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RESEARCH ARTICLE

# Neuromuscular electrical stimulation resistance training enhances oxygen uptake and ventilatory efficiency independent of mitochondrial complexes after spinal cord injury: a randomized clinical trial

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## Abstract

The purpose of the study was to determine whether neuromuscular electrical stimulation resistance training (NMES-RT)-evoked muscle hypertrophy is accompanied by increased $\dot{V}O_2$ peak, ventilatory efficiency, and mitochondrial respiration in individuals with chronic spinal cord injury (SCI). Thirty-three men and women with chronic, predominantly traumatic SCI were randomized to either NMES-RT ($n$ = 20) or passive movement training (PMT; $n$ = 13). Functional electrical stimulation-lower extremity cycling (FES-LEC) was used to test the leg $\dot{V}O_2$ peak, $\dot{V}E/\dot{V}CO_2$ ratio, and substrate utilization pre- and postintervention. Magnetic resonance imaging was used to measure muscle cross-sectional area (CSA). Finally, muscle biopsy was performed to measure mitochondrial complexes and respiration. The NMES-RT group showed a significant increase in postintervention $\dot{V}O_2$ peak compared with baseline ($\Delta\dot{V}O_2$ = 14%, $P < 0.01$) with no changes in the PMT group ($\Delta\dot{V}O_2$ = 1.6%, $P$ = 0.47). Similarly, thigh ($\Delta CSA_{thigh}$ = 19%) and knee extensor ($\Delta CSA_{knee}$ = 30.4%, $P < 0.01$) CSAs increased following NMES-RT but not after PMT. The changes in thigh and knee extensor muscle CSAs were positively related with the change in $\dot{V}O_2$ peak. Neither NMES-RT nor PMT changed mitochondrial complex tissue levels; however, changes in peak $\dot{V}O_2$ were related to complex I. In conclusion, in persons with SCI, NMES-RT-induced skeletal muscle hypertrophy was accompanied by increased peak $\dot{V}O_2$ consumption which may partially be explained by enhanced activity of mitochondrial complex I.

**NEW & NOTEWORTHY** Leg oxygen uptake ($\dot{V}O_2$) and ventilatory efficiency ($\dot{V}E/\dot{V}CO_2$ ratio) were measured during functional electrical stimulation cycling testing following 12–16 wk of either electrically evoked resistance training or passive movement training, and the respiration of mitochondrial complexes. Resistance training increased thigh muscle area and leg $\dot{V}O_2$ peak but decreased $\dot{V}E/\dot{V}CO_2$ ratio without changes in mitochondrial complex levels. Leg $\dot{V}O_2$ peak was associated with muscle hypertrophy and mitochondrial respiration of complex I following training.

*electrical stimulation; oxygen uptake; mitochondrial complexes; skeletal muscle hypertrophy; spinal cord injury*

## INTRODUCTION

Aerobic training has been continuously recommended for improvement of peak oxygen uptake ($\dot{V}O_{2peak}$) and cardiorespiratory fitness (1). Several groups have clearly demonstrated that improvement in peak $\dot{V}O_{2peak}$ is associated with attenuated rates of insulin resistance, glucose intolerance, and cardiovascular mortality (2, 3). Persons with spinal cord injury (SCI) are often severely immobilized and have decreased cardiorespiratory fitness that has been linked to the prevalence of chronic comorbidities including cardiovascular disease (CVD) which exceed that of the general population by 2–4-fold (4, 5); moreover, CVD is among the leading causes of mortality in this population (4, 6). Activities of daily living are not adequate to maintain cardiovascular fitness in persons with SCI (7, 8). Therefore, it is logical to assume that

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rehabilitation protocols that are directed toward enhancing $\dot{V}O_2$ may ameliorate several of the CVD consequences in the SCI population.

Several modalities exist for activity-based rehabilitation protocols that may increase cardiovascular fitness after SCI (9). Functional electrical stimulation (FES)-lower extremity cycling (LEC) has gained considerable attention in enhancing cardiovascular fitness after SCI (10, 11), perhaps in part because FES exercises the large paralyzed muscle groups of the lower extremities (12). Benefits of FES to cardiovascular fitness in persons with SCI vary among studies. For example, a previous review revealed a wide amplitude of changes in $\dot{V}O_2$ peak in response to FES training after SCI, ranging from 0.47 to 2.5 L/min (11, 12), due in part, perhaps to differences in FES techniques or workload applied. Submaximal exercise using FES-LEC may only elicit 40%–60% of measured $\dot{V}O_2$ peak in this population (11). In addition, measuring the $\dot{V}O_2$ peak has been conducted by using arm-cycling ergometer tests in persons with SCI which challenge the specificity of the training of the paralyzed muscles (13, 14). To circumvent these limitations, a submaximal index of measuring ventilatory efficiency relative to $CO_2$ production ($\dot{V}E/\dot{V}CO_2$) has been previously proposed during FES-LEC (15). The utilization of $\dot{V}E/\dot{V}CO_2$ ratio not only provides an indirect assessment of the cardiovascular fitness, but reflects the training specificity of the paralyzed muscles during FES-LEC (15). We reasoned that increasing leg $\dot{V}O_2$ peak during exercises in persons with SCI could be an important means of preconditioning candidates for spinal stimulation technologies to prepare their muscles and cardiovascular system for the workload produced in upright walking (16).

Unlike FES-LEC, the effects of neuromuscular electrical stimulation-induced resistance training (NMES-RT) on cardiovascular fitness are not well studied after SCI. NMES-RT robustly stimulates muscle hypertrophy in persons with motor complete SCI (17–20), decreases intramuscular fat (IMF) and visceral adipose tissue (18, 19), and improves mitochondrial capacity (20, 21). Recent work by Gorgey et al. (18, 22) showed that 16 wk of NMES-RT accompanied by low-dose testosterone replacement therapy increased basal metabolic rate and muscle specific tension and slowed time to peak torque. In this report, NMES-RT also increased mitochondrial density and activity as determined by measuring citrate synthase and succinate dehydrogenase (21). The findings supported earlier relationships between muscle size and biomarkers of mitochondrial health in persons with SCI (23). The effects of NMES-RT on measures of cardiovascular fitness such as $\dot{V}O_2$ peak, or on function of mitochondria located within paralyzed muscle groups, has not been examined. Another alternative to NMES-RT is passive movement training (PMT). Limited evidence exists to support the hypothesis that PMT may improve cardiorespiratory fitness via enhancing afferent drive from the exercising limb (24, 25) Unlike applications of NMES-RT, PMT is easily administered by a therapist, caregiver, or patient (26). However, FES-LEC was found to be superior to continuous PMT in improving measures of cardiovascular fitness ($\dot{V}O_2$ peak, arteriovenous oxygen difference, and cardiac output) in persons with tetraplegia (27). Whether NMES-RT is better than PMT for increasing muscle size and measures of cardiovascular fitness after SCI remains unclear.

To provide SCI-specific measures of fitness, we have measured peak oxygen consumption (leg $\dot{V}O_2$ peak) and $\dot{V}E/\dot{V}CO_2$ ratio (a measure of the workload at which lactate levels and hence respiratory rates rise) during progressive FES-LEC. $\dot{V}E/\dot{V}CO_2$ has been shown to be an effective marker of cardiovascular fitness after SCI (15). Thus, the purpose of the current study was threefold: *1*) to determine whether, NMES-RT evoked muscle hypertrophy as compared with passive movement training (PMT) will increase $\dot{V}O_2$ peak observed during exercise of paralyzed muscles in persons with motor complete SCI; *2*) to determine whether submaximal $\dot{V}E/\dot{V}CO_2$ ratio and substrate utilization can be enhanced following NMES-RT as compared with PMT; *3*) how training alters mitochondrial complexes rates of respiration following NMES-RT as compared with PMT.

