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RESEARCH ARTICLE

Diminished muscle oxygen uptake and fatigue in spinal muscular atrophy

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Abstract

Objective: To estimate muscle oxygen uptake and quantify fatigue during exercise in ambulatory individuals with spinal muscular atrophy (SMA) and healthy controls. **Methods**: Peak aerobic capacity (VO_{2peak}) and workload (W_{peak}) were measured by cardiopulmonary exercise test (CPET) in 19 ambulatory SMA patients and 16 healthy controls. Submaximal exercise (SME) at 40% W_{peak} was performed for 10 minutes. Change in vastus lateralis deoxygenated hemoglobin, measured by near-infrared spectroscopy, determined muscle oxygen uptake (ΔHHb) at rest and during CPET and SME. Dual energy X-ray absorptiometry assessed fat-free mass (FFM%). Fatigue was determined by percent change in workload or distance in the first compared to the last minute of SME ($Fatigue_{SME}$) and six-minute walk test ($Fatigue_{6MWT}$), respectively. **Results**: ΔHHb-PEAK, ΔHHb-SME, VO_{2peak}, W_{peak}, FFM%, and 6MWT distance were lower ($P < 0.001$), and $Fatigue_{6MWT}$ and $Fatigue_{SME}$ were higher ($P < 0.001$) in SMA compared to controls. ΔHHb-PEAK correlated with FFM% ($r = 0.50$) and VO_{2peak} ($r = 0.41$) only in controls. Only in SMA, $Fatigue_{6MWT}$ was inversely correlated with W_{peak} ($r = -0.69$), and $Fatigue_{SME}$ was inversely correlated with FFM% ($r = -0.55$) and VO_{2peak} ($r = -0.69$). **Interpretation**: This study provides further support for muscle mitochondrial dysfunction in SMA patients. During exercise, we observed diminished muscle oxygen uptake but no correlation with aerobic capacity or body composition. We also observed increased fatigue which correlated with decreased aerobic capacity, workload, and body composition. Understanding the mechanisms underlying diminished muscle oxygen uptake and increased fatigue during exercise in SMA may identify additional therapeutic targets that rescue symptomatic patients and mitigate their residual disease burden.

Introduction

Spinal muscular atrophy (SMA) is a recessively inherited neuromuscular disease affecting individuals across the lifespan. Approximately 1 in 50 individuals are carriers resulting in an incidence of 1 in 10,000 live births and an estimated prevalence of 1–4 persons per 100,000.[1] SMA results from homozygous mutations in the survival motor neuron 1 (SMN1) gene and the inadequate production of full-length SMN protein by an imperfect back-up SMN2 gene.[2] Although SMN protein is expressed in all tissues, deficiency predominantly affects lower motor neurons[3] causing muscle weakness, impaired motor function and, frequently, death. Nusinersen, a recently approved

intrathecally administered therapeutic agent, treats SMA by restoring SMN to the central nervous system (CNS) (motor neurons). This results in extended survival and clinically meaningful improvements in motor function,[4] with the best responses seen with early treatment.[5] Still, despite Nusinersen treatment, the clinical signatures of SMA, weakness and fatigue, persist and impact everyday lives. One potential reason is the continued paucity of SMN in peripheral (non-CNS) tissue. Indeed, there is both pre-clinical and clinical evidence to support that structures other than motor neuron cell bodies are vulnerable to low SMN.[6,7]

Prior studies have shown neuromuscular junction (NMJ) development and maturation abnormalities in SMA animal models,[8,9] and there is evidence supporting a relationship between NMJ dysfunction and fatigue in SMA patients.[10] Skeletal muscle is also vulnerable to SMN insufficiency.[6] Muscle tissue from SMA patients exhibits mitochondrial depletion and impaired mitochondrial biogenesis that is inversely related to SMN availability.[7] Moreover, milder SMA patients exhibit attenuated responses to aerobic conditioning programs with only modest improvements in exercise capacity with moderate-intensity training over 6 months.[11] Similar improvements were observed with a shorter, more intensive training program that resulted in severe, debilitating fatigue.[12] Additionally, fatigue observed in children with SMA treated with Nusinersen was not attenuated to the same degree that distance walked increased.[13] Nusinersen is administered intrathecally and does not directly target peripheral tissues and the NMJ. These observations are consistent with the poor motor performance of model mice selectively depleted of SMN in skeletal muscle.[6] Collectively, the data strongly suggest that tissues like muscle, not targeted by Nusinersen, contribute to disease.

SMA is phenotypically heterogeneous disease due, in part, to a disease modifying homologue gene, SMN2.[2,14] Despite the phenotypic heterogeneity, all SMA patients have mutations in the SMN1 gene located on chromosome 5q13.[15] The mild SMA phenotype, type III, also termed Kugelberg–Welander disease, typically presents after age 18 months with symptoms of proximal muscle weakness, mostly involving the lower limbs.[16] SMA type III patients represent an ideal target population because their relatively mild disease burden permits assessments of aerobic capacity and muscle function. Thus, results from this study could help inform our understanding and direct investigations into the more severe SMA phenotypes.

