(H)** according to periods of 6 h from 12 months old sedentary and trained control and SMA mice (n = 12 for sedentary mice, n = 12 for Run CTRL, n = 8 for Run SMA and n = 6 for Swim mice). * and -*- Indicated significance relative to sedentary control and SMA mice, respectively (with $P < 0.05$).

of age (**Figures 4C,F**), except during the high active 12–18 h period (**Figure 4F**). This metabolic shift was confirmed with a significant increase in total fat oxidation during all resting periods (0–6, 12–18, and 18–24 h), when compared to sedentary CTRL mice, and even worsening during the 18–24 h recovery period (**Figure 4G**). In contrast, except during the 0–6 h period, no significant modification of CH oxidation was observed in sedentary SMA mice compared to controls (**Figure 4H**). This confirmed the systemic metabolism alterations in sedentary SMA mice with a predominance of β-oxidation during the resting periods of the day.

Interestingly, if both exercise protocols did not significantly modify CO_2 production in trained SMA mice when compared to sedentary SMA mice (**Figures 4B,E**), an important variability was observed for the low-intensity running protocol (**Figure 4E**). This induced a significant increase in RER (**Figures 4D,F**) in the low active periods (0–6 and 18–24 h), which was confirmed by a significant decrease in fat oxidation (**Figure 4G**) and an associated increase in CH oxidation (**Figure 4H**) when compared to sedentary SMA mice. In contrast, the high intensity swimming protocol did not modify significantly the RER (**Figures 4D,F**), neither fat (**Figure 4G**) nor CH (**Figure 4H**) oxidations during the entire 24 h of recordings when compared to sedentary SMA mice, but reduced fat oxidation during the recovery period (18–24 h). However, for the trained control mice, both protocols induced a significant increase in CO_2 production from 6 to 24 h of recording (**Figure 4E**), which paralleled the increase in activity and O_2 consumption when compared to sedentary controls (**Figures 3E,F**). So, we did not observe any significant shift in RER, only a slight increase (**Figure 4F**). Regarding nutrients oxidations, we confirmed that the low-intensity running protocol favored CH oxidation in trained control mice when compared to sedentary control mice, from 6 to 24 h, while the high-intensity swimming protocol favored fat oxidation, which was significant during the recovery period (18–24 h) (**Figures 4G,H**).

Altogether, these results showed higher fat oxidation levels in sedentary SMA mice when compared to control mice, which was exacerbated during the recovery period, supporting again the hypothesis of a longer period of EPOC in SMA. Importantly, we demonstrated that both exercise protocols were beneficial via exercise-specific modulation in nutrients oxidation, more prominent in SMA compared to controls, which supports the association between low-intensity running and CH oxidation, and between high-intensity swimming and fat oxidation.

Low-Intensity Running and High-Intensity Swimming Reduced Energy Expenditure Defects in SMA-Mice

In sedentary SMA mice, the global alteration in oxidative metabolism with an uncoupling between O_2 consumption and muscular activity led us to hypothesize about a possible enhancement in basal EE and so, a reduction in energy supply. In order to test this hypothesis and determine if exercise protocols could reduce metabolic alterations, we evaluated total energy expenditure (EE) during the four periods for the sedentary and trained control and SMA mice at 12 months of age (**Figure 5A**). Moreover, we refined this analysis by evaluating mice EE during either resting condition (activity below 10, Min) or active condition (activity up to 100, Max) in diurnal and nocturnal phases, pointing out the delta of EE between minimal and maximal EE.

Concerning total EE, sedentary SMA mice showed a significant increase compared to sedentary control mice, whatever the analyzed period (**Figure 5A**). This increase in EE could not be explained by a defect in thermoregulation since sedentary SMA and CTRL mice presented the same rectal temperature during ambient air, cold air and cold water stress tests (**Supplementary Figures S3A,B**). However, during the nocturnal 18–24 h period, the post-active period, we noted that the difference in total EE between sedentary SMA and CTRL mice increased twofold ($<+10\%$ of EE between 0 and 18 h for $>+20\%$ between 18 and 24 h), confirming the persistence of high EE during the low active phase and long after the high active phase (**Figure 5A**). This alteration was also observed by comparing the EE during minimum activity and the EE during maximum activity. Interestingly, SMA mice showed significant increase in minimal EE for both diurnal and nocturnal phases (**Figures 5B,D**) which induced a decrease in the delta of EE for the same periods (**Figures 5C,E**). Taken together, these results suggest a decrease in energy supply in sedentary SMA mice due to an increase in resting EE, limiting their ability to adapt their energy to further elevation in activity.

If both exercise protocols did not modify the total EE in trained SMA mice compared to sedentary SMA mice (**Figure 5A**), both protocols significantly enhanced the delta of EE for both diurnal and nocturnal phases (**Figures 5C,E**). For the running exercise, this energy supply enhancement was due to a non-significant elevation of maximal EE combined with a non-significant decrease in minimal EE. In contrast, for the swimming exercise, this benefit mainly due to a reduction in minimal EE, only significant during nocturnal phase (**Figures 5B,D**). However, for the trained controls, we only observed a significant elevation of total EE during the nocturnal phase (**Figure 5A**). This increase could be associated with the significant increase in spontaneous activity (**Figure 3E**). Finally, training protocols did not alter the delta between minimal and maximal EE for both diurnal and nocturnal phases despite a tendency to increase in CTRL mice (**Figures 5C,E**).

Taken as a whole, our data support a specific alteration in basal EE for an equivalent activity, which reduces energy supply for sedentary SMA mice, and which points out alterations in muscular mitochondria function. However, despite protocol-specific adaptations, both exercise protocols seemed to limit this energy supply reduction, which raised the hypothesis of an exercise-induced enhancement of mitochondrial functions in SMA.

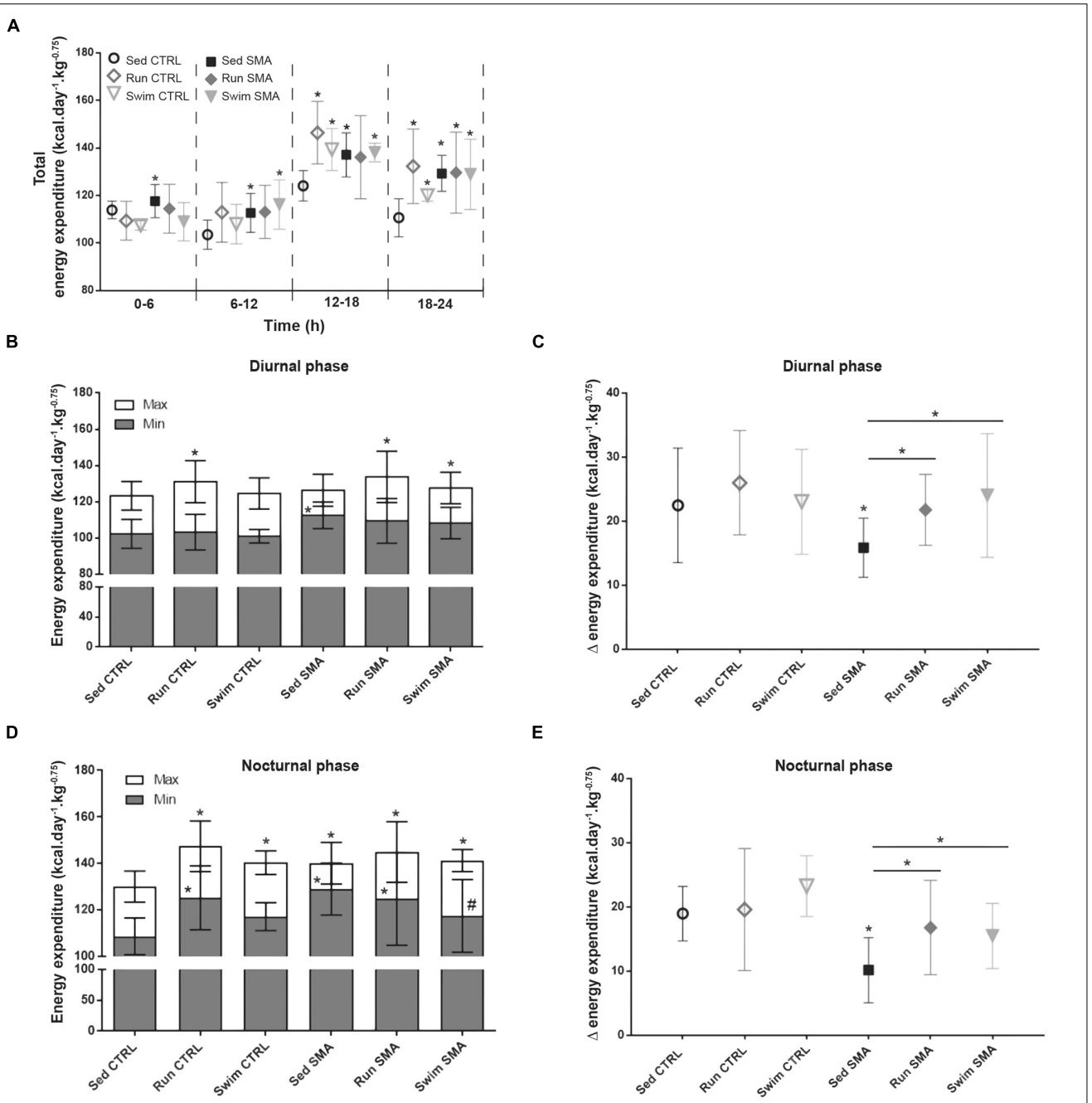

FIGURE 5 | Energy expenditure in sedentary and trained SMA and control mice. **(A)** Quantification of total energy expenditure according to periods of 6 h from 12 months old sedentary and trained control and SMA mice. **(B,D)** Quantification of minimum (<10, Min) and maximum (>100, Max) activity energy expenditure in diurnal **(B)** and nocturnal **(D)** phases of 12 months old sedentary and trained control and SMA mice. **(C,E)** Quantification of the difference between minimum and maximum activity energy expenditure (D) in diurnal **(C)** and nocturnal **(E)** phases from 12 months old sedentary and trained control and SMA mice (n = 12 for sedentary mice, n = 12 for Run CTRL, n = 8 for Run SMA and n = 6 for Swim mice). * and -*- Indicated significance relative to sedentary control and SMA mice, respectively (with $P < 0.05$).

Low-Intensity Running and High-Intensity Swimming Reduced Mitochondria Defects in SMA-Mice

Our systemic and whole-body evaluation of metabolic status in SMA mice pointed out specific alterations in oxidation processes and energy production, confirming muscular mitochondria alterations (Sperl et al., 1997; Berger et al., 2003; Ripolone et al., 2015) also in our mild SMA model mouse. With the elevation of resting EE and long-term O_2 overconsumption post-active phase, we could hypothesize that the efficiency of

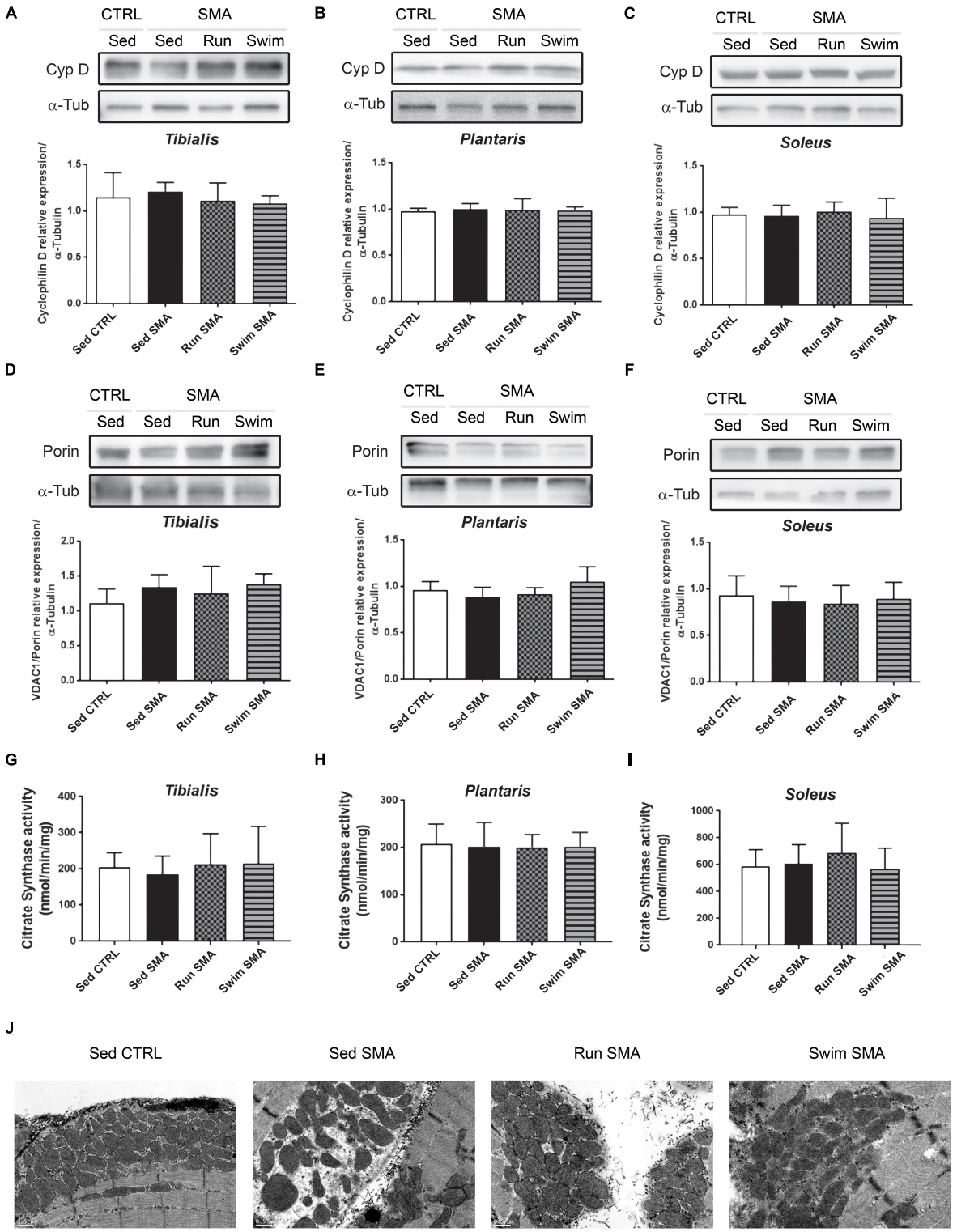

FIGURE 6 | Muscular mitochondria quantification and qualification in sedentary and trained SMA mice. **(A–F)** Western blot analysis and quantification of cyclophilin D **(A–C)** and VDAC1/porin **(D–F)** steady state protein levels in **(A,D)** fast-twitch flexor *tibialis*, **(B,E)** fast-twitch extensor *plantaris* and **(C,F)** slow-twitch extensor *soleus* of sedentary control mice compared to sedentary and trained SMA mice (n = 4 for each group). **(G–I)** Quantification of citrate synthase activity in **(G)** *tibialis*, **(H)** *plantaris,* and **(I)** *soleus* of sedentary control mice compared to sedentary and trained SMA mice at 12 months of age (n = 6). **(J)** Transmission electron microscopy images of submembranar mitochondria in ultrathin (50–90 nm) *tibialis* muscles longitudinal sections from 12 months old sedentary control mice compared to sedentary and trained SMA mice (4000X magnification images).

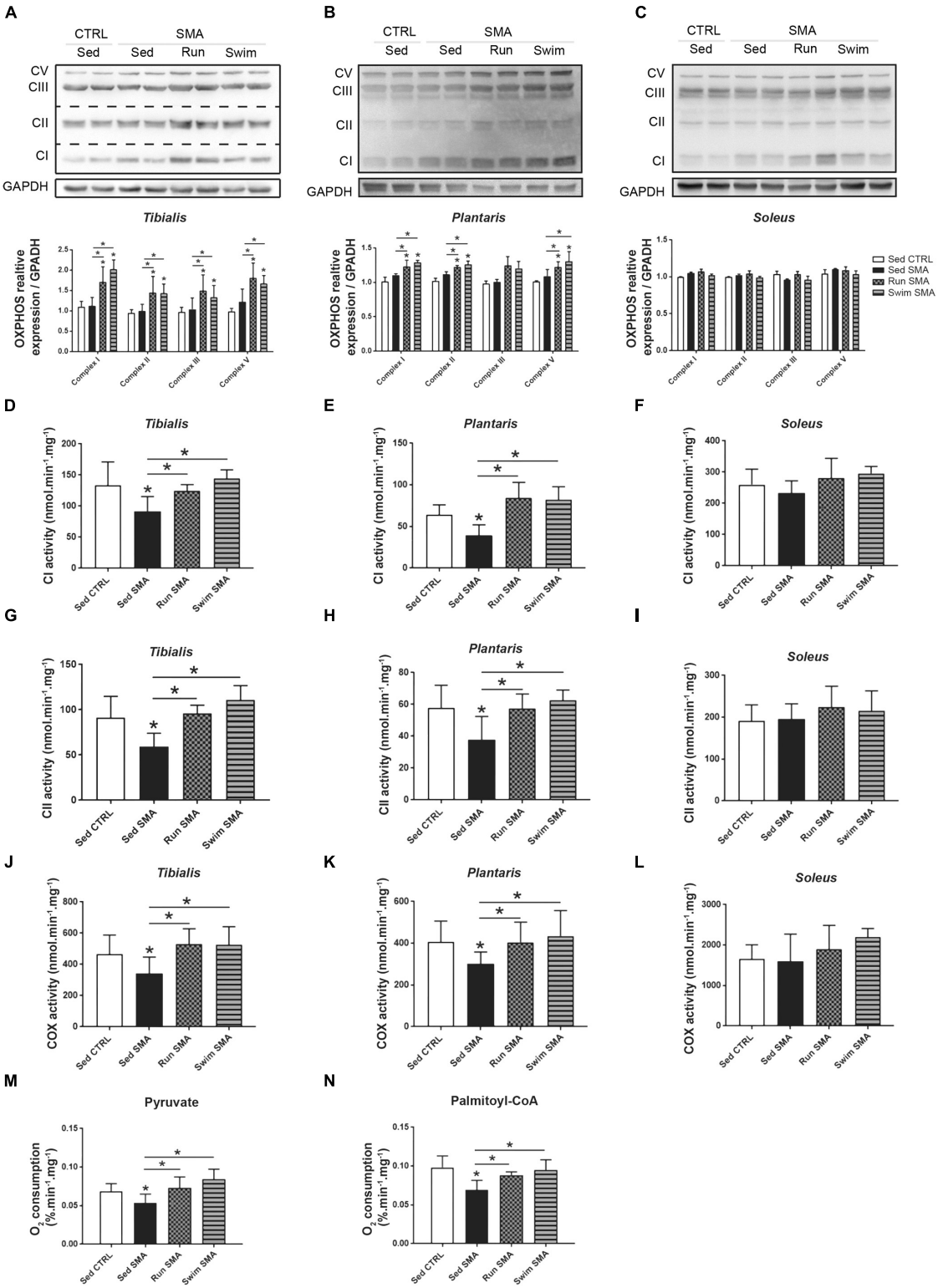

FIGURE 7 | Muscular mitochondrial function in sedentary and trained SMA mice. **(A–C)** Western blot analysis and quantification of CV, CIII, CII, and CI respiratory chain complexes steady state protein levels in **(A)** *tibialis*, **(B)** *plantaris* and **(C)** *soleus* of sedentary control mice compared to sedentary and trained SMA mice ($n = 4$ for each group). **(D–L)** *In vitro* maximal enzymatic activity measurement of **(D–F)** CI, **(G–I)** CII, and **(J–L)** CIV (COX) respiratory chain complexes in **(D,G,J)** *tibialis*, **(E,H,K)** *plantaris* and **(F,I,L)** *soleus* of sedentary control mice compared to sedentary and trained SMA mice ($n = 6$ for each group). **(M,N)** *In vitro* dioxygen consumption measurement of *tibialis* and *plantaris* isolated mitochondria with pyruvate **(M)** and palmitoyl-CoA **(N)** substrates from sedentary control mice compared to sedentary and trained SMA mice ($n = 6$ for each group). * and -*- Indicated significance relative to sedentary control and SMA mice, respectively (with $P < 0.05$).

muscle mitochondria to produce energy is decreased in SMA. To address these issues and to determine how physical exercises could induce metabolic benefits, we evaluated mitochondria status in three different muscles of the calf, the fast-twitch flexor *tibialis*, the fast-twitch extensor *plantaris* and the slow-twitch extensor *soleus*, in sedentary SMA and CTRL mice and in trained SMA mice at 12 months of age.

We analyzed the total amount of mitochondria in sedentary SMA mice compared to control mice via three complementary approaches. Firstly, we quantified by western-blot the amount of Cyclophilin-D, part of the mitochondrial Permeability Transition Pore (PTP) and the outer mitochondrial membrane porin (also called VDAC1) which are usually used as markers of mitochondria enrichment (Webster et al., 2013). No significant differences were observed in the steady state proteins levels in sedentary SMA mice compared to controls (**Figures 6A–F**). Secondly, we measured the citrate synthase activity from the Krebs cycle of the mitochondria matrix and failed to observe any significant differences in activity between sedentary SMA and control mice (**Figures 6G–I**). Finally, no qualitative differences were observed by transmission electron microscopy on mitochondria amount and structure in *tibialis* (**Figure 6J** and **Supplementary Figure S4**) and *soleus* (**Supplementary Figure S4**) longitudinal sections. Our results suggest a preservation of the total amount of mitochondria in analyzed hind limb muscles of our SMA model mouse at 12 months of age. Interestingly, both exercise protocols failed to promote mitochondria biogenesis in SMA muscles, as observed by western blot (**Figures 6A–F**), enzymatic activity (**Figures 6G–I**) or by qualitative transmission electron microscopy (**Figure 6J** and **Supplementary Figure S4**) despite energetic benefits in whole-body measurements.

Taken together, these results suggest that metabolic alterations in SMA and beneficial effects of exercise protocols should rely on mitochondria function. This led us to evaluate the expression and enzymatic activity of the different respiratory chain complexes. Using OXPHOS mix antibody by western blot analysis, we did not observe difference in the steady state protein level of all respiratory complexes and for all analyzed muscles of sedentary SMA mice compared to controls (**Figures 7A–C**). However, using *in vitro* maximal enzymatic activity measurements of the respiratory chain complex I (NADH:ubiquinone oxidoreductase), complex II (succinate dehydrogenase) and complex IV (cytochrome c oxydase), we noted a significant reduction in their activity in *tibialis* and *plantaris* only (**Figures 7D,E,G,H,J,K**), while no significant modification was measured for the slow-twitch *soleus* muscle (**Figures 7F,I,L**). This result suggests a muscle-specific alteration in respiratory chain function, involving phasic fast-twitch muscles and sparing tonic slow-twitch muscle.

Interestingly, both exercise protocols promoted respiratory chain complexes expression in fast-twitch *tibialis* and *plantaris* muscles, but not in slow-twitch *soleus* (**Figures 7A–C**). This muscle-specific overexpression of complexes were associated with a restoration of enzymatic activity in fast-twitch muscles of SMA mice to sedentary control values (**Figures 7D,E,G,H,J,K**). No significant differences in complexes activity were observed in the slow-twitch *soleus* despite a tendency toward an increase (**Figures 7F,I,L**). So, exercise seemed to enhance mitochondrial function through an increased respiratory chain complexes overexpression.

Finally, we sought to understand the link between mitochondrial function alterations and the systemic ones, by evaluating their ability to consume O_2 under either carbohydrate or lipid substrate. We performed mitochondrial isolation of both fast-twitch muscles and we measured their maximal rate of O_2 consumption with either pyruvate or palmitoyl-CoA substrate, *in vitro*. Interestingly, we observed a decrease in O_2 consumption for both substrates in sedentary SMA mice compared to control mice (**Figures 7M,N**), supporting a decrease in mitochondrial efficiency in producing energy. However, after both physical trainings, isolated mitochondria from fast-twitch muscles recovered their ability to consume O_2 under both substrates (**Figures 7M,N**), with a tendency in swimming exerting better effects than running.

Taken together, these experimental results support the hypothesis of a muscle-specific alteration in mitochondrial efficiency, without changes in mitochondrial quantity or in the respiratory chain complexes expression, the latter being restored by both exercise protocols.

DISCUSSION

The high variability in metabolic alterations in SMA patients (Dahl and Peters, 1975; Bruce et al., 1995; Tein et al., 1995; Crawford et al., 1999; Berger et al., 2003; Sproule et al., 2009; Zolkipli et al., 2012; Davis et al., 2015; Bertoli et al., 2017; Kolbel et al., 2017) and mouse models (Bowerman et al., 2012, 2014; Ripolone et al., 2015) can preclude or limit the application, design or efficiency of therapies, including active exercise. Thus, deciphering the origin and mechanisms involved in those metabolic alterations and the way to modulate them appeared crucial. In the present work, we addressed two original points. One, we provide the first lines of evidence indicating mild SMA-like mice are affected by a state of "chronic oxygen debt" due to a decrease in fast-twitch SMA muscle mitochondrial efficiency, which might be at the root of SMA-induced metabolic alterations. This "chronic oxygen debt" in mild SMA-like mice induces elevation in resting O_2 consumption and lipids oxidations, both worsening after long periods of activity. It also induces an elevation in basal EE, and the decrease in energy supply. This metabolic shift may ultimately inflict the systemic metabolic perturbations already reported in SMA mice and patients, including glucose homeostasis impairments and lipid overload. Two, long-term exercise protocols were able to significantly enhance mitochondrial efficiency in SMA fast-twitch muscles, promoting glucose re-use and enhancing lipid metabolism. However, we demonstrated that the high-intensity swimming benefits go through elevated β-oxidation while low-intensity running benefits go through carbohydrate oxidation, showing exercise-specific adaptations.

Usually, the concept of "oxygen debt" is used to characterize the metabolic state of skeletal muscles during physical exertion

(Borsheim and Bahr, 2003). In that case, a cumulative deficit of oxygen results from the high energetic demand imposed to muscles to sustain increased workload during physical exercise. When muscles require more energy than oxidations can deliver, the anaerobic pathway compensates the deficit, resulting in lactate production, in the progressive decrease in strength and then fatigue. When the exercise stops, an excess postexercise oxygen consumption (EPOC) or reimbursement of the oxygen debt occurs, with the body consuming more oxygen than usual at rest for up to 24 h. This recovery period allows the elimination of the produced lactate, replenishment of energy substrate stocks, restoring fuel balance and allowing cellular adaptations through the β-oxidation pathway (Kiens and Richter, 1998; Egan and Zierath, 2013). In the SMA context, for which maximal mitochondria oxygen consumption is significantly reduced from both carbohydrate and lipid substrates (**Figures 7M,N**), the spontaneous activity of mice is sufficient to chronically induce an oxygen debt. The present results show for the first time, an abnormally elevated EE at rest in SMA mice (**Figures 5B,D**), independent of thermoregulation defects, mainly supported by β-oxidation pathway (**Figures 4C,F,G**) and worsening during the post-active period of the nocturnal phase (**Figure 5A**). These results are also highly consistent with previous data reporting a chronic elevation of resting lactatemia in SMA-like mice (Chali et al., 2016). Thus, with a reduced maximal mitochondria oxygen consumption, SMA induces a chronic overuse of oxidation at rest, compared to control mice (**Figure 3C**). A second consequence of this "chronic oxygen debt" is the reduction of muscular energy supply in SMA mice. The increase in basal EE, to reimburse oxygen debt and/or produce sufficient energy for basal behavior, reduces the delta of energy between rest and muscular activity, enhancing in turn the risk of "oxygen debt" when mice will be active. This process appears as a feed-forward negative loop. Finally, this feature of "chronic oxygen debt" and reduced mitochondria efficiency in SMA could explain, at least in part, the muscular fatigue commonly described in SMA patients (Montes et al., 2010; Pera et al., 2017).

Importantly, this state of "chronic oxygen debt" was limited by long-term physical exercise in mild SMA-like mice, but in an exercise-type manner. Both exercise protocols improved mitochondria oxygen consumption from both carbohydrate and lipid substrates, subsequently reducing the resting oxidation function and therefore increasing muscle energy supply (**Figures 5C,E**). However, the swimming-based training induced a decrease in the resting EE while running increases the maximal EE during the nocturnal phase. Thus, in both cases, we observed an increase in the amplitude of energy supply during the nocturnal active phase, allowing better adaptation to any further increased in workload (**Figures 5B–E**). In this study, the effect differences between the two exercise protocols may be related to the differential use of energetic substrates linked to exercise intensity (Chali et al., 2016). Indeed, while the swimming-based training maintained a strong use of the lipid β-oxidation, without modifying the use of carbohydrate, the running-based training shifted the metabolism from β-oxidation toward the use of carbohydrates oxidation (**Figures 4G,H**), as also suggested by the decreased lactatemia in the running-based trained mice (Chali et al., 2016). Interestingly, this exercise-induced difference in energy pathway could explain, by modulating the RER, the elevated resting EE in running-based trained SMA-like mice compared to swimming SMA-like mice, as observed in the trained control mice (**Figures 5B,D**).

Interestingly, we found that only the fast-twitch muscles were affected by these mitochondrial activity defects, suggesting that the state of "chronic oxygen debt" could mainly originate from the limitation of fast-twitch muscles to catabolize carbohydrates by the aerobic pathway. Furthermore, this muscle-specific defect is likely to explain the high lactatemia found in mild SMA-like mice. To sustain the energy demand during the active phase, the fast-twitch muscles may then use lipids as replacement substrates, but with limited energetic power since β-oxidation only occurs in aerobic conditions and with multiple enzymatic steps (Fillmore and Lopaschuk, 2013). The consequence of these muscular mitochondrial defects are reflected at systemic level, with a decrease in glucose use, highlighted by glucose intolerance, and an increase in lipid use, highlighted by the decrease in the RER in SMA-like mice. Consistently, both exercise induced significant increases in the expression of mitochondrial respiratory chain complexes in fast-twitch muscles, resulting in significant improvements in oxidative capacities of muscular SMA mitochondria. Interestingly, the oxidative activity, *per* respiratory chain complex, remained unchanged in exercised muscles compared to sedentary ones, suggesting that the increased oxidative efficiency induced by exercise relates to the increased number of respiratory chain complexes, and not to their intrinsic functional properties. Moreover, the number of mitochondria *per* muscle did not change. Therefore, improving the mitochondrial oxidative capacity could alone explain the beneficial effects of exercise. Indeed, with an aerobic metabolism functioning properly again, fast-twitch muscles can reuse glucose via the aerobic pathway, thus limiting lactate production and, finally, glucose resistance. Ultimately, the state of "chronic oxygen debt" is reduced, as is the resting EE.

Surprisingly, the slow-twitch *soleus* muscle seems spared from SMA-induced alterations in respiratory chain function compared to fast-twitch *tibialis* and *plantaris*. This muscle-specific metabolic alteration has never been reported yet and could be partly explained by two main physiological points. Firstly, slow-twitch muscles are characterized by high amount of mitochondria with an oxidative-based metabolism allowing them to efficiently catabolize lipids, while fast-twitch muscles favor carbohydrate catabolism. So, in the context of SMA with glucose resistance and hyperlipidemia, slow-twitch muscle should suffer less compared to fast-twitch muscles. Secondly, slow-twitch muscles are tonic, that mean continuously contracted to maintain posture against gravity, while fast-twitch muscles are phasic, contracting only for movement. Putting these muscle specificities in parallel with our data supporting the fact that the more active is the muscle, more its function is protected and efficient, could also explain this muscle-specific alteration. Thus, our data reinforce the hypothesis which link neuromuscular activity and protection against SMA (Chali et al., 2016).

Glucose homeostasis impairments have been already reported in other SMA mouse models (Bowerman et al., 2012, 2014).

Consistently with these previous results, we report here a fasting hyperglycemia in mild SMA-like mice compared to controls. This defect occurs as early as 3 months, thus far away before the beginning of MN death in this model (Tsai et al., 2006). Interestingly, SMA tissues remained sensitive to insulin, suggesting a SMA-induced impairment in the equilibrium between blood insulin and glucagon concentrations, as previously shown (Bowerman et al., 2012, 2014). In trained SMA-like mice, although fasting glycemia remained high and unchanged compared to sedentary SMA-like mice, the glucose tolerance was significantly improved, as expected with exercise which improves directly insulin response in muscles (Way et al., 2016; Kleinert et al., 2018).

Moreover, the systemic and muscular metabolic impairments recorded in sedentary mild SMA-like mice, and their adaptations under exercise paradigms are also evidenced at the systemic level. Sedentary mild SMA-like mice displayed the same weight curve and the same levels of food intake as control mice but had yet more adipocytes. High lipid storage is expected in case of glucose resistance and preferential lipid use for insuring energetic metabolism (Samuel and Shulman, 2016). Consistently with these observations, the mRNA expression of lipogenic enzymes such as *Acc1*, *Acc2* and *Fasn* were found significantly increased in SMA fat tissues. Expectedly, both exercise protocols resulted in a significant decrease in fat mass and body weight, with yet a tendency to increase food intake in SMA-like mice. These benefits could be due to the enhancement in glucose tolerance and associated with a significant decrease in the mRNA expression of lipogenic enzymes in fat tissues, demonstrating a whole-body metabolism enhancement.