## MATERIALS AND METHODS

### Participants

Participants were recruited to one of two clinical trials (registered at clinicaltrials.gov: NCT01652040 and NCT02660073 (28, 29; Table 1). The study protocols were approved by Institutional Review Boards at Hunter Holmes McGuire VA Medical Center and Virginia Commonwealth University. Study procedures were explained to each participant before enrollment. Following a written informed consent, a physical examination was performed by a certified physician for each participant. Thirty-three individuals aged between 20 and 61 yr, with chronic (≥1 yr post injury) SCI (C5-L1; American Spinal Cord Injury Classification A or B) were included. Data from these two studies were combined with the goal of maximizing sample size. In the NCT02660073, participants were randomized into either neuromuscular electrical stimulation resistance training (NMES-RT; $n$ = 13) or passive movement training (PMT; $n$ = 13) twice a week for 12 wk, with the goal of studying the effects of evoked muscle hypertrophy on cardio-metabolic risk factors after SCI. Participants ($n$ = 7) in the NCT01652040 trial underwent 16 wk of NMES-RT + testosterone replacement therapy (TRT), twice a week of evoked RT with the goal to study the additive effects of TRT on NMES-RT compared with TRT only on muscle size. Detailed study procedures for both trials were previously published (10, 19). The rationale of combing samples was that participants in both trials followed the same NME-RT protocol as previously highlighted (28, 29). Furthermore, the magnitude of changes in $\dot{V}O_2$ peak in TRT and NMES-RT group was similar to that in the NMES-RT only (data not shown). Finally, participants underwent measurement of $\dot{V}O_{2peak}$ using an FES-LEC ergometer (leg-$\dot{V}O_2$ peak) as previously described by our laboratory (10).

### Interventions

#### *Passive movement training.*

Passive movement training was performed by experienced research staff. Starting with the right leg the knee joint was held with one hand while cupping the leg proximal to the ankle with the other hand to bring the knee from 90° flexion to full extension. Once a full knee extension was achieved, research staff held the leg up for 5 s before bringing it back to

**Table 1.** *Physical and SCI characteristics of all participants enrolled in the PMT and NMES-RT groups*

| | Characteristics | PMT Groupb ($n = 13$) | NMES-RT Group ($n = 20$) | *P Values (within/between)* |
|---|---|---|---|---|
| Physical characteristics | Age, yr | 41.5 ± 13 | 39 ± 11 | –/0.5 |
| | Weight-BL, kg | 69.2 ± 13.1 | 78.9 ± 14.6 | 0.8/0.07 |
| | Weight-PI, kg | 67.4 ± 13.5 | 78.5 ± 16.7 | |
| | Height-BL, cm | 177.1 ± 8.1 | 177.5 ± 8.9 | 0.8/0.4 |
| | Height-PI, cm | 175 ± 11 | 177.2 ± 7.6 | |
| | BMI-BL, kg/m$^2$ | 23.3 ± 5.0 | 25.1 ± 4.3 | 0.99/0.17 |
| | BMI-PI, kg/m$^2$ | 22 ± 4.6 | 25 ± 5.1 | |
| | Sex | Male (10), female (3) | Male (18), female (2) | |
| | Ethnicity | White (6), Black (7) | White (11), Black (9) | |
| | Total caloric intake-*week 1*, kcal/day | 1,738 ± 979 ($n = 13$) | 1,883 ± 638 ($n = 13$) | (0.2/0.57) |
| | Total caloric intake-*week 12*, kcal/day | 1,433 ± 516 ($n = 9$) | 1,567 ± 363 ($n = 7$) | |
| | % Carbohydrate intake *week 1* | 45 ± 7.7 ($n = 13$) | 44 ± 7.3 ($n = 13$) | (0.22/0.59) |
| | % Carbohydrate intake *week 12* | 46.5 ± 7.7 ($n = 9$) | 48.5 ± 6.5 ($n = 7$) | |
| | % Fat intake *week 1* | 36.4 ± 7 ($n = 13$) | 36 ± 5.5 ($n = 13$) | (0.1/0.5) |
| | % Fat intake *week 12* | 34 ± 7 ($n = 9$) | 31.7 ± 5.6 ($n = 7$) | |
| | % Protein intake *week 1* | 17.75 ± 4.2 ($n = 13$) | 17.7 ± 4.4 ($n = 13$) | (0.31/0.37) |
| | % Protein intake *week 12* | 18.0 ± 4.0 ($n = 9$) | 20.0 ± 4.0 ($n = 7$) | |
| SCI characteristics | SCI classification | Paraplegia (9)/tetraplegia (4) | Paraplegia (14)/tetraplegia (6) | |
| | Neurological level of injury | C5–L1 | C5–T12 | |
| | AIS classification | A (7), B (4), C (2) | A (10), B (7), C (3) | |
| | Time since injury, yr | 11 ± 11 | 13 ± 11 | –/0.5 |

Discrepancy in the sample size of caloric intake and percentage macronutrients between BL and PI were attributed to the fact several participants did not turn in their dietary records toward *week 12* in the study. Within-group *P* values indicate statistical differences from baseline to postintervention and between-group *P* values highlight the statistical differences between PMT and NMES-RT groups. AIS, ASIA impairment scale classification; BL, baseline; NMES-RT, neuromuscular electrical stimulation-resistance training; PI, postintervention; PMT, passive movement training; SCI, spinal cord injury.

the starting position. The same procedure was followed for each repetition and a resting period of 3–5 s was allowed between each repetition. Four sets of 10 repetitions/leg were completed during each session (28).

### *Neuromuscular electrical stimulation resistance training.*

Each NMES-RT session lasted for ~45–60 min (18, 29). The goal was to complete 4 sets of 10 repetitions/leg. Participants were instructed to remove their shoes while sitting in a wheelchair. A pillow was placed behind the participant's leg to cushion against hitting the wheelchair. NMES was applied via two surface electrodes placed on the skin over the knee extensor muscle group of each leg. The distal electrode was placed approximately one-third the distance between the patella and inguinal fold and medially over the vastus medialis muscle. The proximal electrode was placed laterally and adjacent to the inguinal fold over the vastus lateralis muscle. A biphasic rectangular waveform having a pulse duration of 450 μs with a 50 μs interpulse interval was used. The stimulator frequency was set to 30 Hz, and the current was gradually increased to achieve a full knee extension. The leg remained extended for 3–5 s to evoke maximum tension in the activated motor units. The current was then gradually decreased to move the leg to its starting position (18, 28, 29). Each leg was allowed 3–5 s of resting between each repetition and ~2–3 min between sets. The first two training sessions were performed without ankle weights to ensure that the participant extend their leg against gravity. The ankle weights were gradually increased once the participant achieved full knee extension to attenuate the occurrence of muscle fatigue. Progression in weights was not allowed until participants were successful in completion 4 sets of 10 reps/each leg.

### *Dietary recalls.*

Each participant met with a dietitian at the start of the study and was asked to maintain a weekly 3- to 5-day food dietary log monitoring their caloric and liquid intake for the duration of the study (30). The dietary logs were administered to ensure controlling for the caloric intake and macronutrients between both groups during the course of the study. No nutritional advice was given on the size of the portion of the food. However, based on their basal metabolic rate, the dietitian recommended percentage macronutrients of 45% carbohydrates, 30% fat, and 25% total protein for both groups during the course of the study. Dietary logs were analyzed on a weekly basis using a nutritional software package (Nutrition Data System for Research v2014) under the supervision of a registered dietitian. After analysis was completed, the average caloric intake (kcal) and percentage macronutrients (carbohydrates, fats, and proteins) were calculated and a monthly feedback was provided via phone call (18, 19, 29).

## Measurements

### *Anthropometric measurements.*

Body weight was determined using a wheelchair scale (Tanita, PW-630U). The participant's weight was calculated by subtracting the weight of the participant's wheelchair from the combined weight of both the participant and wheelchair to the nearest 0.1 kg. The participant's height was measured in a supine position using a stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by height squared ($m^2$).

### FES-LEC peak $\dot{V}O_2$, $\dot{V}E/\dot{V}CO_2$ ratio, and substrate utilization.

FES-LEC was only used in this study for testing purposes. One week before intervention (*week 1*) and postinterventions 1 (*week 14*), FES-LEC peak oxygen uptake ($\dot{V}O_2$) was measured using a COSMED K4b2 (COSMED USA, Chicago, IL) portable metabolic unit (10, 28). After calibration, participants were asked to place the mask on their face to monitor oxygen ($\dot{V}O_2$) consumption and carbon dioxide ($\dot{V}CO_2$) production. A 3-min resting phase was allowed to familiarize participants to use of the breathing mask while connected to the RT-300 bike. After the resting phase, $\dot{V}O_2$ was measured during the 3-min warm-up phase, the resistance of the bike will then be gradually increased by 2 Nm every 2 min until fatigue into incremental stages. The measurements of $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E/\dot{V}CO_2$ ratio were conducted for the last 15 s at the end of each stage (10, 15). $\dot{V}O_2$ and $\dot{V}CO_2$ were monitored throughout exercise to determine total energy expenditure and substrate utilization [carbohydrate utilization (g/day) and fat utilization (g/day)] using the Weir equation. Five minutes of recovery were recorded to determine the efficacy of either intervention on energy expenditure and substrate utilization. Heart rate (via polar HR monitor) was recorded every 30 s and blood pressure (COSMED 740) was recorded before, every 2 min during cycling, and for another 5 min after cycling to ensure full recovery to baseline. Finally, power output of the FES-LEC was measured at baseline and postintervention in both groups.