The purpose of this study was to examine muscle oxygen uptake and quantify fatigue during maximal and submaximal exercise (SME) and its relationship to body composition in ambulatory individuals with SMA and controls with comparable age.

Methods

Study design

This was an observational study of ambulatory children and adults with SMA and healthy controls. All individuals who met eligibility criteria were invited to participate. Participants had to be at least 12 years old, and able to walk at least 25 m without assistance and ride the stationary cycle ergometer. Patients were excluded if they used investigational medications intended for the treatment of SMA or had a contraindication to exercise according to the American College of Sports Medicine (ACSM) criteria.[17] The study was registered with Clin icalTrials.gov (NCT02895789). All participants or guardians of participants signed informed consent forms approved by the Columbia University Irving Medical Center Institutional Review Board.

Exercise testing

Graded and SME tests were performed to evaluate cardiopulmonary responses and muscle function during exercise. Cardiopulmonary exercise testing (CPET) provides an objective evaluation of cardiorespiratory fitness and is considered the gold standard for this purpose in diverse patient populations including those with neuromuscular disease.[18] In SMA, it is safe and complements functional measures that are designed to evaluate exercise interventions in ambulatory SMA patients.[11,12] Participants underwent a CPET using an electronically braked recumbent cycle ergometer (Lode Corival™; Groningen, Netherlands) and an individualized ramping protocol where Standard American College of Sports Medicine test termination criteria were applied.[17] Pedal shoes and straps were used to ensure a stable foot placement and provide posterior and lateral (heel) support. This encouraged neutral hip rotation during exercise and promoted optimal function of the knee extensors. Oxygen uptake and related variables were measured during exercise using a ParvoMedics™ metabolic cart (TrueOne™ model 2400; Sandy, Utah). Peak oxygen uptake (VO_{2peak}) was identified using accepted criteria and expressed relative to total body mass.[17] A 10-min SME test was performed on the recumbent cycle ergometer after a 30-min rest period. The workload for the SME was set at 40% of the peak workload achieved on the CPET corresponding to a target intensity of 3–5 on the OMNI scale of perceived exertion.[19] Workload was adjusted to maintain the target intensity. One-minute warm-up and cool-down periods were included without any resistance (0 W).

Near infrared spectroscopy of muscle

Near infrared spectroscopy (NIRS) is a simple, non-invasive method to measure oxygen saturation in muscle and other tissues in vivo.[20] NIRS quantifies oxyhemoglobin and deoxyhemoglobin levels using light absorption and, indirectly, tissue oxygen uptake. Commercially available devices provide information about relative changes in oxygenated hemoglobin/myoglobin, deoxygenated hemoglobin/myoglobin, and total hemoglobin concentrations. The deoxygenated signal estimates change in muscle microvascular deoxygenation, thus reflecting the balance between local muscle oxygen delivery and muscle oxygen uptake at the site of interrogation.[21] NIRS devices have been validated in humans during exercise[22] and under ischemic conditions.[23] In metabolic myopathies including patients with mitochondrial disease, there was a linear relationship between an NIRS-derived index of muscle oxygen uptake and oxidative capacity from an exercise tolerance test.[24]

Muscle oxygen uptake was measured using NIRS on the left vastus lateralis muscle using a Portamon™ 3-channel NIRS device (Artinis Medical Systems, Elst, Netherlands). Skin fold assessments were performed on the thigh where the device was positioned and the adipose tissue thickness (ATT) was calculated as ½ the average skin fold thickness.[25] The NIRS signal attenuates when the ATT is >1.5 cm.[26] The ATT for all participants did not exceed the 1.5 cm threshold and therefore no correction was required. The Portamon™ software provides real-time feedback on signal quality during evaluation. Good signal quality was confirmed prior to testing.

Relative change in deoxygenated hemoglobin (ΔHHb-PEAK) was calculated by setting all traces to zero at rest, applying a 1-sec moving average filter, and taking the total difference in HHb from rest to peak exercise during maximal exercise (CPET). Similarly, for SME, ΔHHb-SME was calculated by setting all traces to zero during warm-up, applying a 1-sec moving average filter, and taking the total difference in HHb from warm-up to cool down.

Six-minute walk test

The Six-minute walk test (6MWT), an objective evaluation of functional exercise capacity, measures the maximum distance a person can walk in 6 min over a 25-m linear course. It has been shown to be a valid and reliable measure of functional exercise capacity and ambulatory function in SMA.[27] Distance walked over the entire 6-min time period, distance covered each minute, and the time to complete each 25-m interval were recorded. Standard encouragement phrases using even, neutral tones were used for each participant according to the American Thoracic Society (ATS) guidelines adapted for SMA.[28]

Fatigue

Fatigue during SME was calculated as the percent change in workload from the first minute to the 10th minute ($Fatigue_{SME}$). Fatigue during the 6MWT ($Fatigue_{6MWT}$) was calculated by subtracting the distance walked in the sixth minute from the distance walked in the first minute.[29] This difference was divided by distance walked in the first minute and expressed as a percentage. For both variables, a positive value represents fatigue.