Finally, this study opened new avenues in the SMA pathophysiology decryption, pointing out the alteration in intrinsic respiratory chain function as a possible key for a large part of metabolic defects. Without any modification in the total amount of those crucial complexes, this specific decrease in maximum activity should rely to post-translational modifications, mechanism already reported in other neurodegenerative disorders (Nakamura and Lipton, 2017) and need to be deciphered in SMA via a dedicated research project.

Thus, taken together, these findings argue for the use of physical exercise as a therapeutic intervention for SMA patients, in complement to pharmacological or gene-therapies aimed at improving SMN expression in MNs. To do so, two key elements should be taken into account, the metabolic status and the fatigue.

It is admitted today that the metabolic status of SMA patients are diverse, notably in terms of diabetes, obesity or insulinemia (Davis et al., 2015; Kolbel et al., 2017), resulting in different abilities for them to perform active exercise. Therefore, particular attention should be paid to the exercise paradigm used, in order to fit the training protocol to the expected metabolic adaptation of each patient. In this way, it is important to note that the current study has been conducted only on male mice, in accordance with the original work we recently published on the neuroprotective effects of physical exercise in type 3 SMA-like mice (Chali et al., 2016). Nevertheless, it is now well described that the gender can inflict both the metabolic status (Medrikova et al., 2012; Venezia et al., 2016; Reynolds et al., 2019) and the exercise-induced benefits (Veldink et al., 2003; McMullan et al., 2016; Zhou et al., 2018). Thus, it seems crucial to evaluate if female SMA mice with the same genetic background than the males used in the present study could identically adapt to the same exercise protocols.

Finally, the muscular fatigue appears as an important feature in SMA. For instance, the main complaint of type 3 SMA patients during the application of a 12-week aerobic training on ergo cyclometer was an excessive fatigue, even if exercise intensity was low (Madsen et al., 2015). So, the application of an intensive active physiotherapy could appear contradictory. Nonetheless, we demonstrated here that high-intensity exercise i.e., the swimming-based protocol, expected to induce high fatigue levels, has highest beneficial effects than low-intensity exercise i.e., the running-based protocol. These results suggest that a threshold of exercise intensity, and therefore of fatigue, should be exceeded repeatedly throughout the training, in order to obtain beneficial effects on muscular function. Thus, if we take into account the two studies (Madsen et al., 2015 and the present study), a high-intensity exercise would be necessary, even if it induces a high level of fatigue during exercise, to improve muscle function metabolism, ultimately improving resistance to current life fatigue.

All those elements, taken as a whole, support the necessity to use precision and personalized intervention in order to optimize exercise-induced benefits for SMA patient care.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the manuscript/**Supplementary Files**.

ETHICS STATEMENT

Animal handling and experimentation were performed in line with approved Institutional Animal Care and Use Committee protocols at the University of Paris Descartes (CEEA 34, agreement number B75-06-07) and followed the national authority (Ministere de la Recherche et de la Technologie, France) guidelines for the detention, use and the ethical treatment of laboratory animals based on European Union Directive 2010/63/EU.

AUTHOR CONTRIBUTIONS

LH conducted and analyzed the majority of experiments. DD'A conducted the mRNA, adipocytes, and glucose homeostasis experiments. JB supervised, participated, and analyzed the mitochondrial experiments. FaC and CD participated in the animal care, glucose homeostasis, and tissues collection. VR conducted and analyzed the Western blot experiments. JS and CO participated and analyzed the enzymatic activity measurements. TR and BB conducted and analyzed the physiocages experiments and participated in the writing of the manuscript. JR supervised

the physiocages experiments. DS helped in animal care and tissues collection. LW and PL helped in the writing of the manuscript. FD helped in analyzing the data and writing of the manuscript. CB participated in the mRNA experiments and animal care. FrC helped in supervising the project and writing of the manuscript. OB supervised the project, conducted the mitochondrial experiments, analyzed the data, and wrote the manuscript. All authors have approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

FUNDING

LH is the recipient of a Ph.D. fellowship from the Ministry of Research and Technology. This work was supported by AFM (Association Française contre les Myopathies), Paris Descartes University, and INSERM fundings.

ACKNOWLEDGMENTS

All the authors wish to thank C. Mader for animal care, J. Coppet for animal training, and Cochin Institute electron microscopy facility.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.01258/full#supplementary-material

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Conflict of Interest: CB was employed by company Biophytis. TR, BB, and JR were employed by company Biomeostasis. A patent on active physiotherapy for SMA patients is pending.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 01 October 2019
doi: 10.3389/fphys.2019.01243

Can Proprioceptive Training Reduce Muscle Fatigue in Patients With Motor Neuron Diseases? A New Direction of Treatment

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Muscle fatigue is a serious problem in patients with motor neuron diseases (MNDs). It commonly disturbs both daily life activity and rehabilitation tolerance. A particular concern should be taken when MNDs occur in older ages. Older patients with MNDs usually have a worse clinical presentation and a lower survival rate. This could increase the occurrence of muscle fatigue. Muscle fatigue occurs due to a dysfunction in either motor or sensory systems. Current exercise interventions performed to decrease the occurrence of muscle fatigue focused only on treating motor causes of muscle fatigue. It has been demonstrated that these interventions have a high debate in their effectiveness on decreasing the occurrence of muscle fatigue. Also, these exercise interventions ignored training the affected sensory part of muscle fatigue, however, the important role of the sensory system in driving the motor system. Thus, this review aimed to develop a novel exercise intervention by using proprioceptive training as an intervention to decrease the occurrence of muscle fatigue in patients with MNDs particularly, older ones. The physiological effects of proprioceptive training to decrease the occurrence of muscle fatigue could include two effects. The first effect includes the ability of the proprioceptive training to increase the sensitivity of muscle spindles as an attempt to normalize the firing rate of α-motoneurons, which their abnormalities have major roles in the occurrence of muscle fatigue. The second effect includes its ability to correct the abnormal movement-compensations, which develop due to the biomechanical constraints imposed on patients with MNDs.

Keywords: motor neuron diseases, muscle fatigue, older patients, proprioceptive, training

OPEN ACCESS

Edited by:
Francesca Lanfranconi, Victoria University, Australia

Reviewed by:
Andrea Rigamonti, ASST Lecco, Italy
Juergen Konczak, University of Minnesota, United States

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *24 November 2018*
Accepted: *10 September 2019*
Published: *01 October 2019*

Citation:
Mohamed AA (2019) Can Proprioceptive Training Reduce Muscle Fatigue in Patients With Motor Neuron Diseases? A New Direction of Treatment. Front. Physiol. 10:1243. doi: 10.3389/fphys.2019.01243

INTRODUCTION

Muscle fatigue is a common problem in patients with motor neuron diseases (MNDs) (Gibbons et al., 2013). MNDs are defined as a group of diseases in which there is a progressive degeneration of motor neurons (Quansah and Karikari, 2015). MNDs include three main subtypes. The first subtype includes disorders which affect lower motor neurons, such as spinal muscular atrophy (SMA) and spinobulbar muscular atrophy (SBMA or Kennedy's disease). The second subtype includes disorders which affect upper motor neurons, such as spastic paraplegias and primary

lateral sclerosis (PLS). The last subtype includes disorders which affect both upper and lower motor neurons, such as amyotrophic lateral sclerosis (ALS) (Figlewicz and Orrell, 2003).

One of the common complaints in patients with MNDs is muscle fatigue (McElhiney et al., 2009; Abraham and Drory, 2012; Gibbons et al., 2018). Muscle fatigue is defined as the time-related reduction in the maximum force-production capacity of the muscle. Muscle fatigue is one of the most common complaints occur in patients with MNDs. McElhiney et al. (2009) have demonstrated that muscle fatigue affects approximately 44% of patients with MNDs. It has been reported that there are several causes for the occurrence of muscle fatigue in MNDs. Most studies have demonstrated that neurodegenerative cause is the main cause of muscle fatigue in patients with MNDs, while the neuromuscular transmission, and muscle metabolism are normal in those patients (Abraham and Drory, 2012). In contrast, other studies have stated that patients with ALS usually have a neuromuscular junction disassembly and muscle denervation and this abnormality of the neuromuscular junction is trademark feature of ALS onset and progression. Other studies have shown that there is a severe abnormality in the mitochondrial function in patients with ALS (Bowling et al., 1993; Dupuis et al., 2005; Vandoorne et al., 2018).

However, MNDs are common in adults, this previous debate increases in older ages because older patients with MNDs usually experience more complex clinical presentation and lower survival rate (Eisen et al., 1993; Forbes et al., 2004; Broussalis et al., 2018). It has been demonstrated that the prognosis is much worse in older patients with MNDs than younger ones (Tysnes et al., 1994). Older patients with MNDs usually have a higher incidence of bulbar symptoms than younger ones and this bulbar onset presents in at least half of all patients with MNDs over 80 years (Eisen et al., 1993; Forbes et al., 2004; Broussalis et al., 2018). Also, older patients with MNDs could experience more occurrence of muscle fatigue during exercises than younger ones because aging produces an abnormality in normal function and firing rate of the α-motoneurons (Soderberg et al., 1991; Knight and Kamen, 2007; Watanabe et al., 2016), which is considered one of the main causes of muscle fatigue.

Furthermore, it has been shown that elderlies usually experience a high degree of muscle fatigue during exercise rather than younger ones (Klass et al., 2007; Mcneil and Rice, 2007; Kent-Braun, 2009). Klass et al. (2007) investigated the effect of aging on the fatigability of ankle dorsiflexor muscles throughout concentric and eccentric contractions. They found that fatigability increased progressively with aging and they argued this increase in fatigability to peripheral alterations occurred in Ca^{2+}-controlled excitation-contraction coupling process and neuromuscular propagation. Thus, the occurrence of MNDs in older ages could cause further alteration in the Ca^{2+}-controlled excitation-contraction coupling process and neuromuscular propagation.

Moreover, it has been demonstrated in the literature that there is a change in muscle fibers with both aging and MNDs. With aging, type I muscle fibers usually transform to type II, which increases the occurrence of muscle fatigue during exercises (Mcneil and Rice, 2007; Kent-Braun, 2009). Also, it has been shown that with MNDs, there are atrophic changes in muscle fibers with mild denervation. Thus, older patients with MNDs could experience more changes in muscle fibers than younger ones due to the combination of aging and MNDs effects. Thus, older patients with MNDs should not be neglected from future revisions.

Current exercise interventions performed to treat muscle fatigue in patients with MNDs are few and their qualities are very low (Gibbons et al., 2018). Thus, it is impossible to reach strong conclusions about the effectiveness of these interventions to reduce the occurrence of muscle fatigue in patients with ALS/MND (Gibbons et al., 2018). Generally, exercise interventions performed to treat muscle fatigue have assumed that muscle fatigue occurs due to dysfunction in motor control. This dysfunction occurs due to failure in one or more mechanisms included in the voluntary muscle contraction. This failure can occur in any area along the neuromuscular system, including the motor cortex, signals from the motor cortex to motoneurons, signals from motoneurons to muscle, neuromuscular junction coupling in muscle, or actin-myosin links (Light et al., 2010).

Also, current exercise interventions included either grading exercise intensity (Wallman et al., 2004), increasing rest period (Nogueira et al., 2012), using mild training intensity (Dennett et al., 2016), or using massage for the fatigued muscle (Nunes et al., 2016). The effectiveness of these exercise interventions is still in debate. Some studies (Morriss et al., 1996; Friedberg, 2002; Wallman et al., 2004) have demonstrated that increasing physical activity or grading exercise intensity is beneficial in decreasing the occurrence of muscle fatigue. In contrast, other studies (Black et al., 2005; Oh et al., 2016) have shown that increasing physical activity or grading exercise intensity has no effect on reducing the occurrence of muscle fatigue.

It has been shown in the literature that muscle fatigue does not occur due to dysfunction in the motor control only, however, it occurs due to dysfunction in both motor and sensory systems (Light et al., 2010). Several studies have shown that MNDs affect sensory neurons besides motor neurons (Anagnostou et al., 2005; Pugdahl et al., 2007; Vaughan et al., 2015). Pugdahl et al. (2007) conducted a study to detect the presence of any dysfunction in sensory neurons in patients with ALS. They found that about 22.7% of the included patients had an abnormality in the conduction time of at least one sensory nerve. Anagnostou et al. (2005) conducted a study to detect the presence of any dysfunction in sensory neurons in children with SMA. They found that children with SMA had dysfunctions in the conduction time of sensory nerves. Recently, Vaughan et al. (2015) conducted a study to detect the presence of any dysfunction of the proprioceptive system in mice with ALS. They found that these mice had significant degenerations in nerve endings of the proprioceptive system.

Also, it has been demonstrated in several studies that muscle fatigue has sensory receptors responsible for sensing and developing muscle fatigue (Light et al., 2010; Boyas and Guével, 2011; Staud, 2012; Nunes et al., 2016; Kuppuswamy, 2017). However, the vital role of the sensory system in driving motor control (Riemann and Lephart, 2002), till now

there is no exercise intervention focused on improving the function of the sensory element of muscle fatigue. It has been shown in the literature that the sensory mechanism of muscle fatigue starts from mechanoreceptors and metaboreceptors. These receptors are responsible for generating the sensation of muscle fatigue (St Clair Gibson et al., 2003; Light et al., 2010). Mechanoreceptors are primary receptors of muscle fatigue and they are sensitive to changes in muscle strain (St Clair Gibson et al., 2003; Light et al., 2010). While metaboreceptors are secondary receptors of muscle fatigue and they are sensitive to changes in the number of metabolites created by muscle contraction (St Clair Gibson et al., 2003; Light et al., 2010).

Mechanoreceptors are also the same receptors of the proprioception. The sensitivity and function of mechanoreceptors can be improved both neurologically or morphologically by performing proprioceptive training (Kaya, 2016). Thus, it is reasonable to suppose that performing a proprioceptive training to the fatigued muscle could improve the function of mechanoreceptors within this muscle. Consequently, this could be an effective modality to reduce the occurrence of muscle fatigue in patients with MNDs, particularly older patients who have a worse clinical presentation, and a low survival rate. Thus, this review aimed to demonstrate possible physiological mechanisms of proprioceptive training as an exercise intervention to treat muscle fatigue in patients with MNDs, particularly older ones.

This review included seven subtopics, the previous mechanisms of muscle fatigue in MNDs, the proprioception dysfunctions in MNDs, the mechanism of the sensation of muscle fatigue, the possible physiological effects of proprioceptive training on decreasing muscle fatigue in MNDs, the effects of proprioceptive training to correct the imposed biomechanical constraints in MNDs, the effects of aging on pathological degeneration of motor and sensory neurons in patients with MNDs, and the physiological effects of proprioceptive training to create theoretical bases to fight the MNDs in elderlies.

The Previous Mechanisms of Muscle Fatigue in MNDs

It has been reported that muscle fatigue in MNDs occurs as a result of a defect in lower motor neurons. This defect causes a failure of motor units to provide the needed levels of activity, consequently, peripheral muscle fatigue occurs (Abraham and Drory, 2012). In the literature, neurodegenerative causes are the main causes of muscle fatigue. These neurodegenerative causes include any dysfunction of microglia, glutamate excitotoxicity, misfolded proteins, mitochondrial dysfunction, or oxidative stress (Abraham and Drory, 2012).

Vucic et al. (2007) investigated the alteration of axonal excitability occurred after an induced voluntary contraction to recognize peripheral mechanisms of muscle fatigue in patients with ALS. They found that patients with ALS had a membrane hyperpolarization. This membrane hyperpolarization caused an increase in the threshold occurred after the voluntary contraction in patients with ALS compared with controls. They argued this membrane hyperpolarization to the abnormality in either the Na^+/K^+ pump or firing rate of motor neurons. They also found that there was a dysfunction in the Na + /K + ATPase, which might cause a loss of motor neurons.

Sharma et al. (1995) examined possible mechanisms of muscle fatigue in patients with ALS. They measured muscle force, energy metabolism, and muscle activation pattern. They used the phosphorus-3 1 magnetic resonance spectroscopy to measure muscle force and energy metabolism, and the neurophysiological measures and magnetic resonance imaging to measure the muscle activation pattern. These measures were collected through a 25 min intermittent isometric contraction of the tibialis anterior muscle. They found that both tetanic and maximum voluntary force decayed in those patients more than controls. Also, they found that muscular activation impaired due to small proton signal intensities and amounts of energy metabolites. Lastly, they found that the neuromuscular transmission was nearly normal because amplitudes of the evoked compound of the muscle action potential were steady throughout the contraction.

However, the common belief that the neuromuscular transmission and muscle metabolism are normal in patients with MNDs, several studies have demonstrated that patients with MNDs experience abnormalities in the neuromuscular transmission, and mitochondrial function (Bowling et al., 1993; Dupuis et al., 2005; Rocha et al., 2013; Cappello and Francolini, 2017; Vandoorne et al., 2018). Cappello and Francolini (2017) have stated that patients with ALS usually have a neuromuscular junction disassembly and muscle denervation. Additionally, Rocha et al. (2013) have demonstrated that the degeneration of the neuromuscular junction is a trademark feature of ALS onset and progression. Also, It has been shown in several studies that there is a severe abnormality in the mitochondrial function in patients with ALS (Bowling et al., 1993; Dupuis et al., 2005; Vandoorne et al., 2018). Thus, this abnormality in the neuromuscular transmission and mitochondrial function should be considered as a source of muscle fatigue in patients with MNDs in the future.

The Proprioception Dysfunctions in MNDs

Motor neuron diseases significantly disturb the whole proprioceptive system. Several studies have demonstrated that MNDs usually disturb a variety of cells, such as Renshaw and Glial cells in the spinal cord (Haidet-Phillips et al., 2011; Mochizuki et al., 2011; Philips and Rothstein, 2014; Vaughan et al., 2015). Some studies used neurophysiological and neuroimaging analyses to detect the presence of any abnormality in the sensory neurons in patients with ALS. They found that about 20–60% of sensory neurons showed an abnormality in those patients (Hammad et al., 2007; Pugdahl et al., 2007). Other studies used a histological analysis to detect any abnormality in the sensory neurons. Also, they found that there was a significant degree of degeneration in these neurons and their axons (Dyck et al., 1975; Hammad et al., 2007).

One of the major sensory systems which has a vital role in driving motor control is the proprioceptive system

(Vaughan et al., 2015). The degeneration of proprioceptive neurons, mainly Ia/II proprioceptors, would possibly have a significant effect on increasing the deterioration of α-motoneurons. It has been demonstrated that proprioceptive (sensory) and α-motor neurons are structurally and functionally connected. Proprioceptive information detected by mechanoreceptors delivers to α-motoneurons via monosynaptic connections to adjust their actions. Thus, any loss of the proprioceptive mechanism could highly affect α-motoneurons function (Vaughan et al., 2015).

Two animal studies have demonstrated that MNDs significantly affect the proprioceptive mechanism. Mentis et al. (2011) conducted a study to detect the presence of any early signs of malfunction in the sensory-motor connectivity in mice with SMA. They found that mice with SMA experienced a decrease in proprioceptive reflexes and a decrease in function and number of proximal dendrites and motor neuron synapses. These abnormalities raised early through the disease and they accompanied the affection of motor neurons. Also, Vaughan et al. (2015) conducted a study to detect the presence of any degrees of degeneration in nerve endings of the proprioceptive system in mice with ALS. They found that peripheral nerve endings of the proprioceptive system experienced a significant degree of degeneration, particularly types Ia/II. This degeneration occurred early prior to the presence of any neurological symptoms or loss of any central projecting nerve branches.

To the best of our knowledge, there was no human study in the literature demonstrated the effect of MNDs on the proprioceptive system. Only a human study conducted by Hammad et al. (2007) to detect the presence of any degree of sensory involvement in patients with ALS. They found that approximately 32% of those patients had affection in the sensory system, about 27% of those patients had abnormalities in the amplitudes of nerve action potentials of the sural sensory nerve, and 91% of those patients had pathological anomalies in the sensory system. Also, they found that large-caliber myelinated fibers got the most affection (73%), while small-caliber myelinated fibers got the least affection (23%). Furthermore, they found that there was a significant degree of degeneration in both axons and myelin sheaths.

The Mechanism of the Sensation of Muscle Fatigue

The sensation of muscle fatigue is a complex phenomenon. It occurs on both conscious and unconscious perceptions. The sensation of fatigue occurs in the same way by which the body senses any change in its functions, such as the decrease in the heart rate occurs as a consequence to any increase in cardiac output, feeling of an elevated muscle activity rate occurs as a consequence to any elevation in the power generation, which occurs in response to a rise in its physical activity levels, the breathlessness occurs in response to any increase in the ventilation, and the sensation of warm and gummy occurs with any increase in the temperature or sweating (St Clair Gibson et al., 2003).

The sensation of muscle fatigue starts with a change in a particular component during the physical activity, such as a change in sensation of strain in working muscles and/or joints (St Clair Gibson et al., 2003), accumulation of muscle metabolites (Fitts, 1994; Green, 1997), or depletion of substrates (Balsom et al., 1999; McConell et al., 1999). These peripheral changes are sensed by either mechanoreceptors (Pandolf et al., 1975; Mihevic, 1981) or metaboreceptors (Rotto and Kaufman, 1988; Bongiovanni and Hagbarth, 1990). Then, this sensory data reaches the brain to inform it by the level of exertion or fatigue in working muscles (St Clair Gibson et al., 2003).

Mihevic (1981) has demonstrated that the perception of exertion relies on input data from both "muscle and cardiorespiratory system." This data includes a feedback data about changes in the muscle strain (the primary source for the sensation of fatigue) and a feedback data from the cardiorespiratory system about the depletion in the number of metabolites or substrates (the secondary source of the sensation of fatigue). This study came in accordance with the study of Stamford and Noble (1974), who found that the proprioceptive feedback, precisely from the Golgi tendon organ, was the primary mechanism of the perception of exertion.

Hutton and Nelson (Nelson and Hutton, 1985; Hutton and Nelson, 1986) also conducted two studies to investigate the activity of mechanoreceptors in the fatigued gastrocnemius muscle during ramp stretch in cats. The first study (Mochizuki et al., 2011) investigated the sensitivity of Golgi tendon organs in fatigued gastrocnemius muscle. They found that with ramp stretch, there was a significant decrease in response latencies of Ib nerve types. This decrease presented regardless of any rise in twitch tension or change in peak and static tension. Also, White and Hall (2018) reached the same results and they added that during muscle fatigue the Golgi tendon organs had a tendency to preserve a fixed level of force which could be the cause of the continuous reduction in muscle force.

The second study (Nelson and Hutton, 1985) investigated the sensitivity of muscle spindles in the fatigued gastrocnemius muscle. They found that during static stretching of the fatigued muscle, there was a decline in response latency to any displacement, a rise in the mean frequency, and an increase in resting discharge. While at rest, the frequency of firing to vibration significantly increased in both Ia and IIa nerve fiber types. Also, they found that the sensitivity of cats to different positions significantly decreased with the occurrence of muscle fatigue.

The association between proprioceptive dysfunction and muscle fatigue has been demonstrated in the literature (Ribeiro et al., 2007; Gear, 2011). Ribeiro et al. (2007) investigated the effect of induced muscle fatigue on the knee position sense in elderlies. They performed 30 successive maximal gravity adjusted concentric contractions to knee flexors and extensors using an isokinetic dynamometer. They found that with muscle fatigue, the absolute angular error significantly increased, and the peak torque of knee muscles significantly declined. Gear (2011) investigated the effect of various levels of muscle fatigue of the hamstring muscle on the position sense of the knee joint. In this study, an isokinetic exercise through an angular range of motion used to produce muscle fatigue. Three levels of muscle fatigues were examined, including 90% (mild fatigue), 70% (moderate

fatigue), and 50% (maximum fatigue) of hamstring peak torque. He found that there was a significant decrease in the position sense of the knee joint at 90% and 50% of muscle fatigue.

The Possible Physiological Effects of Proprioceptive Training on Muscle Fatigue in MNDs

It has been reported in the literature that the proprioceptive system has three functions (Jha et al., 2017). It protects joints from excessive and injurious movements via a reflexive mechanism in response to proprioception afferent feedback. It assists in the stabilization of joints during a static posture. Finally, it promotes a better performance of complex movements in more precise coordinated manners. Several studies have stated that proprioceptive training can achieve significant effects on improving motor control dysfunctions in almost musculoskeletal or neurological disorders. These improvements occurred in balance control (Tibone et al., 2013), pain level (McCaskey et al., 2014), motor learning (Aman et al., 2014), and walking parameters (Yong and Lee, 2017).

However, the high value of proprioceptive training in the field of rehabilitation, its effect on decreasing the occurrence of muscle fatigue in patients with MNDs has not been demonstrated yet. The main effect of proprioceptive training on reducing the occurrence of muscle fatigue includes its ability to normalize the firing rate of motor neurons. It has been demonstrated that the decrease or stoppage of the firing of motor neurons has a significant role in reducing the muscular force and developing muscle fatigue (Wan et al., 2017). One of the major causes of the decrease in muscle spindle activity is the decrease in the motor neuron firing rate, which reduces the firing rate of group Ia muscle afferents. Thus, an increase in the presynaptic inhibition and decreasing the firing rate of the motor neurons occur (Brerro-Saby et al., 2008; Vie et al., 2013). Improving the muscle spindle activity by proprioceptive training could help in renormalizing the firing rate of group Ia muscle afferents, presynaptic inhibition and firing rate of motor neurons (Ribot-Ciscar et al., 2000; Hospod et al., 2007; De Luca and Kline, 2012).

Hospod et al. (2007) examined the effect of proprioceptive training on the muscle spindle activity arising from the common peroneal nerve. They found that Ia afferent responses changed significantly after the performance of proprioceptive training. The change in Ia afferent included an increase in the variability of discharge, a decrease in depth of modulation, and a change in spontaneous activity. Potvin and Fuglevand (2017) developed a phenomenological model of motor unit fatigue as a controllable resource to expect muscle fatigue for several tasks and to demonstrate different contractile responses of motor units. This phenomenological model demonstrated that normalization of the firing rate of motor neurons caused an increase in muscle performance and a decrease in the occurrence of muscle fatigue.

Normalization of the firing rate of motor neurons consequently could help in normalizing the amount of calcium released from calcium channels in the sarcoplasmic reticulum and skeletal muscles. The normal release of calcium helps in decreasing the incidence of muscle fatigue because the depletion of calcium is considered one of the main causes of muscle fatigue (Fryer et al., 1995). Muscle spindles activate intrafusal muscle fibers through the activation of gamma motoneurons, which increases strain on the sensory region. Then, through a reflex action intermediated by muscle spindle afferents, an increase in α-motoneurons activity, stimulation of the extrafusal muscle fibers, and occurrence of muscle contraction occur afterward (Edman et al., 2002).

Kuo and Ehrlich (2015) have demonstrated that the contraction of the extrafusal muscle fibers occurs due to the activation of α-motoneurons which stimulates the release of the acetylcholine at the neuromuscular junction. The released acetylcholine spreads across the synaptic cleft and activates nicotinic acetylcholine receptors over the motor endplate. The activation of nicotinic acetylcholine receptors causes an influx of cations (sodium and calcium) then the depolarization of the muscle cell membrane occurs afterward. This depolarization triggers high numbers of voltage-gated sodium channels over the muscle membrane and causes initiation of the action potential.

The action potential spreads along the surface membrane and transverse tubules. In transverse tubules, the action potential is sensed by the dihydropyridine receptors (voltage-sensor molecules). This mechanism sequentially opens the calcium release channels in the sarcoplasmic reticulum and skeletal muscles. These channels release calcium into the sarcoplasm (Fichna et al., 2015). Then, calcium binds with the troponin to move the tropomyosin far away of the myosin-binding area on actin. This initiates the cross-bridge cycling and muscle contraction (MacIntosh et al., 2012). After muscle contraction, the calcium is removed from the cytoplasm by Ca2+ATPase enzyme. This causes a return of tropomyosin to its blocked location and the relaxation to occur (MacIntosh et al., 2012). Using proprioceptive training could help in the normalization of calcium release mechanism by increasing the muscle spindle activity; this could assist significantly in reducing the incidence of muscle fatigue. The pathological mechanisms responsible for the occurrence of muscle fatigue in patients with MNDs and the effect of proprioceptive training on the renormalization of these mechanisms are illustrated in **Figure 1**.

The Effects of Proprioceptive Training to Correct the Imposed Biomechanical Constraints in MNDs

One of the common signs of MNDs is muscle weakness. The pattern of weakness can be either distal to proximal (upper motor neuron disease) or proximal to distal (lower motor neuron disease) (Statland et al., 2015). This weakness occurs due to the degeneration of motor units of certain muscles according to the pattern of weakness for each type. Assuming that force requirements are the same to keep the body erect or produce any movement, thus the load increases in a pattern opposite to the weakness pattern of each type. Thus, movement compensations develop and a further increase in muscle fatigue occurs with any small load or activity.

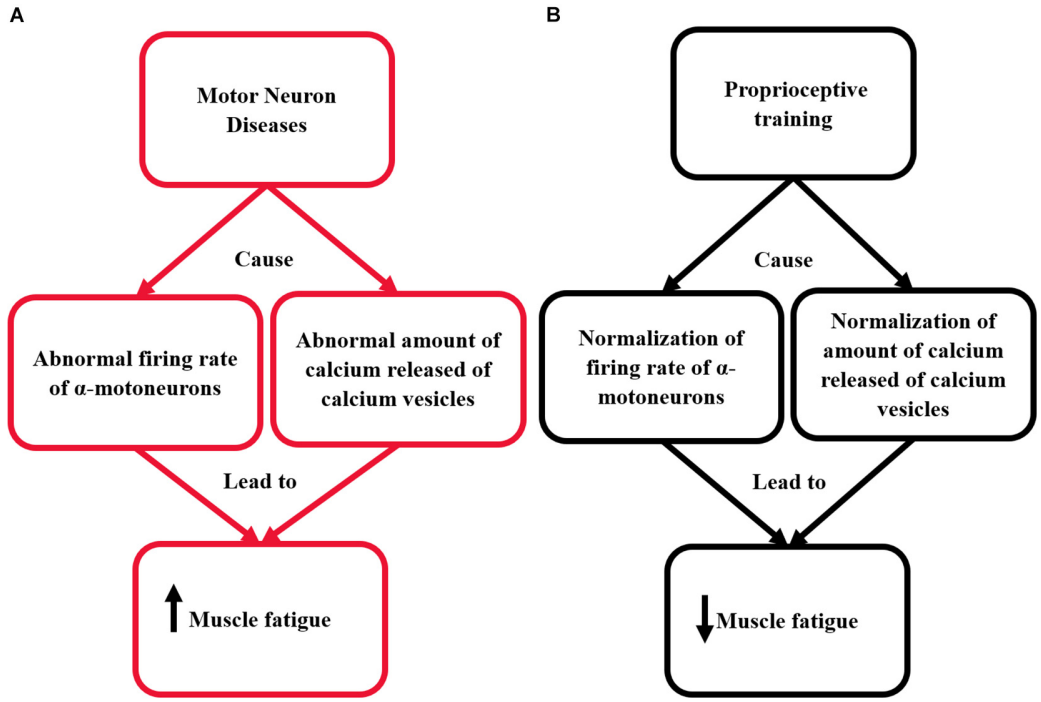

FIGURE 1 | This diagram illustrates the effect of proprioceptive training on re-normalizing the pathological mechanisms responsible for the occurrence of muscle fatigue in patients with MNDs. **(A)** The pathological mechanism responsible for the occurrence of muscle fatigue in patients with MNDs. **(B)** The effect of proprioceptive training on renormalizing the pathological mechanisms responsible for the occurrence of muscle fatigue in patients with MNDs.

Several studies (Wu and Ng, 2010; Wu and Shi, 2011; Radovanović et al., 2014; Witiuk et al., 2014; Grunseich and Fischbeck, 2015; Hausdorff et al., 2019) have demonstrated that patients with MNDs have functional movement-compensations. Grunseich and Fischbeck (2015) have demonstrated that patients with SMA usually have progressive leg weakness. This weakness is symmetrical and causes more muscle fatigue in order to keep the balance on uneven surfaces. Radovanović et al. (2014) have demonstrated that patients with ALS have a longer gait cycle and smaller stride length compared to controls. Hausdorff et al. (2019) have shown also that patients with ALS have a longer cycle time and more decrease in cadence, stride length, and velocity compared to controls. They also have shown that patients with ALS spend less time on one leg (swing time) and more time on two legs (double support time).

Functional movement-compensations present in patients with MNDs can be improved by proprioceptive training. Proprioceptive training can adjust the motor control and correct these functional movement-compensations through both increasing patient awareness about the normal movements and correcting the abnormal ones (modulating motor control).

Proprioceptive training can modulate motor control through either central or peripheral mechanisms (Kaya, 2016). Centrally, Eriksson (2001) and Kaya (2016), have shown that proprioceptive training modifies proprioceptive input by modulating muscle spindle control and inducing plastic adjustments in the central nervous system (Vallbo and Al-Falahe, 1990). Peripherally, Hutton and Atwater (1992) have shown that proprioception training causes morphological adaptations in the muscle spindles themselves. These morphological adaptations occur due to micro-adaptations occur to the intrafusal muscle fibers due to some metabolic alterations. Also, these macro-adaptations can occur due to a decline in the response latency of the stretch reflex and a rise in its amplitude.