FES-LEC was performed at the beginning (baseline) and end of training (postintervention) for both the PMT and NMES-RT groups (10, 14, 15). Full details of the FES-LEC protocol have been previously published elsewhere. Briefly, two adhesive large size electrodes ($8 \times 10$ $cm^2$) were applied bilaterally to the knee extensor, knee flexor ($7.5 \times 10$ $cm^2$), and gluteus maximus ($5 \times 9$ $cm^2$) muscle groups with a frequency set at 33.3 Hz and a pulse duration of 350 μs. The amplitude of the current was maximally set to 140 mA, 100 mA, and 80 mA for knee extensors, flexors, and gluteus maximus groups, respectively. Parameters were adjusted with the notion that knee extensors may elicit 75%–80% of the torque required to evoke one complete cycling revolution and accumulation of the electrical charges under the electrodes that were placed to activate both knee flexors and gluteus maximus muscle groups may elicit autonomic dysreflexia in persons with SCI. The stimulation parameters of FES-LEC remained unchanged between baseline and postintervention measurements because we were concerned that changing the stimulation parameters could alter muscle recruitment and confound the measured outcome variables.

FES-LEC was performed as follows. Although seated in a wheelchair, the participant was positioned and centered in front of the FES bike. The participant's feet were then placed gently inside the bike pedals and secured with crossed elastic Velcro straps. The participant's wheelchair was then secured to the FES bike by hooking two extendable hooks located at the base of the bike to stabilize the wheelchair during cycling. Two wooden bars were placed under the participant's wheelchair to prevent unnecessary movements during FES-LEC.

The testing protocol was as follows: 3 min of resting while attached to the bike, and 3 min of warm up with the stimulation turned off, during which the participant passively cycled at 30–35 revolutions/min (rpm) with the assistance from the motor of the bike. This was followed by 2-min exercise stages with the stimulation gradually ramping up to maintain the target cycling pattern (40–45 rpm). The resistance of the bike in each exercise stage was manually adjusted starting with 1 Nm (*stage 1*) and increased by 2 Nm for every 2 min of cycling [*stage 2* (3 Nm), *stage 3* (5 Nm)] until fatigue. The goal was to cycle against resistance without the motor support of the bike. The target speed during each exercise stage was set at 45 rpm. Resistance was only increased if the participant was able to cycle for the entire 2 min without attaining the preset fatigue threshold. Once a fatigue threshold of 18 rpm was attained, a 1-min cool down period at 30–35 rpm was immediately initiated by the assistance of the servo motor with the stimulation turned off. After the end of the cooldown period, the participant was asked to completely relax, and each participant underwent a recovery period of 5 min to collect oxygen uptake, heart rate, and ensure recovery of blood pressure (28). Blood pressure was monitored every 2 min during FES-LEC. In the event that participants developed signs of autonomic dysreflexia, the active FES-LEC was immediately ceased and blood pressure was measured to ensure recovery to an acceptable physiological range. If the condition persisted, the entire session was then terminated, and participants were then moved to the recovery phase. The leg $\dot{V}O_2$ peak, $\dot{V}E/\dot{V}CO_2$ ratio, and the other outcome variables were considered based on the last FES-LEC stage before developing autonomic dysreflexia.

### *Magnetic resonance imaging.*

Magnetic resonance imaging (MRI) was performed at baseline and postintervention from the hip joint to the knee joint (thigh) using a whole body coil (18, 19, 34, 35). A fast spin-echo sequence was implemented using a General Electric Signa 1.5-Tesla magnet (Waukesha, WI) and localized GE body array flex coil to ensure an adequate signal-to-noise ratio and image resolution (repetition time, 850–1,000 ms; echo time, 6.7 ms; field of view, 20 cm; matrix, $256 \times 256$). Trans-axial images (0.8 cm thick, 1.6 cm apart) were captured from the femoral head to knee joint to measure cross-sectional area (CSA) of thigh muscle groups and intramuscular fat (IMF) within each muscle group (i.e., absolute muscle CSA).

Image analysis was performed using Win-Vessel software (Ronald Meyer, Michigan State University, East Lansing, MI). Images were automatically segmented into fat, skeletal muscle, and background/bone (high, medium, and low intensity, respectively). To correct for intensity variations

caused by radio frequency heterogeneity, a first pass segmentation was used. The corrected image was then resegmented to the three intensity components using a fuzzy c-mean clustering algorithm. For each selected image, the anatomical regions of interest were manually traced pixel-by-pixel to quantify CSA ($cm^2$). Absolute IMF CSA was then quantified using a bimodal histogram and finding the midpoint between the muscle and fat peaks.

***Skeletal muscle biopsy and tissue preparation.***

Muscle biopsy samples were obtained from all participants following an overnight fast to measure mitochondrial respiration via using high-resolution respirometry (14, 21). Briefly, muscle biopsy specimens were collected from the right vastus lateralis (VL) using a 14-gauge Tru-Cut needle (Merit Medical Systems, South Jordan, UT) under local anesthesia (2% lidocaine). Immediately following the biopsy procedure, ~20 mg was placed in ice-cold biopsy preserving solution (BioPS media, 2.77 mM $CaK_2EGTA$, 7.23 mM $K_2EGTA$, 5.77 mM $Na_2ATP$, 6.56 mM $MgCl_2{\cdot}6H_2O$, 20 mM taurine, 15 mM $Na_2$ phosphocreatine, 20 mM imidazole, 0.5 mM dithiothreitol, and 50 mM MES hydrate, pH 7.1) for high-resolution respirometry. Muscle fibers were separated along the longitudinal axis using needle-tipped forceps under magnification yielding between 1 and 10 mg (average 4.9 ± 0.5 mg) of viable tissue. Due the presence of fat infiltration that is observed in muscles in individuals with SCI, an excess amount of muscle tissue was utilized (36, 37). The plasma membrane of muscle fibers was permeabilized by gentle agitation for 20 min at 4°C in BioPS containing 50 μg/mL saponin (38) followed by two 4-min washes in mitochondrial respiration buffer (miR05, 0.5 mM EGTA, 3 mM $MgCl_2{\cdot}6H_2O$, 60 mM lactobionic acid, 20 mM taurine, 10 mM $KH_2PO_4$, 20 mM HEPES, 110 mM D-sucrose, 1 g/L bovine serum albumin, pH 7.1).

***High-resolution respirometry measurement of permeabilized muscle fibers.***

High-resolution $O_2$ consumption measurements of separated muscle fibers suspended in 2 mL of miR05 buffer were performed at 37°C using the OROBOROS Oxygraph-2k (Oroboros, Innsbruck, Austria). Oxygen concentration and flux were recorded with DatLab software (Oroboros, Innsbruck, Austria). Respiration was measured using the following protocol: 10 mM glutamate + 5 mM malate (complex I substrates), followed by sequential additions of 0.1–1 mM ADP, 10 μM cytochrome c (to test for membrane integrity), 1 μM rotenone (complex I inhibitor), 10 mM succinate (complex II substrate), 40 μM 2-thenoyltrifluoroacetone (TTFA) (complex II inhibitor), 0.5 mM tetramethyl-p-phenylenediamine (TMPD), 10 mM sodium ascorbate (complex IV substrates), and 10 mM sodium azide (complex IV inhibitor); oxygen flux was expressed as pmol/s normalized to mg wet weight of the fiber bundle (39). Respiration rates were corrected for nonmitochondrial respiration by using the oxygen consumption rates determined following the addition of the corresponding inhibitors for complexes I, II, and IV. Respiration rates were expressed as inhibitor-sensitive rates to eliminate the contribution of oxygen consumption not related to oxidation of the specific substrate (39, 40).