Dual energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DEXA) is a method of estimating bone and lean body mass (LBM) by comparing the absorption of two distinct energy level beams at 46.8 and 80 keV, which are effective at differentiating soft tissue and bone.[30] A standard DEXA scan was performed in the supine position. A total body image was obtained, with computer-generated estimates for the percentage of total body fat, LBM, and bone. Similar to a recent study of body composition in SMA patients, fat-free mass was calculated as the sum of lean mass and bone mineral content divided by total body mass and expressed as a percentage.[31] Whole-body oxygen consumption (VO_2) relative to LBM was also calculated as absolute VO_2 at peak exercise (mL/min) divided by LBM (kg) from DEXA.

Statistical methods

Descriptive statistics were used to present the clinical characteristics of the participants and their performance on the assessments. Wilcoxon rank-sum tests and Fisher's exact test were used to determine differences between the SMA and healthy control groups. Pearson correlation coefficients were used to evaluate bivariate relationships among the measures.

Results

Nineteen ambulatory SMA and 16 healthy children and adults were evaluated. The participants with SMA were mostly male (73.7%) with a median age of 32.7 years (range 12.7–56.8). The control group participants were similar (81.2% male and median age 23.6 years; range 13.3–53.6). Of the 19 participants with SMA, 13 (68.4%) were treated with Nusinersen with a median treatment duration of 1.07 years (range 0.54–6.02). The clinical characteristics of the participants are described in Table 1.

Table 1. Clinical characteristics of participants.

Characteristic	SMA (n = 19)	Control (n = 16)	P-value[1]
Age (years)	32.7 (12.7–56.8)	23.6 (13.3–53.5)	0.27
Sex (male)	14 (73.7%)	13 (81.3%)	0.70
Disease duration (years)	22.9 (5.2–54.8)		
Age at symptom onset (years)	7 (1.5–16.0)		
Weight (kg)	78.2 (40.9–134.5)	71.5 (44.4–98.1)	0.35

Statistics presented as median (range), or n (%), as appropriate. SMA, spinal muscular atrophy.
[1]From Wilcoxon rank-sum test (for age and weight) or Fisher's exact test (for sex).

Compared to healthy controls, individuals with SMA had lower VO_{2peak} relative to total body mass and VO_{2peak} relative to LBM, achieved lower Wpeak and had lower ΔHHb-PEAK from rest to maximal effort during the CPET ($P < 0.001$) (Table 2). Relative changes of deoxygenated hemoglobin during the CPET from rest to cool down for the healthy control and SMA participants are presented in Figure 1. During SME, ΔHHb-SME was lower in participants with SMA ($P < 0.001$) (Table 2, Fig. 2). Distance walked on the 6MWT was lower in SMA than in healthy controls ($P < 0.001$) (Table 2). $Fatigue_{6MWT}$ (median = 16.2%; range −1.4 to 55.6) was similar to that in previous reports[32] (Fig. 3A) and fatigue was also measurable during SME ($Fatigue_{SME}$; median = 0.0%; 0.0–80.0) in SMA participants but minimal in controls ($Fatigue_{SME}$; median = 0.0%; range −4.6 to 29.4) ($P < 0.001$) (Fig. 3B). Healthy controls had greater FFM% (median 77.7; range 68.0–86.4) than individuals with SMA (median 57.5; range 42.1–83.3) ($P < 0.001$) (Table 2).

VO_{2peak} correlated with FFM% in controls ($r = 0.58$; $P = 0.02$) and SMA ($r = 0.46$; $P = 0.05$), and with W_{peak} in controls ($r = 0.65$; $P = 0.007$) and SMA ($r = 0.66$; $P = 0.002$). ΔHHb-PEAK correlated with FFM% ($r = 0.50$; $P = 0.05$) only in controls. The observed moderate correlation between ΔHHb-PEAK and VO_2peak in controls did not reach statistical significance ($r = 0.41$; $P = 0.12$). In SMA, $Fatigue_{6MWT}$ was inversely correlated with W_{peak} ($r = -0.50$; $P = 0.03$), and $Fatigue_{SME}$ was inversely correlated with FFM% ($r = -0.55$; $P = 0.02$) and VO_2peak ($r = -0.69$; $P = 0.002$), but fatigue measures were not associated with VO_{2peak}, W_{peak}, or FFM% in controls.