A study conducted by myself and others (Mohamed et al., 2019) to correct the shrug sign which is a type of movement compensations usually develops in patients with adhesive capsulitis. However, adhesive capsulitis is a self-limiting disorder, this sign can prevent its full recovery. In our study, we developed a new proprioceptive training to correct the shrug sign. We found that this proprioceptive training decreased the shrug sign and helped in gaining more shoulder and scapular range of motion. The effects of proprioceptive training on decreasing movement-compensations responsible for increasing the occurrence of muscle fatigue in patients with MNDs are illustrated in **Figure 2**.

The Effects of Aging on Pathological Degeneration of Motor and Sensory Neurons Occur in Patients With MNDs

Several studies have shown that the incidence of MNDs among elderlies increases and those patients experience a worse clinical presentation and low survival rate than younger adults (Eisen et al., 1993; Norris et al., 1993; Forbes et al., 2004; Terao et al., 2006; Broussalis et al., 2018). Broussalis et al. (2018)

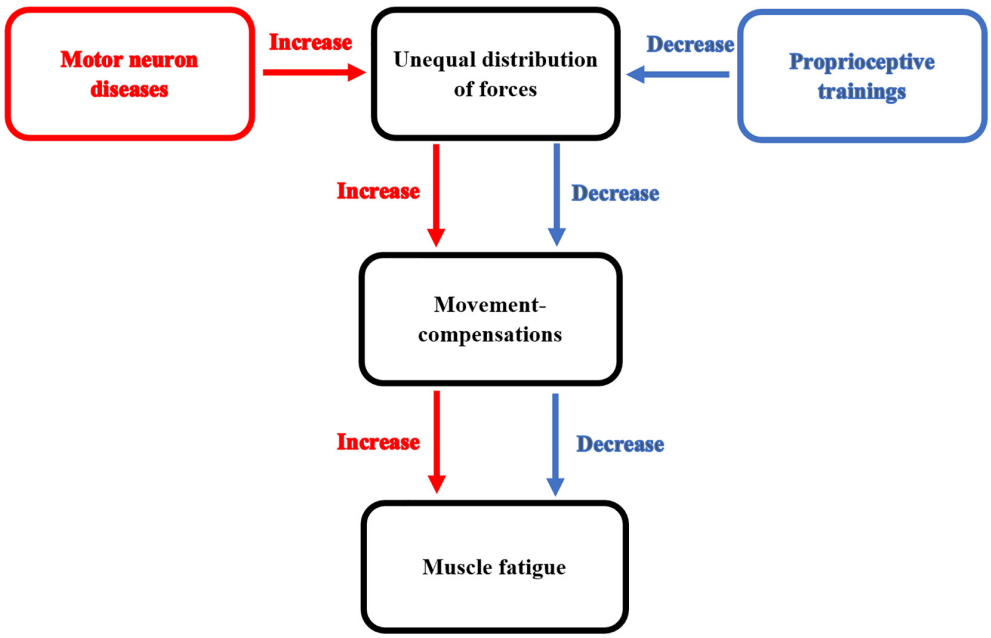

FIGURE 2 | This diagram illustrates the effect of proprioceptive training on decreasing movement-compensations responsible for increasing the occurrence of muscle fatigue in patients with MNDs.

conducted a study to investigate the onset of ALS among elderlies. They found that the majority of admitted patients were elderlies (age of older than 70). Forbes et al. (2004) conducted another study to investigate the clinical presentation of ALS in elderlies over 80 years. They found that clinical presentation and survival rate in elderlies with ALS were worse than younger adults. These results correspond with the study of Terao et al. (2006), who conducted a study to investigate the clinical presentation, and survival rate of ALS among older Japanese patients. They found older patients with ALS had worse survival rates and more complications than younger adults.

Aging is considered one of the key risk factors for the development of MNDs (Kurtzke, 1991; Hospital et al., 1993). Causes of the worse survival rate among older patients with MNDs are not clear yet. These causes might be mainly due to the deteriorating effects of aging on musculoskeletal and neurological systems (Kurtzke, 1991; Hospital et al., 1993). Aging usually causes atrophic changes in extrafusal muscle fibers (McKenzie et al., 2002; Hepple, 2003; Marzetti et al., 2012), degenerations of neuromuscular junctions (Valdez et al., 2010; Tintignac et al., 2015), physiological and cellular modifications in motor axons (Apel et al., 2009; Kang and Lichtman, 2013), and changes in the expression of genes that could critically change normal functions of neuromuscular junctions and skeletal muscles (McKenzie et al., 2002; Weisleder et al., 2006; Jang et al., 2011). Aging causes a decline in both the peripheral and central nervous system processing of sensory information (Nusbaum, 1999). Thus, these mechanisms could significantly cause more complication and worse survival rate among older patients with MNDs.

Furthermore, several studies (Herndon et al., 2002; Pan et al., 2011; Sann et al., 2012; Toth et al., 2012; Li et al., 2016) have examined the effect of age on neuronal tissues using animal models. Understanding these animal models can offer a vision into the bases of selective neuronal susceptibility in neurodegenerative disorders in humans. These animal studies have demonstrated that aging causes abnormal changes in neural axons within the spinal cord. These changes include swelling, waviness, defasciculation, and shrinkage of their diameter (Herndon et al., 2002). Also, aging causes abnormal changes in neurons. These changes include soma distortion, development of abnormal branches, and novel neurite-like projections from the soma (Pan et al., 2011; Tank et al., 2011; Toth et al., 2012). Furthermore, aging causes extensive structural changes in mechanosensory neurons and their microtubule networks (Pan et al., 2011; Toth et al., 2012). These structural changes can disorganize with distorted somas (Pan et al., 2011).

Other types of neurons also exhibit age-related morphological changes, such as dopaminergic neurons, chemosensory neurons, interneurons, and motor neurons. Aging causes morphological changes in the soma of the dopaminergic neuron (McCaskey et al., 2014), axon edging of GABAergic neurons, defasciculation of cholinergic axons in the anterior nerve cord (Pan et al., 2011), and ectopic branches from GABAergic axons (Tank et al., 2011). Aging causes a synaptic decline in the aged neurons, this occurs due to the decrease in the number of synaptic vesicles and size of presynaptic concentrations in the spinal cord (McCaskey et al., 2014).

Aging causes proteins such as SNB-1/synaptobrevin and RAB-3 GTPase, to ectopically collect in synaptic axonal regions and dendrites (Li et al., 2016). Endosomal membrane compartments in aged GABAergic motor neurons disorganize too.

These GABAergic motor neurons are important for constructing and reprocessing of synaptic vesicles (Sann et al., 2012). With aging, the presynaptic release of substances decreases in motor neurons and gradually deteriorates afterward, these substances are important for muscle contraction (Liu et al., 2013). Aging causes deterioration of the synaptic organization in the form of a decrease in the number of dendritic spines and the axonal transport, which is vital for synaptic maintenance (Chen and Hillman, 1999; Valdez et al., 2010). With aging, there is a malfunction in neuronal transporters released from synaptic vesicles. The malfunction of these transporters increases the speed of the synaptic decline and motor circuit malfunction. This might explain the chief role of axonal transport in the preservation of synaptic structural integrity through human life (Li et al., 2016).

The Physiological Effects of Proprioceptive Training to Create Theoretical Bases to Fight the MNDs in Elderlies

However, MNDs significantly affect human life quality and mobility (Simmons, 2013), abnormal structural, and morphological changes occur with aging could aggravate these adverse effects, and speed the degeneration rate occurs to α-motoneurons in patients with MNDs. Normalization of α-motoneurons using proprioceptive training might be a good intervention to fight the occurrence of MNDs in older ages. This can be accomplished by increasing the sensitivity of mechanoreceptors particularly, muscle spindles and Golgi tendon organs which significantly decrease in older ages (Kararizou et al., 2005; Liu et al., 2005; Ribeiro and Oliveira, 2007). It has been demonstrated that increasing the sensitivity of mechanoreceptors could normalize the firing rate of α-motoneurons. This could decrease the disruption of the function of α-motoneurons, the calcium release, and the ATPase enzyme; these mechanisms mainly present with MNDs (Sharma et al., 1995; Ellis et al., 2003; Masson et al., 2014; Nijssen et al., 2017). Thus, using proprioceptive training could be a useful tool to slow down the deterioration in the function of α-motoneurons.

The effectiveness of proprioceptive training on modulating the abnormal tone and improving manual control has been shown with other neuro-degenerative disorders (Shumaker, 1980; Bieñkiewicz et al., 2013; Shih et al., 2016; Wang et al., 2018). Bieñkiewicz et al. (2013) conducted a study to investigate the effect of proprioceptive training by using visual biofeedback on bradykinetic movements of the hand in patients with Parkinson's disease. They found that temporal features of hand movements significantly moderated by using visual biofeedback. These improvements included an improvement of muscle tone, movement time, and peak velocity.

Shih et al. (2016) examined the effect of using proprioceptive training in the form of game-based training with a Kinect sensor on postural stability in patients with Parkinson's disease. They found that proprioceptive training-induced improvements in both static and dynamic stability. Wang et al. (2018) studied the effect of using proprioceptive training in the form of game-based training on lower limb function and gait control in patients with spinocerebellar ataxia. They found that proprioceptive training caused an improvement in limb stability, limb-kinetic function, and gait-posture after 4 weeks.

CONCLUSION

Proprioceptive training can be an effective method for decreasing the incidence of muscle fatigue in patients with MNDs, particularly elderlies. This can be accomplished through the ability of proprioceptive training to normalize the firing rate of the α-motoneurons and the amount of calcium released from calcium release channels, which has a major role in the occurrence of muscle fatigue. The normalization of α-motoneurons and the amount of calcium released could be helpful to decrease the incidence of development of MNDs or to slow down the progression on presented MNDs in elderlies. Also, proprioceptive training decreases the occurrence of muscle fatigue by correcting the abnormal movement-compensations, which develop due to the biomechanical constraints imposed on patients with MNDs.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 26 December 2019
doi: 10.3389/fphys.2019.01537

Tailored Exercise Training Counteracts Muscle Disuse and Attenuates Reductions in Physical Function in Individuals With Amyotrophic Lateral Sclerosis

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OPEN ACCESS

Edited by:
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Reviewed by:
Chien-Liang Chen, I-Shou University, Taiwan
Riikka Kivelä, University of Helsinki, Finland

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Specialty section:
This article was submitted to Clinical and Translational Physiology, a section of the journal Frontiers in Physiology

Received: *26 September 2019*
Accepted: *05 December 2019*
Published: *26 December 2019*

Citation:
Ferri A, Lanfranconi F, Corna G, Bonazzi R, Marchese S, Magnoni A and Tremolizzo L (2019) Tailored Exercise Training Counteracts Muscle Disuse and Attenuates Reductions in Physical Function in Individuals With Amyotrophic Lateral Sclerosis. Front. Physiol. 10:1537. doi: 10.3389/fphys.2019.01537

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, characterized by the progressive loss of motor neurons, which leads to a reduction in strength and exercise capacity. Although the concept of "Exercise is Medicine" is accepted for many diseases, the role of exercise in individuals with ALS is still debated. The aim of this study was to propose a tailored exercise training program that was both safe and effective for individuals with ALS, and to evaluate the effects of this combined, moderate-intensity, aerobic and strength training program on aerobic capacities, strength, and physical function. Sixteen individuals with ALS were randomly assigned to either a training (three times a week for 12 weeks; TRAIN, $n = 8$) or usual care (continued their usual standard of care and served as control; UC, $n = 8$) group. Peak power, peak oxygen uptake, as well as the gas exchange threshold (GET) during a cardiopulmonary exercise test (CPET) on a cycle ergometer, and the maximal strength (1RM) of the knee extensor muscles, were evaluated before and after 12 weeks. Participants also performed the "Timed Up and Go" (TUG) and the "6-min walking" (6MWT) tests. The ALS Functional Rating Scale revisited (ALSFRS-R), the ALS Severity Scale (ALS-SS), and the McGill quality of life (QoL) questionnaire were also measured. The GET increased from 0.94 ± 0.08 to 1.06 ± 0.10 L min^{-1} in TRAIN ($p = 0.009$) and decreased from 0.79 ± 0.17 to 0.72 ± 0.17 L min^{-1} in UC ($p = 0.001$). There was a significant difference between groups for changes in TUG (9.1 ± 5.5% improvement in TRAIN and 56.8 ± 18.5% worsening in UC, $p = 0.002$), ALSFRS-R (4.7 ± 2.6% decrease in TRAIN and 23.0 ± 5.6% decrease in UC, $p = 0.007$), and for the ALS-SS (2.2 ± 2.1% decrease in TRAIN and 12.4 ± 4.4% decrease in UC, $p = 0.04$). Even if the 1RM of the knee-extensor muscles showed a tendency to increase in TRAIN (70.1 ± 30.0%, $p = 0.07$), there was not a statistically significant difference ($p = 0.57$) with respect to the

changes in the UC group (44.9 ± 20.7% increase, $p = 0.11$). This study showed that a combined moderate-intensity aerobic and strength training program, tailored to the physical capacities of each individual, can improve aerobic fitness and maintain physical function in individuals with ALS.

Keywords: amyotrophic lateral sclerosis, exercise training, aerobic capacity, muscle strength, physical function

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive degeneration and death of upper and lower motor neurons, which leads to a reduction in muscle mass and strength and ultimately death. Patients with ALS have a reduced exercise tolerance (Mezzani et al., 2012; Lanfranconi et al., 2017), which is associated with impaired physical function and quality of life (QoL; Korner et al., 2015). Currently there is no cure for this disease, and the lack of effective treatments able to alter the pathophysiological pathways that modulate disease progression has led to suggestions that the muscle may be a strategic therapeutic target (Shefner, 2009), in order to counteract the inevitable loss of function in patients with ALS. Increasing the strength and the oxidative capacity of muscle fibers whose innervation is intact could preserve function in these patients and potentially also impact survival rates. Due to the well-known beneficial effects of exercise in improving muscle strength and oxidative capacity in healthy individuals, tailored exercise training could represent a possible therapeutic intervention in patients with ALS.

Even if exercise has been scientifically proven to be beneficial in maintaining/increasing aerobic fitness, physical function, and independence in a wide range of clinical conditions, such as cancer (Iyengar and Jones, 2019), as well as neurodegenerative (Motl and Pilutti, 2012; LaHue et al., 2016), muscle (Voet et al., 2013), and cardiovascular (Pryzbek et al., 2019) diseases, the role of physical activity in patients with ALS is still debated. As a result, patients with ALS are usually advised to avoid or reduce their physical activity, which can further exacerbate the decrease in exercise tolerance and muscle strength due to cardiovascular deconditioning and muscle disuse, leading to a deleterious cycle of reduced function. Recently, a few published studies have shown the tolerability and safety of an exercise regimen in patients with ALS (Clawson et al., 2018; Merico et al., 2018), and the positive effects exercise has in reducing the global function decline evaluated by disease-specific rating scales (Drory et al., 2001; Dal Bello-Haas et al., 2007; Lunetta et al., 2016). While these results are encouraging, this evidence is not sufficiently detailed to recommend a specific exercise training program. In particular, there is a lack of data on the effects of exercise on objective physiological outcomes, such as maximal aerobic capacity and muscle strength.

The increasing recognition of "Exercise is Medicine" highlights the need to determine the exact type and dose of exercise to improve a specific physical capacity for a specific population. The main challenge when prescribing an exercise training program for patients with ALS is to find the right balance between an exercise that is both safe and effective. In this study, our purpose was to propose exercises that provided a sufficient stimulus to increase muscle aerobic capacity and strength, with minimal or no muscle damage, and without fatiguing the muscle and the patients. Strength training, in particular if proposed between a moderate and a high intensity (around 60–80% of 1 Repetition Maximum, 1RM) and in sets of 6–12 repetitions (Kraemer et al., 1996), has the main benefit to increase muscle mass and strength. Aerobic training at a moderate intensity (near the anaerobic threshold, the point where the lactate starts to be produced and fatigue appears) represents the optimal intensity for improvement of endurance fitness (resulting from an increase in the oxygen uptake at the anaerobic threshold; Jones and Carter, 2000) and can have a beneficial effect on exercise tolerance and fatigue during daily living activities. In the light of these observations, our strength training was set at the lower effective intensity (60% of 1RM) and the aerobic training below the anaerobic threshold (80% of the anaerobic threshold), so that the patients could exercise without producing lactate and with less fatigue.

The purpose of this study was to gain knowledge on the effects of a 12-week combined aerobic and strength training program at a moderate intensity in individuals with ALS on muscle strength, aerobic capacity, and physical function. We hypothesized that the individuals with ALS who exercised would present a reduced decline in muscle strength and aerobic capacity by counteracting muscle disuse. We hypothesized also that the reduced impairment of these characteristics would translate to a reduced deterioration of their physical function and QoL.

MATERIALS AND METHODS

Participants

Sixteen individuals with a diagnosis of possible, probable, or definite ALS (according to the revised El Escorial criteria; Brooks et al., 2000) were recruited through four Italian neurological centers specialized in the treatment of individuals with this disease. A neurological assessment was made in order to verify the patient's eligibility for this project and to stratify the phenotype: patients with a time since diagnosis of less than 48 months and able to pedal on a cycle ergometer were included in the study. All participants underwent a medical evaluation to exclude the presence of any acute cardiopulmonary and/or infectious diseases. A careful collection of their physical activity history was also recorded. Most participants (70%) were on riluzole therapy (100 mg daily) for an average time of 6 months, and continued the medication for the entire intervention period. Participants were assigned randomly based on age, sex, BMI and

time elapsed from diagnosis to two groups: (1) training (TRAIN, n = 8, 2 bulbar onset; 2 females), who conducted a tailored exercise training program three times per week for 12 weeks; (2) usual care (UC, n = 8, 3 bulbar onset; 2 females), who received passive manual therapy once weekly/fortnight for 12 weeks, and who served as a control group. **Table 1** shows the demographic and clinical characteristics of patients in both the TRAIN and UC groups. Patients in both groups were not significantly different for age, time from diagnosis, BMI, fat free mass or peak oxygen uptake ($\dot{V}O_{2peak}$). The $\dot{V}O_{2peak}$, which can be considered an objective measure of exercise tolerance in patients with ALS (Lanfranconi et al., 2017) and the motor ALS functional rating scale revised version (ALSFRS-R) sub-score were not statistically different between the two groups. The ALSFRS-R is a validated rating instrument to monitor the progression of disability in patients with ALS, evaluating three different domains (bulbar, motor, and respiratory function) (Cedarbaum et al., 1999). There was a tendency toward a significant difference for the total ALSFRS-R scores, probably because in the UC group there was a greater proportion of the bulbar sub-type (3 vs. 2) that impacted on the total score. Patients with a bulbar onset develop initial symptoms in the bulbar-innervated muscles that control speech and swallowing, and present the worst survival rate (Chio' et al., 2011). The higher proportion of patients with bulbar onset could have also sped up the disease progression of the UC group, with a quicker physical deterioration, with respect to the TRAIN group.

Participants were informed about the risks of testing and training and provided informed consent to participate in the study. The study conformed to the standards set by the latest revision of the Declaration of Helsinki. The procedures were approved by the ethics committee of the University of Milano-Bicocca (protocol #129, 10-June-2014, University of Milano-Bicocca). Personal data were treated according to standard principles of confidentiality. The data presented in this manuscript are part of the "ME&SLA" project (ClinicalTrials.gov Identifier: NCT02548663).

TABLE 1 | Demographic and clinical characteristics of patients with amyotrophic lateral sclerosis (ALS) in the training (TRAIN) and usual care (UC) groups before the 12-week intervention (T0).

	TRAIN (n = 8)	UC (n = 8)	p
Age (years)	50.7 ± 3.3	55.5 ± 5.95	0.33
Sex (♀/♂)	2/6	2/6	
Onset (B/S)	2/8	3/8	0.30
*Duration (months)	20.5 ± 20.3	13.4 ± 6.6	0.36
BMI (kg m^{-2})	25.2 ± 3.4	26.0 ± 2.8	0.63
FFM (kg)	60.9 ± 5.12	59.1 ± 4.9	0.75
NIV (no/yes)	6/2	5/3	0.59
ALSFRS-R	40.4 ± 1.5	35.0 ± 3.4	0.09
ALSFRS-R motor	16.9 ± 1.3	13.8 ± 2.5	0.16
$\dot{V}O_{2peak}$ (mL kg^{-1} min^{-1})	21.3 ± 2.2	17.3 ± 2.1	0.16

*Values are mean ± SE. BMI = body mass index; FFM = fatty free mass; onset B = bulbar or S = spinal; NIV = non-invasive ventilation; ALSFRS-R = ALS functional rating scale revised; $\dot{V}O_{2peak}$ = peak oxygen uptake. *From diagnosis to start of study.*

Experimental Design

At baseline (T0) and after 12 weeks of training or standard of care (T1) each participant underwent the following assessments:

- Cardiopulmonary exercise test (CPET) on a cycle ergometer (Monark-LC6: Monark, Varberg, Sweden) under medical supervision and with 12-lead electrocardiography monitoring (Quark C12x: COSMED, Roma, Italy). After 2 min of unloaded cycling, the power was increased every minute by step increments of 3, 5, or 15 W, depending on the fitness level of each subject, and participants were encouraged to exercise until exhaustion. Exhaustion was defined as the inability to maintain the pedaling frequency (60 revolutions min^{-1}, RPM), despite vigorous encouragement by the experimenters. Pulmonary ventilation ($\dot{V}E$, in BTPS), O_2 consumption ($\dot{V}O_2$, in STPD) and CO_2 output ($\dot{V}CO_2$, in STPD) were determined breath-by-breath by a computerized metabolic cart (Vmax SPECTRA 229: SensorMedics Corporation Yorba Linda, Yorba Linda, CA, United States). Expiratory flow was recorded at the mouth of the subject by a mass flow sensor (hot wire anemometer). $\dot{V}O_2$ and $\dot{V}CO_2$ were determined through continuous monitoring of PO_2 and PCO_2 at the mouth throughout the respiratory cycle and from established mass balance equations. The gas exchange threshold (GET), which represents the transition from aerobic metabolism to anaerobic (with consequent lactate production) plus aerobic metabolism, was calculated by conventional methods (Beaver et al., 1986). Arterial blood O_2 saturation (SaO_2) was checked continuously through pulse oximetry at the finger (RAD 9 Signal Extraction Pulse Oximeter: Masimo Corporation, Irvine, CA, United States). The environmental temperature during exercise was standardized to 20°C using an air-conditioning system and the current barometric pressure was recorded. The rating of perceived exertion (RPE) was obtained through the Borg scale. Blood lactate was determined on capillary blood samples obtained from an ear lobe, at rest and at 1, 3, 5, 7 min after the termination of CPET by a dual-channel analyzer (BIONSEN C-line, EKF Diagnostics, Cardiff, United Kingdom). The highest value obtained during the 7 min of recovery was considered the peak lactate concentration (La^{+}_{peak}).
- The "Timed Up and Go" Test (TUG; Podsiadlo and Richardson, 1991) and the 6-min Walking Test (6MWT, Guyatt et al., 1985).
- Maximal strength of the quadriceps muscles by a ten repetition maximum (10RM) test during a bilateral leg extension (LE) exercise performed on an isotonic machine (Leg Extension Alpha pro, multi-function bench, Kettler, Ense, Germany). The value obtained was then used to estimate the 1RM (Brzycki, 1993) – an indication of the maximal strength of the quadriceps muscle.
- ALS functional rating scale-revised version (ALSFRS-R, Cedarbaum et al., 1999). The ALSFRS-R includes 12 questions, which are rated on a five-point scale from 0 = can't do to 4 = normal ability. Individual

scores are summed to produce a total score between 0 = worst and 48 = best.
- ALS-SS (Hillel et al., 1989) is a complimentary scale of ALSFRS-R, and has the same purpose to assess function in four categories (speech, swallowing, lower- and upper-extremity abilities).
- McGill Quality of Life Questionnaire (Cohen et al., 1995), to assess the QoL of patients with a life-threatening illness.
- Body fat-free mass (FFM) by measuring skinfolds at seven sites (C10Plicometer Tanner – Whitehouse; Holtain, Ltd., Crymych, United Kingdom), and applying the Jackson Pollock body density equation (Jackson and Pollock, 1978).

Training Program

Participants in the TRAIN group visited a gym located at Clinica S. Pietro, La Meridiana, Monza, Italy, three times/week for 12 weeks. Each session, of 60 min duration, was supervised by two sport scientists, a medical doctor, and a student from Medical School; there was a patient/therapist ratio of 1:1. The training program was characterized by aerobic, resistance, balance, and stretching exercises, distributed as follows:

- 15 min of cycling at an intensity corresponding to 80% between baseline and the GET calculated during CPET.
- 25 min of strength exercises at an intensity corresponding to 60% of 1RM. Three sets of 10 repetitions (2 min rest between sets) of upper (biceps curl and arm lateral raise) and lower (squat, calf raise, and LE) body exercises were performed with dumbbells; the LE exercise was performed on an isotonic machine. TheraBand™ elastics were also used to perform chest press and seated row exercises. Strength exercises were alternatively performed during the week. To reduce the possibility of muscle damage, the eccentric phase of the exercises was avoided.
- 10 min of proprioceptive exercises, most of which were performed on the BOSU® Pro balance trainer.
- 10 min of upper and lower extremity stretching exercises realized on a Pancafit®.

Safety parameters such as blood pressure, heart rate, and oxygen saturation, as well as training intensity and attendance at training sessions, were documented in a patient diary.

After 6 weeks of training the 10RM test was repeated to adjust the load to the new values obtained, so the load of 60% of 1RM was maintained throughout the training. A 200-ml hyperproteic supplement was given immediately after each training session (300 kcal, 12 g of proteins, 37 g of carbohydrates, 12 g of fat, 0.1 g of fibers, and 12 g of minerals) along with 200 ml of water (eventually thickened).

Patients randomized to the UC group were instructed to maintain their usual daily activities. They were given the same supplement as the participants in the TRAIN group three times/week for 12 weeks.

Statistical Analysis

Values are expressed as mean ± Standard Error (SE). Effect size (ES) was estimated with Cohen's *d*. A Shapiro–Wilk test was performed to test for normality of the data. The statistical significance of the difference in mean values between groups (TRAIN vs. UC) was evaluated by paired two-tailed Student's *t*-test, while the difference in the proportion of drop-outs, non-invasive ventilation (NIV) and disease onset between groups was calculated by a two proportion *z*-test. Regression and correlation analyses were performed using the least squared residuals method. The level of significance was set at $p < 0.05$, and tendency was noted for $0.05 < p < 0.09$. All statistical analyses were performed using a commercially available software package (Prism 6.0: GraphPad, La Jolla, CA, United States).

RESULTS

Participation

The flow chart (**Figure 1**) shows the drop-out rate for both groups ($p = 0.10$), with 1 drop-out for the TRAIN group (one patient showed the first signs of depression and felt that exercise was not

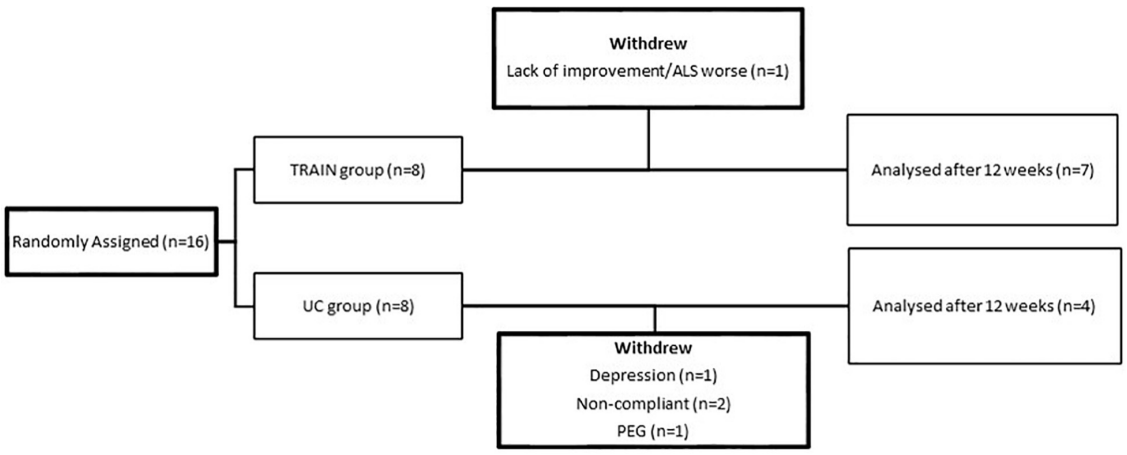

FIGURE 1 | Flow chart of participants from random assignment to interventions, withdrawals, and completion of the project.

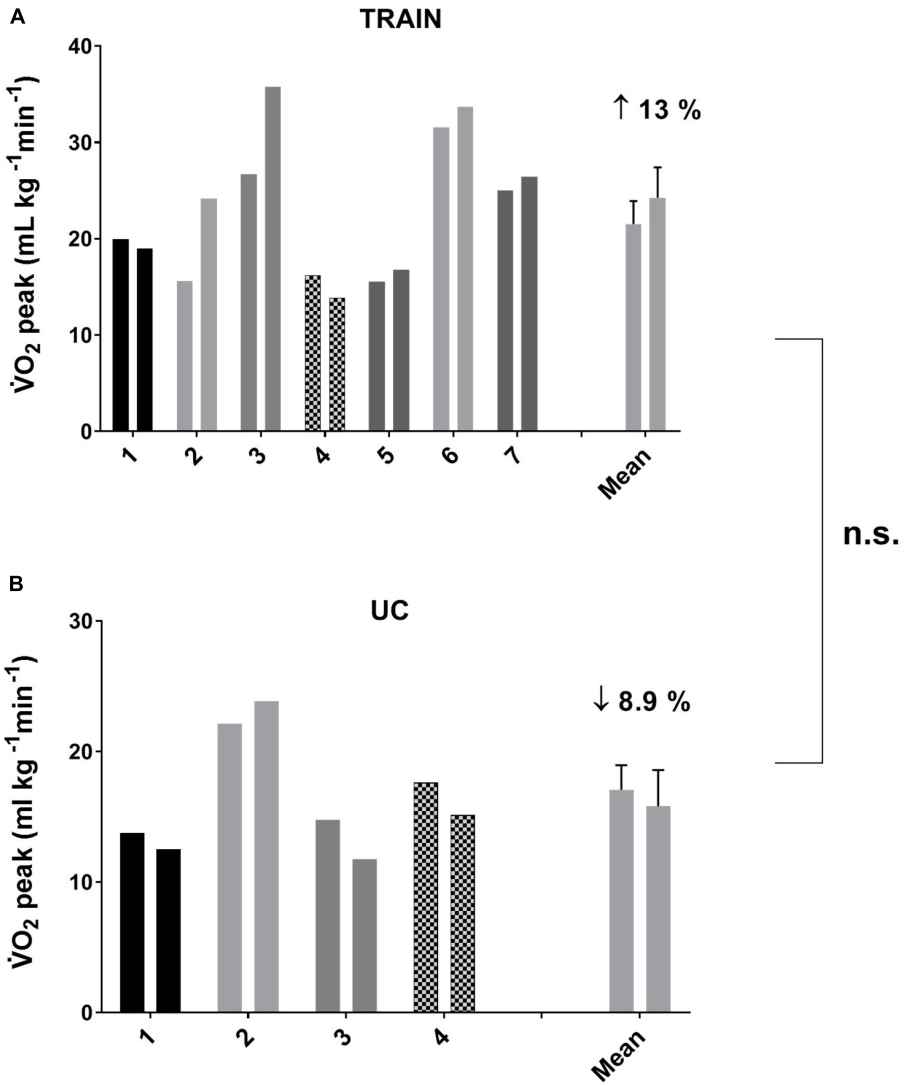

FIGURE 2 | Individual and average values for peak oxygen uptake ($\dot{V}O_{2peak}$) before and after 12 weeks of exercise training (**A**, TRAIN) and usual care (**B**, UC). Average data are expressed as mean + SE. n.s., not significantly different between TRAIN and UC.

good for him), and four drop-outs for the UC group (one patient required percutaneous endoscopic gastrostomy at the time of the final test evaluation; two found it difficult to come to the hospital for the test sessions, and one had severe depression). In the TRAIN group the adherence to the training program was 84.8 ± 6.3%. The satisfaction regarding the training program, evaluated by the Visual Analog Scale (VAS), was 8.8 ± 0.8. No adverse events happened during the 12 weeks of training.

Cardiopulmonary Exercise Testing (CPET)

All participants terminated the CPET due to their inability to maintain the pedaling frequency of 60 RPM. The respiratory exchange ratio (RER) and [La^+] values at the end of CPET were 1.12 ± 0.02 and 4.3 ± 0.6 mmol L^{-1} for TRAIN, and 1.13 ± 0.07 and 4.4 ± 1.1 mmol L^{-1} for UC. The TRAIN and UC groups reached 81.2 ± 2.9 and 80.2 ± 6.4% of the age-predicted maximum HR, respectively.