### Statistical Analysis

All data were tested for normality using the Shapiro–Wilk tests. Outliers were detected using normal Q–Q plots at different time points (BL, PI) for each group. A mixed model ANOVA test (intervention × time) was used to determine if there were any interactions or main effects of NMES-RT or PMT on muscle CSA, $\dot{V}O_2$ peak, $\dot{V}E/\dot{V}CO_2$, substrate utilization, and rate of oxygen consumption of mitochondrial complexes I, II, and IV. When appropriate, a Bonferroni post hoc adjustment for multiple comparisons was performed to control for type II error. For mitochondrial complexes, missing data were estimated using the SPSS missing data package. Linear regression analyses were used to test the association between the $\dot{V}O_2$ peak and different mitochondrial complexes. The study was powered based on preliminary $\dot{V}O_2$ peak data following NMES-RT and yielded an effect size of 0.432 and a power of 99.82%. A sample size of minimum 19 participants/group was needed to detect differences between both groups. Partial eta squared ($\eta^2_p$) measurements were reported for the primary outcome variables. Statistical analyses were performed using IBM-SPSS v26.0 (SPSS, Chicago, IL). Statistical significance was set at an alpha level of 0.05 and all values are presented as means ± SD.

## RESULTS

Participant demographics, injury characteristics, and anthropometric measurements are presented in Table 1. There were no differences in participants' physical and SCI characteristics between both NMES-RT and PMT groups. Participants in the NMES-RT were 14% heavier than those in PMT group ($P$ = 0.07). Total caloric intake, percentage macronutrients of carbohydrate, fat, and protein for both NMES-RT and PMT are presented in Table 1.

### Whole Muscle and Knee Extensor CSAs Following NMES-RT or PMT

Figure 1 presents muscle CSA of absolute thigh (Fig. 1*A*) and knee extensor (Fig. 1*B*) for both the PMT and NMES-RT groups (Table 2). In the PMT group, no significant changes were observed for the thigh (ΔCSA = −6.0 $cm^2$, $P$ = 0.06) and knee extensor muscles (−4.7 $cm^2$, $P$ = 0.09). Following 12–16 wk of NMES-RT, maximum weights lifted were 15 ± 9 lbs. (6.8 ± 4.1 kg)/leg. NMES-RT resulted in 19% increase ($P$ = 0.002; $\eta^2_p$ = 0.28) in whole thigh muscle CSA whereas there was a 6% decrease following PMT. A significant interaction was noted in whole thigh muscle CSA between NMES-RT and PMT groups ($P$ = 0.0001; $\eta^2_p$= 0.59). In postintervention, whole thigh muscle CSA was greater following NMES-RT compared to PMT by 14.51 $cm^2$ ($P$ = 0.009; $\eta^2_p$ = 0.077).

Furthermore, NMES-RT resulted in a 30% increase ($P$ = 0.0001; $\eta^2_p$= 0.37) in absolute knee extensor muscle CSA, with a significant interaction between groups ($P$ = 0.0001; $\eta^2_p$ = 0.54). In postintervention, knee extensor muscle CSA was greater in NMES-RT compared to PMT by 10.36 $cm^2$ ($P$ = 0.029; $\eta^2_p$ = 0.036).

### Effects of NMES-RT or PMT on FES-LEC $\dot{V}O_2$ Peak

Following NMES-RT, maximum power of the FES-LEC ($P$ = 0.046) increased by 35.7% without any changes in the PMT

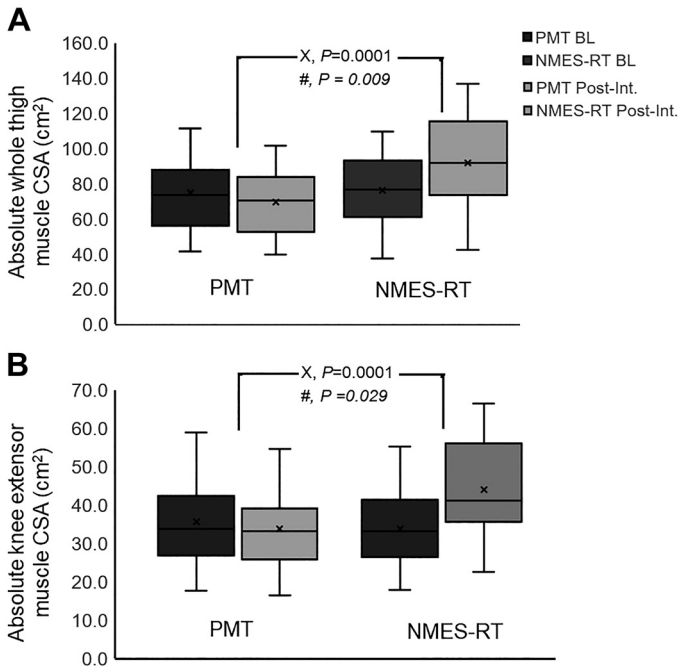


**Figure 1.** Distribution of muscle cross-sectional area (means ± SD) during baseline (BL) and postintervention (postint.) for both the PMT and NMES-RT groups for thigh muscle CSA (*A*) and knee extensor muscle CSA (*B*) in persons with SCI. CSA, cross-sectional area; NMES-RT, neuromuscular electrical stimulation-resistance training; PMT, passive movement training; SCI, spinal cord injury.

group (Table 3). At baseline and postintervention measurements, both groups demonstrated significant increase ($P < 0.0001$; $\eta^2_p = 0.65$) in FES-LEC $\dot{V}O_2$ peak compared with resting and warm-up $\dot{V}O_2$ (Fig. 2). The NMES-RT group showed a 14.2% increase in postintervention $\dot{V}O_2$ compared with baseline; whereas the PMT group demonstrated a $\Delta\dot{V}O_2$ of only 1.6%.

There were no between group differences ($P > 0.1$) in resting, warm-up, exercise, and recovery $\dot{V}O_2$ between NMES-RT and PMT. Exercise FES-LEC $\dot{V}O_2$ peak demonstrated interaction ($P = 0.023$; $\eta^2_p = 0.15$) between NMES-RT and PMT. A follow-up independent $t$ test indicated that there was a trend of greater (23%; $P = 0.09$) leg $\dot{V}O_2$ peak in NMES-RT compared with PMT. Delta $_{Excs\text{-}Rest}$ $\dot{V}O_2$ peak indicated an interaction effect ($P = 0.013$) following NMES-RT (BL: 257.7 ± 136.3 mL/min to PI: 299.5 ± 173.2 mL/min) compared with PMT (BL: 264.3 ± 180 mL/min to PI: 224.4 ± 158.4 mL/min).

The ratio of exercise $\dot{V}O_2$ peak to whole thigh muscle CSA indicated a trend toward interaction ($P = 0.09$) between NMES-RT and PMT groups (Table 2). However, a significant interaction ($P = 0.036$) was noted for the ratio of exercise $\dot{V}O_2$ peak to knee extensor muscle CSA between NMES-RT and PMT groups.

### Relationships between Muscle CSA and $\dot{V}O_2$ at Baseline and Postintervention

Postintervention, a positive relationship was observed between whole muscle CSAs and resting $\dot{V}O_2$ (whole muscle CSA: $r = 0.54$, $P = 0.01$ and knee extensor muscle CSA: $r = 0.49$, $P = 0.03$), warm-up $\dot{V}O_2$ (whole muscle CSA: $r = 0.38$, $P = 0.026$ and knee extensor muscle CSA: $r = 0.29$, $P = 0.09$), exercise $\dot{V}O_2$ peak (whole muscle CSA: $r = 0.45$, $P = 0.008$ and knee extensor muscle CSA: $r = 0.37$, $P = 0.03$), and recovery $\dot{V}O_2$ (whole muscle CSA: $r = 0.48$; $P = 0.04$ and knee extensor muscle CSA: $r = 0.39$, $P = 0.02$).