Discussion

This study provides further support for mitochondrial dysfunction in SMA contributing to the clinical manifestations of reduced exercise capacity and fatigue. We observed a correlation between aerobic capacity and fat-free mass in SMA and control participants. In contrast, muscle oxygen uptake, representing local examination, was only associated with fat-free mass in controls. Individuals with SMA demonstrated increased fatigue that was associated with decreased aerobic capacity, workload, and fat-free mass. Since fatigue was not experienced during SME in healthy controls, no such relationships existed in this group.

Aerobic capacity reflects the integrative function of several body systems including the respiratory, cardiovascular, musculoskeletal, and neuromuscular systems. Aerobic capacity (VO_{2peak}) is the product of cardiac output and the arterio-venous oxygen (a-VO_2) difference, so functionally oxygen delivery and oxygen uptake at the tissue level are its primary determinants. Furthermore, each of these variables is affected by exercise training and deconditioning. Thus, it is likely that the attenuated oxygen uptake responses in SMA reflect a combination of deconditioning due to insufficient physical activity and abnormal muscle oxygen uptake. SMA patients have lower muscle mass compared to healthy people and, because muscle tissue is the largest contributor to energy expenditure during exercise, this undoubtedly contributes to the decreased VO_{2peak} in SMA. Our results demonstrated clear and

Table 2. Group comparisons on clinical assessments.

Variable	SMA (n = 19)	Control (n = 16)	P-value[1]
VO_{2peak} (mL/kg per min)	13.0 (7.9–25.6)	44.1 (24.8–53.8)	<0.001
VO_{2peak}/LBM (mL/kg×min)	25.2 (16.8–45.7)	56.6 (34.3–77.1)	<0.001
Workload peak (W)	25.0 (10.0–65.0)	202.5 (145.0–324.0)	<0.001
ΔHHb-PEAK (μmol)	−0.5 (−8.4 to 4.6)	10.0 (3.4–31.4)	<0.001
ΔHHb-SME (μmol)	0.5 (−2.7 to 2.3)	6.8 (1.7–17.9)	<0.001
6MWT distance (m)	354.0 (137.0–557.0)	680.5 (591.0–767.0)	<0.001
FFM – whole body (%)	57.5 (42.1–83.3)	77.7 (68.0–86.4)	<0.001

Values presented are median (range). LBM, lean body mass; 6MWT, six-minute walk test; FFM, fat-free mass, expressed as a percentage of total mass; ΔHHb, change in deoxygenated hemoglobin; Peak, CPET; SME, submaximal exercise; SMA, spinal muscular atrophy.
[1]From Wilcoxon rank-sum test.