In TRAIN, the GET increased significantly from 0.94 ± 0.08 at T0 to 1.06 ± 0.10 L min^{-1} at T1 (p = 0.009, ES = 0.47), while in UC the GET decreased significantly from 0.79 ± 0.17 at T0 to 0.72 ± 0.17 L min^{-1} at T1 (p = 0.001, ES = 0.19), indicating that the aerobic training produced an improved exercise tolerance in individuals with ALS. In TRAIN, $\dot{V}E_{peak}$ showed a tendency to increase from 62.32 ± 10.44 to 78.64 ± 14.91 L min^{-1} (27.3 ± 11.5%, p = 0.09, ES = 0.49), while there was no change in $\dot{V}E_{peak}$ for the UC (−6.3 ± 4.0%, p = 0.29, from 49.17 ± 8.61 to 45.53 ± 7.78 L min^{-1}). We also found significant between groups differences in percentage for changes in GET, and a tendency toward a change for W_{peak} (p = 0.08) and $\dot{V}E_{peak}$ (p = 0.09). Despite a 13.0% increase in $\dot{V}O_{2peak}$ in TRAIN (with 5 out of 7 patients improving their value) and an 8.9% decrease in UC

TABLE 2 | Mean values before (T0) and after (T1) 12 weeks of exercise training (TRAIN) and usual care (UC), and percentage changes of different variables measured during a cardiopulmonary exercise test (CPET).

	TRAIN	TRAIN	TRAIN	UC	UC	UC
	T0	T1	% Change	T0	T1	% Change
W_{peak} (W)	111.1 ± 19.0	117.6 ± 23.2	↑ 3.0 ± 5.4 ES = 0.12	70.2 ± 13.3	59.7 ± 19.7	↓ 20.12 ± 10.4 $ ES = 0.28
FFM (kg)	59.6 ± 5.1	59.9 ± 4.4	↑ 1.3 ± 2.0 ES = 0.02	59.8 ± 4.9	58.5 ± 4.7	↓ 2.0 ± 1.1 ES = 0.12
W_{peak}/FFM (W kg^{-1})	1.77 ± 0.2	1.85 ± 0.3	↑ 2.1 ± 5.2 ES = 0.12	1.15 ± 0.14	1.00 ± 0.24	↓ 17.9 ± 9.4 ES = 0.30
$\dot{V}O_{2peak}$ (mL kg^{-1} mm^{-1})	21.5 ± 2.4	24.3 ± 3.1	↑ 13.0 ± 8.4 ES = 0.37	17.1 ± 1.9	15.8 ± 2.8	↓ 8.9 ± 5.4 ES = 0.27
GET (L min^{-1})	**0.94 ± 0.08**	**1.06 ± 0.10***	**↑ 11.9 ± 3.6 ES = 0.47**	**0.79 ± 0.17**	**0.72 ± 0.17***	**↓ 19.9 ± 3.4# ES = 0.19**
$\dot{V}E_{peak}$ (L min^{-1})	62.3 ± 10.4	78.6 ± 14.9	↑ 27.3 ± 11.5 ES = 0.49	49.2 ± 8.6	45.5 ± 7.8	↓ 6.3 ± 4.0 $ ES = 0.20
La^{+}_{peak} (mmol L^{-1})	4.3 ± 0.6	4.6 ± 1.0	↑ 4.3 ± 14.4 ES = 0.15	4.4 ± 1.1	3.9 ± 1.0	↓ 11.6 ± 4.6 ES = 0.22

*Values are expressed as mean ± SE. W_{peak} = peak power output; FFM = fatty free mass; $\dot{V}O_{2peak}$ = peak oxygen uptake; GET = gas exchange threshold; $\dot{V}E_{peak}$ = peak ventilation; La^{+}_{peak} = peak lactate concentration. *$p < 0.05$ statistically different from T0. #$p < 0.05$ statistically different from TRAIN. $$0.05 < p < 0.09$ tendency to a difference from TRAIN. Only the row indicated with bold is statistically significant between T0 and T1 for both groups.*

(with 3 out of 4 participants worsening their value), we did not find significant group differences for the change in $\dot{V}O_{2peak}$ (**Figure 2**). Even if the differences were not statistically significant for most of the parameters evaluated (with the exception of the GET), due in part to the underpowered samples and the differential dropout rates, it is interesting to note the different direction of the arrows (indicating the changes) in the two groups (**Table 2**).

Physical Function

The individual values for the TUG test are shown in **Figure 3**. In the TRAIN group, even if 6 of 7 patients performed the TUG test at T1 faster than at T0, the mean group change was not significantly different between T0 and T1 (mean reduction of time of 9.1 ± 5.5%, $p = 0.12$, ES = 0.22). In the UC group, 4 out of 4 patients showed an impaired performance in the TUG test (increase in time of 56.8 ± 18.5%, $p = 0.05$, ES = 1.29). The percentage changes between TRAIN and UC were significantly different ($p = 0.002$). The TRAIN and UC groups did not differ significantly at T0 and T1 in the distance covered during the 6MWT, with a small non-significant increase at T1 in TRAIN (+4.5 ± 7.9%) and decrease in UC (−10.7 ± 10.2%). When the data of both groups were plotted together, there was a significant correlation between the GET and the 6MWT (**Figure 4A**), indicating that the individuals that had higher GET values had a better performance in the 6MWT. A similar correlation was also found between $\dot{V}O_{2peak}$ and 6MWT (**Figure 4B**).

Body Fat-Free Mass (FFM) and Strength Measurements

There was no statistical difference ($p = 0.18$) between changes in FFM in TRAIN (+1.4 ± 2.0%) and in UC (−2.0 ± 1.1%). While in TRAIN the power expressed relative to the metabolically active body mass (W_{peak}/FFM in W kg^{-1}) was maintained after the 12 weeks of training (+2.1 ± 5.2%, $p = 0.4$), in UC this value was reduced (even if not significantly, $p = 0.46$) by 17.9 ± 9.4%. In TRAIN, 1RM LE showed a tendency to increase from 39.8 ± 8.2 to 63.8 ± 16.2 kg (70.1 ± 30.0%, $p = 0.07$, ES = 0.74) without showing a significant difference ($p = 0.57$) with respect to the changes in the UC group (+44.9 ± 20.7% from 36.8 ± 8.9 to 53.2 ± 13.4 kg, $p = 0.11$).

ALSFRS-R and ALS-SS Scores and Quality of Life

The ALSFRS-R and ALS-SS scores decreased significantly in UC by 23.0 ± 5.6% ($p = 0.01$, ES = 0.80) and by 12.4 ± 4.4% ($p = 0.04$, ES = 0.49) (**Figure 5**). In the TRAIN group there was not a significant reduction of these scores (−4.7 ± 2.6%, $p = 0.11$, ES = 0.41, and −2.2 ± 2.1%, $p = 0.33$, ES = 0.23, for the ALSFRS-R and ALS-SS, respectively). The changes in percentage of the ALSFRS-R and ALS-SS scores between the TRAIN and UC groups were significantly different ($p = 0.007$, ES = 2.04 and $p = 0.04$, ES = 1.4).

When separating the ALSFRS-R results into the single sub-scores (bulbar, respiratory and total motor functions), the changes in percentage between the TRAIN and UC groups were significantly different for bulbar ($p = 0.01$) and total motor ($p = 0.02$), with UC showing greater decreases in these sub-scores (**Figure 6**). In UC, the total motor sub-scores decreased significantly by 31.6 ± 10.5% from 13.6 ± 2.5 to 10.2 ± 2.7 ($p = 0.02$), while in the TRAIN the decrease was only of 4.4 ± 4.1% ($p = 0.25$). When considering all the data from the TRAIN and UC groups, we found that the GET was significantly correlated to ALSFRS-R score, indicating that higher GETs in individuals with ALS are associated with higher functional score

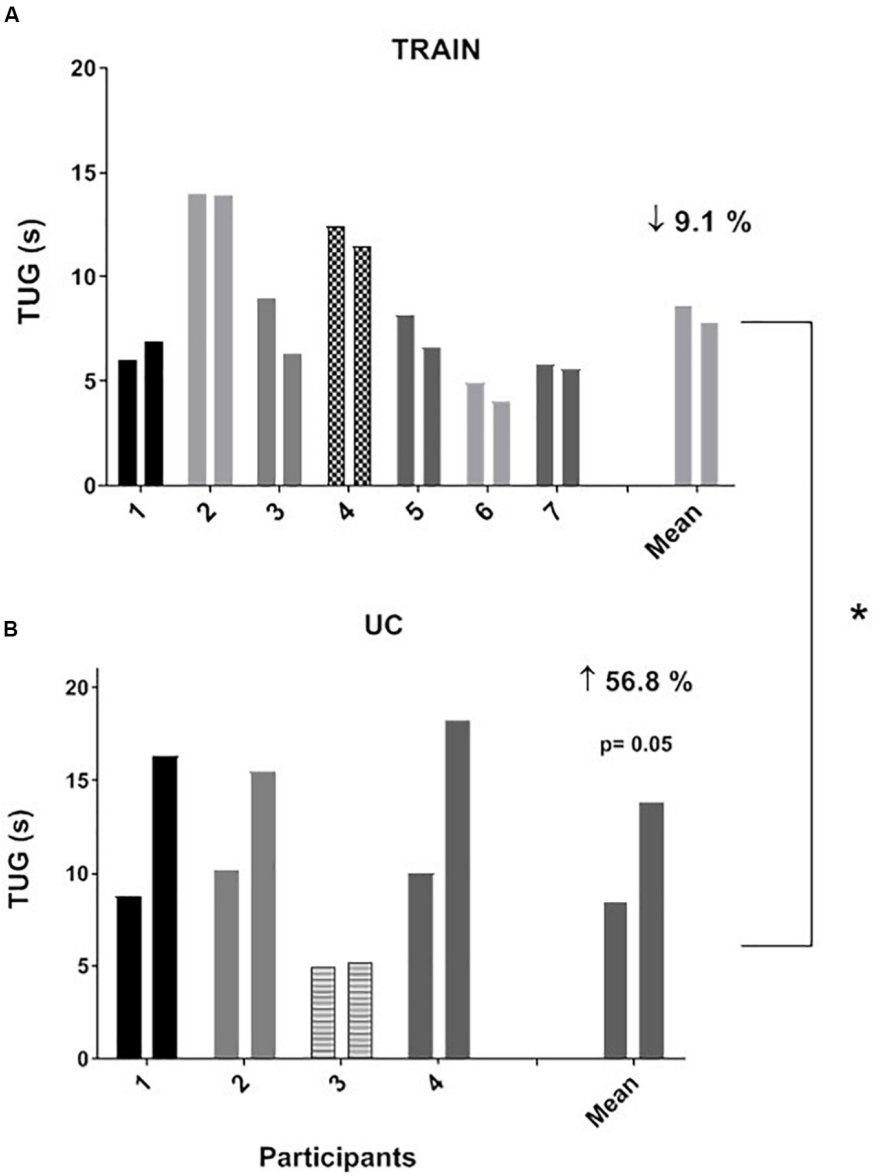

FIGURE 3 | Individual and average values for "Timed Up and Go" test (TUG) before and after 12 weeks of exercise training (**A**, TRAIN) and usual care (**B**, UC). Average data are expressed as mean + SE. *Significantly different between TRAIN and UC, $p < 0.05$.

in the ALSFRS-R (**Figure 7**). QoL was not significantly different in the TRAIN and UC groups at T0 (7.0 ± 1.1 vs. 8.0 ± 1.2), but while the value was maintained in the TRAIN group at T1 (7.0 ± 0.8, $p = 0.99$), in UC the value showed a tendency to be lower (5.5 ± 1.5, $p = 0.09$).

DISCUSSION

This study shows that a combined moderate aerobic and strength training program is not only safe and enjoyable for participants, but also has beneficial effects on physical function (as measured by the TUG test) and aerobic fitness (as measured by GET). These positive effects were translated to the maintenance of the ALSFRS-R and ALSS scores in individuals with ALS who were training; on the contrary, the same scores decreased significantly in those who continued their standard of care.

The GET represents the point at which the energy requirement to sustain a determined effort cannot be fulfilled only by aerobic metabolism but there is also the need to use the anaerobic metabolism, with the consequent production of lactate and the development of muscle fatigue. The submaximal (at 80% of GET) aerobic exercise on the bicycle three times/week for 12 weeks had the effect to elevate the GET, which could improve the ability to sustain submaximal daily activities, such as walking or gardening, at a higher intensity or for longer time than before

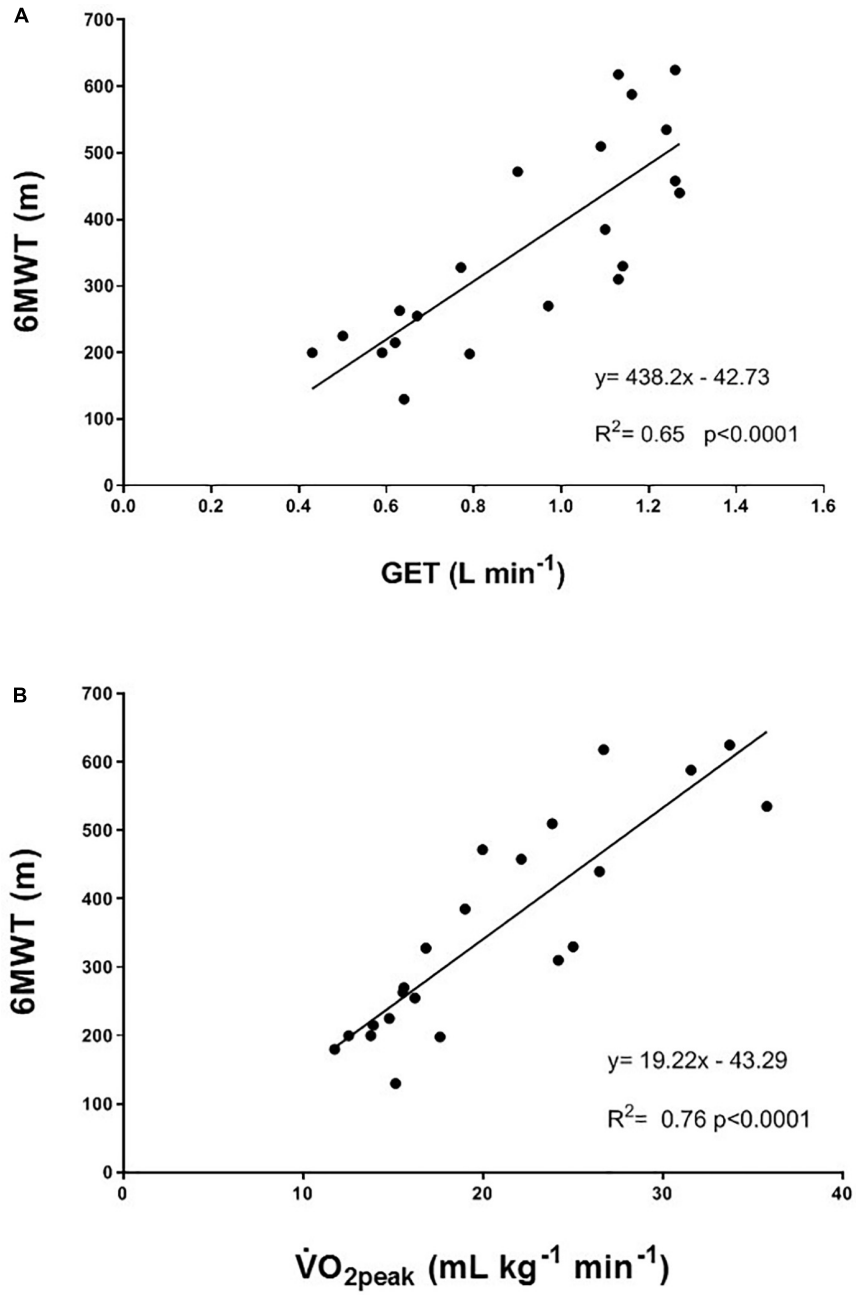

FIGURE 4 | Correlation between **(A)** gas exchange threshold (GET) and the 6-min walking test (6MWT); and **(B)** peak oxygen uptake ($\dot{V}O_{2peak}$) and the 6-min walking test (6MWT). Participants from both the TRAIN and UC groups are considered in the correlation.

the training, and with less fatigue. This result suggests that part of the impaired aerobic capacity was not only due to the disease itself, but also to the muscle disuse, and that the right dose of exercise could help to restore (at least in part) the reduced aerobic fitness. Interestingly, **Figure 4A** shows a significant correlation between GET and 6MWT, but the improvement in aerobic capacity (as shown by GET) was not translated in a higher distance covered during the 6MWT after training. Because the 6MWT is also significantly correlated to the $\dot{V}O_{2peak}$ (**Figure 4B**), the effect of an aerobic exercise with an intensity higher than that used in our study (around GET), may have a greater effect on this performance. This hypothesis should be investigated in future studies, without forgetting to ensure the right balance between exercise intensity and safety, and considering the high level of fatigue experienced by this population. The 13% non-significant increase in $\dot{V}O_{2peak}$ in a population comprising very heterogeneous sub-entities, which carry the same expression (the ALS phenotype) but harbor widely different activated pathways,

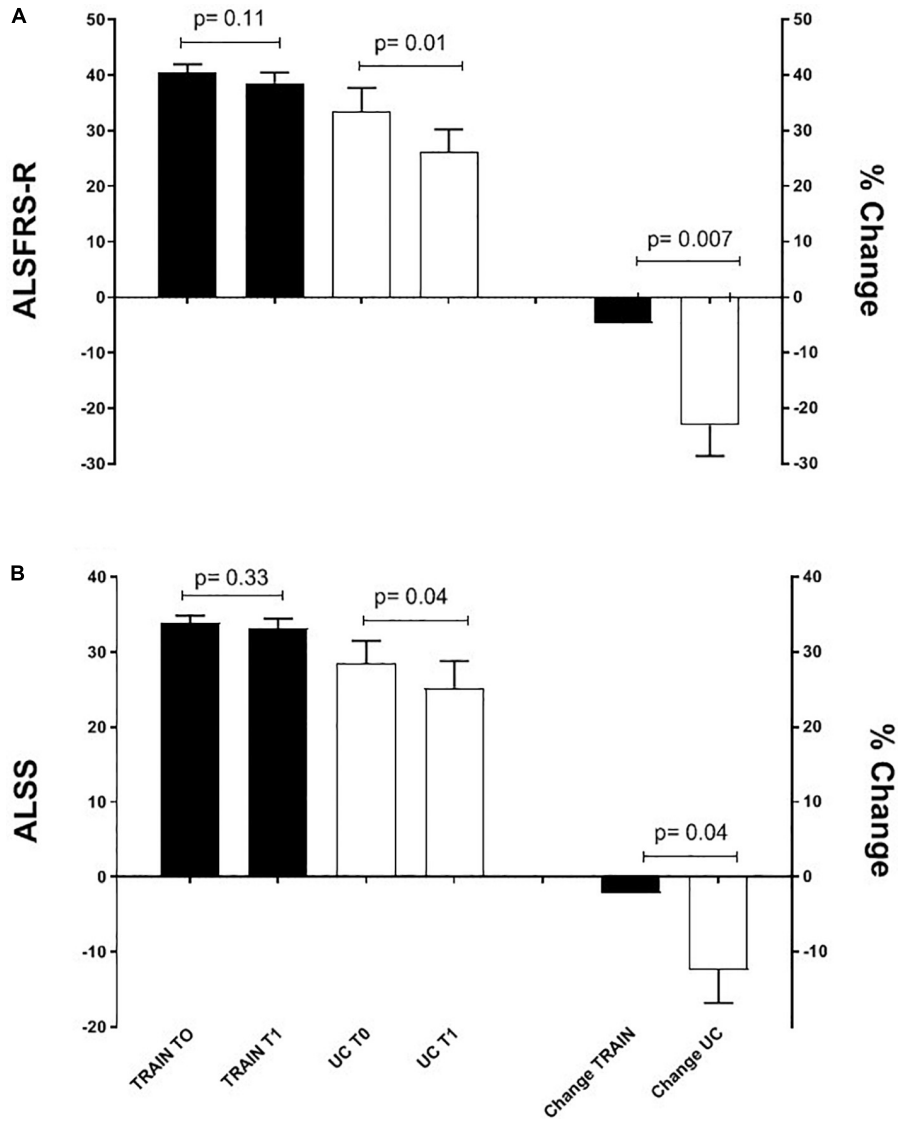

FIGURE 5 | Mean ALSFRS-R **(A)** and ALS-SS **(B)** scores before (T0) and after 12 weeks (T1) of exercise training (TRAIN) and usual care (UC), and mean changes expressed as a percentage. Average data are expressed as mean + SE.

should be considered representative of a real positive change in the maximal aerobic capacity due to the training, particularly if compared to the 9% decrease in the UC group. Indeed, in patients with multiple sclerosis (another fragile population), changes in $\dot{V}O_{2peak}$ were considered reliable if they were >10% (Langeskov-Christensen et al., 2014).

Even if not significant, due in part to the differential drop-outs and the intrinsic heterogeneity of the disease, it is interesting to note the direction of the arrows toward an increase in TRAIN and a decrease in UC for most of the physiological parameters evaluated during the CPET (**Table 2**). In particular, the tendency toward a significantly different change in the power output between TRAIN and UC seems due to the decrease (∼20%) of this parameter in the UC group. Although we cannot quantify how much of this decrease can be attributed to the disease and/or the disuse, our data support the hypothesis that tailored exercise training is able to counteract this reduced performance whatever the cause (as shown by a 3% increase of this parameter in the TRAIN group). The tendency toward a significantly different change in the peak ventilation between TRAIN and UC seems particularly due to an improved respiratory mechanics (∼27%) in the TRAIN group. A previous study by our group (Lanfranconi et al., 2017) has shown that the decrease in maximal ventilation in patients with ALS depends principally on the inability to increase the tidal volume, because of the weakness/fatigability of the respiratory muscles in individuals with ALS. The tendency toward a significant increase in peak ventilation after 12 weeks of moderate exercise training seems due to the concurrent increase in tidal volume (∼9%) and respiratory frequency (∼11%), and suggests that the submaximal aerobic

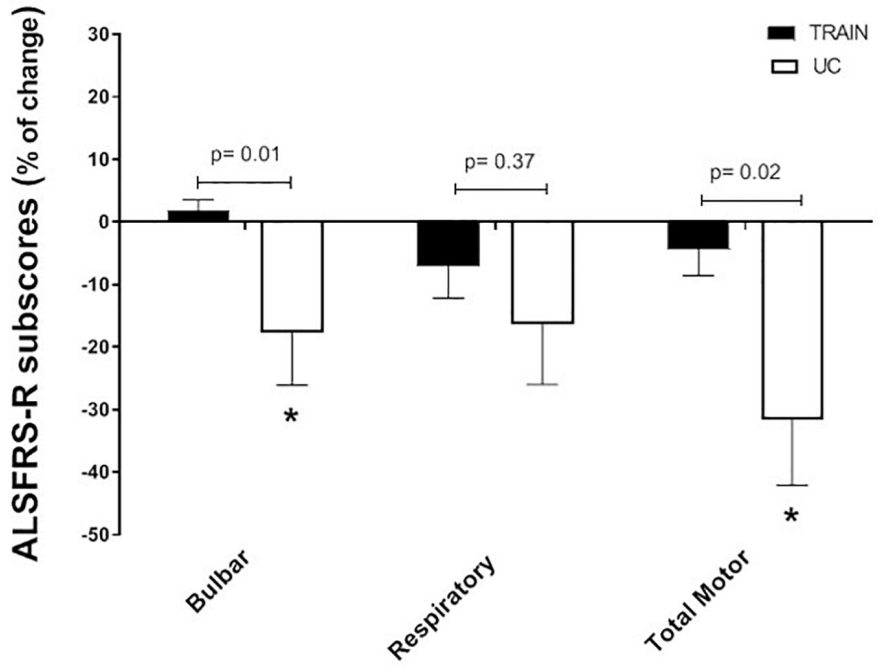

FIGURE 6 | Changes in percentage for the ALSFRS-R subscores (bulbar, respiratory and total motor function) for the training (TRAIN) and usual care (UC) groups. Average data are expressed as mean + SE. $*p < 0.05$ between T0 and T1 in the UC group.

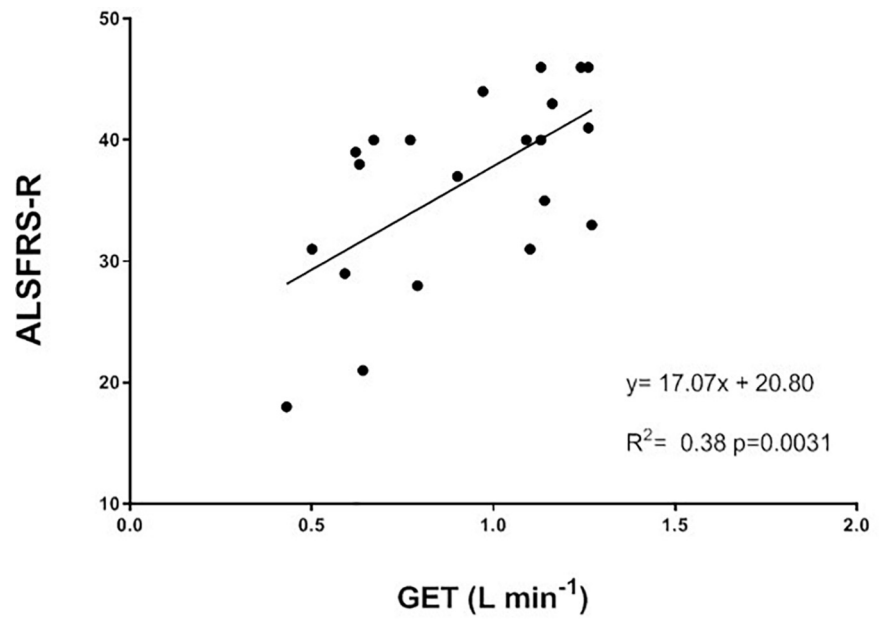

FIGURE 7 | Correlation between gas exchange threshold (GET) and the ALS functional rating scale revised (ALSFRS-R) for all the participants in this study (TRAIN and UC groups).

training can maintain or even improve the efficiency of the respiratory muscles in supporting the O_2 requirement during an intense exercise. Further studies, focusing on the effects of submaximal aerobic exercises on specific respiratory functions, should be performed in the future to reveal if this kind of training could provide an effective stimulus to the respiratory muscles of individuals with ALS.

The real challenge of an exercise training program for any clinical population is to produce an effect not only on the capacities that are specifically trained (i.e., strength,

aerobic capacity, etc.), but to have an effect on more complex movements that incorporate different capacities and that define the ability to maintain physical independence. The maintenance of the ability to perform the TUG test (which incorporates strength, balance, and mobility) indicates the effectiveness of this type of exercise training in attenuating the decrease in physical function in individuals with ALS. Even if the changes in strength of the knee extensor muscles were not significantly different between TRAIN and UC, when translated to more complex movements related to the daily life activities, and associated probably to an improvement in balance and mobility (that was not tested in this project), there was a positive impact in maintaining physical function.

The beneficial impact of exercise training on physical function is also demonstrated by the attenuated reduction of the ALSFRS-R scores in TRAIN with respect to UC. The significant relationship between GET and the ALSFRS-R suggests that efforts should be directed toward trying to maintain the aerobic fitness in patients with ALS, which in turn will ensure the maintenance of physical independence for the longest possible time. Our results are in agreement with other reports that evaluated the impact of different type of exercise training on ALSFRS in individuals with ALS (Drory et al., 2001; Dal Bello-Haas et al., 2007; Lunetta et al., 2016). While Drory et al. (2001) reported only the total ALSFRS score (significant less decrease after 3 months of light training), Dal Bello-Haas et al. (2007) also presented the values of the motor ALSFRS sub-score. They found significant differences between a resistance exercise training group and a usual care group after 3 months (only motor ALSFRS sub-score) and 6 months (both total and motor ALSFRS scores) of intervention. Similar to our study, Lunetta et al. (2016) presented the results for the total ALSFRS score plus each sub-score (bulbar, motor, and respiratory). They showed a significantly higher score in the total ALSFRS after 180 days, but not after 90 days. While they did not find any difference in the respiratory and bulbar sub-scores, they found that the exercise training had its major effect on the motor domain. Interestingly, the exercise training proposed to our participants had an effect on not only the motor function (as expected) but also the bulbar function (not expected). The latter effect seems promising considering the difficulties faced by patients with a bulbar phenotype and the poorer outcomes reported. The ALS-SS scale confirmed, in a more articulated manner, the results found by the ALSFRS-R scale and supports the idea that exercise attenuates the disease-induced reduction in physical function in different areas of daily living in individuals with ALS.

The role of physical activity in improving the QoL has been demonstrated in various diseases. Recently, a meta-analysis conducted in six chronic brain disorders (Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, schizophrenia, and unipolar depression) confirmed that exercise is superior to pharmacological treatment in improving QoL (Dauwan et al., 2019). The maintenance of QoL found in this study in individuals with ALS who exercised, and the tendency toward lower values of QoL in individuals with ALS who did not exercise, is probably due to the fact that participants in the TRAIN group felt that their motor skills were maintained and that they could do something about it. In fact, the reduced QoL has been linked particularly to the physical status and anxiety of individuals with ALS (Young et al., 2019). In a disease where there is no cure and the only pharmacological treatment has limited efficacy, the exercise training represented a viable therapy.

Participation

The differential drop-outs in TRAIN and UC could have biased our results (Bell et al., 2013), as some data could be lost due to non-random effects (i.e., patients withdraw for reasons related to their disease or treatment), particularly as patients with lower physical function or QoL values are more likely to drop out so as to leave the stronger patients in the UC group. Interestingly, the higher drop-out rate was in the UC and not in the TRAIN group, which was more challenged (e.g., travel time, the availability of a caregiver to take the participants to the gym) by having to travel three times/week for 12 weeks to the Clinica San Pietro where the gym was located. The high satisfaction in the exercise training program showed by the VAS (∼9), the high adherence by the individuals in the TRAIN group (∼85%), and the lower drop-outs indicate that the patients enjoyed the training program and the relationship with the human resources involved.

CONCLUSION

Despite the small number of patients, this study supports the idea that tailored moderate-intensity exercise is not detrimental for patients with ALS and can counteract muscle disuse. The idea of non-exercising as a safe recommendation for individuals with ALS should be reconsidered in the light of these and other authors' results. ALS care requires an integrated approach, and our results indicate that moderate-intensity exercise training strictly monitored by clinical exercise physiologists represents a real opportunity to maintain physical function and QoL in individuals with ALS, and should be introduced in the usual management and care of this disease.

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Milano-Bicocca Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AF, FL, and LT contributed to the conception and design of the manuscript. AF, FL, GC, RB, SM, and LT contributed to the collection and analysis of the data. AF, FL, GC, RB, and SM contributed to the training of the participants. AM contributed to the provision of the study materials.

ACKNOWLEDGMENTS

We thank our patients and their caregivers for their enthusiastic participation in this study. We are grateful to Prof. D. J. Bishop for his careful English revision and editing, and the rest home "San Pietro," Cooperativa La Meridiana (Monza, Italy) for providing appropriate spaces for this project.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

CASE REPORT
published: 04 February 2020
doi: 10.3389/fneur.2020.00057

Beneficial Effects of Ketogenic Diet on Phosphofructokinase Deficiency (Glycogen Storage Disease Type VII)

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Background: A deficiency of muscle phosphofructokinase (PFKM) causes a rare metabolic muscle disease, the Tarui disease (Glycogen storage disease type VII, GSD VII) characterized by exercise intolerance with myalgia due to an inability to use glucose as an energy resource. No medical treatment for GSD VII currently exists. The aim of this study was to determine whether a dietary intervention with excessive fat intake would benefit GSD VII.

Patient and Methods: A ketogenic diet (KD) intervention implemented as a modified Atkins diet was established for one patient with PFKM deficiency, with a low late lactate response and very high ammonia levels associated with exercise. We recorded the KD intervention for a total of 5 years with clinical and physiotherapeutic evaluations and regular laboratory parameters. Cardiopulmonary exercise testing, including breath gas analysis and venous lactate and ammonia measurements, was performed before KD and at 3, 8 months and 5 years after initiation of KD.

Results: During the 5 years on KD, the patient's muscle symptoms had alleviated and exercise tolerance had improved. In exercise testing, venous ammonia had normalized, the lactate profile remained similar, but oxygen uptake and mechanical efficiency had increased and parameters showing ventilation had improved.

Conclusions: This study is the first to show a long-term effect of KD in GSD VII with an alleviation of muscle symptoms, beneficial effects on breathing, and improvement in exercise performance and oxygen uptake. Based on these findings, KD can be recommended under medical and nutritional supervision for selected patients with GSD VII, although further research of this rare disease is warranted.

Keywords: Tarui disease, GSDVII, ketogenic diet, cardiopulmonary exercise capacity, lactate, ammonia, glykogen storage disease

OPEN ACCESS

Edited by:
Mauro Marzorati, Institute of Biomedical Technologies, (CNR), Italy

Reviewed by:
Priya Kishnani, Duke University, United States
Rossella Parini, Fondazione MBBM, Italy

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Specialty section:
This article was submitted to Neuromuscular Diseases, a section of the journal Frontiers in Neurology

Received: *18 October 2019*
Accepted: *15 January 2020*
Published: *04 February 2020*

Citation:
Similä ME, Auranen M and Piirilä PL (2020) Beneficial Effects of Ketogenic Diet on Phosphofructokinase Deficiency (Glycogen Storage Disease Type VII). Front. Neurol. 11:57. doi: 10.3389/fneur.2020.00057

INTRODUCTION

Tarui disease or muscle phosphofructokinase (PFKM) deficiency belongs to the glycogen storage diseases (GSD VII, OMIM#232800). It is a rare autosomal recessive disorder caused by mutations in the *PFKM* gene and characterized by exercise intolerance, muscle cramping, and myoglobinuria associated with compensated hemolysis and later nascent muscle weakness and mild myopathy

(1, 2). Phosphofructokinase is the rate-limiting enzyme in the glycolytic pathway and catalyzes the phosphorylation of fructose 6-phosphate to fructose-1, 6-bisphosphate. In Tarui disease, typically the total lack of phosphofructokinase enzyme in muscle tissue causes the oxidative pathway from glucose to pyruvate to be blocked at this point of the glycolysis pathway, and glucose cannot be used in muscle energy metabolism normally. Therefore, the muscle tissue in Tarui disease must utilize alternative oxidative substrates other than glucose in energy metabolism. In McArdle's disease (GSD V, #232600), a condition resembling Tarui disease, increased fat oxidation during exercise has been reported (3). The importance of free fatty acids for muscle oxidative metabolism has also been revealed in Tarui disease, and patients with this disease have been shown to benefit from substrates available after overnight fasting or triglyceride infusion during aerobic exercise (4). Indeed, glucose would not have a favorable effect on their cardiorespiratory capacity, and glucose ingestion could even worsen the condition (4, 5). Patients with Tarui disease, unlike in McArdle's disease, do not show the second-wind phenomenon, an exercise-related increase in the capacity for muscle oxidative phosphorylation (6, 7). In both diseases, an excess of purine metabolites, e.g., uric acid, hypoxanthines, and ammonia, as an indicator of increased utilization of proteins has been found (8).