Delta changes in whole thigh muscle CSA (Fig. 3*A*) explained 31.2% of the variance in $\dot{V}O_2$ peak ($r^2 = 0.31$, $P = 0.01$) and absolute knee extensor muscle CSA (Fig. 3*B*) explained 15.2% of the variance in $\dot{V}O_2$ peak ($r^2 = 0.15$, $P = 0.04$)

### $\dot{V}E/\dot{V}CO_2$ Ratio Following NMES-RT or PMT

Both groups demonstrated significant decreases ($P < 0.0001$; $\eta^2_p = 0.16$) in exercise $\dot{V}E/\dot{V}CO_2$ ratio compared with resting, warm-up, and recovery n persons with SCI (Table 3) at baseline and postintervention FES-LEC. There were no

**Table 2.** *Physiological effects of NMES-RT and PMT on muscle CSA, ratio of $\dot{V}O_2$ peak to muscle CSA and mitochondrial complexes*

| | Baseline | Postintervention | *P* Values (within/interaction/between) |
|---|---|---|---|
| NMES-RT whole thigh muscle CSA, $cm^2$ | 76.2 ± 20 | 92 ± 25 | 0.002/0.0001/0.12 |
| PMT-whole thigh muscle CSA, $cm^2$ | 74.7 ± 20 | 70 ± 18 | |
| NMES-RT knee extensor muscle CSA, $cm^2$ | 33.8 ± 9.4 | 44.1 ± 13.7 | 0.0001/0.0001/0.29 |
| PMT- knee extensor muscle CSA, $cm^2$ | 35.6 ± 11.5 | 33.7 ± 11.0 | |
| NMES-RT exercise $\dot{V}O_2$ peak to whole thigh muscle CSA, $mL/min^{-1}/cm^{-2}$ | 7.0 ± 1.9 | 6.67 ± 2.1 | 0.8/0.09/0.1 |
| PMT-exercise $\dot{V}O_2$ peak to whole thigh muscle CSA, $mL/min^{-1}/cm^{-2}$ | 6.60 ± 2.5 | 6.9 ± 2.5 | |
| NMES-RT exercise $\dot{V}O_2$ peak to knee extensor muscle CSA, $mL/min^{-1}/cm^{-2}$ | 15.8 ± 4.5 | 14.0 ± 4.8 | 0.25/**0.036**/0.77 |
| PMT-exercise $\dot{V}O_2$ peak to knee extensor muscle CSA, $mL/min^{-1}/cm^{-2}$ | 14.1 ± 5.8 | 14.6 ± 5.7 | |
| NMES-RT complex I ($n = 9$)^, $pmol/s^{-1}/mg^{-1}$ | 10.3 ± 5.3 | 11.03 ± 6.8 | 0.8/0.87/0.67 |
| PMT complex I ($n = 10$)^, $pmol/s^{-1}/mg^{-1}$ | 11.62 ± 6.4 | 11.8 ± 6.64 | |
| NMES-RT complex II ($n = 9$)^, $pmol/s^{-1}/mg^{-1}$ | 8.0 ± 2.0 | 8.2 ± 4.1 | 0.72/0.57/0.74 |
| PMT complex II ($n = 10$)^, $pmol/s^{-1}/mg^{-1}$ | 8.2 ± 2.66 | 7.23 ± 4.45 | |
| NMES-RT complex IV ($n = 9$)^, $pmol/s^{-1}/mg^{-1}$ | 52.65 ± 26.2 | 62.0 ± 31.4 | 0.69/0.65/0.57 |
| PMT complex IV ($n = 10$)^, $pmol/s^{-1}/mg^{-1}$ | 52.5 ± 32.3 | 51.7 ± 33.3 | |

^Intent to treat analysis approach was used by accounting for missing data using missing value analysis-SPSS for the mitochondria complexes data. Originally 11 subjects (NMES-RT = 5 and PMT = 6; data are included in Supplemental Table S2) out of the 19 had complete mitochondrial data for both baseline and postintervention measurements. The remaining 8 subjects had only either baseline or postintervention mitochondrial data owing to poor muscle quality, technical issues with the oxygraph unit and data loss as a result of crashing of the hard drive. CSA, cross-sectional area; NMES-RT, neuromuscular electrical stimulation-resistance training; PMT, passive movement training; $\dot{V}O_2$, oxygen uptake.

**Table 3.** *Physiological effects of NMES-RT and PMT on FES-LEC $\dot{V}O_2$ peak, RER, total energy expenditure, substrate utilization, and cardiovascular response in persons with SCI*

| | Baseline | | | | Postintervention | | | |
|---|---|---|---|---|---|---|---|---|
| | Resting | Warm-up | Exercise | Recovery | Resting | Warm-up | Exercise | Recovery |
| NMES-RT $\dot{V}O_2$, mL/min | 255.5 ± 83.5 | 295.3 ± 121.5 | 513.3 ± 143.5* | 320.14 ± 85.7 | 280.0 ± 140 | 293.0 ± 106 | 579.3 ± 169$^{x}$* | 309.6 ± 91.5 |
| PMT-$\dot{V}O_2$, mL/min | 230 ± 59 | 247.3 ± 57 | 494.02 ± 218.2* | 274.03 ± 86 | 247.0 ± 88 | 260.3 ± 122 | 471.5 ± 178* | 268.4 ± 58.8 |
| NMES-RT $\dot{V}E/\dot{V}CO_2$ | 48 ± 11 | 46 ± 9 | 36 ± 6 | 43 ± 9 | 45 ± 7 | 44.5 ± 8 | 33 ± 5$^{\#}$* | 42 ± 7 |
| PMT-$\dot{V}E/\dot{V}CO_2$ | 47 ± 9.0 | 50 ± 10 | 39 ± 7 | 47 ± 9 | 45 ± 8 | 48 ± 10 | 38.5 ± 5$^{\#}$* | 44 ± 8 |
| NMES-RT RER | | | | | | | | |
| *Excs-S1* | 0.92 ± 0.10 | 0.85 ± 0.07 | 0.84 ± 0.14 | 1.17 ± 0.18 | 0.91 ± 0.12 | 0.85 ± 0.09* | 0.86 ± 0.09* | 1.28 ± 0.23* |
| *Excs-S2* | | | 0.99 ± 0.18 | | | | 1.1 ± 0.15* | |
| PMT RER | | | | | | | | |
| *Excs-S1* | 0.85 ± 0.12 | 0.84 ± 0.1 | 0.85 ± 0.12 | 1.17 ± 0.3 | 0.91 ± 0.12 | 0.88 ± 0.13 | 0.85 ± 0.11* | 1.3 ± 0.18* |
| *Excs-S2* | | | 1.06 ± 0.16 | | | | 0.99 ± 0.16 | |
| NMES-RT energy exp., kcal/day | | | | | | | | |
| *Excs-S1* | 1,678 ± 345 | 1,802 ± 400 | 2,204 ± 452 | 2,538 ± 560 | 1,685 ± 437 | 1,810 ± 481 | 2,351 ± 732 | 2,484 ± 882 |
| *Excs-S2* | | | 2,924 ± 1559 | | | | 3,417 ± 1525 | |
| PMT energy exp., kcal/day | | | | | | | | |
| *Excs-S1* | 1,686 ± 572 | 1,695 ± 351 | 2,120 ± 565 | 2,463 ± 942 | 1,617 ± 437 | 1,810 ± 543 | 1,986 ± 446 | 2,324 ± 599 |
| *Excs-S2* | | | 3,079 ± 1330 | | | | 3,086 ± 1169 | |
| NMES-RT max. carbohydrate utilization, g/day | 273 ± 108 | 210 ± 116 | 582 ± 387 | 575 ± 154 | 252 ± 117 | 207 ± 135 | 719 ± 380 | 567 ± 219 |
| PMT-max. carbohydrate utilization, g/day | 182 ± 146 | 164 ± 76 | 645 ± 297 | 519 ± 254 | 257 ± 152 | 218 ± 145 | 592 ± 253 | 539 ± 177 |
| NMES-RT max. fat utilization, g/day | 57.5 ± 38 | 99 ± 31.5 | 170.5 ± 199 | 15 ± 32 | 67.5 ± 56 | 101 ± 61 | 113.5 ± 73 | 12.5 ± 54 |
| PMT-max. fat utilization, g/day | 99 ± 53 | 108 ± 51 | 115 ± 70 | 31.5 ± 62 | 58 ± 44 | 96 ± 67 | 110 ± 68 | 7.5 ± 24 |
| NMES-RT maximum resistance of the bike, N | | | 3.2 ± 1 | | | | 3.8 ± 1.9 | |
| PMT maximum resistance of the bike, N | | | 7.4 ± 7.5 | | | | 4.9 ± 4.9 | |
| NMES-RT maximum power, W | | | 6.6 ± 3.3 | | | | 9 ± 4.4* | |
| PMT maximum power, W | | | 16 ± 18 | | | | 12 ± 16 | |
| NMES-RT HR, beats/min | 82 ± 32 | 77 ± 16 | 119 ± 55 | 90 ± 39 | 84 ± 34 | 90 ± 35 | 146 ± 52 | 92 ± 39 |
| PMT HR, beats/min | 88 ± 37 | 86 ± 36 | 116 ± 38 | 103 ± 55 | 98 ± 37 | 99 ± 41 | 127 ± 49 | 93 ± 34 |
| NMES-RT systolic BP, mmHg; *n* = 13 | 101 ± 21.5 | 102 ± 14.5 | 129 ± 28 | 109.5 ± 20 | 99 ± 17 | 102.5 ± 17.5 | 136 ± 46 | 117 ± 17 |
| NMES-RT diastolic BP, mmHg; *n* = 13 | 65 ± 12.5 | 62 ± 12.5 | 70 ± 14 | 70 ± 12.5 | 63 ± 10 | 67 ± 7 | 79 ± 24 | 74.5 ± 11 |
| PMT systolic BP, mmHg; *n* = 13 | 112 ± 20 | 111 ± 20 | 135.5 ± 28 | 118.5 ± 17 | 106 ± 19 | 1,110 ± 19 | 138 ± 27 | 114.5 ± 19 |
| PMT diastolic BP mmHg; *n* = 13 | 67 ± 12.5 | 67 ± 13.5 | 76 ± 18.5 | 73 ± 11 | 66.5 ± 14 | 68 ± 14 | 80 ± 23 | 67 ± 14 |