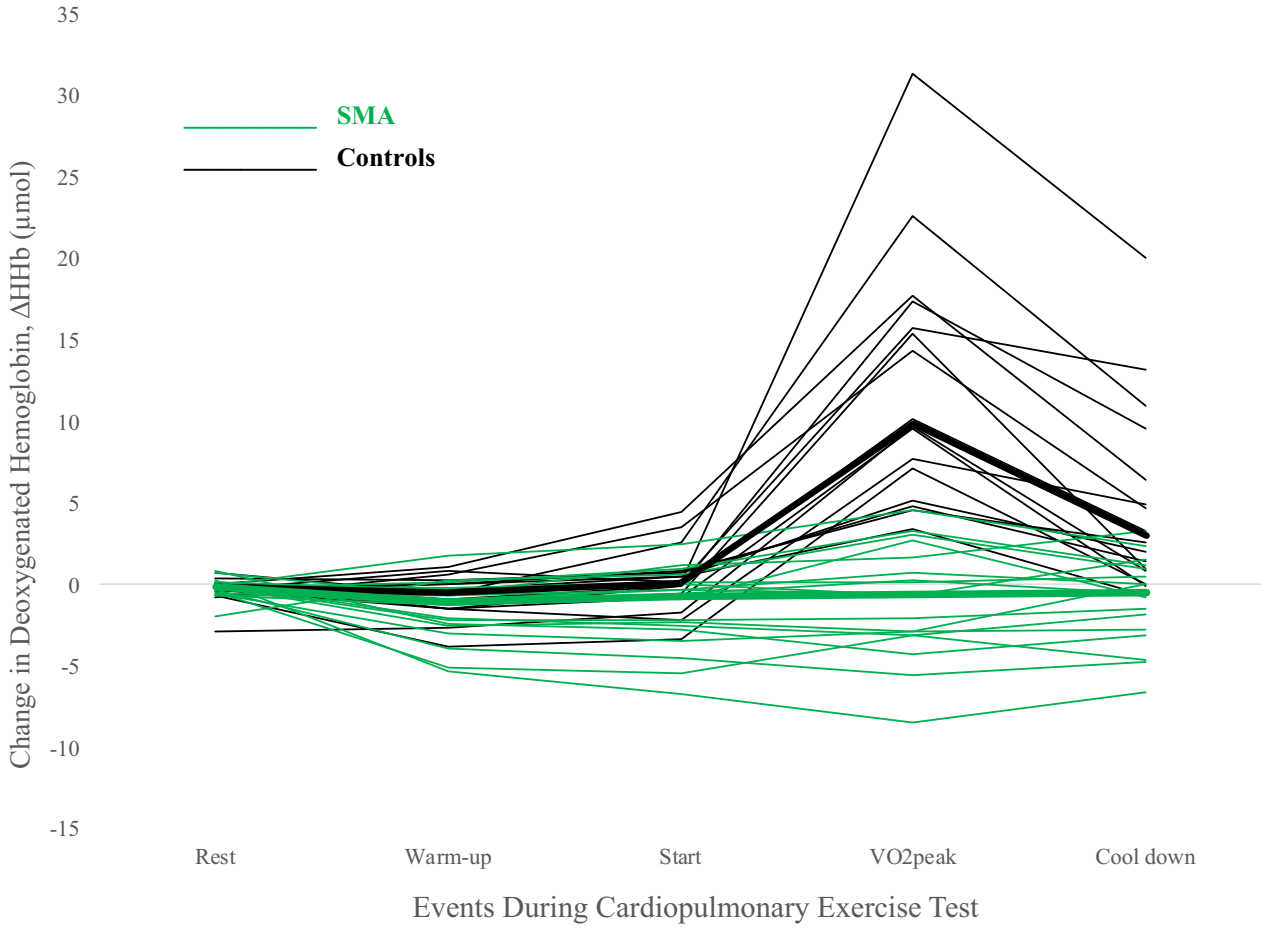

Figure 1. Muscle oxygen uptake measured as the relative change in deoxygenated hemoglobin (ΔHHb) during a CPET from rest to cool down in SMA (n = 19) and control (n = 16) participants. CPET, cardiopulmonary exercise tolerance test; SMA, spinal muscular atrophy. Light-weight lines indicate ΔHHb for individual participants; heavy-weight lines represent the median for SMA (green) and controls (black). Start, start of incremental exercise.

substantial abnormalities in muscle oxygen uptake, supporting the likelihood that individuals with SMA may not experience the expected widening of a-VO_2 difference during exercise that is typical in healthy individuals.

In healthy controls, deoxygenated hemoglobin increased with increasing workloads reflecting muscle oxygen uptake. In contrast, there was little or no increase in deoxygenated hemoglobin during exercise to peak aerobic capacity in SMA participants despite increasing workloads. While this study did not focus on oxygen delivery, no between-group differences in changes in total hemoglobin were observed, further supporting a deficiency in muscle oxygen uptake in SMA. These findings suggest that SMA patients may rely to a greater extent on anaerobic metabolic pathways (e.g., glycolysis) early in exercise compared with healthy people.

Unlike local examination using NIRS, VO_{2peak} reflects the aerobic capacity of all muscle groups during exercise, the sum of which can help support the relationship with fat-free mass.

Mitochondrial depletion has been associated with neurogenic atrophy in SMA.[33] Down-regulation of mitochondrial biogenesis has also been implicated in SMA and other neurodegenerative disorders.[34,35] Muscle tissue from SMA patients showed reduced mitochondrial DNA content and reduced PGC1-alpha, the primary cofactor of mitochondrial biogenesis, which was positively correlated with SMA disease severity.[7] Impaired mitochondrial proliferation may be a downstream result of SMN insufficiency. Mitochondria may also be implicated in the dysfunction of motor neurons in SMA. Axonal transport of mitochondria is impaired in motor neurons in SMA mouse models.[36] Mitochondria are also enriched at the presynaptic nerve terminals, modulate efficacy and plasticity of mature synapses,[37] and play an essential role in presynaptic differentiation.[38] Moreover, mitochondria