Currently, no specific treatment options exist for Tarui disease. Regular physiotherapy is important, although a benefit from slowly progressive exercise training has not been established (6, 7). However, a diet low in carbohydrates and high in fat and protein (ketogenic diet, KD) could hypothetically be beneficial for patients. In order to understand the benefit of KD in the long term we present the results of a careful 5 years follow-up with blood specimens, clinical visits, and cardiopulmonary exercise testing with blood gas, ammonia, and lactate examinations on a male patient suffering from Tarui disease (9, 10).

PATIENT AND METHODS

Patient

The patient is a 59 years-old man carrying a homozygous *PFKM* gene mutation, c.329G>A. The histological and genetic findings (9) and the analyses of the exercise lactate profile (10) have been published earlier. In childhood, he had normal motor development and normal exercise tolerability, but was not very keen on sports. As a young boy he could run up to 100 meters but around the age of 12 years he started to develop symptoms, including muscle weakness, and attacks of muscle pain, weakness, cramping, and vomiting, during extensive physical activity. He was never hospitalized due to the muscle symptoms or due to raise in creatine kinase (CK) values.

At age 59, there was mild muscle weakness in hip flexors and extensors and ankle flexors and extensors on the right side [MRC scale 4 out of 5 (11)], mild reticulocytosis without anemia, and normal EMG. No muscle atrophy was evident. Medication for high blood pressure had been recently introduced (amlodipin 5 mg and valsartan 160 mg once a day). The patient's weight was 71 kg and height 177 cm (BMI 23.0 kg/m^2). He could walk with a slow pace about 10 km but walking on an incline was limited by muscle pain. He could not run, because of muscle cramping and feeling unwell, and could walk only one flight of stairs. He did regular hunting and hiking trips in the forest. He annually spent 4–5 days in Lapland with a group of friends hiking 15–20 km daily but he was always the last in the group and other members had to wait for him.

Ethical approval for the study was granted by the Medical Ethics Committee of Helsinki University Central Hospital, Finland. Informed consent was provided by the patient and the controls.

Diet

The patient's diet was evaluated using a 3 days food record prior to KD initiation, and the evaluation was repeated 6 months after starting KD. Dietary intakes were calculated using the national food composition database of the National Institute for Health and Welfare, Finland (12). The KD was guided by the same dietitian (M.S.) throughout the study period. Nutrition and diet were assessed and counseled at clinic visits or by phone or email contacts several times during the initiation period and later at least yearly. Daily energy intake was planned to be at the same level as before the KD, the amount of carbohydrates was restricted to 10 g per day, and the consumption of fat and protein was encouraged, aiming at a ketogenic ratio of ∼1:1. Consumption of unsaturated fats was recommended to avoid unfavorable changes in serum lipids. Multivitamin, additional vitamin D, and calcium supplementations were introduced. Temporal mitigation of carbohydrate restriction (using small or moderate amounts of e.g., rye bread) was allowed during the treatment when disadvantage of the diet was considered (strong increase of LDL-cholesterol).

Laboratory Assessment

Laboratory parameters were followed up regularly; e.g., β-hydroxybutyrate, glucose, cholesterol, CK, and liver function were measured almost monthly during the first year and, after stabilization of the diet, twice during the second year and once a year thereafter, except for cholesterol values, which were measured more often. The laboratory results from the time points of exercise testing are presented in **Table 1**. In addition, concentrations of some vitamins (vitamins D and A), minerals (such as selenium and zinc), carnitine (total and free), prealbumin, and urine calcium and creatinine were measured discretionarily to optimize nutrient intakes. Blood β-hydroxybutyrate was also measured by the patient at home to ensure ketosis (target level 2.5–5), at the beginning every morning and evening, and later on demand.

Cardiopulmonary Exercise Test

Cardiopulmonary exercise test with breath gas analysis (spiroergometry) was performed as described earlier before KD and at 3, 8 months, and 5 years after start of KD (13). The test was started with a 40 W work load, with an increase of 40 W in 3 min increments (40 W/3 min). A maximal subjective level of at least 17/20 on the Borg scale was attained. The venous blood specimens were taken via a vacuum technique, and the analysis of lactate and ammonia specimens as well as the venous blood

TABLE 1 | Laboratory parameters before ketogenic diet (KD) and during the 5 years follow-up at the time points of cardiopulmonary exercise testing.

Parameter assayed reference range	Before KD	KD 3 months	KD 8 months	KD 5 years
Plasma β-hydroxybutyrate (mmol/L) <0.2 mmol/L	0.22	1.30	0.59	0.4**
Plasma glucose (mmol/L) 4.0–6.0 mmol/L	5.8	5.2	5.3	5.6**
Serum insulin (mU/L) (2–20 mU/L)	4.1	1.6	1.2	3.5**
Plasma creatine kinase (U/L) 40–280 U/L	96	74	160	159
Plasma alkaline phosphatase (U/L) 35–105 U/L	55*	34	34	Not measured
Plasma alanine aminotransferase (U/L) 10–70 U/L	33	36	25	27
Plasma glutamyltransferase (U/L) 15–115 U/L	41*	18	24	18**
Plasma triglycerides (mmol/L) <2.0 mmol/L	2.39	0.64	0.59	0.67
Plasma cholesterol (mmol/L) <5.0 mmol/L	4.6	5.2	5.6	4.4
Plasma LDL[#] cholesterol (mmol/L) <3.0 mmol/L	2.7	3.5	3.5	2.1
Plasma HDL[##] cholesterol (mmol/L) >1.0 mmol/L	1.52	1.88	2.15	1.83

Laboratory tests were studied once a month during the first year, three times in the second year, and annually in years 3–5.
**Measured during KD initiation.*
***Measured 6 months before exercise test (values from the time of exercise test are missing).*
[#]Low density lipoprotein.
[##]High density lipoprotein.

gases and electrolytes have been reported earlier (10). The results of four healthy men with a mean age of 48 (range 35–60) years and BMI 23.0 kg/m^2 (SD 1.4) were selected from an earlier study (10) and served as a control. Younger men and women from the original controls were excluded.

Statistical Methods

Spearman's correlation test was performed between the baseline and diet phases for the patient as well as for the controls.

RESULTS

Diet and Laboratory Tests

Before KD, the calculated total energy intake was 2,660 kcal per day and consisted of 177 g of available carbohydrates (27 E%, percentage of total energy intake), 107 g of protein (16 E%) and 164 g of fats (55 E%). Six months after beginning KD, the calculated total energy intake was ∼2,880 kcal per day and consisted of 229 g of fats (72 E%), 189 g of protein (26 E%) and 12 g of available carbohydrates (2 E%), with a ketogenic ratio of ∼1:1.

The β-hydroxybutyrate concentrations measured in laboratory ranged between 0.4 and 2.9 mmol/L during the follow-up period mostly being below 2.5 mmol/L (**Table 1**). During periodical mitigation of carbohydrate restriction β-hydroxybutyrate concentration was lower. An increase was initially seen in cholesterol values, with the highest concentrations measured after 2 years on KD, total cholesterol 8.2 mmol/L, LDL-cholesterol 6.2 mmol/L and HDL-cholesterol 2.01 mmol/L. Ezetimibe 10 mg (Ezetimibe accord®) medication was started due to high cholesterol values. Finally, during medication, total cholesterol, and LDL-cholesterol were below baseline values and HDL-cholesterol was slightly increased at 5 years control. Plasma glucose and insulin values were within normal limits before and during KD and both decreased after beginning of the diet (**Table 1**).

Vitamin D concentration (serum 25-hydroxyvitamin D_3 and D_2) was at the beginning of dietary therapy below reference values, 32 nmol/L. After vitamin D supplementation, the concentration increased to 63 nmol/L.

Clinical Examination

Already after 6 months' exposure to KD, the patient began to experience a subjective alleviation of muscle symptoms, manifesting as more rapid recovery and less muscle discomfort. He was able to increase daily exercise and could gradually spend extended periods of time hiking and hunting in the forest. However, he still often suffered from stiffness.

At 5 years, his muscle strength (MRC) was within the normal range (5 of 5) except for ankle extension forces (4 of 5). Deep tendon reflexes were present excluding Achilles. He felt that exercise tolerance had improved during KD and experienced less cramping and nausea during exercise than before KD. He could now walk longer without stopping and could ski 10–15 km/day at the same speed as his friends, which was not possible before KD. During KD he could participate in long hiking trips, moving at a similar speed as his peers, also not possible before KD. He felt that especially capability to walk on an incline was better than before KD.

The patient's weight had decreased from 71 to 58 kg (height 177 cm), with BMI falling from 22.7 to 18.5 kg/m^2 in 5 years.

Results of Cardiorespiratory Exercise Testing

The main results of the cardiorespiratory exercise testing during the follow-up at the time points of 3, 8 months and 5 years are presented in **Table 2**. The maximal working capacity was moderately reduced before KD, increasing slightly during KD, but it remained lower than the values of age-matched controls. The maximal oxygen uptake by body weight had a mild increase during KD and the mechanical efficiency ($Wmax/VO_2max$) increased from 13.1 to 16.5% (normal value ≥20%).

During KD a decrease occurred in the very high maximal breathing frequency in exercise, from 68 to 46/min and an increase in tidal volume from 44.6 to 60.3% of predicted value. However, slight hyperventilation was found, as assessed in slightly increased minute ventilation vs. CO_2 production (VE/VCO_2) and O_2 consumption (VE/VO_2) and also in slightly decreased $FetCO_2$.

TABLE 2 | Results of cardiorespiratory exercise testing of the patient before and during KD.

	Before KD	KD 3 months	KD 8 months	KD 5 years	Control subjects ($N = 4$) mean (SD)
Heart rate maximum percentage of predicted (%)*	98.3	94.6	94.9	94.8	97.9 (4.6)
Borg subjective scale 6–20	19	19	17	20	18.3 (2.1)
RQ ($V'CO_2/V'O_2$) in maximal exercise	0.91	0.88	0.92	0.75	1.13 (0.05)
Wmax/3 min (maximal working capacity) (W)	93	90	99	102	285.2 (81)
$V'O_2$ max (maximal oxygen uptake) (L/min)	2.045	1.838	2.159	1.778	3.79 (1.1)
$V'O_2$/kg max (maximal oxygen uptake/weight) (ml/min/kg)	28.8	25.9	34.8	30.7	52.1 (12.1)
Wmax/$V'O_2$ max (%)	13.1	11.1	14.1	16.5	21.7 (1.4)
Breathing frequency	68	61	56	46	43.0 (12.8)
Tidal volume, % of predicted	44.6	41.7	56.1	60.3	109.8 (24.8)
Fraction of end tidal CO_2 ($FetCO_2$) (%)	4.16	3.82	3.8	4.0	5.4 (0.51)

RQ, respiratory quotient; $V'CO_2$ is maximal CO_2 production.
**205–0.5 × age.*

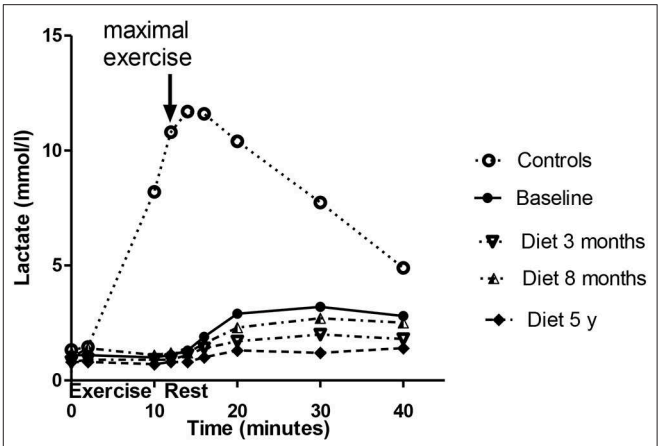

FIGURE 1 | Lactate levels associated with exercise tests of the patient before diet and during the follow-up. The values of the control subjects without dietary intervention are also given for comparison. The baseline lactate data has been published earlier (10), the controls matched to the age and gender of the patient were obtained from the forementioned publication. A strong correlation existed between the patient's baseline lactate values and his diet curves at 3, 7 months, and 5 years, rho being 0.914 ($p = 0.006$), 0.885 ($p = 0.015$), and 0.90 ($p = 0.001$), respectively.

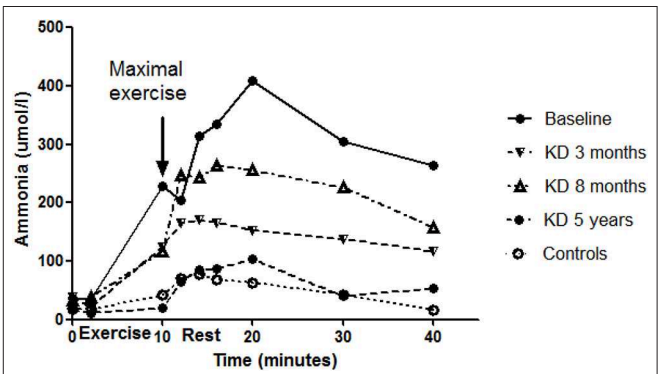

FIGURE 2 | Ammonia levels associated with exercise tests of the patient before the diet and during the follow-up. Values of the control subjects without dietary intervention are also given for comparison. The baseline ammonia data has been published earlier (10), and the controls matched to the age and gender of the patient were obtained from the aforementioned publication. No correlations existed between the baseline curve of the patient and those of the controls or the diet curves of the patient.

Results of Venous Blood From Exercise Testing

The venous lactate level at rest remained at pre-KD level (1.1 mmol/L) in the follow-up (0.8 mmol/L) (**Figure 1**). The ammonia level at rest decreased from 36 to 27 μmol/L. The maximal ammonia level (measured 4–10 min after exercise) was 409 μmol/L before KD (10) and was reduced at 3 months to half of the pre-KD level. However, a new increase was seen at 8 months (after the periodical mitigations of carbohydrate restriction), which at 5 years had returned to normal level, 111 μmol/L (**Figure 2**). Before KD, metabolic alkalosis was seen after exercise (pH 7.46 and BE +4.2 mmol/L at 2 min after exercise), but at 5 years the pH value after exercise was within normal limits (pH 7.38 and BE + 2.6 mmol/L) (**Figure 3**).

DISCUSSION

During the 5 years follow-up of KD the patient remained clinically stable with subjective alleviation of muscle pain symptoms and better exercise tolerance. In cardiopulmonary exercise testing, working capacity and mechanical efficiency had increased. In venous blood, lactate levels had decreased from the low pre-KD levels, and the very high ammonia levels associated with exercise testing detected in the measurements before KD had decreased to normal. KD had a beneficial impact also on respiratory parameters during exercise, reflected as lowering of breathing frequency and the ventilatory equivalent for O_2 ($V'E/O_2$) and as increasing tidal volume, suggesting diminished hyperventilation. However, the end tidal CO_2 level ($FetCO_2$) remained rather low, indicating permanent hyperventilation tendency.

Increasing evidence is emerging for a benefit of dietary therapy, especially in metabolic muscle diseases (14), but also in dystrophic disorders (15). However, among GSDs specific enzyme replacement therapy exists only in Pompe's disease (GSD II) (16). In McArdle's disease (GSD V), some data suggest a benefit of KD in relieving symptoms (17). In one child with PFKM deficiency presenting with congenital arthrogryposis and severe myopathy, KD starting at age 4 months alleviated clinical symptoms, enhanced motor skill development, and improved muscle strength (18). Before KD, our patient's diet fat content

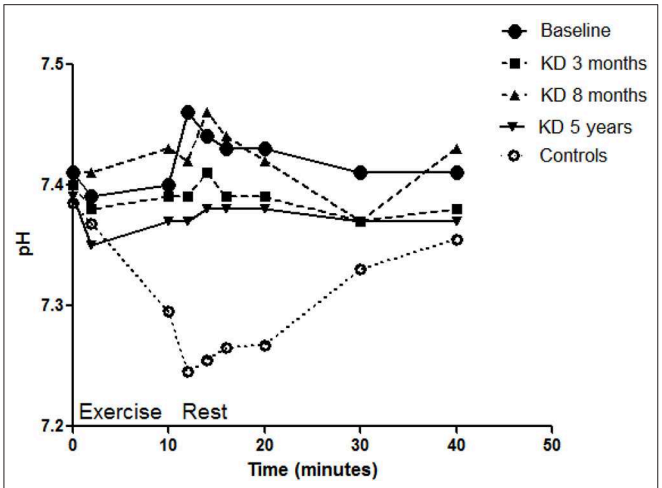

FIGURE 3 | pH levels associated with exercise tests of the patient before the diet and during the follow-up. Values of the control subjects without dietary intervention are also given for comparison. The baseline ammonia curve has been published earlier (10), and the controls matched to the age and gender of the patient were obtained from the aforementioned publication. A high negative correlation existed between the patient's baseline curve and mean diet curve (rho = −0.826, $p = 0.006$).

(55 E%) was relatively high and carbohydrate content (27 E%) low compared with the typical diet of Finnish men (fat 36 E%, carbohydrates 42 E%) (12), possibly attenuating our findings on the beneficial effects of KD. The patient's original diet was evaluated for the first time before the initiation of KD, but had remained similar for years.

KD was fairly well-tolerated by the patient. About 7 months after adopting KD, high LDL-cholesterol level was measured (4.9 mmol/L). Since cardiovascular disease exists among the patient's immediate family, the disadvantage of the KD for the patient was reconsidered, and the restriction of carbohydrates was mitigated (e.g., rye bread was allowed), which led to a decrease in ketosis. Based on the patient's subjective experience of lowered exercise capability after the increase of carbohydrates, however, the KD was again tightened. With rising LDL-cholesterol values (up to 6.2 mmol/L) cholesterol-lowering medication was started after 3 years of KD. The LDL-cholesterol level was at the 5 years control lower than before KD (2.1 vs. 2.7 mmol/L). During follow-up, no abnormalities were detected in the liver or kidney functions. The patient's weight decreased by 13 kg during KD. He was satisfied with the weight decrease, although it was not the original aim of the treatment. Interestingly, the reported energy intake during KD was greater than before KD. This may be due to the better working capacity, leading to increased physical activity during the diet. However, it is also possible that the difference between these calculated intakes is explained by normal day-to-day variation in food intake since the food record periods were short (3 days).

The patient's maximal oxygen uptake (L/min) actually decreased in the follow-up, but relative to weight oxygen uptake mildly increased from the pre-KD values [from 91 to 103% of predicted values (19)]. The benefit of KD was more clearly seen in the mild increase in maximal working capacity, which rose from 56 to 68 W, with a simultaneous decrease in maximal heart rate. The mechanical efficiency, which is the maximal working power during the exercise test relative to the simultaneous maximal oxygen consumption (uptake), also increased during KD, reflecting improved exercise performance. However, at 5 years on KD, his exercise capacity remained at 68.1% of healthy controls' values (**Table 2**). The decrease in the respiratory quotient (RQ, relation of CO_2 production to O_2 consumption) at 5 years may more be associated with decreased glucose oxidation and increased fat oxidation (20) than with submaximal effort. This would be in line with former findings of inverse connection between free fatty acid concentration and oxidation with insulin concentration (21) since insulin adequately decreased after beginning of the KD.

Before KD, a very low lactate level was found in the cardiopulmonary exercise test, with a delayed increase occurring 10–20 min after the exercise (10). During KD the lactate curve associated with exercise testing had a similar shape as before the diet, but was situated lower, which is explained by less ingested carbohydrates. The level of ammonia increased to very high levels in exercise testing before KD compared with control subjects (**Figure 2**) as well as with earlier data on exercise tests in healthy subjects (22). High levels of ammonia might cause the patient's exercise fatigue and short-term neurological discomfort, as reported also in sport medicine (23, 24), being one explanation for the increased ventilation, especially during exercise. With KD, the level of ammonia during exercise was drastically decreased, attaining normal levels. In line with our finding, elevated ammonia has earlier been reported in Tarui disease during exercise testing (8).

A common route to catabolize proteins and nucleotides is deamination of adenosine monophosphate (AMP) to inosine monophosphate (IMP) (23–25) which generates ammonia. In Tarui disease, the increase of ammonia during exercise has in some reports been explained by impaired cellular adenosine diphosphate (ADP) phosphorylation and its increased degradation to AMP (6). The results of our earlier study (10) suggested that in Tarui disease, glucose passing the normal glycolytic route could be used in other anabolic biosynthetic reactions leading to increase of ammonia (26–28).

Just before the exercise test at 8 months the patient returned to ketosis, and in the exercise test, a new increase in ammonia was detected. This suggests that even a short period of more carbohydrates may increase the ammonia level during the exercise test (**Figure 2**) highlighting the importance of carbohydrates in the high ammonia levels associated with Tarui disease.

Since convincing evidence between higher whole-grain consumption, which is a substantial contributor to carbohydrate intake, and lower risk of coronary heart disease, type 2 diabetes, colorectal cancer and all-cause mortality, is growing (29), extreme restriction of carbohydrates has to be well-founded. The risk factors of chronic diseases must be considered during long-term KD as was done in our case, with cholesterol-lowering medication started due to the increase in LDL-cholesterol levels. The

patient felt that his exercise capability was decreased in conjunction with increased carbohydrate intake and wanted to continue the strict KD with subsequent initiation of cholesterol medication.

During KD associated with the exercise test, pH and base excess values remained rather high, indicating that no metabolic acidosis had developed. Carbohydrate intake restriction and ingested fat and proteins produce acidic ketones, which have a tendency to advance to metabolic acidosis; however, this was not seen here. This is probably explained that there still would have been release of alkaline ammonia from protein catabolism, although less than before KD.

In our previous study (10), ventilation during exercise of the patient was markedly increased before KD, which may arise from lower exercise tolerance and increased ammonia levels during and after exercise causing neural discomfort. In the present study, better cardiopulmonary condition and lower ammonia production possibly lowered the ventilation. Correspondingly, in an earlier study of Tarui disease, exercise tests during triglyceride infusion were associated with decreases in ventilation and respiratory exchange ratio and increases in work intensity and oxygen uptake (4).

The main strength of this study is that KD was carefully implemented and followed in a very cooperative patient. Laboratory examinations were performed continuously, the follow-up intervals increasing as the patient achieved a balance with KD. Also the cardiorespiratory exercise testing was first performed with shorter intervals, and the last test at 5 years to obtain data on long-term KD treatment. A study limitation is that we had only one patient, and further studies are needed.

In conclusion, KD intervention in our patient with Tarui disease seemed to have a beneficial effect, as measured by the patient's subjective condition with diminished muscle symptoms and increases in exercise capacity and oxygen uptake, which also had a favorable effect on the patient's ventilation. Cholesterol values increased on the diet, but with statin medication the levels were kept under control. Our results thus encourage the implementation of KD in patients with Tarui disease as the risk factors of chronic diseases are considered.

DATA AVAILABILITY STATEMENT

The datasets for this article are not publicly available because of legislation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee of Helsinki and Uusimaa, 199/13/03/01/11. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PP: spiroergometric testing of patient, interpretation and analysis of data, and drafting of the article. MA: treatment of the patient with Tarui disease, data analysis, and drafting of the article. MS: treatment of the patient, the dietary planning and follow-up, data analysis, and drafting of the article.

ACKNOWLEDGMENTS

The authors thank Ritva Luukkonen, Ph.D., for statistical consultation and Carol Ann Pelli, Hon. B.Sc., for language revision.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

OPINION
published: 30 April 2020
doi: 10.3389/fphys.2020.00350

Metabolic Myopathies: "Human Knockout" Models and Translational Medicine

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Keywords: metabolic myopathies, mitochondrial myopathies, McArdle disease, functional evaluation, skeletal muscle oxidative metabolism

Metabolic myopathies are rare diseases characterized by derangements of glycogen or lipid metabolism or mitochondrial function, caused by genetic mutations leading to defects of the main pathways of energy provision in skeletal muscle fibers.

The patients can be considered a sort of "human knockout" model, and present unique opportunities to investigate fundamental physiological mechanisms. One of the first authors to exploit this possibility was James Hagberg in the early 1980's, in a classic paper (Hagberg et al., 1982) carried out on patients with McArdle disease (McA). In McA patients the absence of myophosphorylase activity substantially impairs the flux of substrates along the glycolytic pathway (Lewis and Haller, 1986). At the end of an incremental exercise these patients typically reach exhaustion without any increase in blood lactic acid levels vs. those determined at rest (Hagberg et al., 1982), and no metabolic acidosis ensues during exercise. Hagberg et al. (1982) demonstrated that the "ventilatory threshold" (pulmonary ventilation [$\dot{V}E$] and CO_2 output [$\dot{V}CO_2$] "disproportionate increases" vs. that of O_2 uptake [$\dot{V}O_2$], occurring at about 50–70% of peak work rate) (Beaver et al., 1986) was not different in McA patients vs. controls, providing evidence against a causative role for blood lactic acid in the $\dot{V}E$ and $\dot{V}CO_2$ responses.

Mechanisms of metabolic regulation often present a redundancy, and within a complex system several factors may concur in determining a response. Thus, in strict terms the experimental "elimination" of one factor, and the observation of a normal response, does not mean that the factor, in physiological conditions, does not have a regulatory role. Nonetheless, in the example mentioned above [identification of the factor[s] responsible for the ventilatory threshold], McA patients represent indeed an ideal experimental model. Let's summarize the *scenario*. Experimental question: are the disproportionate increases in $\dot{V}E$ and $\dot{V}CO_2$, vs. that of $\dot{V}O_2$, observed during an incremental exercise, attributable to an increased elimination of CO_2 generated in tissues and blood as a consequence of H^+ buffering by bicarbonate? The experimental approach was straightforward: what happens in McA patients, in whom (without the need of any experimental intervention) no H^+ accumulation in muscles and blood occurs during exercise? The experimental data were clear: no differences for the $\dot{V}E$ or $\dot{V}CO_2$ vs. $\dot{V}O_2$ responses in the McA patients vs. the controls. Take home message: H^+ accumulation in blood is not necessary for the $\dot{V}E$ or $\dot{V}CO_2$ vs. $\dot{V}O_2$ disproportionate increases observed at 50-70% of peak work rate during an incremental exercise (Hagberg et al., 1982).

In McA patients the reduced flux of substrates along the glycolytic pathway limits the supply of substrates to the tricarboxylic acid cycle, thereby impairing oxidative metabolism, as also suggested by the slow $\dot{V}O_2$ kinetics during the transition from rest to exercise (Grassi et al., 2009) and by the slow phosphocreatine recovery kinetics during the recovery from exercise (Siciliano et al., 1995). A similar impairment, although by a different and more "downstream" cause (mutations leading to impairment[s] of enzyme[s] of the mitochondrial respiratory chain) is described in patients affected by another type of metabolic myopathy, the heterogeneous series of diseases termed "mitochondrial myopathies" (MM). Also in this respect McA and MM patients may function as human knockout models, allowing to elucidate basic physiological mechanisms.

OPEN ACCESS

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *28 October 2019*
Accepted: *26 March 2020*
Published: *30 April 2020*

Citation:
Grassi B, Porcelli S and Marzorati M (2020) Metabolic Myopathies: "Human Knockout" Models and Translational Medicine. Front. Physiol. 11:350. doi: 10.3389/fphys.2020.00350

An excellent example is represented by a set of compensatory responses occurring in the framework of a physiological adaptation, described over the years, thanks to the elegant work of different groups. In MM and McA patients the impaired oxidative metabolism [considered as whole body "peak" pulmonary $\dot{V}O_2$ (Linderholm et al., 1969; Lewis and Haller, 1986; Taivassalo et al., 2003; Grassi et al., 2007), or as muscle peak $\dot{V}O_2$ (Taivassalo et al., 2002)] is almost exclusively attributable to an impaired peak capacity of fractional O_2 extraction, i.e., to an impairment of the second term of the right-hand portion of the equation describing the Fick principle of conservation of mass (systemic arterial—mixed venous O_2 concentration difference, C(a-$\bar{v}$)O_2; or "local" arterial—venous O_2 concentration difference, C(a-v)O_2). After putting fractional O_2 extraction in the equation expressing the Fick principle we obtain, at the whole body level:

$$\text{Pulmonary } \dot{V}O_2 \;=\; \text{cardiac output} \times \text{C(a} - \bar{v})\text{O}_2 \qquad (1)$$

On the other hand, if we consider the Fick principle of conservation of mass across a specific skeletal muscle group we obtain:

$$\text{Muscle } \dot{V}O_2 = \text{muscle blood flow} \times \text{C(a} - \text{v})\text{O}_2 \qquad (2)$$

Several groups have documented decreased submaximal and peak C(a-$\bar{v}$)O_2 in MM (Linderholm et al., 1969; Taivassalo et al., 2003) and McA (Lewis and Haller, 1986) patients, mainly by measuring cardiac output and $\dot{V}O_2$ during an incremental test. Other groups demonstrated a decreased submaximal and peak C(a-v)O_2 across an exercising limb (Taivassalo et al., 2002), by invasive measurements of CaO_2 and CvO_2, or indirectly by determining submaximal and peak fractional O_2 extraction in muscle by near-infrared spectroscopy (NIRS, see the review by Grassi and Quaresima, 2016) (Grassi et al., 2007).

These authors also described a highly significant and negative linear correlation between peak $\dot{V}O_2$ and the NIRS-determined peak fractional O_2 extraction (Grassi et al., 2007). Although a correlation does not imply cause-effect, within the patho-physiological context the data strongly suggest that in MM and McA patients the impaired peak fractional O_2 extraction is presumably the cause of the impaired peak $\dot{V}O_2$ (Grassi et al., 2007).

Oxidative metabolism is essential to sustain activities of exeryday life. Thus, it is reasonable to expect some compensatory mechanisms entailed by MM and McA patients in order to restore pulmonary or muscle $\dot{V}O_2$ values, or at least to attenuate the $\dot{V}O_2$ impairments attributable to the disease. How to accomplish this? The answer lies again in the equations expressing the Fick principle of conservation of mass mentioned above (Equations 1, 2): if the second term of the right-hand portion of the equations (fractional O_2 extraction) is impaired, the system tries to compensate for this impairment by increasing the first term of the right-hand portion of the equations (O_2 delivery), that is cardiac output or muscle blood flow.

This compensatory mechanism has been repeatedly demonstrated in MM and in McA patients, starting from the description, in the pioneering work by Linderholm et al. (1969), of the "exaggerated" or "hyperkinetic" cardiovascular response to exercise. Higher O_2 delivery values vs. controls, for the same work rate, were subsequently described both in MM (see e.g., Taivassalo et al., 2002, 2003) and in McA patients (see e.g., Lewis and Haller, 1986). Grassi et al. (2007) considered the heart rate (HR) response a reasonable "proxy" of the cardiac output response, and described steeper HR vs. work rate relationships in McA and MM patients vs. controls. Interestingly, the slopes of the HR vs. work rate relationships were linearly related to the impairment of fractional O_2 extraction, as estimated by NIRS (Grassi et al., 2007). In other words, in the presence of a more severe impairment of oxidative metabolism, a more pronounced cardiovascular response was described; the signals involved in this "metaboreflex" are presumably different in MM and in McA patients. Enhanced systemic norepinephrine vasoconstriction and enhanced functional sympatholysis in working muscles have been described in MM patients (see e.g., Jeppesen and Vissing, 2019); in these patients a preminent role in peripheral vasodilation would be played by the ATP released by red blood cells (Jeppesen and Vissing, 2019).

The compensatory mechanism of enhanced O_2 delivery would manifest itself also at a morphological level. Taivassalo et al. (2012) have indeed demonstrated that in MM patients the iperkinetic cardiovascular response was associated with increased capillary and vascular angiogenic growth factor levels in skeletal muscles, the increased capillarity being more pronounced around the fibers with the most pronounced oxidative impairment. In other words, besides inducing an enhanced cardiovascular response the impaired skeletal muscle oxidative metabolism would also promote angiogenesis. Another beautiful example of physiological mechanisms uncovered by the study of the human knockout models.