$^{x}$Statistical interaction compared with baseline exercise $\dot{V}O_2$; #significant differences between groups (NMES-RT and PMT); *significant increase in $\dot{V}O_2$ from resting or decrease in $\dot{V}E/\dot{V}CO_2$ ration from resting. S1, *stage 1* of FES-LEC testing with a resistance set at 1 Nm; S2, *stage 2* of FES-LEC testing with a resistance set at 3 Nm. FES-LEC, functional electrical stimulation-lower extremity cycling; HR, heart rate; NMES-RT, neuromuscular electrical stimulation-resistance training; PMT, passive movement training; RER, respiratory exchange ratio; RT, resistance training; $\dot{V}CO_2$, ventilatory efficiency relative to $CO_2$; $\dot{V}O_2$, oxygen uptake; SCI, spinal cord injury.

between ($P > 0.1$) group differences in resting, warm-up, and recovery periods between NMES-RT and PMT. There were group differences ($F$ = 4.3; $P$ = 0.046; $\eta^2_p$ = 0.12) in exercise $\dot{V}E/\dot{V}CO_2$ ratio between NMES-RT and PMT. Follow-up independent $t$ tests indicated postintervention exercise $\dot{V}E/\dot{V}CO_2$ ratio was lower in NMES-RT compared with PMT (mean difference: −5.3; $P$ = 0.01).

### Effects of NMES-RT Versus PMT on Whole Body Substrate Utilization

Neither intervention appeared to influence respiratory exchange ratio (RER), substrate utilization of fat or carbohydrates, or total energy expenditure. There was significant increase in RER values of the cooldown ($P$ = 0.021) and the recovery ($P$ = 0.013) periods compared with resting phase during FES-LEC testing in both groups (Table 3). Postintervention, RER showed a remarkable decrease in *exercise-stage 1* ($P$ = 0.027–0.12) followed by an increase in *exercise-stage 2* ($P$ = 0.004–0.12) and recovery ($P < 0.0001$).

Total energy expenditure (kcal/day) significantly increased in *exercise-stage 1* ($P < 0.0001$) and exercise-*stage 2* ($P < 0.0001$) and remained elevated in the recovery phase ($P < 0.0001$) following both interventions. Compared with resting phase, fat utilization (g/day) significantly increased during warm-up ($P$ = 0.001) and *exercise-stage 1* ($P$ = 0.009) and remarkably decreased during *exercise-stage 2* ($P$ = 0.001) and recovery ($P$ = 0.0001) phases. *Exercise-stage 2* ($P < 0.0001$) and recovery ($P < 0.0001$) phases increased carbohydrate utilization (g/day) without any changes in the warm-up ($P$ = 0.2) and *exercise-stage 1* ($P$ = 0.4) compared with the resting phase following either interventions (Table 3).

### Effects of NMES-RT and PMT on Mitochondrial Respiration for Complexes I, II, and IV

The rate of oxygen consumption for substrates that selectively donate reducing equivalents to complexes I, II, or IV did not change for either NMES-RT or PMT (Table 2 and Supplemental Table S2; all Supplemental material https://doi.org/10.6084/m9.figshare.14424188). At baseline, none of the $\dot{V}O_2$ measurements at rest and exercise were correlated to mitochondrial respiration. At postintervention ($n$ = 17), resting ($r$ = 0.57, $P$ = 0.015), exercise ($r$ = 0.68, $P$ = 0.002), and delta (difference in $\dot{V}O_2$ peak postpre) $\dot{V}O_2$ were positively correlated to respiration with complex I substrate ($r$ = 0.54, $P$ = 0.025). Finally, the rate of oxygen consumption of complex I was positively related to complex II ($r$ = 0.59, $P$ = 0.008).

## DISCUSSION

The current work investigated the hypothesis that evoking muscle hypertrophy may increase in $\dot{V}O_{2peak}$ during FES-LEC testing in persons with SCI and that the increase in leg

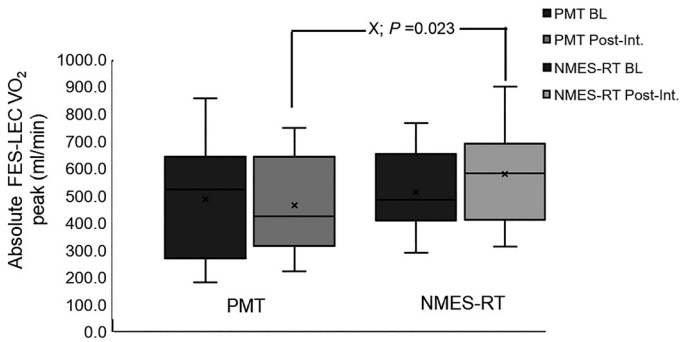


**Figure 2.** Distribution of absolute peak FES-LEC $\dot{V}O_2$ during baseline (BL) and postintervention (postint.) for both PMT and NMES-RT groups. X, interaction effects as a result of changes in postintervention measurements. Note that FES-LEC testing was only used for measuring cardiometabolic performance following NMES-RT and PMT. FES-LEC, functional electrical stimulation-lower extremity cycling; NMES-RT, neuromuscular electrical stimulation-resistance training; PMT, passive movement training; $\dot{V}O_2$, oxygen uptake.

$\dot{V}O_2$ peak is partially explained by the improvement in the rate of oxygen consumption of skeletal muscle mitochondria in persons with SCI. The major findings indicated that NMES-RT for 12–16 wk resulted in a remarkable increase in $\dot{V}O_2$ peak compared with PMT. In addition, there was a decrease in $\dot{V}E/\dot{V}CO_2$ ratio, a submaximal index of cardiovascular performance. The delta increases in thigh and knee extensor muscle CSAs explained 30% and 15%, respectively, of the variance in $\dot{V}O_{2peak}$ in persons with SCI. Finally, in a subset of the studied sample, $\dot{V}O_2$ peak was associated with complex I-mediated respiration independent of either intervention. However, neither intervention influenced the rate of oxygen consumption of mitochondrial complexes I, II, or IV.