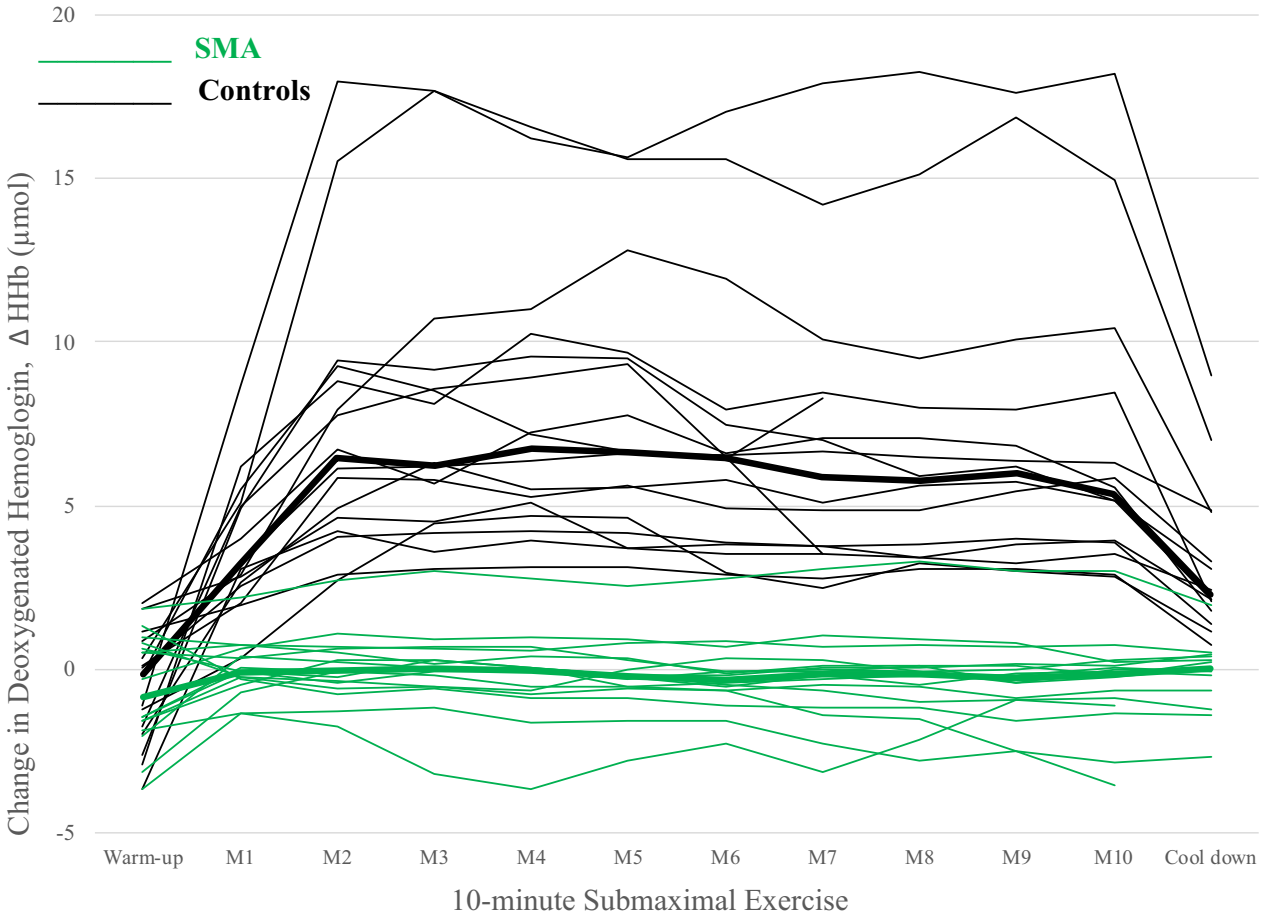

Figure 2. Muscle oxygen uptake measured as the relative change in deoxygenated hemoglobin (ΔHHb) during 10 minutes of submaximal exercise (SME) from warm-up to cool down in SMA (n = 19) and control (n = 16) participants. Light-weight lines indicate ΔHHb for individual participants; heavy-weight lines represent the median for SMA (green) and controls (black). M, minute; SMA, spinal muscular atrophy.

have shown to have a specific role in NMJ transmission specifically under intense stimulation in animal models.[39] Therefore, motor neuron defects in SMA patients may also be affected by the reduced or impaired mitochondrial function.

Exercise capacity measured by peak oxygen uptake (VO_{2peak}) is lower in ambulatory SMA patients compared to other myopathic[40–42] and motor neuron disorders,[43] perhaps reflecting in part a disease-specific mitochondrial dysfunction of the motor unit. The clinical manifestations of reduced mitochondrial biogenesis may be reflected in SMA patients' response to aerobic conditioning exercise. Muscle mitochondrial proliferation occurs with aerobic conditioning in healthy populations.[44] Adaptations in the oxidative capacity as a response to cycling exercise have previously been studied in SMA and other neuromuscular conditions. In our randomized controlled study of exercise in ambulatory SMA patients, the average baseline VO_{2peak} was 13.0 mL/kg per min[11] compared to a range of 17–36 mL/kg per min found in other neuromuscular conditions. Moreover, there were minimal average increases (1.8%) in VO_{2peak} following 12 weeks of exercise in SMA. In other studies, most other disease groups had significant positive training effects (up to 47%) using similar training protocols.[40,42] Ambulatory SMA patients took 6 months to elicit only modest improvements (6.6%) in exercise capacity (VO_{2peak}).[11] Similar improvements in VO_{2peak} were observed in a shorter time frame with an intensive training program but limited by a severe, debilitating fatigue.[12] SMA is thus characterized by apparently reduced muscle oxygen uptake, impaired response to exercise training, and debilitating fatigue, some of which may stem from intrinsic defects in the motor unit.