Apart from the algebraic approach (see above), what would the physiological rationale be of increasing O_2 delivery to muscle fibers, which have problems in utilizing the O_2 they receive from the cardiovascular system? Porcelli et al. (2019) recently proposed an answer to this question by applying to MM patients the "Wagner's approach" (Wagner, 1996) evaluating perfusive and diffusive limitations in the O_2 pathway. In Figure 3 of that paper (Porcelli et al., 2019), peak O_2 delivery (cardiac output × CaO_2) in MM patients was considered to be normal (see Linderholm et al., 1969; Taivassalo et al., 2003). In the model peripheral O_2 diffusion is dictated by the Fick law of diffusion:

$$\dot{V}O_2 \;=\; \text{D}m\text{O}_2 \;\times\; (\text{P}m\text{v}O_2 - PiO_2) \qquad (3)$$

in which D$m$$O_2$ represents peripheral O_2 diffusive capacity, PmvO_2 microvascular partial pressure of O_2 and P$i$$O_2$ intracellular PO_2. In MM and McA patients $\dot{V}O_2$ at peak exercise is lower than normal because peripheral O_2 diffusion is impaired (see Figure 3 in Porcelli et al., 2019). This impairment occurs because PiO2 is presumably higher than normal (impaired intracellular oxidative metabolism), thereby decreasing the PO_2 gradient from the microvascular to the intracellular compartment. In this *scenario*, how can the partial pressures gradient be increased, thereby enhancing peripheral O_2 diffusion? The answer: an enhanced O_2 delivery would increase

$P_{mv}O_2$, thereby increasing the driving pressure for the peripheral diffusion of the gas.

Whereas, at submaximal work rates the increased O_2 delivery can fully compensate for the impaired fractional O_2 extraction ($\dot{V}O_2$ being therefore substantially normal, for the same submaximal work rate, between patients and controls), the compensation may not be complete in "maximal" or "peak" conditions, that is when the patient reaches exhaustion. This translates into a lower peak $\dot{V}O_2$, with the associated impaired exercise tolerance. Peak $\dot{V}O_2$ values in MM and McA patients, although presenting a substantial variability among patients, are typically around 10–20 ml kg^{-1} min^{-1} (see e.g., Lewis and Haller, 1986; Taivassalo et al., 2003; Grassi et al., 2007; Porcelli et al., 2016, 2019), i.e., in a range compatible with patients belonging to NYHA class II-IV heart failure.

In conclusion, we hope we have convinced the reader that the human knockout models of MM and McA patients are ideally suited to be evaluated within a translational approach, which brings basic science methods and tools "to the bed of the patient." This can be accomplished by utilizing non-invasive experimental approaches developed over the years in the field of exercise physiology. In these patients the aims are to address specific issues related to basic pathophysiological mechanisms, to identify and quantify the impairment of skeletal muscle oxidative metabolism and the factors limiting exercise tolerance, and ultimately the patients' quality of life. The non-invasiveness of the adopted methods facilitates serial measurements, allowing clinical course of the diseases to be examined, as well as the efficacy of rehabilitation or therapeutical interventions.

Physiology and physiological research remain the essential link between genes, molecules and clinical care (Joyner, 2011; Wagner and Paterson, 2011). The –omics world may identify concepts and mechanisms, but only physiology can give a meaning to these concepts and mechanisms within the general picture of a human body, healthy or ill (Grassi et al., 2019).

AUTHOR CONTRIBUTIONS

BG, SP, and MM participated to the studies mentioned in the Opinion. BG wrote the first draft of the present manuscript. SP and MM participated in the discussion and in the editing of the manuscript.

FUNDING

This work stems from studies originally funded by Telethon-UILDM Grants GUP 030534 and GUP 08007.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 20 May 2020
doi: 10.3389/fphys.2020.00451

Exercise-Related Oxidative Stress as Mechanism to Fight Physical Dysfunction in Neuromuscular Disorders

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Neuromuscular diseases (NMDs) are a group of often severely disabling disorders characterized by dysfunction in one of the main constituents of the motor unit, the cardinal anatomic-functional structure behind force and movement production. Irrespective of the different pathogenic mechanisms specifically underlying these disease conditions genetically determined or acquired, and the related molecular pathways involved in doing that, oxidative stress has often been shown to play a relevant role within the chain of events that induce or at least modulate the clinical manifestations of these disorders. Due to such a putative relevance of the imbalance of redox status occurring in contractile machinery and/or its neural drive in NMDs, physical exercise appears as one of the most important conditions able to positively interfere along an ideal axis, going from a deranged metabolic cell homeostasis in motor unit components to the reduced motor performance profile exhibited by the patient in everyday life. If so, it comes out that it would be important to identify a proper training program, suitable for load and type of exercise that is able to improve motor performance in adaptation and response to such a homeostatic imbalance. This review therefore analyzes the role of different exercise trainings on oxidative stress mechanisms, both in healthy and in NMDs, also including preclinical studies, to elucidate at which extent these can be useful to counteract muscle impairment associated to the disease, with the final aim of improving physical functions and quality of life of NMD patients.

Keywords: neuromuscular diseases, oxidative stress, physical exercise training, aerobic and anaerobic exercise, quality of life

OPEN ACCESS

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Reviewed by:
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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *17 January 2020*
Accepted: *09 April 2020*
Published: *20 May 2020*

Citation:
Siciliano G, Chico L, Lo Gerfo A, Simoncini C, Schirinzi E and Ricci G (2020) Exercise-Related Oxidative Stress as Mechanism to Fight Physical Dysfunction in Neuromuscular Disorders. Front. Physiol. 11:451. doi: 10.3389/fphys.2020.00451

INTRODUCTION

Neuromuscular disease (NMD) is a quite broad nosographic term, which refers to different disorders either affecting the spinal cord anterior motoneuron horn cell, the peripheral nervous system, or neuromuscular junction and skeletal muscle (Iolascon et al., 2019), i.e., the components of what is termed the motor unit, an anatomic-physiological entity fundamental to realize the voluntary movement. In the case of conditions affecting the peripheral nervous system, also the sensitive pathways are involved in the pathological process, accordingly enriching clinical appearance with additional specific features.

From an etio-pathogenic point of view, NMDs are inherited or acquired conditions affecting both children and adults and ultimately leading to a motor impairment with different severity

degree and time-course characteristics, sometimes with a more or less rapidly progressive evolution, sometimes with time-elapsed clinical recurrence, also life threatening, mostly implying a significant everyday life burden of disease (Silva et al., 2019). Cardiac involvement, respiratory failure, and joint contractures are among the most frequent associated features that contribute to increased disability and impaired quality of life of the patient (Mercuri and Muntoni, 2013; Morrison, 2016). Although quite homogeneous in clinical appearance, different pathogenic mechanisms (structural, metabolic, inflammatory) are involved in the process of determining cell damage in NMDs, influencing the disease course as well as its susceptibility to the available therapies. Within the chain of events that downstream follow the primary cause of the disease, several other mechanisms are thought to be important in modulating the severity of the disease; among them, a disequilibrium in the redox status, the so-called oxidative stress, has widely been considered (Bouzid et al., 2018). Such a dysfunctional toxic–metabolic state, at some extent rather common to each living cell also with protective effects in physiological contexts, can acquire a relevant pathogenic role in these conditions, in particular for its role and close relationships to the skeletal muscle contraction process and, more generally, to the physical motor activity.

Generally speaking, any type of incongruous physical activity, either physical inactivity or opposite strenuous sports, may be harmful for any organism, both in normal and illness conditions. Increased tissue peroxidative processes can be part of those dangerous scenarios through an imbalance between production of reactive oxygen species (ROS) and inactivation of antioxidant system, mostly related to a triggered mitochondrial dysfunction; this in turn leads not only to parental tissues but also to systemic, deleterious consequences that can make worse the clinical condition (Bouzid et al., 2018). NMD patients, including those with primary mitochondrial disorders as paradigmatic condition of altered redox status, are often more prone to damaging exercise, as muscle contraction itself, for different reasons, is defective and can add further damage to an already debilitated system; it turns out that they are discouraged to perform physical activity for fear of overwork weakness, rather conducing a quite sedentary lifestyle (Voorn et al., 2019). Unfortunately, however, this condition further decreases fitness and reduces individual general sense of health, as well as physical functions (Voorn et al., 2019), by favoring a deconditioning process that includes also the redox mechanism within the skeletal muscle. As a consequence of these considerations, whether or not and with which characteristics a supervised and individualized physical exercise training can be beneficial or not in NMDs is still a matter of debate, and it is even more unclear which can be its effects on muscle contractile-related redox mechanisms (Bouzid et al., 2018).

The review is aimed to focus the attention on the effects of different exercise training programs on oxidative stress, analyzing both the health status and the different pathological conditions of the neuromuscular system, with the exception of canonic mitochondrial myopathies here deliberately omitted for their peculiar significance in this context, to clarify if this may be useful to counteract muscle impairment, improving physical functions and quality of life of the affected patients.

MATERIALS AND METHODS

A literature search on PubMed was performed using the following keywords: "oxidative stress," combined with "physical exercise," "aerobic exercise," "anaerobic exercise," "resistance/strength exercise," "training," "healthy," "neuromuscular diseases," "Duchenne muscular dystrophy," "facioscapulohumeral dystrophy," "myotonic dystrophy," "spinal muscular atrophy," "amyotrophic lateral sclerosis," and "peripheral neuropathies." We included both *in vivo* (in animal models and humans) and, when available, *in vitro* studies, including papers up to December 2019.

OXIDATIVE STRESS AND ROS GENERATION

Oxidative stress describes a condition of imbalance between the production of ROS (**Table 1**) and the ability of the antioxidant system (**Table 2**) to detoxify these reactive chemical species (RCS) (Sies, 2015).

Several intracellular sources of ROS have been recognized; among them, the main one is the mitochondrial respiratory chain that generates ROS as by-products of the electron transfer. Cytosolic sources, including NADPH oxidases (NOX), xanthine oxidase, lipoxygenases (LOX), cyclooxygenases (COX), and cytochrome P450 enzymes, contribute to the intracellular ROS pool. ROS are also produced by fatty acids beta-oxidation, xenobiotic components metabolism, and after the activation of phagocytosis (Mancuso et al., 2008).

Under physiological conditions, ROS are required to control biochemical processes, including cell differentiation (Vieira et al., 2011), neurogenesis (Kennedy et al., 2012), antioxidant genes expression (Allen and Tresini, 2000; Ma, 2010), as well as regulation of the immune system (Siciliano et al., 2007). However, pathological conditions in which an excessive ROS production can result in oxidative stress lead to a damage in lipids, proteins, nucleic acids, cells, and tissues, with consequent alteration of signaling pathways, inducing necrosis and apoptosis (Yan et al., 2006; Feissner et al., 2009).

In normal conditions, an efficient antioxidant defense system in the muscle fibers prevents, counteracts, or cancels the potentially damaging action of ROS on the musculoskeletal

TABLE 1 | Main reactive oxygen species.

	Symbols
Radicals	
Superoxide anion	$O_2 \cdot^-$
Hydroxyl radical	·OH
Peroxyl, alkoxyl radical	ROO·, RO·
Hydroperoxyl	HO_2^-
Non radicals	
Hydrogen peroxide	H_2O_2
Singlet oxygen	$^1O_2$
Trioxygen (Ozone)	O_3
Hypochlorous acid	HOC1

TABLE 2 | Main intracellular antioxidants.

	Symbols
Enzymatic antioxidants	
Superoxide dismutases	SODs
Catalase	CAT
Glutathione peroxidase	GPx
Glutathione reductase	GR
Glutathione-S-transferase	GST
Thioredoxin peroxidase	TrxPx
Thioredoxin reductase	TrxR
Non enzymatic antioxidants	
Glutathione	GSH
Glutaredoxin	Grx
Thioredoxin	Trx
Cytochrome c oxidase	
Coenzyme Q	
Ascorbic Acid	
Tocopherol	
Vitamins A, C, E	
Carotene	

system (Cooper et al., 2002). Nevertheless, physiological levels of ROS are useful to modulate the muscle force production and to control the signaling and the genetic expression pathways in the muscle cells (Dröge, 2002).

PHYSICAL EXERCISE AND OXIDATIVE STRESS

The physical exercise should not be mistaken with the physical activity. The term physical activity is defined as any bodily movement, produced by skeletal muscles and that requires energy expenditure. Physical exercise is a planned, structured, repetitive, and purposeful movement to improve or maintain the physical fitness (cardiorespiratory, muscular strength, muscular endurance fitness) (Fisher-Wellman and Bloomer, 2009).

Dillard et al. (1978) for the first time observed a correlation between physical exercise and oxidative stress more than 40 years ago, demonstrating that the physical exercise can lead to an increase in lipid peroxidation. After this initial report, several studies confirmed that a prolonged and intensive exercise induces oxidative stress (Judge and Dodd, 2003; Fisher-Wellman and Bloomer, 2009; Powers et al., 2011).

It is generally assumed that an excessive physical exercise can cause oxidative stress both favoring the RCS generation, including ROS, and decreasing the antioxidant defense system. It may seemed paradoxical that, although an intense and excessive physical exercise promotes oxidative stress, a moderate and routinely exercise is associated with numerous health benefits (Powers et al., 2011), such as reducing the risk of cardiovascular, endocrine, and neuromuscular diseases (Judge and Dodd, 2003).

While an adequate exercise training is able to enhance endogenous antioxidant defense systems, reducing the harmful effect of the peroxidation processes after an intense muscle activity (Reid, 2016; Ismaeel et al., 2019), during an intense and strenuous exercise, RCS are overproduced and they are free to attack and damage any cellular component (Powers et al., 2011). It can be assumed that the physical exercise can have positive or negative effects on the oxidative stress depending on the load, specificity, and on the basal level of training (Margonis et al., 2007).

Aerobic Exercise and Oxidative Stress

Several studies have tested the effects of the aerobic exercise (e.g., running, swimming, and cycling) on oxidative stress. An intense aerobic exercise increases oxygen consumption (VO_2), a measure of the volume of oxygen that is used to produce adenosine triphosphate (ATP), with consequent rise in ROS production. This overproduction is not found in healthy people undergoing low exercise intensity [<50% of maximal oxygen uptake (VO_2max)], which have a better antioxidant activity (Finaud et al., 2006).

The effect of an acute swimming protocol on the oxidative damage biomarkers was investigated in trained children (n = 22), who were subjected to 12 bouts of 50 m distance, at a pace corresponding to 70–75% of the maximum velocity reached, each bout separated by 1 min of rest. A significant increase in thiobarbituric-acid-reactive substances (TBARS), protein carbonyls (PC), catalase (CAT) activity, total antioxidant capacity (TAC), and oxidized glutathione (GSSG) concentration, as well as a significant decrease in reduced glutathione (GSH) concentration and GSH/GSSG ratio, were found post-exercise with respect to pre-exercise. The authors concluded that an acute swimming bout resulted in blood oxidative stress (Nikolaidis et al., 2007).

However, other studies do not confirm the evidence that oxidative stress increases with intense exercise. Inal et al. (2001) evaluated the effects of the swimming on the antioxidant status in short (100 m) and long-distance (800 m) swimmers (n = 10 and n = 9, respectively), founding that, particularly in the second ones, the antioxidant CAT, glutathione peroxidase (GPx), and GSH enzyme activity were increased.

Kouvelioti et al. (2019) observed that oxidative stress biomarkers (TBARS and PC) varied similarly after a running or cycling training. Specifically, 20 healthy men (22.3 ± 2.3 years) performed two high-intensity interval exercise trials (crossover design), running on treadmill and cycling on cycle ergometer. Trials consisted of eight running or cycling intervals (lasting 1 min) at ≥90% of the maximum heart rate (HR_{max}), separated by of passive recovery intervals (1 min). The duration but not the type of the exercise influenced the level of oxidative stress markers; in particular, TBARS and PC did not change from pre- to 5 min post-exercise but significantly decreased from 5 min to 24 and 48 h post-exercise (Kouvelioti et al., 2019).

This could be explained based on the time required to activate the biological pathways underlying the cellular redox state after an aerobic exercise. It is well known that physical exercise induces, in the immediate, an increase in ROS; appreciable positive changes in redox status cannot occur during, or immediately after, the exercise but are required several hours (e.g., 9, 24, 48 h) after the exercise end. It can be speculated

that this timeframe is compatible and is required for antioxidant gene transcription activation, the messenger RNA (mRNA) maturation, and its translation into protein, [i.e., superoxide dismutase (SOD), CAT, GPx]. Exercise induces a pleiotropic adaptive response in skeletal muscle, through the activation of transcription factors (e.g., peroxisome proliferator-activated receptor g coactivator 1a, PGC-1a) that regulate mitochondrial biogenesis and activate the transcription of antioxidant enzymes (Pasquinelli et al., 2016). Thus, if the aerobic training is performed for a long time, not only for a limited period (a few weeks), its positive effects on oxidative stress can persist over time, since it keeps the antioxidant enzymatic machinery active.

Nonetheless, the contrasting results of the literature can be explained by different antioxidant nutritional status or by different intensity, duration and frequency of the training, type of the exercise (Finaud et al., 2006), previous training experience, inclusion of an untrained control group, number of participants, as well as anthropometric parameters such as body mass index (BMI), age, and sex (Celik et al., 2019). It is possible that sex may influence the degree of oxidative stress; women can be less susceptible to oxidative stress than men considering the antioxidant properties of estrogens, especially during periods when estrogens levels are high (e.g., ovulation) (Bloomer et al., 2009). Nevertheless, only few studies regarding the aerobic exercise-related oxidative stress, in healthy human, evaluated and found differences among sexes in oxidative stress biomarkers (Bloomer and Fisher-Wellman, 2008; Kabasakalis et al., 2014; Souglis et al., 2018; Celik et al., 2019).

Anaerobic Exercise and Oxidative Stress

It is known that the anaerobic exercise, such as sprints, intermittent running, jumps, or resistance exercises, is a source of free radicals, produced by xanthine oxidase (XO), an enzyme that generates ROS during the ischemia reperfusion (Heunks et al., 1999). XO, in the presence of hypoxanthine or xanthine, reduces molecular oxygen in superoxide anion (O_2-) and hydrogen peroxide (H_2O_2), which, in turn, is reduced into hydroxyl radical (OH), inducing oxidative damage (Heunks et al., 1999). ROS are also produced during myocyte contraction by NADPH oxidase and nitric oxide synthase activation (Powers and Jackson, 2008). Elevated lactate levels, as well as an alteration of the oxidative status, assayed by an increase in the lipid peroxide concentration were found after anaerobic exercise (Powers and Jackson, 2008). In addition, gender differences need to be considered when anaerobic activity is evaluated. The myocyte metabolic activity and mass and histology of the skeletal muscles are different in men with respect to women (Janssen et al., 2000; Esbjornsson-Liljedahl et al., 2002). Men show higher peak of anaerobic power that is correlated positively with post-exercise oxidative stress that, in turn, is influenced by the type II muscle-fiber composition. In fact, the type II fibers have intrinsic properties that favor ROS production, with a consequent increase of PC (Quindry et al., 2011).

Studies evaluating enzymatic antioxidant status have not yet provided clear evidence that anaerobic exercise prevents the trigger of oxidative stress. A study with healthy male volunteers (n = 8) demonstrated that short-term supramaximal anaerobic exercises (the 30 s Wingate test) decreased the SOD activity, suggesting that this type of exercise induces oxidative stress, although no changes in GPx activity were detected (Groussard et al., 2003). Conversely, Berzosa et al. (2011), in healthy untrained male subjects (n = 34) undergoing to cycloergometric tests, including maximal and submaximal episodes, found an increase in CAT, GPx, glutathione reductase (GR), and SOD enzyme activities in plasma after both maximal and submaximal exercise periods.

A study by Wiecek et al. (2015) aimed to evaluate the changes in non-enzymatic antioxidants in 20 healthy individuals (both man and women) undergoing an anaerobic exercise (20 s bicycle sprint). In both sexes, total oxidative status (TOS), TAC, TOS/TAC, vitamin A, vitamin E, vitamin C, uric acid, and GSH concentrations raised after exercise, suggesting that also the anaerobic exercise may play a beneficial role because of its ability to increase the antioxidant defenses.

The effect of a resistance training (RT) on oxidative stress biomarkers depends substantially on the type of training carried out. A moderate-intensity RT, rather than a maximal exercise, is able to improve adaptive responses to oxidative stress by upregulation of antioxidant defenses, limiting the formation of free radicals and preserving the reducing power after a moderate-intensity and low-frequency training (Jürgenson et al., 2019).

When an anaerobic training protocol is proposed, several confounding factors should be taken into consideration, e.g., type, intensity of exercise performed, sex, and training status of the participants.

OXIDATIVE STRESS AND EXERCISE TRAINING IN NEUROMUSCULAR DISORDERS

It is widely known that oxidative stress is linked to the pathogenesis of NMDs (Wehling et al., 2001). Several studies were conducted both in animal models and humans affected by NMD, covering a wide spectrum of pathologies: from muscular dystrophies to spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), and peripheral neuropathies (PN) (Cassereau et al., 2020).

Despite that oxidative stress is involved in NMDs, and exercise induces ROS production, some types of training increase mRNA levels and the expression of metabolic muscle proteins. In particular, a moderate and regular physical exercise has been suggested as non-pharmacological treatment for the NMDs (Sjøgaard et al., 2013).

Muscular Dystrophies

Duchenne Muscular Dystrophy

Oxidative stress has been proposed as a secondary effect in the Duchenne muscular dystrophy (DMD) (Serra et al., 2018). An alteration of the oxidative balance was detected both in *mdx* mice and in muscle biopsies or blood from DMD patients. TBARS, a by-product of lipid peroxidation, was found higher in *mdx* mice *versus* controls muscles (Ragusa et al., 1997), and also protein oxidation levels were reported in *mdx* mice (Dudley et al., 2006).

Similarly, TBARS (Kar and Pearson, 1979), carbonyl-proteins (Petrillo et al., 2017), and 8-OHdG (Rodriguez and Tarnopolsky, 2003) were found elevated in the muscle and blood of DMD patients *versus* healthy controls. While the literature data agree on the increased oxidative damage DNA, proteins, and lipids, data related to antioxidant status in DMD are conflicting. Some studies reported higher levels of SOD-2 and CAT, while decreased SOD-1 activity was found in *mdx* mice (Ragusa et al., 1997). In addition, DMD patients showed an increased muscle enzymatic activity of CAT and GR than controls, while SOD activity was not altered (Kar and Pearson, 1979). Another study found decreased levels of GSH with an increased GSSG/GSH ratio, and a greater activity of GPx and GR in *mdx* mice muscles (Dudley et al., 2006). Recently, Petrillo et al. (2017), analyzing the antioxidant status in DMD patients with respect to healthy subjects, observed a decrease in GSH levels and GPx activity, as well as an imbalance of GSSG/GSH ratio in muscle and plasma.

An eccentric exercise can damage the contractile and cytoskeletal components of the muscle fibers; it may have dangerous effects resulting in muscle deterioration (Markert et al., 2011), In addition, high-resistance strength training is not recommended in DMD, but submaximal aerobic exercises can be helpful to improve the muscle functions (Yiu and Kornberg, 2015).

Exercise-related oxidative stress in DMD animal models

Voluntary running exercise for 7 weeks performed by *mdx* mice improved citrate synthase and succinate dehydrogenase activities, both markers of muscle aerobic capacity, and it enhanced the expression of aerobic metabolism genes to levels similar or higher than those observed in healthy mice (Hulmi et al., 2013).

Sedentary *mdx* mice, with downregulated levels of SOD-1, when subjected to low-intensity endurance exercises (run on a motorized rotarod for 5 days/week for 6 weeks), showed restored normal levels of SOD-1, suggesting that specific programs of training could contribute to a significant recovery of the damaged muscle, counteracting oxidative stress and reducing the muscle degeneration (Fontana et al., 2015).

In addition, Hyzewicz et al. (2015) agree that low-intensity training may have a beneficial effect on dystrophic muscle in *mdx* mice. In fact, a voluntary swimming training for 30 min, 4 days/week for 4 weeks, improved the expression of proteins involved in energy metabolism and in muscle contraction, as well as decreased oxidized proteins (Hyzewicz et al., 2015). Low-intensity treadmill training (9 m/min, 30 min/day, three times/week, for 8 weeks) reduced collagen deposition, enhancing both enzymatic and total antioxidant capacity (Fernandes et al., 2019).

These results could be useful to design correct exercise training protocols for DMD patients.

Clinical trials in DMD patients

Few human studies have focused on exercise training in DMD. An important purpose in DMD is delaying the loss of the motor functions, which negatively impacts DMD patients' life. As asserted by Jansen et al. (2010, 2013), physical training could be effective to limit the deterioration of muscle tissue. The authors examined the effects of a low-intensity physical training (5 days/week for 24 weeks), on muscle endurance and functional abilities, in 30 young DMD patients (18 ambulant and 12 wheelchair dependent; mean age, 10.5 $\pm$ 2.6 years), divided into two groups: a trained group (n = 17) and a control group (n = 13) of untrained patients. The intervention group trained their limbs in 30 min sessions (15 min for arms and 15 min for legs) by a bicycle training equipment used actively or with electrical motor support, depending on their physical limitations (Jansen et al., 2010). In the trained group, the total Motor Function Measure (MFM) score remained stable, whereas it significantly decreased in the control group. No significant differences were found for the Assisted 6-Min Bicycle Test (A6MCT). A low-intensity cycling training could be beneficial, feasible, and safe for both ambulant and wheelchair-dependent children, perhaps delaying the functional muscle deterioration due to disuse in DMD patient (Jansen et al., 2013).

A clinical trial with adult Becker muscular dystrophy patients (n = 11) subjected to an aerobic and moderate-intensity training (training session of 50, 30 min on a stationary cycle ergometer at 65% of VO_{2max}) confirmed the effectiveness of the exercise protocol with persisting positive effects after 3 months of follow-up. Six patients continued the same training protocol for the next 12 months, highlighting the importance of the proposed rehabilitation exercises in dystrophinopathies (Sveen et al., 2008).

Alemdaroğlu et al. (2015) investigated the effects of exercise programs for strengthening range of motion with an arm ergometer, in early-stage DMD patients. Ambulation scores, endurance, arm functions, and proximal muscle strength were improved after 8 weeks of training.

Facioscapulohumeral Dystrophy

The involvement of oxidative stress in facioscapulohumeral dystrophy (FSHD) is predominantly supported by *in vitro* studies; myoblasts from FSHD patients are susceptible to oxidative insults early during FSHD myogenesis (Winokur et al., 2003), contributing to an aberrant differentiation of myoblasts (Dmitriev et al., 2016). This condition can be reversed to a normal state by treatment with antioxidants, reducing the oxidative damage and the morphological defects during the myogenic differentiation (Dmitriev et al., 2016).

Using myocytes differentiated from induced pluripotent stem cells (iPSCs) derived from FSHD patients, Sasaki-Honda and collaborators (Sasaki-Honda et al., 2018) demonstrated that oxidative stress increased DUX4 expression by a DNA damage response signaling to the opened FSHD 4q35 region. Using isogenic controls of FSHD2 iPSCs in order to correct the SMCHD1 mutation, basal DUX4 expression was suppressed and heterochromatic markers at 4q35 were partially recovered, suggesting that oxidative stress could represent a risk factor in FSHD progression (Sasaki-Honda et al., 2018).

Abnormal mitochondrial functions and increased levels of oxidative stress biomarkers (e.g., lipid peroxidation, PC, and lipofuscin accumulation) were detected in FSHD compared to healthy muscles (Turki et al., 2012).

In patients with FSHD, some strategies, including antioxidant supplementation and physical exercise training, could be

considered to improve the muscle function performance compromised by oxidative stress.

A clinical randomized controlled trial with FSHD patients reported, after antioxidant supplementation with vitamin C, vitamin E, zinc gluconate, and selenium (once a day, for 17 weeks), an enhanced muscle function evaluated by 2-min walking test (2-MWT), maximal voluntary contraction (MVC), and endurance limit time. MVC is the expression of an isometric muscle contraction expressed as the percent of strength for the design person. Non-enzymatic antioxidant and lipid peroxidation biomarkers were also improved in treated patients *versus* placebo group, concluding that the supplementation of antioxidant substances can really help to improve physical performance by enhancing antioxidant defenses and reducing oxidative stress (Passerieux et al., 2015).

How exercise training can be useful in FSHD has been observed by Voet et al. (2014), who subjected 28 FSHD patients to an aerobic exercise training, lasting 16 weeks, which consisted of three sessions/week of cycling exercises, for 30 min with additional warming-up (5 min) and cooling-down (3 min) periods. During the training period, cardiovascular load was monitored with a heart rate belt and watch and adjusted to the individual participant's level, reaching an increase of 50–65% in heart rate reserve (HRR), normally calculated as the difference between the maximum heart rate (HR_{max}) and resting heart rate (HR_{rest}). After training, the patients showed a reduction in fatigue, assessed by the fatigue subscale of the Checklist Individual Strength (CIS fatigue), and the beneficial effects persisted over time, at 16 and 28 weeks of follow-up.

One study, aimed to assess in FSHD the efficacy of 6 months of home-based exercise training program (cycling three times/week, for 35 min), consisting in a combination of strength, high-intensity interval, and low-intensity aerobic exercise, has shown improvement on VO_{2max}, MVC, and muscle endurance without the evidence of muscle tissue damage (Bankolé et al., 2019).

Studies evaluating the effect of training on muscle performance did not find evidence of enhancing by isometric strength testing, supporting the idea that strength-training exercise is probably ineffective for significantly improving muscle strength in FSHD (Tawil et al., 2015). Therefore, a stationary bicycle rather than a treadmill should be recommended for patients with leg weakness (Tawil et al., 2015). Although no data suggest that strength training is detrimental in FSHD and considering these evidence, patients should be encouraged to perform low-intensity aerobic exercises (Tawil et al., 2015).

Myotonic Dystrophy

Type 1 myotonic dystrophy (DM1) is a neuromuscular disease characterized by multisystemic involvements, in which oxidative stress may be associated with muscular signs of disease, contributing to muscle atrophy (Toscano et al., 2005; Miljević et al., 2010). An increase in protein oxidation (Siciliano et al., 2005) that correlates with extramuscular signs of the disease, as well as an increase in lipid peroxidation (Kumar et al., 2014), was found in plasma of patients with DM1. In addition, antioxidant status is compromised, as demonstrated by lower levels of GPx, glutathione S-transferase (Kumar et al., 2014), SOD, and CAT (Nikolić-Kokić et al., 2016) detected in DM1 patients than in controls.

The effects of exercise and physical training to counteract the progressive loss of maximal muscle strength and muscle wasting have been well documented (Roussel et al., 2019).

In DM1 patients, strength testing (single session exercise, at 50% of MVC until exhaustion) showed lower maximal strength and longer recovery time compared to healthy controls, which are in line with muscle weakness and myotonic phenomenon (Esposito et al., 2017). In a 12-week aerobic training protocol on a cycle ergometer, in which each training session consisted of 5 min of warm-up and 30 min of exercise at 65% of the VO_{2max}, positive effects on the aerobic capacity, fitness performance, and self-reported improvement in daily activities were observed. In addition, a post-training histological analysis showed a muscle fiber remodeling, with an increase in types I and IIa fiber diameter, with respect to the basal condition, whereas fiber density was not changed (Orngreen et al., 2005).

Nevertheless, according to a single-case study design, five DM1 patients underwent a hand exercise program focused on endurance training at low resistance, with a duration of ∼45 min. The training sessions, three times/week, for 12 weeks, included isolated sets of different exercises in 3 or 5 repetitions and mass sets of movements with a Theraputty in 10 or 15 repetitions, followed by a stretching session. A significant increase in muscle force and an improved fine motor control were reported, whereas no statistical significant difference was found in grip force. A higher occupational performance was self-reported when asked (Aldehag et al., 2005).

These data have been confirmed in patients ($n = 20$) subjected to a composite rehabilitation program consisting of 15 sessions spread out over a 6-week period. Stretching exercises for muscle stiffness and strengthening, balance, and endurance training were performed. In particular, the endurance training consisted of walking on a treadmill (a 20 min session, at 60% of the VO_{2max}).

The results on endurance were not conclusive, probably because the proposed endurance training program was insufficient to improve the walking perimeter, while muscle strength and locomotor and posture locomotion improved (Missaoui et al., 2010).

Type 1 myotonic dystrophy patients ($n = 20$) underwent an aerobic exercise protocol with a hand grip, consisting of three sets of intermittent fatiguing contractions, at incremental working load up to 60% of MVC, lasting 1 min, each separated by 1 min of rest. The lactate acid levels increased at 60% of MVC, while oxidative stress markers did not differ from baseline (Baldanzi et al., 2017).

Spinal Muscular Atrophy

An abnormal deposition of 4-hydroxy-2-non-enal-modified protein (4-HNE), a product of membrane lipid oxidation, was observed at immunohistochemical staining in the spinal motor neurons of patients with SMA type 1. In addition, an immunoreactivity for 8-hydroxy-2-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, was observed in SMA (Hayashi et al., 2002). Mano et al. (2012) subsequently confirmed that urinary 8-OHdG levels in SMA patients were higher

compared to controls and that they were correlated with the motor function scores and the disease duration.

In SMA, physical exercise training might improve muscle and cardiorespiratory function. As described by Bartels et al. (2019), aerobic exercise training optimized the aerobic capacity, counteracting the muscle deterioration that occurs secondarily to motor neuron loss and inactivity.

Spinal muscular atrophy type 3 patients, because of a milder progression of the disease, could represent an ideal target population for applying strengthening and endurance exercise protocols. Nevertheless, no conclusive data are available regarding the protective role of the physical exercise in this type of patients (Madsen et al., 2015).