### Rationale of the Work

The novelty of the work relies on the fact that, unlike published work that focused primarily on measuring submaximal or peak $\dot{V}O_2$ following exercise in persons with SCI (14, 37), we measured cardiovascular performance at three different sites including the cellular level (mitochondria), muscular level (leg-$\dot{V}O_2$ peak), and whole body ($\dot{V}E/\dot{V}CO_2$ ratio). The work originated from the research question of whether exercising paralyzed muscles would potentially provide additional cardiometabolic benefits to persons with SCI. Previous trials clearly indicated that exercising the lower extremity muscles was likely to drive improvement in insulin sensitivity, glucose tolerance, whole body $\dot{V}O_2$ peak, and markers of mitochondrial oxidation (41, 42). Others have shown that exercising lower extremity muscle of persons with SCI results in transformation of fiber types from fatigable fast-glycolytic to fast-oxidative glycolytic with greater mitochondrial density (43, 44). Following SCI, the shift in fiber type from slow oxidative to fast glycolytic with muscle atrophy may explain the increase in IMF, impaired glucose tolerance, and reliance on carbohydrates as the primary source of fuel (15, 36, 37, 45). NMES-RT has been shown to decrease IMF, and improves glucose tolerance and insulin sensitivity in persons with SCI (18, 19). Furthermore, Ryan et al. (20) also noted that 16 wk of NMES-RT was accompanied by a 25% increase in mitochondrial capacity as measured by phosphocreatine rate of recovery using magnetic resonance spectroscopy. Finally, recent work demonstrated that 16 wk of NMES-RT with low-dose androgen replacement resulted in muscle hypertrophy associated with increased in basal metabolic rate, faster time to peak torque, and elevation of markers of mitochondrial density (18, 21). These findings collectively indicated that exercising the paralyzed muscle to evoke muscle hypertrophy is accompanied by favorable fiber type switching from fast fatigable to fast-oxidative fatigue resistance fibers. Therefore, it was plausible to hypothesize that, as compared with PMT, NMES-RT may result in improvement in leg-$\dot{V}O_2$ peak, ventilatory efficiency, and substrate utilization after SCI.

### $\dot{V}O_2$ Peak and Exercise after SCI

FES-LEC was used to test for the effects of muscle hypertrophy on cardiovascular parameters to account for specificity of the training (10). Improvement in FES-LEC technology allowed development of exercise testing that ensures progression of the cycling resistance in a similar fashion to measuring $\dot{V}O_2$ max in healthy able-bodied controls. After 3-

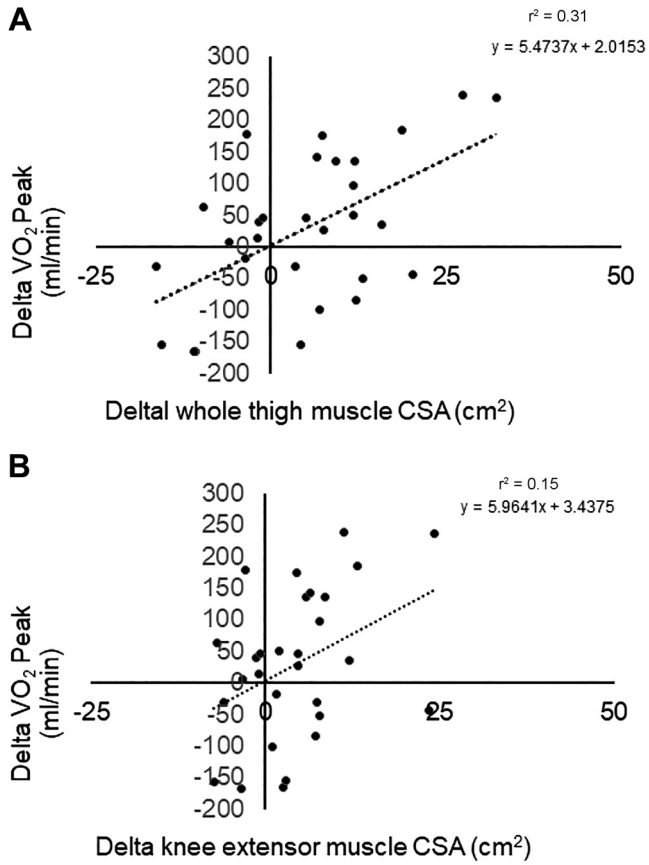


**Figure 3.** Relationships between changes in $\dot{V}O_2$ with respect to changes in thigh and knee extensor muscle CSAs at the start and end of training for both PMT and NMES-RT groups. $\Delta\dot{V}O_2$ vs. Δthigh muscle CSA (*A*) and $\Delta\dot{V}O_2$ vs. Δknee extensor muscle CSA (*B*). $\dot{V}O_2$, postintervention $\dot{V}O_2$ - baseline $\dot{V}O_2$; Δthigh muscle CSA, postintervention thigh muscle CSA - baseline thigh muscle CSA; Δknee extensor muscle CSA, postintervention knee extensor muscle CSA - baseline knee extensor muscle CSA; CSA, cross-sectional area; NMES-RT, neuromuscular electrical stimulation-resistance training; PMT, passive movement training; $\dot{V}O_2$, oxygen uptake.

min of warmup, the bike servomotor was turned off and the resistance gradually increased by 2 Nm increments during subsequent stages until fatigue. The increase in leg $\dot{V}O_2$ peak of 14% following NMES-RT was in line with a previous systematic review that noted an increase in $\dot{V}O_2$ peak by 11% and 28% following with FES-rowing and FES-LEC, respectively, in persons with SCI (12).

Persons with SCI are at the lowest end of the spectrum of physical activity with very low cardio-respiratory fitness (46). We and others have clearly shown that a submaximal acute bout of FES-LEC resulted in negligible increase in $\dot{V}O_2$ of 0.2 L/min in persons with SCI (10, 11). Previous work demonstrated that arm cycling exercise (ACE) is superior to FES-LEC in increasing submaximal $\dot{V}O_2$ at 40%, 60%, and 80% of $\dot{V}O_2$ peak in persons with SCI (11). The study demonstrated that hybrid exercise (ACE + FES-LEC) is the recommended mode of exercise to enhance cardiorespiratory fitness in persons with SCI (11). The study based this conclusion on the ability of hybrid exercise to increase sympathetic drive (ACE only), engage more muscle mass during exercise (ACE + FES-LEC), and enhance venous return and ventricular filling (FES-LEC only) (11, 12). Previous work suggested that reduced power output, as a result of altered mechanical efficiency, may be a primary factor for such low submaximal $\dot{V}O_2$ peak during FES-LEC (11, 12). Collectively, these findings have led to the hypothesis that evoking muscle hypertrophy of the paralyzed muscle may increase $\dot{V}O_2$ peak during FES-LEC testing. This conclusion was supported by the current findings which indicated that NMES-RT resulted in 35.7% increase in the maximum power output during FES-LEC.

### Leg $\dot{V}O_2$ Peak Versus Whole Body $\dot{V}O_2$ Peak

A novel aspect of the current work is the concept of leg $\dot{V}O_2$ peak that has not previously been addressed in persons with SCI. This concept was previously investigated in healthy controls following an acute bout of dynamic leg extension via using surface NMES. The delta $\dot{V}O_2$ for dynamic leg extension exercise was only 0.24 L/min (47). A similar increase in submaximal $\dot{V}O_2$ was reported following an acute bout of FES-LEC in persons with motor complete tetraplegia (10). Sympathetic dysfunction is likely to contribute to major difference between whole body $\dot{V}O_2$ peak and leg $\dot{V}O_2$ peak in persons with SCI (48, 49). In persons with SCI whose injury is at or above the sixth thoracic vertebra (T6), sympathetic dysfunction limits the rise in $\dot{V}O_2$ peak owing to attenuated effects on heart rate, stroke volume, and cardiac output. Therefore, a clear distinction should be made between whole body and leg-$\dot{V}O_2$ peaks, owing to the sympathetic nervous system decentralization above T6 level of injury. It should be noted that only 5 out of the 20 participants (25%) in the NMES-RT and 3 participants out of 11 in the PM-T (23%) were injured below T6.

### Ventilatory Efficiency ($\dot{V}E/\dot{V}CO_2$ Ratio)

The $\dot{V}E/\dot{V}CO_2$ ratio was used as submaximal index of cardiovascular performance following training in persons with SCI (15). Compared with PMT, NMES-RT resulted in a decrease of 5.3 of $\dot{V}E/\dot{V}CO_2$ ratio. Previously, the lowest $\dot{V}E/\dot{V}CO_2$ ratio during exercise has been suggested to be the most stable and reproducible marker of ventilatory efficiency in patients with heart failure (3, 50). The study clearly showed that $\dot{V}E/\dot{V}CO_2$ ratio has a comparable sensitivity (79% vs. 73%) and specificity (52% vs. 54%) with $\dot{V}O_2$ peak (50). Owing to the specificity of training, we primarily explored the possibility of using FES-LEC to test $\dot{V}O_2$ in persons with SCI. However as result of premature neuromuscular fatigue that is likely to occur with electrical stimulation, there is a skepticism that persons with SCI may attain a peak $\dot{V}O_2$ with FES-LEC testing (10). Therefore, the reliance on the $\dot{V}E/\dot{V}CO_2$ ratio provided a submaximal index that suggested improvement in cardiovascular performance following NMES-RT in persons with SCI (15). In addition, during low-intensity FES-LEC, the decrease in $\dot{V}E/\dot{V}CO_2$ ratio may reflect an increase in $CO_2$ or bicarbonate production (15). The decrease in $\dot{V}E/\dot{V}CO_2$ following NMES-RT and not following PMT may suggest that skeletal muscle hypertrophy has increased the buffering capacity for lactate production that commonly occurs during FES-LEC testing.