Fatigue was measured as % change in workload or distance in the first compared to the last minute of SME and the 6MWT as reported previously.[32] Notably, fatigue demonstrated on the 6MWT was inversely correlated with fat-free mass and aerobic capacity in SMA. Since fatigue is

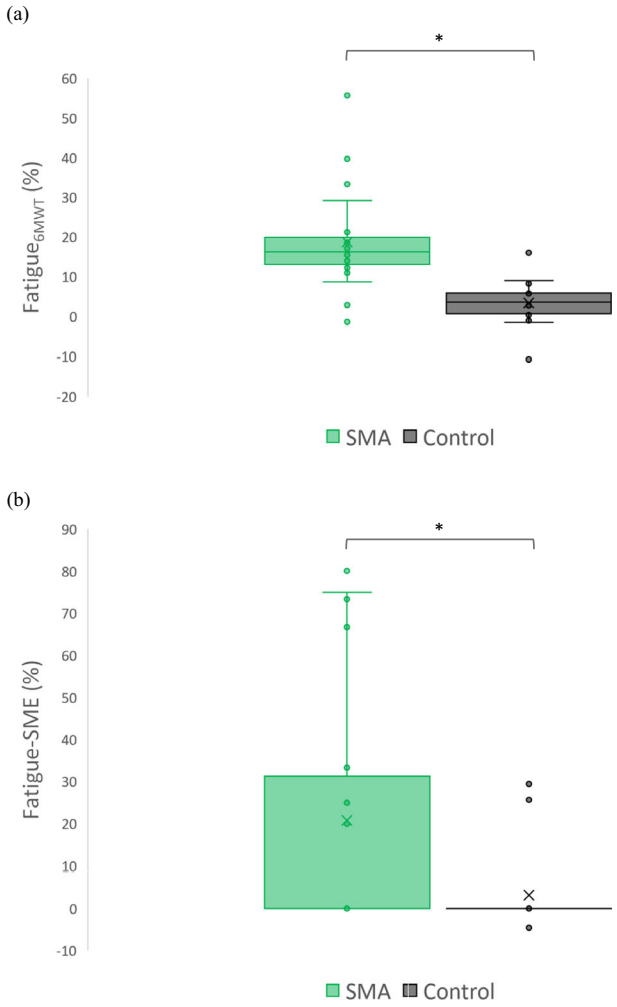

Figure 3. (A and B) Fatigue represented as percent change in (A) distance walked from 1st minute to the 6th minute of the 6MWT and (B) workload from the 1st minute to the 10th minute of submaximal cycle exercise in SMA participants (n = 19) and healthy controls (n = 16). 6MWT, six-minute walk test; SME, submaximal exercise; SMA, spinal muscular atrophy. *P < 0.001.

not experienced or minimal during SME in healthy controls, there were no relationships with FFM% or VO_{2peak}. Fatigue is a complex phenomenon including both psychological and physiological components. The two domains of fatigue include perceptions of fatigue (subjective) and performance fatigue or fatigability (objective).[45] Perceived fatigue can be assessed with patient-reported assessments investigating feelings of mental and/or physical tiredness, including momentary perceptions of fatigue and more "chronic" aspects. Performance fatigue is the decline in one or more aspects of performance during a continuous or prolonged task. These two domains of fatigue are not only different but potentially independent of each other,[45] including in SMA.[46]

Performance fatigue may be a sign of synaptic transmission failure in SMA. Functional deficits and fatigue may occur at the NMJ as suggested by pre-clinical studies in transgenic models where repetitive stimulation at high frequency resulted in intermittent neurotransmission failures.[8] Similar findings in SMA patients highlighted dysfunction at the NMJ using repetitive nerve stimulation[47] and in a separate study was related to decrement in performance during the 6MWT.[10] Similarly, performance fatigue is a clinical signature of myasthenia gravis (MG) resulting from the impaired neuromuscular transmission and is observed during the 6MWT in untreated MG patients.[48]

In SMA, performance fatigue has been quantified using functional outcome measures.[32,49] Using the 6MWT, fatigue was demonstrated by a 17% decrease in gait velocity from the first to the last minute. Similar degrees of fatigue (16.2%) were observed in this study despite Nusinersen treatment in more than 2/3 of the SMA participants. In contrast, performance fatigue on the 6MWT was not observed in patients with other neuromuscular conditions and weakness, despite similar functional ability, perhaps representative of a mechanistic defect of neurotransmission unique to SMA.[48]

In patients treated with Nusinersen, improvements in walking distance on the 6MWT were observed with only stabilization of performance fatigue.[13] Ambulatory children with SMA in this early phase, open-label clinical trial (NCT01703988) demonstrated clinically meaningful increases in walking distance on the 6MWT and modest decreases or stabilization in fatigue.[13] The fatigue observed in SMA was not reduced to the same degree that distance walked with Nusinersen improved/increased. Investigating mechanisms underlying performance fatigue in SMA and understanding why Nusinersen treatment fails to mitigate it to the extent desired may be key to improving current therapies for the human disease.