Six SMA type 3 patients subjected to 3 months of training, two to four sessions for a week, on a cycle ergometer for 30 min at 60–70% of the VO_{2max}, and a control group of nine sedentary subjects were compared, and it was found that the patients' oxidative capacity and the endurance improved after the training. On the contrary, a significant fatigue was reported, confirming that SMA patients are susceptible to exercise-induced fatigue (Bartels et al., 2019).

Other studies found that, in SMA patients trained with the same exercise protocol, which differed only in the duration (6 *versus* 12 months in the two patient groups), no significant differences were reported regarding fatigue (Montes et al., 2015).

Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis is considered a multisystem and multifactorial pathology in which several mechanisms play important roles in the development and progression of the disease (Cozzolino et al., 2008).

The etiology and pathogenesis of the neuronal apoptosis in ALS are currently largely unknown. Among the several hypotheses, oxidative stress could be a driver in the neurodegenerative processes (Cozzolino et al., 2012; Gagliardi et al., 2012; Mao et al., 2012), including ALS. High levels of oxidative stress biomarkers, such as 4-HNE (Beal et al., 1997; LoGerfo et al., 2014), mitochondrial dysfunction (Shaw et al., 1995; Cutler et al., 2002), and, on the contrary, a downregulation of glutathione S-transferases (GSTs) (Curti et al., 1996; Simpson et al., 2004), peroxiredoxins (Kong and Xu, 1998), and nuclear factor erythroid 2-related factor 2 (Nrf2) (Wiedemann et al., 2002; Kato et al., 2005; Kirby et al., 2005; Usarek et al., 2005) were observed in ALS patients (Barber et al., 2006; Sarlette et al., 2008; Strong, 2010). Furthermore, Nrf2 activators have been shown to protect against oxidative stress and cell death induced by SOD1-mutant protein (Rossi et al., 2012). The *NRF2* overexpression in glial cells increases the resistance to oxidative stress and, by the increase in GSH levels, the ability of the motor neurons to neutralize the toxic effects caused by SOD1-mutant protein (Gitcho et al., 2008). In addition, a reduction in Nrf2 protein was found in ALS patients than in controls (Vargas et al., 2008; Kanno et al., 2012; Mead et al., 2013).

In the last decade, genome-wide association studies identified two genes associated with sporadic and non-SOD1 familial ALS: RNA/DNA-binding proteins, 43 kDa transactive response (TAR) DNA-binding protein (TDP-43), and fused in sarcoma/translocated in liposarcoma (FUS/TLS) (Kato et al., 2000; Neumann et al., 2006; Guo et al., 2013; Milani et al., 2013). Both TDP-43 and FUS, which predominantly nuclear proteins involved in RNA metabolism, are observed as aggregates in the cytosol of ALS neurons (Mackenzie et al., 2007).

In addition, *in vitro* studies, NSC34 motor neuronal cell lines, expressing TDP-43 mutants, exhibited shortened neuritis and higher oxidative stress. These effects were reversed by the UPS inhibitor MG132, but not by the Nrf2 activator sulforaphane (Vance et al., 2009; Vargas et al., 2013). This evidence was attributed to an increase in heme oxygenase 1 (HO-1), following MG132 treatment, apparently independent from Nrf2 activation. While the protective role of Nrf2 in SOD1-related toxicity is clear, the effect on other ALS-associated genes (e.g., TDP-43 and FUS) needs to be clarified (Vance et al., 2009; Vargas et al., 2013).

To date, few studies evaluated the correlation between oxidative stress and exercise in ALS. Among them, Flis et al. (2018) demonstrated that a swimming training is able to extend the lifespan of ALS G93A mice. The transgenic mice performed an exercise protocol that started at the presymptomatic stage (70 days of age) and ended when the mice were 115 days of age. The protocol consisted of a swim for 30 min, in 30°C water, in a swimming pool with an adjustable flow of water (maximum rate of 5 L/min), for five times/week. On the 105th day of life, the frequency of training was reduced to three times/week, and the daily swimming time (maximum of 30 min), and water flow (maximum of 5 L/min) was set individually, according to the abilities of the ALS mice. This exercise protocol could have had beneficial effects on the lifespan of ALS mice, probably because it may induce changes in oxidative stress and bioenergetics. In particular, higher levels of CAT activity were observed in the trained ALS mice than in sedentary ALS group of mice, although higher thiols (SH) oxidation was found both in the ALS sedentary group and in the trained group. Moreover, the COX activity and the RCR increased in the trained group *versus* the sedentary group, evaluated in the skeletal muscle of ALS mice. Additionally, also markers of lipid peroxidation were measured in the crude mitochondrial fraction isolated from the skeletal muscle, and it was found that there was a reduction in lipid peroxidation in the mitochondria of the ALS-trained *versus* ALS no-trained groups. These protective changes induced by swimming could be explained as the result of a reduction in the cholesterol content and an increase in the caveolin-1 protein level in the crude mitochondria of the trained ALS mice skeletal muscle.

These positive results could encourage swimming-based training programs that may be helpful in ALS patients. Swimming exercises produce physically and psychologically positive effects, as well as neuroprotective effects improving the motor functions as observed in ALS mice (Just-Borràs et al., 2019), suggesting a possible future use of well-structured swimming-based training protocols also in ALS patients. ALS mice subjected to swimming (in an adjustable-flow swimming pool) or to running (on a treadmill) protocols (for 30 min a day, 5 days/week, for 12 weeks) showed delayed spinal motoneuron death and preserved the motoneurons with large soma area. In contrast, a substantial motoneuron loss and an increased proportion of motoneurons with small soma area were observed after running-based training

(Just-Borràs et al., 2019). Running is a high-impact exercise, which recruits only small motoneurons and generates more oxidative stress than swimming, which is a low-impact exercise that recruits both small and big motoneurons, probably mediated by exacerbated fast-to-slow functional transition by running, while swimming preserves the normal muscle phenotype (Just-Borràs et al., 2019). Therefore, it can be assumed that, although both protocols induce changes in the proportion of motoneurons, swimming mitigates the vigorous loss of big and fast motoneurons unlike running (Just-Borràs et al., 2019).

Although preclinical studies (Flis et al., 2018; Just-Borràs et al., 2019) suggest that swimming and aquatic training could be very encouraging exercise interventions in ALS, clinical studies are needed to translate these mouse exercise results to humans, considering also that personalized aquatic training programs will be needed.

To our knowledge, only two studies that evaluated the exercise-induced oxidative stress remodulation in ALS have been conducted in humans (Pasquinelli et al., 2016; Chico et al., 2018). In both studies, the exercise protocol, on the forearm muscles with a myometer, consisted in several steps in which the contractile force increased incrementally at 30, 50, and 70% of the MVC, each step including 1 min of intermittent contractions (∼1/s) and 2 min of rest.

In the first of these two studies Pasquinelli et al. (2016) found that the plasma levels of oxidative protein products (AOPPs), ferric-reducing ability (FRAP), and total thiol groups (t-SH) remained stable during this short-lasting exercise protocol, suggesting a more delayed, if any, exercise-related kinetic curve for these redox biomarkers. On the contrary, the levels of lactate during each step of the exercise protocol increased, although no difference was observed when comparing the curves of ALS patients and healthy subjects. Interestingly, patients genotyped for the Gly482Ser PGC-1α gene polymorphism (during exercise, muscle contraction causes PCG-1α protein activation, increases mitochondrial biogenesis and oxidative capacity, leading to fast-to-slow muscle fiber conversion) showed a different response on oxidative stress-related biomarkers, according to their genotype. In particular, patients harboring the Ser428Ser genotype showed, during exercise, higher levels of AOPP (at 50% of MVC and at recovery) and lactate (at 30 and 50%) compared to Gly482Gly patients, highlighting a possible role of PGC-1α in exercise-related oxidative stress.

In the second study, Chico et al. (2018) evaluated, over a period of 6-month trial and compared to clinical scores, the exercise-related effects, on the same redox parameters, of the oral administration of a curcumin-based compound (600 mg/day, Brainoil), a natural antioxidant nutraceutical, in ALS patients. Patients were randomized into two groups: group A (n = 24) received placebo for 3 months, then Brainoil for the following 3 months; group B (n = 18) took Brainoil for 6 months. Evaluations were conducted at basal (T0), after 3 months of double-blinded Brainoil/placebo treatment (T1), and after 3 months of open-label phase (T2). After curcumin administration, an improvement in oxidative stress biomarkers evaluated during the incremental forearm exercise test was observed. In particular, the intragroup analysis showed a significant reduction in AOPPs at 30 and 50% of the MVC at T2 > T0 and T2 > T1 in ALS patients belonging to group B who consumed Brainoil for 6 months. Moreover, at T2, group B showed, at 50% of MVC, lower AOPP levels than group A. In group A, a significant reduction in FRAP values both at 70% of MVC and at recovery was observed at T1 > T0, suggesting that during placebo administration, there was worsening of FRAP. At T2, when Brainoil was introduced in the diet of group A, a FRAP restore was observed and rising FRAP levels, at different steps of the exercise-related curve, comparable to those of T0. Finally, in group B, relative lactate levels decreased at 50% of MVC at T1 > T0. Comparing groups A and B, there were no significant differences both in absolute and relative lactate levels, at T0 and T2, while at T1 in group A, lactate absolute values increased with respect to group B at 50 and 70% of MVC. The evaluated biomarkers improved in the treated patients group, suggesting that curcumin oral administration could have a protective effect during muscle exercise (Chico et al., 2018).

However, conclusive data regarding the effect of oxidative stress on physical performance in ALS patients, and conversely, if the muscle activity can modify the redox status, are still lacking. While some studies highlight the positive effect of physical exercise on the quality of life in ALS patients (Lehman et al., 2012), others argue that exercise has a harmful effect on muscle function (Lunetta et al., 2016).

In previous studies, strenuous exercise was associated with higher ALS incidence, as observed in athletes (Strickland et al., 1996; Scarmeas et al., 2002; Gotkine et al., 2014) and professional football and American football players (Chio et al., 2005; Lehman et al., 2012). Probably, an intense and prolonged exercise could induce both a massive increase in ROS and calcium concentration, with consequent motoneurons degeneration (Harwood et al., 2009), as observed in ALS patients in an extra 10 kJ/kg/day of physical activity, equivalent approximately to 45 min of brisk walking. This was consistently associated with an increased risk of ALS, with the strongest association observed for adulthood exercise-related physical exercise (Harwood et al., 2016). Furthermore, neurons, more than other cells, are particularly susceptible to oxidative stress due to high oxygen demand and low amount of antioxidants (Halliwell, 2006). Nevertheless, epidemiological data on the association between sport and ALS are contrasting. A study conducted by Belli and Vanacore (2005) observed a high risk for ALS among Italian soccer players. In this scenario, also drugs and food supplements intake should be discussed (Luna et al., 2017), considering that they are used to enhance sports performance. A high consumption of dietary supplements containing branched chain amino acids (BCAAs) and a chronic misuse of anti-inflammatory drugs could play an important role in the etio-pathogenesis of ALS among susceptible athletes (Belli and Vanacore, 2005). In fact, a dangerous interaction between genetic susceptibility and environmental factors can occur (Roche et al., 2012), but no data regarding doping and ALS are given in the literature.

However, it is important to emphasize that physical activity promotes the production of neurotrophic factors, as observed in adult rats subjected to exercise training (5 days, 30 min/day,

at a speed of 27 m/min, at a 3% incline using a motor-driven treadmill belt) (Gomez-Pinilla et al., 2001), with a protective effect on spinal cells, synaptic remodeling, and hyperinnervation of neuromuscular junctions (Nguyen et al., 1998; Wehrwein et al., 2002). Furthermore, it has been observed that endurance exercise is able to reactivate pathways interrupted by the disease, as it triggers remodeling mechanisms in the muscle fiber cells. In particular, endurance exercises protect skeletal muscle against the excessive activation of autophagy and ubiquitin–proteasome system, act by upregulating mitochondrial metabolism and fiber-type transformation, and stimulate the mitochondrial biogenesis and the expression of mitochondrial respiration genes (Ferraro et al., 2014). Considering these conflicting data, it can be assumed that there could not be a direct association between sport activity and the onset of ALS.

It is well known that regular and moderate physical activity protects neuronal cells from ischemic damage as demonstrated in hippocampal tissues of ALS mouse models (Tsitkanou et al., 2019). A study conducted by Pinto et al. (1999) showed that a moderate-intensity training on a treadmill (12 months), coupled with the use of a non-invasive ventilator, reduced the percentage of respiratory deterioration compared to an untrained group.

Similar results were obtained by Sanjak et al. (2010), showing that 8 weeks of repetitive rhythmic exercise mediated by supported treadmill ambulation training (STAT) for 30 min, 3 days a week, had positive effects on the ALS Functional Rating Scale (ALSFRS), distance and length of the step during 6 min of walking tests in ALS patients, confirming the beneficial role of physical exercise. Clawson et al. (2018) demonstrated that a fitness program of 24 weeks with resistance exercises using a cyclette did not promote any effect on the muscle strength and functionality and on the quality of life. Furthermore, a study conducted by Lunetta et al. (2016) showed that an active and/or passive exercise program (twice weekly) applied to the four limbs did not produce positive effects on the ALSFRS score, but it induced an improvement in the mood of ALS patients (Lunetta et al., 2016).

On the other hand, a resistance exercise program can be considered a good non-pharmacological adjuvant therapeutic approach in ALS, improving muscle force, inducing muscle hypertrophy, and maintaining skeletal muscle function (Tsitkanou et al., 2019). A randomized controlled trial in ALS patients showed that a moderate resistance training (6 months) can induce significantly better function, less decline in leg strength, and higher quality of life without adverse effects (Bello-Haas et al., 2007).

No conclusive data regarding the protective or detrimental role of physical activity in ALS population are available because of limited number of trials with low sample size and the difficulty to apply a standardized exercise protocol because of the heterogeneous progression rate of the disease clinical course.

Peripheral Neuropathies

Conceptually, oxidative stress in PN not only can be part, as for the other NMD, of the underlying pathogenic mechanism but also can be an expression of it and may, conversely, modulate an important aspect of PN, the reinnervation process. This aspect can accompany at least those diseases in which treatment is able to slow down or remit the disease process. Experimental studies in mice model of olfactory degeneration show how acute *N*-acetylcysteine (NAC) administration is able to ameliorate loss of olfactory neurons *in vivo* and, in an olfactory cell culture model, to alter the expression of several genes involved in oxidative stress pathways (Goncalves and Goldstein, 2019), suggesting a putative therapeutic effect of antioxidant compounds.

This also can happen by the involvement of nitric oxide (NO) and nitric oxide synthases (NOS) system, as shown in experimental models of denervation and reinnervation in rats. In his paper, the author (Tews, 2001) discussed how downregulation of sarcolemmal motor neuronal NOS in denervated muscle may contribute to axonal regeneration and attraction to muscle fibers by reducing the detrimental effects of NO nerve fiber growth. These mechanisms, therefore, stimulate the formation of new motor endplates for reinnervation process. However, decreased NO production in denervation process reduces the scavenger function of NO, promoting oxidative stress by the increase in $O_2 \cdot -$.

Free radicals cause oxidative damage that induces axonal degeneration and segmental demyelination.

Apart from diabetic neuropathy (DN), in which pathophysiological mechanisms due to hyperglycemia are well known and characterized by increase in oxidative stress through different metabolic pathways, in other types of PN, data are still lacking. In particular, polyol metabolism increases the formation of advanced glycation end products (AGEs), the activation of protein kinase C (PKC), and the hexosamine pathway, which are primarily linked to the development of DN.

In some inherited neuropathies, such as Charcot-Marie-Tooth-2K (CMT2K), p.C240Y mutation in GDAP1-GST is associated to a mitochondrial complex I defect with a greater oxidative stress (Cassereau et al., 2020).

Nevertheless, in inflammatory peripheral neuropathies, the presence of ROS is known to be a central feature in the processes of myelin rearrangement for immune reactions. In fact, the therapeutic effect of intravenous immunoglobulins (IVIg) is related to the modulation of ROS activity on removal of the autoreactive T cells by NOX pathway (Marrali et al., 2016).

In Guillain–Barré syndrome (GBS), Tang et al. (2017) found that redox status was altered, as demonstrated by decreased levels of lipophilic antioxidant defenses, mainly γ-tocopherol and δ-tocopherol concentrations in plasma of patients. In addition, also lower serum uric acid and albumin levels, which are markers that correlate with oxidative stress, were found to be decreased in patients than in healthy controls (Su et al., 2017). Based on our knowledge, studies on the role of physical training in GBS are still lacking.

Regarding chemotherapy-induced peripheral neuropathy, some antitumoral agents, such as oxaliplatin, induce oxidative stress reactions. These mechanisms, on the one hand, lead to cancer's cell death; on the other hand, it is well known to lead to neurotoxicity (Laurent et al., 2005).

It has been demonstrated that physical exercise, including combined exercise protocols of endurance, strength, and

sensorimotor training, in cancer patients suffering peripheral neuropathy improves physical functions (Duregon et al., 2018), but no data are available regarding the possible relation with oxidative stress. Similarly, it is not possible to establish in a conclusive manner if physical exercise could counteract the detrimental effect of oxidative stress in the natural course of other different pathogenic forms of neuropathies.

CONCLUSION

An imbalance between ROS production and antioxidant capacity can be a consequence of a sedentary lifestyle on the one hand and an excessive physical activity on the other hand, both in healthy people and in patients with NMD.

To improve motor functions, muscle weakness, and fatigue, targeted training protocols, which should take into consideration the planning type, workload, and session duration of the exercise, should be part of the motor regimen that can be advised within the different pathological frames of NMDs. Considering the expected natural history of each disease, aerobic and low-intensity exercise, due to its capacity to limit the detrimental effect of oxidative stress, could be considered, for different reasons, a potential non-pharmacological approach to induce beneficial adaptations in the neuromuscular system. It promotes muscle endurance, muscle strength/power, muscle trophism sparing or growth, and physical and respiratory functions in several forms of NMDs, improving the quality of life in NMD patients. Further studies are, however, necessary to optimize these therapeutic strategies in the setting of a neuromuscular disorder, likely supported by the use of reliable biomarkers, in order to better understand the context within which and at which extent the single disease mechanism can interact with tissue redox signaling, also in view of targeting their possible focused therapeutic interventions.

AUTHOR CONTRIBUTIONS

GS contributed to the planning and assembly of the manuscript. LC contributed to the writing and bibliography review for the chapter on muscle diseases. AL contributed to the writing and bibliography review for the chapter on ALS. CS contributed to the manuscript criticism. ES contributed to the writing and bibliography review for the chapter on peripheral neuropathies. GR contributed to the revision.

FUNDING

The authors are grateful to the following supporting and funding subjects: European Reference Network for Neuromuscular Diseases (ERN EURO-NMD), Duchenne Parent Project (2019 Research Project: Phenotypic variability in Becker Muscular Dystrophy), AIG (Associazione Italiana Glicogenosi: 2019 Research Project: The development of a Mobile App for Pompe's disease), UILDM (Unione Italiana Lotta alla Distrofia Muscolare) and AFM-Telethon (trampoline project 21611) for the Patient Associations side, and Rotary Foundation (Global Grant no. 1862130) as Charity, Aliveda and CSL Behring for the Drug Companies interested in this work.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 21 May 2020
doi: 10.3389/fphys.2020.00349

Aerobic Exercise Training in Patients With mtDNA-Related Mitochondrial Myopathy

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In patients with mitochondrial DNA (mtDNA) mutation, a pathogenic mtDNA mutation is heteroplasmically distributed among tissues. The ratio between wild-type and mutated mtDNA copies determines the mtDNA mutation load of the tissue, which correlates inversely with oxidative capacity of the tissue. In patients with mtDNA mutation, the mutation load is often very high in skeletal muscle compared to other tissues. Additionally, skeletal muscle can increase its oxygen demand up to 100-fold from rest to exercise, which is unmatched by any other tissue. Thus, exercise intolerance is the most common symptom in patients with mtDNA mutation. The impaired oxidative capacity in skeletal muscle in patients with mtDNA mutation results in limitation in physical capacity that interferes with daily activities and impairs quality of life. Additionally, patients with mitochondrial disease due to mtDNA mutation often live a sedentary lifestyle, which further impair oxidative capacity and exercise tolerance. Since aerobic exercise training increase mitochondrial function and volume density in healthy individuals, studies have investigated if aerobic training could be used to counteract the progressive exercise intolerance in patients with mtDNA mutation. Overall studies investigating the effect of aerobic training in patients with mtDNA mutation have shown that aerobic training is an efficient way to improve oxidative capacity in this condition, and aerobic training seems to be safe even for patients with high mtDNA mutation in skeletal muscle.

Keywords: mitochondrial DNA, mitochondrial myopathies, treatment, training, oxidative capacity

OPEN ACCESS

Edited by:
Lucio Tremolizzo, University of Milano-Bicocca, Italy

Reviewed by:
Massimiliano Filosto, Civil Hospital of Brescia, Italy
Dominik H. Pesta, Deutsche Zentrum für Diabetesforschung (DZD), Germany

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *05 November 2019*
Accepted: *26 March 2020*
Published: *21 May 2020*

Citation:
Jeppesen TD (2020) Aerobic Exercise Training in Patients With mtDNA-Related Mitochondrial Myopathy. Front. Physiol. 11:349. doi: 10.3389/fphys.2020.00349

INTRODUCTION

Mitochondria are small dynamic organelles that, with the exception of mature red blood cells, exist in all cells throughout the human body. Mitochondria contain the electron transport chain where ATP is produced through conversion of oxygen into water. Besides energy production, mitochondria are essential for cell signaling particularly apoptosis, and mitochondria host several metabolic pathways. Given these fundamental roles, it is not hard to imagine that defects in mitochondrial function can have catastrophic consequences for the cell. In patients with mitochondrial DNA (mtDNA) mutations, only a fraction of the mtDNA harbors mutation (**Figure 1**). Studies have demonstrated that oxidative capacity and the mtDNA mutation load of the tissue correlates, at least when measured in skeletal muscle (**Figure 2**; Tatuch et al., 1992; Jeppesen et al., 2003, 2006a; Frederiksen et al., 2006), indicating a close relationship between mtDNA mutation and the impact of mitochondrial dysfunction on different tissues.

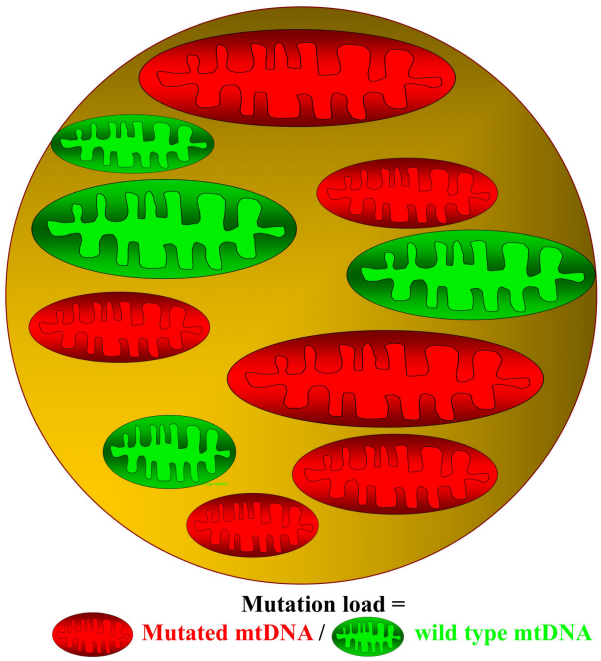

FIGURE 1 | Cell with mutated and wild-type mitochondrial DNA. In patients with mitochondrial DNA (mtDNA), mutations contain both mutated (red) and wild-type (green) mitochondria. The ratio between mutated mtDNA and wild-type mtDNA denotes the mutation load of the cell.

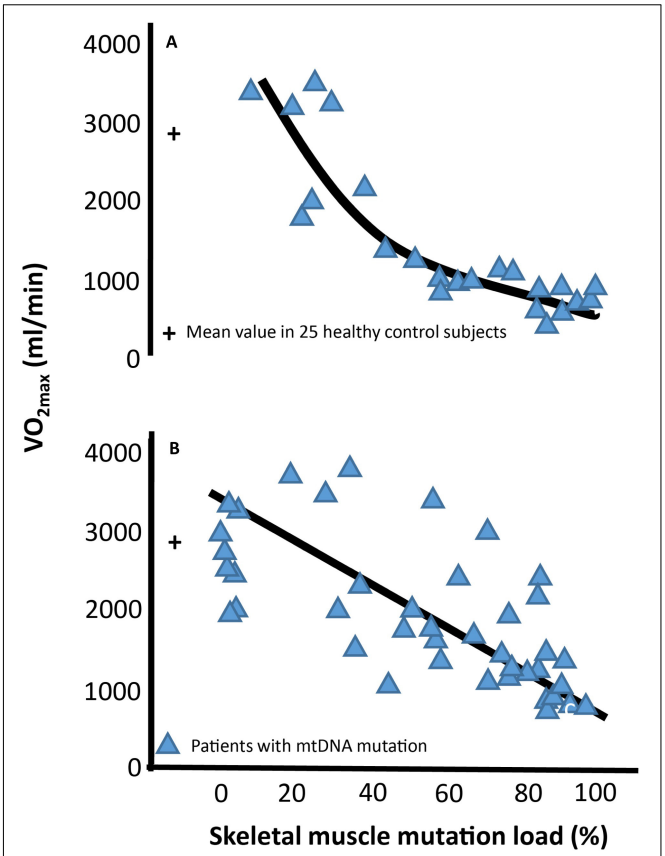

FIGURE 2 | Association between maximal oxidative capacity and skeletal muscle mitochondrial DNA mutation load Correlation between level of percentage mitochondrial DNA (mtDNA) mutation load in skeletal muscle and maximal oxygen uptake (VO_{2max}) in 24 subjects with different mtDNA mutation **(A)** and 51 subjects with the 3,243 A > G point mutation of mtDNA **(B)**. Blue triangles each represent a subjects with mtDNA mutation. The cross at 0% muscle mutation represents the mean ±SE of 25 age- and gender matched healthy subjects.

In patients with mtDNA mutations, mutation load is often high in skeletal muscle, and since oxidative demand is higher in skeletal muscle than in any other tissue, exercise intolerance is the most common symptom in patients with mtDNA mutations. This exercise intolerance relates to a low oxidative capacity in many patients, where the maximal oxidative capacity is lower than what is needed for cycling and jogging (**Figure 3**). Thus, the low oxidative capacity seriously interferes with trivial daily activities, which reduces quality of life and results in a sedentary lifestyle that increases the risk of secondary diseases such as diabetes and cardiovascular diseases. Muscle is a highly adaptable tissue that responds to changes in nutrition, hormones, and training (Booth and Watson, 1985; Babij and Booth, 1988). Aerobic training induces mitochondrial function and volume density (Holloszy, 1975; Andersen and Saltin, 1985; Hoppeler and Fluck, 2003), and result in higher anaerobic threshold and enhanced functional work capacity in both healthy individuals (Holloszy, 1967, 1975; Andersen and Saltin, 1985; Turner et al., 1997; Hoppeler and Fluck, 2003; Tarnopolsky et al., 2007) and patients with chronic disorders (Bruce and Hawley, 2004; Sveen et al., 2007, 2008; Cornelissen et al., 2013; Di Meo et al., 2017). Taken that quality of life is reduced due to impaired exercise tolerance (**Figure 3**) and the increased risk of secondary diseases that a sedentary lifestyle results in, studies have focused on using aerobic training as treatment for impaired oxidative capacity in patients with mtDNA mutations. In the following, the physiological consequences of mtDNA mutations and effect of aerobic training is described.

THE IMPACT OF AEROBIC TRAINING ON OXYGEN DELIVERY AND UPTAKE

Maximal oxygen uptake (VO_{2max}) is used as a parameter for exercise capacity, since oxygen uptake from rest to maximal exercise is attributed to skeletal muscle oxygen uptake alone (Mitchell et al., 1958; Hoppeler and Weibel, 1998; di Prampero, 2003). VO_{2max} depends on the ability to deliver oxygen from air to blood (lung conductance), the capacity to deliver oxygen (cardiac output), and the capacity to extract oxygen and produce ATP (Saltin, 1986; Bangsbo et al., 2000). In healthy individuals, cardiac output is the rate-limiting step for VO_{2max} (Saltin, 1986; Berglund and Ekblom, 1991; Saltin and Strange, 1992; **Figure 4**). In contrast, mitochondrial capacity and function seems to be the rate-limiting step for VO_{2max} in patients with mtDNA mutations (Haller et al., 1978; Haller and Lewis, 1984; **Figure 4**). This notion is emphasized by a consistent finding of inverse correlation between VO_{2max} and mtDNA mutation load in skeletal muscle of patients with mtDNA mutation (Jeppesen et al., 2003,

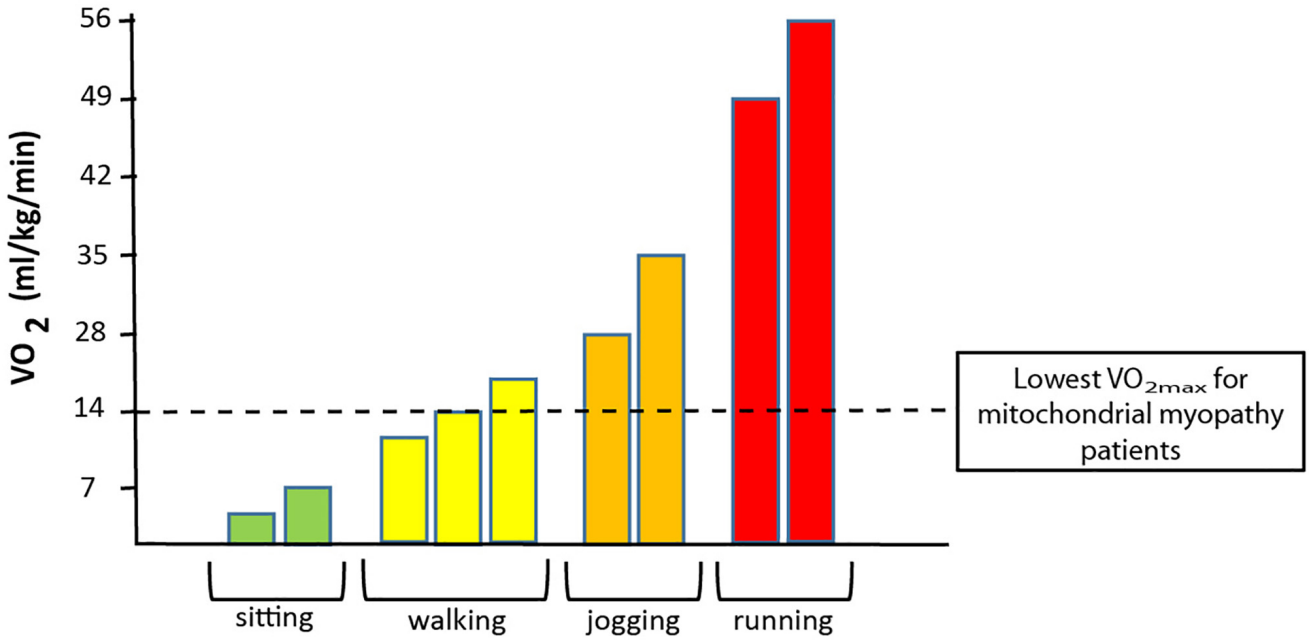

FIGURE 3 | Association between oxygen uptake and different physical activities. The graph demonstrates the range of oxygen demand demonstrated in VO_2 (oxygen uptake) during no activity (sitting) to walking (slow to brisk walking speed), jogging and to running at maximal speed. The dotted black line denotes the maximal oxidative capacity that is found in patients with a high level of mtDNA mutation load in skeletal muscle.

2006a; Taivassalo et al., 2003; **Figure 2**). This finding indicates that VO_{2max} is a good marker of the oxidative dysfunction caused by mtDNA mutation. Since endurance training increases mitochondrial function in healthy individuals, studies have investigated if aerobic training could be used as treatment for the impaired oxidative capacity and exercise intolerance in patients with mtDNA mutation (Taivassalo et al., 1996, 1998, 1999, 2001, 2006; Siciliano et al., 2000, 2012; Cejudo et al., 2005; Jeppesen et al., 2006b, 2009a). Studies have investigated 8–48 weeks of aerobic training (treadmill and cycle training) in 4–20 patients with different mtDNA mutations, exercising 30–45 min three to five times per week at workloads of 60–85% of VO_{2max}. The conclusion from the different studies was overall the same: Patients with mtDNA mutations, irrespective of mutation type are able to increase VO_{2max} to the same extent as that found in healthy subjects (24%; 20–28%) (Taivassalo et al., 1998, 2001, 2006; Siciliano et al., 2000, 2012; Cejudo et al., 2005; Jeppesen et al., 2006b, 2009a; Adhihetty et al., 2007; Porcelli et al., 2016; **Figure 5**).