### Effects on Substrate Utilization

Persons with SCI suffer from impaired fat utilization as a source of fuel during exercise (15, 51). The sympathetic nervous system stimulates lipolysis in adipose tissue during exercise. Impairment of the sympathetic nervous system and diminished release of hormone sensitive lipase may be responsible for such findings. Prior work demonstrated impaired lipolysis during FES-LEC exercise in persons with SCI (15, 51). Kjaer et al. (51) noted impaired free fatty acid uptake during FES-LEC in persons with SCI. On the other hand, an acute bout of FES-LEC resulted in a remarkable increase in carbohydrate utilization with limited changes in fat utilization in persons with complete SCI (15). However, the study measured whole body substrate utilization during the entire duration of FES-LEC without considering different stages (15). Based on the current findings, low-intensity FES-LEC testing (i.e., *exercise-stage 1* set at 1 Nm) resulted in a significant increase in fat utilization whereas only high intensity FES-LEC (i.e., *exercise-stage 2* set at 3 Nm) resulted in a significant increase in carbohydrate utilization. The findings may suggest that mitochondrial beta-oxidation may be inadequate to meet demands for energy at high exercise intensity in persons with chronic SCI.

On the other hand, interest in training lower extremity muscles compared with upper extremity may rely on the fact the upper extremity muscles favor carbohydrate utilization compared with the large lower extremity training muscles that favors fat utilization (52). Previous work indicated that NMES-RT decreased IMF CSA; one interpretation of these changes is a possible effect on fat utilization (18, 19). The lack of directs effects on substrate utilization may be explained by the fact that current study used indirect calorimetry to assess whole body substrate utilization in response to both interventions. Future studies should consider measuring the rate of clearance and disappearance of fatty acids as a primary source of substrate utilization than using the whole body strategy (51).

### NMES-RT and Mitochondrial Complex-Specific Respiration

Mounting evidence indicates that skeletal muscle mitochondrial activity is impaired by up to 50%–60% after SCI

(53, 54). A positive association between muscle size and biomarkers of mitochondria density and activity has been noted (23). We have recently reported that 16 wk of NMES-RT with testosterone replacement therapy resulted in an increase in the enzymatic activities of citrate synthase and succinate dehydrogenase (21). However, we have noted no changes in the activity of complex III of the mitochondrial electron transport chain (21).

In the current study, we have expanded in our previous work by measuring the effect of inhibitors of three different mitochondrial complexes on mitochondrial oxygen consumption rate. Specifically, after muscle fiber permeabilization, we measured the rate of oxygen consumption of intact (31) mitochondria present in muscle biopsy samples using the appropriate substrate and inhibitors for each complex. Compared with published reports (31, 55), complex I had a very low respiration rate suggesting a mitochondrial deficit in complex I activity after SCI. A recent study showed that complex I respiratory rates in young healthy males were approximately ~40 pmol $s^{-1}$ $mg^{-1}$ and after 7 days of immobilization, the rates dropped to 30–35 pmol $s^{-1}$ $mg^{-1}$ (31). The study had a small sample size ($n$ = 4–5 subjects/group), suggesting that recruiting a large sample size for these types of studies is challenging even for healthy young individuals (31). Excessive reactive oxygen species production in skeletal muscle in persons with SCI may contribute to the complex I defect (30, 56). Another important aspect is that the content of the mitochondria that present in the muscle biopsy tissue in persons with SCI may be lower than what has been extracted in heathy able-bodied controls. Rasmussen et al. (57) reported that the yield of mitochondria extracted was lower in older (37 ± 7%) than in young adults (47 ± 5%) in a similar amount of skeletal muscle tissues, suggesting that altered muscle composition after SCI as characterized by increasing IMF may have impact upon the content of mitochondria present is the skeletal muscle samples. The findings indicated that there is an association between $\dot{V}O_2$ peak and complex I only after training; one interpretation of this finding is that that enhancement in $\dot{V}O_2$ may be linked to change in the electron transport chain activity of complex I. It is also possible to assume that with training may be improvement in upstream signaling pathway that stimulate mitochondrial biogenesis (i.e., PGC1-α), to increase mitochondrial content, similar to our recent findings (21).

### Limitations and Clinical Implications

There are number of limitations that need to be acknowledged in the current report. The participants included were pooled from 2 different clinical trials. Both trials implemented a similar NMES-RT protocol for 12 or 16 wk. In the 16-wk trial ($n$ = 7), participants were administered daily testosterone treatment and sub hoc analysis (Supplemental Table S1) indicated no additional effects of androgen replacement on $\dot{V}O_2$ peak or other parameters in the current study. Pooling of participants from two similar studies was done to ensure that the study was adequately powered and to yield the appropriate effect size of NMES-RT on $\dot{V}O_2$ peak. However, it is possible that addition of testosterone treatment and longer study duration in the 7 participants from previous study may inadvertently confound the current findings. A previous trial showed that $\dot{V}O_2$ peak did not change following 3-yr of testosterone treatment in elderly men; however, the treatment group did not experience decline in $\dot{V}O_2$ peak similar to the placebo group (58).

Owing to data loss and challenges obtaining muscle biopsies, postintervention measurements of mitochondrial complexes were estimated in 5 participants using the missing value feature available in our statistical software package (Supplemental Table S2). This may have masked the true changes of evoking muscle hypertrophy on mitochondrial complexes. This is unlike previous work that demonstrated that 12 wk of RT training resulted in changes in increase in a mitochondrial respiratory capacity of the trained muscles (59). Furthermore, in our respiration protocols, we did not measure state 4 respiration via the addition of oligomycin to inhibit ATPase synthase (55). Measuring state 4 respiration would have required us to concurrently run two separate protocols in two different chambers of the Oxygraph-2k (55). This approach was not possible due to the limited amounts of tissue available and the quality of the muscle tissue. The findings suggest that the current study was underpowered to reveal changes in mitochondrial respiration after SCI.

The clinical implications of the current work rely on the fact that conditioning paralyzed muscles may enhance cardiometabolic fitness, attenuate the effects of circulating cytokines and maximize the function before enrollment in clinical trials that are directed toward overground locomotion (60). The increase in muscle size and leg $\dot{V}O_2$ peak reported herein reflects the ability to overcome premature fatigue that commonly leads to task failure and limits functional activities in persons with SCI. At this point, there is no established minimal clinically important difference for $\dot{V}O_2$ peak in persons with SCI; clinically important differences in this population aligns more with symptoms and a function-centric approach (61).

### Conclusions

Overall, the current findings clearly indicated that 12–16 wk of NMES-RT resulted in a remarkable muscle hypertrophy that was accompanied by an increase in leg $\dot{V}O_2$ peak and a decrease in $\dot{V}E/\dot{V}CO_2$ as a submaximal index of cardiovascular performance. The findings did not support that improvement in $\dot{V}O_2$ is primarily linked to adaptions of the mitochondrial respiratory chain as measured by respirometry. Furthermore, NMES-RT or PMT did not influence substrate utilization. However, low intensity FES-LEC is likely to augment fat utilization compared with higher intensity FES-LEC that predominantly enhances carbohydrate utilization. The findings are of clinical significance to this population considering the need to attenuate cardiovascular problems and to enhance muscle quality before enrollment in future clinical trials targeting locomotion restoration in persons with SCI.

## SUPPLEMENTAL DATA

Supplemental Tables S1 and S2: https://doi.org/10.6084/m9.figshare.14424188

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

A.S.G. conceived and designed research; A.S.G., R.E.L., R.E.K., and J.R. performed experiments; A.S.G., R.E.L., C.C., Q.C., and E.J.L. analyzed data; A.S.G., C.C., Q.C., and E.J.L. interpreted results of experiments; A.S.G. prepared figures; A.S.G., C.C., and E.J.L. drafted manuscript; Q.C. edited and revised manuscript; A.S.G., C.C., and E.J.L. approved final version of manuscript.

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