To date, approved treatments include Nusinersen, targeting the dysfunctional SMN2 gene, and Risdiplam, a small molecule SMN2 gene splicing modifier for infants, children, and adults. Onasemnogene abeparvovec-xioi, which replaces the absent SMN1 gene using an AAV9 viral vector, is also available and is administered intravenously in children <2 years old. Both Risdiplam and Onasemnogene abeparvovec-xioi have the ability to cross the blood-brain barrier and still reach vulnerable spinal cord motor neurons while exposing muscle tissue. The initiation and thus timing of intervention with currently available SMA therapies can, for the most part, be controlled, limiting opportunity for claims that one agent is more effective than the other. However, the manner in which the agents are administered and thus the range of tissues in which repletion is effected remains a factor that can be used not only to distinguish one therapy from

another but also to understand the impact of SMN insufficiency of muscle on the residual symptoms of fatigue and reduced exercise capacity.

Limitations

This study is not without limitations. The vastus lateralis muscle was chosen because it is typically affected in SMA and its role in normal biomechanics of cycling. However, because it is usually among the weakest muscles in the lower extremity, other muscle groups (e.g., plantar flexors) may have played a greater role in pedaling during exercise. While knee extensors are not associated with gait function in SMA,[32] their role in cycling has not been evaluated. Our choice in this study was not because of its role in gait, rather its primary role in cycling and previous studies incorporating NIRS of the vastus lateralis in other conditions. It is possible that the observed minimal changes in muscle oxygen uptake in SMA were a result of the insufficient viable muscle of the vastus lateralis to contribute meaningfully to the NIRS signal. Future studies should include muscle imaging or other techniques to determine the volume and viability of the muscles evaluated using NIRS. Furthermore, understanding the contributions of different muscles to lower extremity muscle function during exercise and evaluating muscle oxygen uptake of both more and less impaired muscles will be informative.

Muscle oxygen uptake was examined by non-invasive means using relative changes in deoxygenated hemoglobin. Direct measures of oxidative metabolism during exercise were not performed. In healthy individuals, lactic acid is a product of glycolysis and plasma lactate concentrations typically rise with increasing intensity of exercise. Inter- and intra-lactate shuttles facilitate the use of lactic acid as an energy source by skeletal muscle and other tissues (e.g., myocardium) during and following exercise.[50] Patients with mitochondrial myopathy have high resting plasma lactate levels attributed to skeletal muscle production that outpaces clearance.[51] It is possible that, due to mitochondrial dysfunction, there is a limited ability of the muscle to oxidize lactate in SMA. Blood lactate has not been studied in vivo during exercise in SMA but may serve as an informative biomarker of skeletal muscle metabolism and aid in our understanding of the underlying mechanisms of fatigue.

Motor unit and muscle fiber recruitment influences muscle activation and thus influences oxygen demand and uptake. This study focused on the metabolic function of muscle during exercise incorporating near-infrared spectroscopy (NIRS) for the first time in participants with SMA. Future studies could incorporate surface electromyography to investigate the impact of motor unit recruitment during exercise on muscle oxygen uptake. Additionally, investigating the spinal reflex arc using the H-reflex may help to better understand central factors contributing to fatigue.

We used DEXA as a measure of body composition to estimate fat-free mass. Fat-free mass includes lean mass and bone. A more precise, volumetric assessment of muscle mass may be more informative in understanding whether there is an attenuation of VO_2 per unit of muscle, which would further support the dysfunction of the skeletal muscle in SMA. Muscle MRI has been used in SMA to evaluate individual muscle involvement, atrophy, and fatty infiltration[52] and should be used in future studies to better understand its impact on muscle function during exercise.

Lastly, the present investigation involved small numbers of SMA participants and healthy controls. Multi-center studies involving larger numbers of participants are needed to confirm our findings, especially in light of the number of statistical tests performed.

Conclusion

The results from this study add to the growing body of evidence suggesting that mitochondrial dysfunction contributes to the clinical phenotypes and symptoms experienced by individuals with SMA, including fatigue and reduced exercise capacity. This includes preclinical and clinical evidence of muscle, NMJ, and motor neuron mitochondrial dysfunction. This is further supported by other clinical observations of persistent fatigue related to impaired neurotransmission and blunted responses to exercise conditioning. Understanding the subcellular mechanisms underlying diminished muscle oxygen uptake and increased fatigue during exercise in SMA may reveal additional cellular targets for therapeutic intervention. Treating such tissues, for example, muscle, may result in better outcomes than achieved following intrathecal (CNS) restricted therapies such as Nusinersen.

Acknowledgments

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Author Contributions

JM, AMG, MPM, AKR, CEG, and DCD contributed to the conception and design of the study; JM, AMG, MPM, DU, FMH, KC, KMC, JC, MH, AKR, UM, CEG, and

DCD contributed to data acquisition, analysis and interpretation of the data, and to drafting the text and preparing the figures.

Conflicts of Interest

JM: advisory boards for Biogen, Roche, and Scholar Rock; consultant for Biogen. UM: consultant for Genentech/Roche. AMG, MPM, DU, FMH, KC, KMC, JC, MH, AKR, CEG, and DCD have no commercial relationships that are relevant to this study.

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