Oxygen delivery during exercise is strictly regulated. The relationship between oxygen delivery (cardiac output) and utilization in healthy individuals is 5 L of blood for every 1 L of VO_2 irrespective of age, gender, or physical condition (Andersen and Saltin, 1985; Saltin and Strange, 1992). Cardiac output is regulated by different factors, including muscle (local vasomotor effect), central factors like systemic vasoconstriction and respiratory pump, systemic vasodilatation, and CNS stimuli of heart rate and contractility and redistribution of blood from non-active muscle cells (Andersen and Saltin, 1985; Saltin and Strange, 1992; Bangsbo et al., 2000). Since the rate-limiting factors for VO_{2max} in healthy individuals is cardiac output (**Figure 4**), exercise-induced increase in VO_{2max} is related to improvement of cardiac output and, to a much lesser extent, increased oxygen extraction for exercising muscle (Saltin, 1986; Bangsbo et al., 2000). Larsson et al. (1964) were the first to demonstrate arterialization of venous blood from contracting muscle during cycle exercise in patients with mtDNA mutation. Since then, many studies have confirmed that delivery of oxygen does not seem to be rate-limiting for VO_{2max}, and instead, there is a hyperemic response to exercise in patients with mtDNA mutations (Bank and Chance, 1994; Ozawa et al., 1995; Abe et al., 1997; Bank and Chance, 1997; Taivassalo et al., 2002). The only study that has investigated oxygen delivery and consumption directly in these conditions showed that while oxygen extraction was normal at rest, patients with mtDNA mutation were unable to increase extraction levels during exercise, along with findings of a workload-adjusted maximal exercise leg hyperemia up to ∼twofold (mean 65%) higher than that found in healthy controls (Jeppesen et al., 2012; **Figure 6**). Interestingly, the hyperemic response seemed to be induced by an excessive unloading of vasodilating substance ATP (Jeppesen et al., 2012). Four studies have investigated the physiological mechanisms behind increases in VO_{2max} with aerobic exercise in patients with mtDNA mutation, and interestingly, the driving factors for improvement in VO_{2max} was different than that found in healthy subjects (Taivassalo et al., 1998; Taivassalo et al., 2001, 2006; Porcelli et al., 2016). The studies demonstrated that cardiac output did

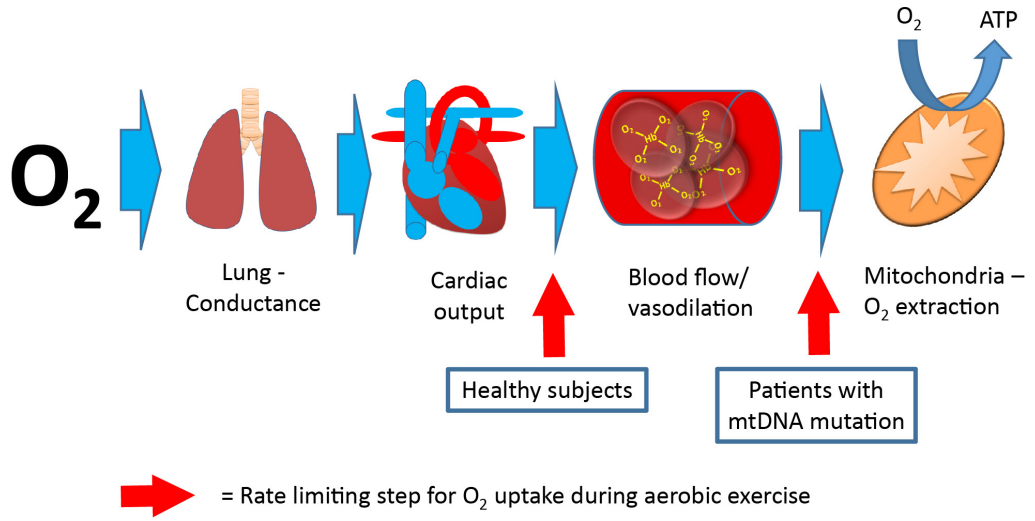

FIGURE 4 | Pathway for oxygen, from air to skeletal muscle. Oxygen uptake from lungs to final destination, the mitochondria. The rate-limiting step (red arrow) for maximal oxygen uptake is oxygen delivery (cardiac output) in healthy subjects, whereas the rate-limiting step for maximal oxygen uptake is oxygen extraction in patients with mitochondrial myopathy (final step).

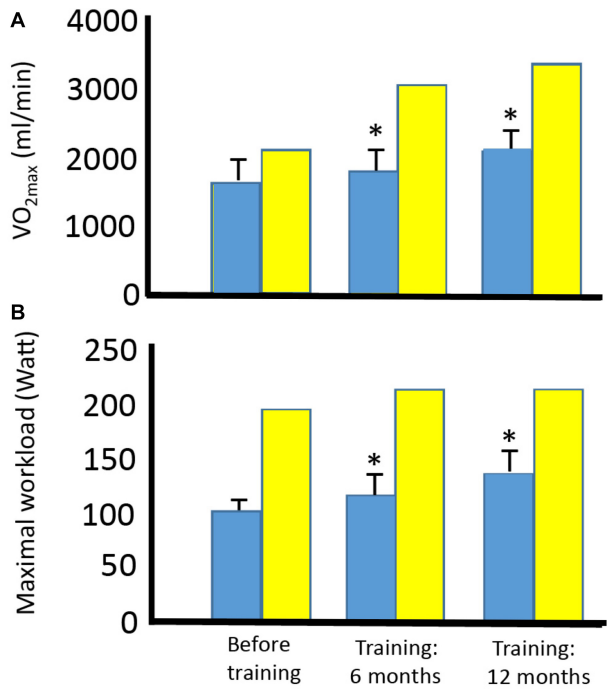

FIGURE 5 | Effect of aerobic training on maximal oxygen uptake and maximal workload in four patients with mtDNA mutation and one healthy subject. Maximal oxygen uptake **(A)** and maximal workload **(B)** before training and after 6 and 12 months of training in four patients with different mtDNA mutations (blue bars) and one healthy subject (yellow bar). *Different compared to before training ($p < 0.05$). #Different compared to before training and after 6 months of training.

not increase linearly to the increase in VO_{2max} as seen in healthy subjects. Instead, cardiac output either stayed unchanged or only improved, to a small extent, despite a substantial increase in VO_{2max}. With near-infrared spectroscopy (Porcelli et al., 2016), ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) (Taivassalo et al., 1998, 2001), and calculation a-vO_2 extraction (Taivassalo et al., 1998, 2001, 2006; Porcelli et al., 2016), authors demonstrated that the improvement in VO_{2max} in patients with mtDNA mutation was driven by an increase in mitochondrial capacity related to training-induced improvement in half-time recovery of ADP, the initial rate of phosphocreatine resynthesis, and maximum rate of ATP synthesis (Taivassalo et al., 1998, 2001). In line with these findings, another study demonstrated, with near infrared-spectroscopy technique, that the mismatch between oxygen delivery and oxygen consumption was partly ameliorated after aerobic exercise training in patients with mtDNA mutations (Porcelli et al., 2016). These findings indicate that improvement in oxygen extraction from exercising skeletal muscle may dominate the training response in patients with mtDNA mutations. Additionally, Porcelli et al. (2016) demonstrated that in patients with mtDNA mutations that had impaired pulmonary VO_2 pre-training, pulmonary VO_2 kinetics increased after 12 weeks of aerobic training. Since VO_2 kinetics reflect performance of skeletal muscle oxidative metabolism, the improvements in pulmonary VO_2 kinetics indicate that some of the patients with mtDNA mutation obtained lower O_2 deficit and higher exercise tolerance after 12 weeks of moderate intensity cycle training (Porcelli et al., 2016).

The rate of pulmonary ventilation (VE) increases linearly with exercise intensity until the VE threshold is reached (Goldstein et al., 1975; Martin and Weil, 1979; Davis et al., 1982). From this point, VE exceeds oxygen uptake, and lactate is accumulated (Davis et al., 1983). VE is tightly regulated by areas in the central nervous system, peripheral nervous system, including feedback from mechanical breathing pattern, arterial carbon dioxide, and oxygen tension (Dempsey et al., 1985; Forster and

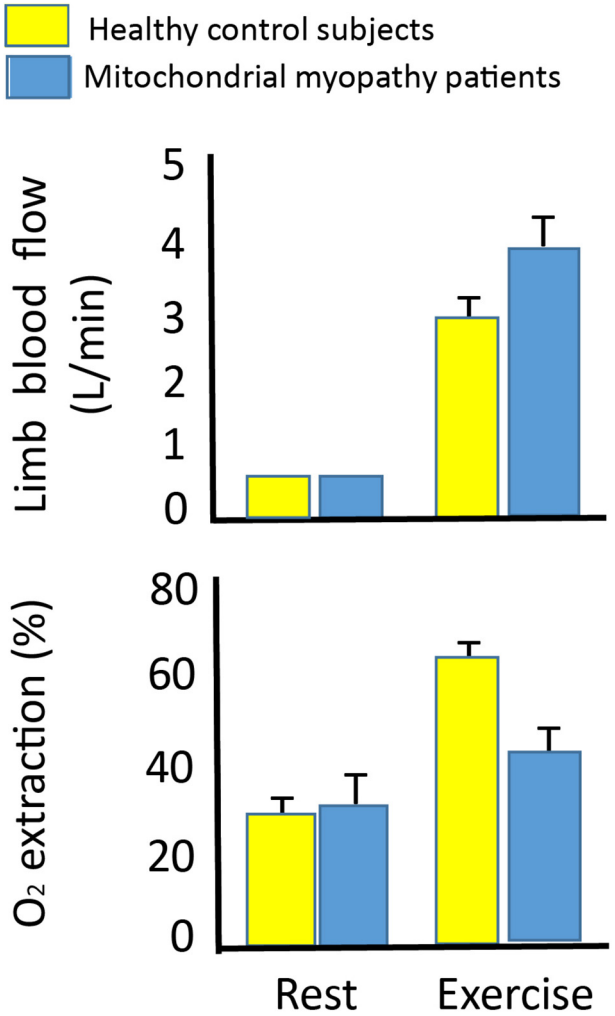

FIGURE 6 | Leg blood flow and oxygen extraction at rest and during leg exercise in 10 patients with mtDNA mutation and 10 healthy subjects. Leg blood flow **(upper graph)** and oxygen extraction **(lower graph)** in 10 patients with mtDNA mutation (blue bars) and 10 healthy subjects (yellow bars) at rest and during one-legged knee exercise at maximal workload (27 ± 3 vs. 55 ±6 W). There was a higher leg blood flow in the patients with mtDNA mutation compared to healthy subjects, while oxygen extraction did not change from rest to exercise indicating a hyperemic response to exercise in patients with mtDNA mutation. *Different from healthy subjects ($p < 0.05$).

Pan, 1988), and a direct feedback from muscle due to a decrease in ATP:ADP ratio, lactate accumulation, and fall in pH (Davis et al., 1983; **Figure 7A**). Studies have demonstrated that VE is exaggerated in patients with mtDNA mutation during exercise (Haller and Lewis, 1984; Haller et al., 1989; Flaherty et al., 2001; Taivassalo et al., 2003; Heinicke et al., 2011), and the level of VE correlated with the muscle mtDNA mutation load (Taivassalo et al., 2003; Taivassalo and Haller, 2005). Increased motor unit recruitment and excessive build-up of ADP, lactate, and other metabolites in exercising muscle may very likely be responsible for the excessive VE found in patients with mtDNA mutation (**Figure 7B**). The few studies that have reported VE adaptations to aerobic exercise in patients with mtDNA mutation, overall, did not find a change in VE with aerobic training, which indicates that factors responsible for enhanced VE in patients with mtDNA mutation do not change with aerobic training. Thus, the excessive VE in patients with mtDNA mutations during exercise may be one of the factors responsible for premature fatigue. Only one study has reported VE during constant workload test, and found that VE rate decreased after 12 weeks of training, indicating that aerobic training might have a positive effect on the otherwise excessive VE rate seen in patients with mtDNA mutation (Cejudo et al., 2005).

THE IMPACT OF AEROBIC TRAINING: FUEL METABOLISM DURING EXERCISE IN PATIENTS WITH mtDNA MUTATION

During exercise the relative contribution of carbohydrate and fat for energy (ATP) synthesis varies with intensity and duration of the exercise bout (Saltin and Astrand, 1993; Hawley et al., 2011). From rest to exercise intensity of 50% of VO_{2max}, the principle substrate for ATP production is free fatty acids (FFA) (Saltin and Astrand, 1993). From exercise intensity of 50% of VO_{2max}, oxidation of carbohydrate increases linearly with increase in exercise intensity, but from exercise intensities beyond 85% of VO_{2max}, anaerobic metabolism becomes increasingly important until peak VO_2 (Van Hall et al., 2003). Since oxidation of glucose releases more ATP per molecule of O_2 that is utilized compared to oxidation of FFA, it has been speculated if carbohydrate could be the preferred energy substrate in patients with mtDNA mutation in skeletal muscle. However, two studies have shown that FFA and carbohydrate are oxidized to the same extent at rest and during exercise as that seen in healthy individuals (Jeppesen et al., 2009b, 2013). In healthy individuals, aerobic training changes fuel turnover from carbohydrate toward increased fatty acid oxidation (Bylund et al., 1977; Saltin and Astrand, 1993). This change is met by an increase in mitochondrial biogenesis, including increased respiratory chain enzyme levels (Holloszy, 1975; Andersen and Saltin, 1985; Hoppeler and Fluck, 2003), which lowers muscle lactate production and respiratory exchange ratio (Davis et al., 1983). The rate and slope of these changes depend on pre-training status, duration, and intensity of training. No study has, to date, investigated changes in carbohydrate and FFA turnover in patients with mtDNA mutation. However, since FFA and carbohydrate turnover is the same at rest and during exercise in patients with mtDNA mutation compared to healthy subjects, training-induced changes in fuel turnover would not be expected to be different than that found in healthy subjects.

In patients with mtDNA mutation, resting plasma lactate levels correlate with mtDNA mutation load in skeletal muscle (Jeppesen et al., 2003), which has prompted the idea that net lactate release from skeletal muscle depends on oxidative capacity in skeletal muscle. However, only one study has investigated lactate turnover in patients with mtDNA mutation. This study demonstrated that the capacity to oxidize lactate is not limited in patients with mtDNA mutations, not even in patients with

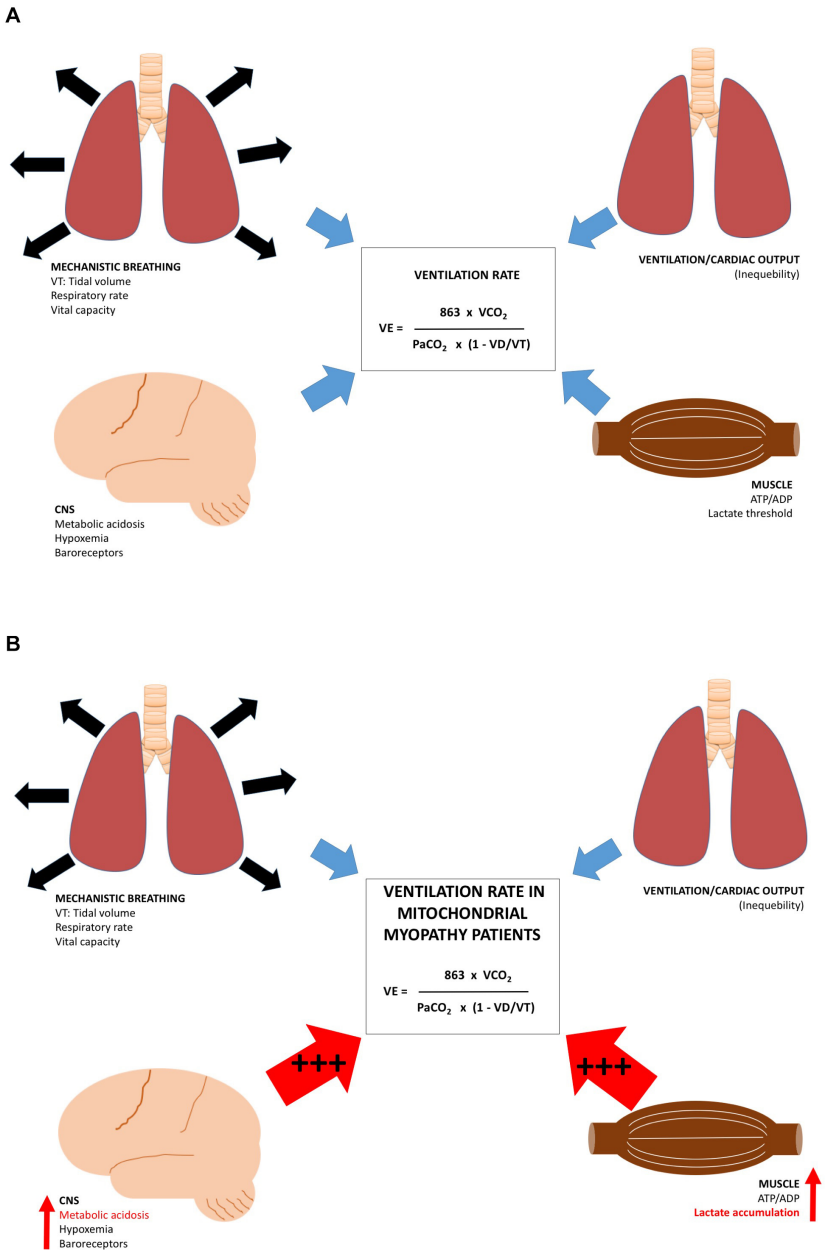

FIGURE 7 | Regulating factors for ventilation rate at rest and during exercise. Different regulating factors for rate of ventilation. (1) factors responsible for lung function, (2) Central nervous system, (3) relationship between cardiac output, and oxygen uptake in the lungs, and (4) ATP/ADP, lactate and pH in skeletal muscle in **(A)** healthy subjects and **(B)** patients with mtDNA mutation. During exercise, patients with mtDNA mutation have an excessive metabolic acidosis increasing the CNS-mediated drive on ventilation rate (+++), the ATP/ADP ratio (+++), pH drop, and lactate concentration accumulate (+++), which result in increased skeletal muscle drive on ventilation rate.

high level of mtDNA mutation in skeletal muscle (Jeppesen et al., 2013). The authors argued that the substantial high lactate level found at rest and during exercise in patients with mtDNA mutation may be a result of a constant production of lactate in fibers with high levels of mtDNA mutation along with intact lactate oxidation capacity in muscle fibers with no or low levels of mtDNA mutation (**Figure 8**). This hypothesis is related to the heterogeneous distribution of mtDNA mutation load among skeletal muscle in patients with mtDNA mutation irrespective of type I or type II fibers resulting in a situation where some fibers may rely solely on anaerobic glycolysis, while adjacent muscle fibers with lower mtDNA mutation load will be able to oxidize lactate (**Figure 8**). Siciliano et al. (2000, 2012) investigated the effect on aerobic training on lactate levels during constant workload test, and in one study, the concomitant catecholamine production was investigated. The authors showed that patients

with mtDNA mutation had a lower plasma lactate level during a constant workload test after 10 weeks of aerobic training compared to healthy subjects. Interestingly, the authors found that the catecholamine levels decreased to the same extent as those seen in healthy subjects, indicating that the higher catecholamine level was not the driving factor for exaggerated lactate level found in patients with mtDNA mutation (Siciliano et al., 2012). Instead, the study implied that decreased lactate level after aerobic training in patients with mtDNA mutation could be a result of increased capacity to oxidize lactate on skeletal muscle level.

The premature fatigue that is often seen in patients with mtDNA mutation has been linked to the excessive build-up of lactate (Dengler et al., 1996; Finsterer et al., 1998). However, in one study where resting lactate level was reduced pharmacologically, exercise capacity did not improve in patients with mtDNA mutation. This finding indicated that premature fatigue in patients with mtDNA mutation may not be related to plasma lactate levels (Vissing et al., 1998). Another potential explanation for premature fatigue could be that a relative higher fraction of muscle fibers is recruited during exercise in patients with mtDNA performing the same workload as healthy subjects. This hypothesis is based on the heterogeneous distribution of mtDNA mutation load among fibers in the same muscle (**Figure 8**). Thus, muscle fibers with a high mtDNA mutation load rely heavily on anaerobic glycolysis and are, thus, prone for depleting glycogen storage fast, which inevitably result in recruitment of additional muscle fibers for muscle contraction (**Figure 8**). The higher skeletal muscle fiber recruitment result in a sense of fatigue and, thus, could induce premature fatigue in patients with mtDNA mutation compared to healthy subjects exercising at the same relative workload (Saltin, 1981). Training studies investigating the effect of aerobic training on resting and peak-exercise-induced lactate levels have shown that exercise-induced fatigue was reduced after 8–14 weeks of aerobic training (Taivassalo et al., 1998, 2006; Siciliano et al., 2000; Cejudo et al., 2005; Porcelli et al., 2016). At the same time, peak-lactate level remained unchanged (Siciliano et al., 2000, 2012; Cejudo et al., 2005; Jeppesen et al., 2006b, 2009a; Porcelli et al., 2016) or was higher (Taivassalo et al., 1998, 2001) in patients with mtDNA

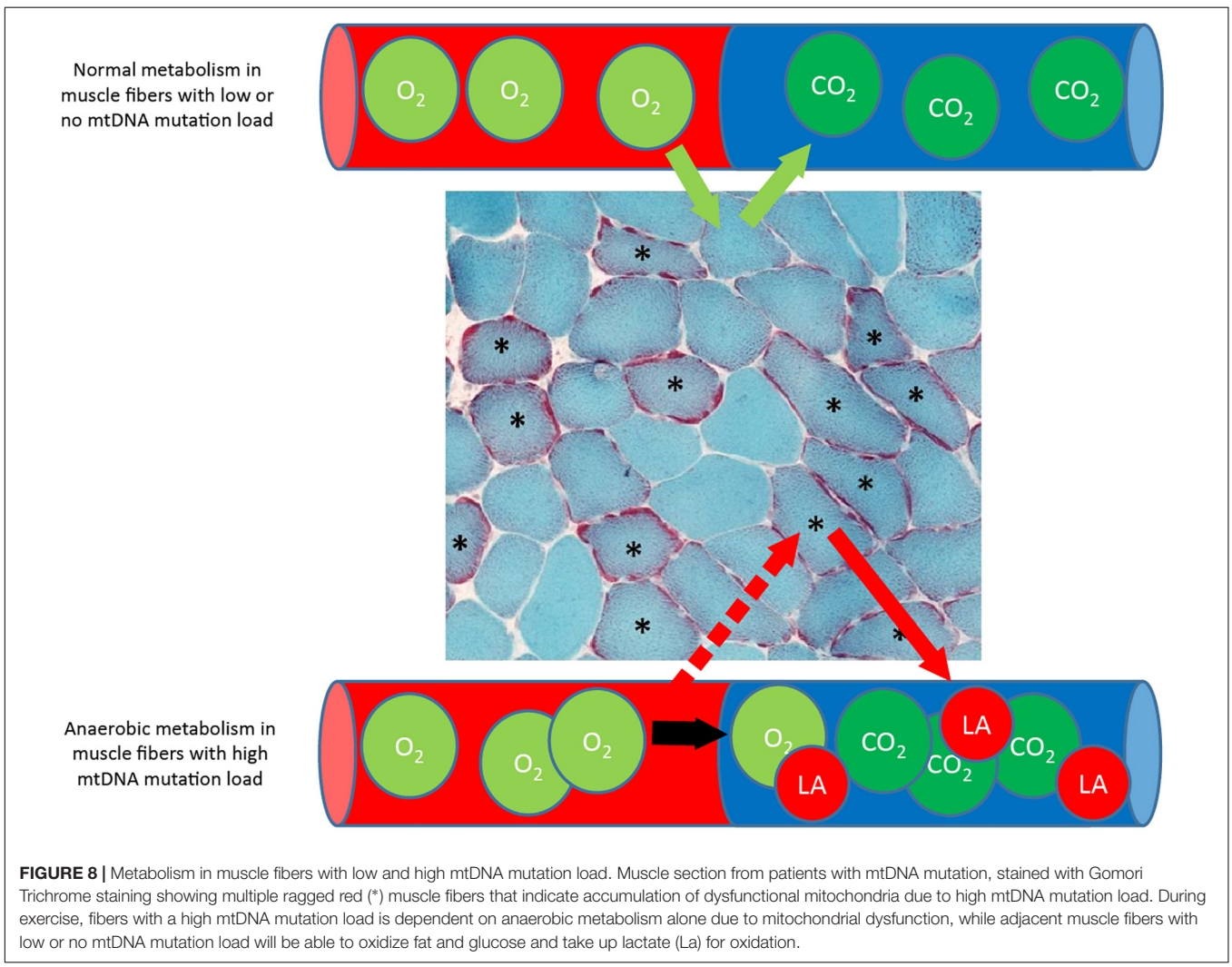

FIGURE 8 | Metabolism in muscle fibers with low and high mtDNA mutation load. Muscle section from patients with mtDNA mutation, stained with Gomori Trichrome staining showing multiple ragged red (*) muscle fibers that indicate accumulation of dysfunctional mitochondria due to high mtDNA mutation load. During exercise, fibers with a high mtDNA mutation load is dependent on anaerobic metabolism alone due to mitochondrial dysfunction, while adjacent muscle fibers with low or no mtDNA mutation load will be able to oxidize fat and glucose and take up lactate (La) for oxidation.

mutation. This finding underscores that the absolute plasma lactate level does not, itself, induce premature fatigue in patients with mtDNA mutations.

IMPACT OF AEROBIC TRAINING: ADAPTATION OF MITOCHONDRIAL VOLUME AND MITOCHONDRIAL DNA

Although only a few studies have examined the effect of aerobic training on mitochondrial content directly (Hoppeler and Weibel, 1998; Hoppeler and Fluck, 2003), it is widely accepted that mitochondrial volume density increases with aerobic training. Mitochondrial content is usually assessed with indirect measures like citrate synthase, cardiolipin, porin, and mtDNA copy number. Citrate synthase, which is the rate-limiting step in the Krebs cycle, correlates closely with oxidative capacity (Schwerzmann et al., 1989; Kiens et al., 1993; Rasmussen et al., 2001; Jacobs et al., 2013); cardiolipin, which is a phospholipid located in the inner mitochondrial membrane, has been found to be a good biomarker of the total mitochondrial cristae surface area in human skeletal muscle (Pfeiffer et al., 2003; Larsen et al., 2012); and porin, which is the most abundant protein of the mitochondrial outer membrane, correlates with the mitochondrial volume in skeletal muscle (van Moorsel et al., 2016). It has been a general notion that mtDNA copy number is replicated along with an increase in mitochondrial content, and therefore, mtDNA content has also been used as a marker of mitochondrial volume (Menshikova et al., 2006; Civitarese et al., 2007; Balakrishnan et al., 2010; Perry et al., 2010; Zoladz et al., 2013). It has been a concern if shorter mtDNA copies (mutated mtDNA) could have a replicative advantage over larger mtDNA copies (wild type) in patients with mtDNA mutations (Moraes et al., 1989; Larsson et al., 1990; Fu et al., 1996; Weber et al., 1997; Chinnery and Samuels, 1999), which inevitably would result in increase in mtDNA mutation load in skeletal muscle with continuous training. When a study demonstrated that mtDNA mutation load increased in six of nine patients after 14 weeks of cycle exercise, the safety of aerobic exercise in patients with mtDNA mutation was questioned (Taivassalo et al., 2001). Only one study has investigated mtDNA copy number in trained vs. untrained limb of the same subjects and demonstrated that mtDNA copy number does not change with aerobic training (Fritzen et al., 2019). This finding demonstrates that changes in mitochondrial volume do not result in changes in mtDNA copy number and, thus, prove that mtDNA mutation load will not increase in skeletal muscle in patients with mtDNA mutations because of increase in oxidative capacity. In line with this, none of the following training studies conducted in patients with mtDNA mutations found any evidence of change in mtDNA mutation load, indicating that aerobic training in patients with mtDNA mutations is safe (Jeppesen et al., 2006b, 2009a; Taivassalo et al., 2006).

It is a general concern that patients with mtDNA mutation, due to uncoupling of complexes in the respiratory chain, produces higher levels of reactive oxidative species (ROS) than healthy patients with intact mitochondria (**Figure 9**). ROS is harmful to the cell due to induction of mtDNA mutation, activation of mitochondrial permeability pore and direct damage to lipid membranes (Lu et al., 2003; Ikawa et al., 2009; Liu et al., 2009; Wu et al., 2010; Siciliano et al., 2012). During exercise, there is an increased flux through the respiratory chain, and

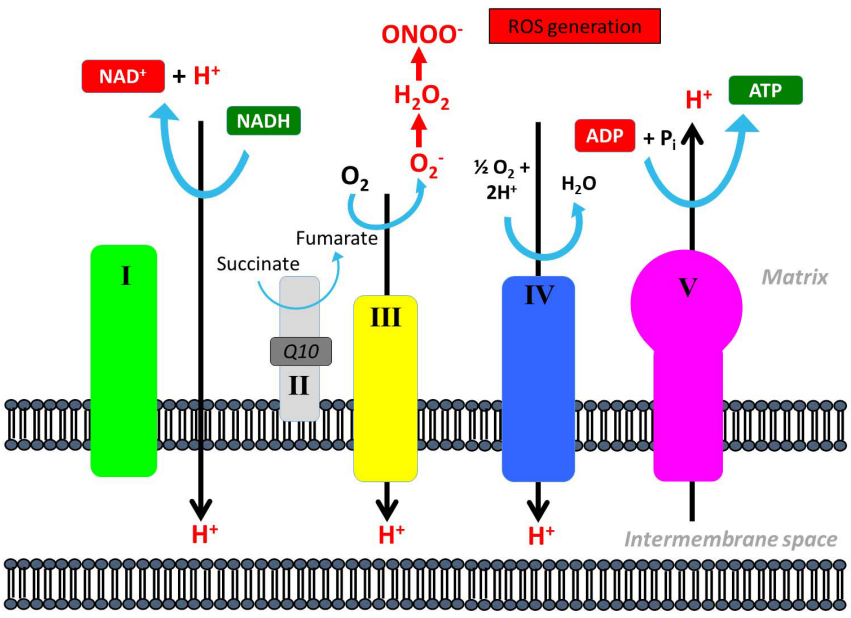

FIGURE 9 | Reactive oxidative species production in the mitochondrial respiratory chain. Reactive oxidative species (ROS) can be generated through conversion of oxygen (O_2) to superoxide anion ($O2^-$) that can be converted further into peroxynitrite ($ONOO^-$) that is a highly reactive species and can oxidize DNA, proteins, and lipids and interfere with important vascular signaling pathways.

therefore, studies have questioned the safety of long-term training in patients with mtDNA mutations (Adhihetty et al., 2007; Siciliano et al., 2012). Only two studies have directly investigated differences in oxidative stress and compensatory mechanisms related to training in patients with mtDNA mutation compared to healthy subjects (Adhihetty et al., 2007; Siciliano et al., 2012). One study found that there was an increased ROS production in patients with mtDNA mutation, but this was counteracted by compensatory mechanisms. These compensatory mechanisms included increased antioxidant levels, increased DNA repair capacity, and elevated levels of Bcl-2, which serve as a protective mechanism by lowering pro-apoptotic proteins (Adhihetty et al., 2007). However, the authors found that there was a reduced expression of DNA repair machinery along with an increased oxidative damage in skeletal muscle after 14 weeks of aerobic training (Adhihetty et al., 2007). Siciliano and authors showed that 10 weeks of aerobic training resulted in a decrease in blood lipoperoxide, which indicated that training induced a "one-step scaled down" on the conventional four-step scale adopted for oxidative stress (Siciliano et al., 2012). Taken together, training seems to induce factors that counteract potentially harmful effects of training on mitochondria in patients with mtDNA mutation, but whether the harmful effect is counterbalanced on a long term still remains unclear and warrants further investigation.

IMPACT OF AEROBIC TRAINING: QUALITY OF LIFE AND FUNCTIONAL TESTING

A typical consequence of low oxidative capacity in patients with mtDNA mutation is impaired physical performance including difficulties in walking. Thus, it seems straightforward that along with indices of training-induced increases in oxidative capacity, quality of life, and functional assessment should also be performed. However, only a few studies have investigated the impact of training on quality of life (Taivassalo et al., 1998, 2001, 2006; Cejudo et al., 2005; Porcelli et al., 2016). Moreover, only one study aimed to convert an increase in oxidative capacity into clinical meaningfulness by a functional test (Cejudo et al., 2005). Improvements in quality of life ranged from no-change to 25% improvement after 8–14 weeks of aerobic training. Interestingly, an improvement of 25% in oxidative capacity did not result in a higher physical activity level during or after the training period (Porcelli et al., 2016), indicating that increase in oxidative capacity of 25% not necessarily translates into a more physical active lifestyle.

PERSPECTIVE

All individuals, even sedentary, experience repeated periods where physical activity level differs. In line with this, patients with mtDNA mutation also experience periods with a higher physical activity than others. With the potential risk that physical exercise may increase mtDNA mutation load, physical exertion could potentially result in mitochondrial dysfunction with time in patients with mtDNA mutation. No study has, to date, investigated if the level of new mtDNA mutations increases with time in patients with mtDNA mutation and whether this could be related to the level of physical activity. A cross-sectional study of a large cohort of patients with the common 3,243 A > G mutation have indicated that the inherited mtDNA mutation does no increase with time (Frederiksen et al., 2006). However, this finding does not rule out that patients with inherited mtDNA mutations may be more prone to develop new mtDNA mutations with time. In theory, an increase in new mtDNA mutations and, thus, increased dysfunctional mitochondria with time, could be the driving factor for the progressive nature of disease in patients with mtDNA mutations. Thus, one scope for future long-term training studies could be assessment of new mtDNA mutations along with assessment of the level of ROS, mtDNA repair activity, and antioxidant levels.

AUTHOR CONTRIBUTIONS

TJ drafted the manuscript.

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