The Effect of Body Weight-Supported Treadmill Training on Muscle Morphology in an Individual With Chronic, Motor-Complete Spinal Cord Injury: A Case Study

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Abstract

Objective: The purpose of this pilot study was to examine the effects of 4 months of thrice-weekly body weight-supported treadmill training (BWSTT) on skeletal muscle morphology in a woman (age 27 y) with chronic, motor-complete (ASIA B) spinal cord injury (SCI).

Methods: The participant performed passive thrice-weekly BWSTT for 4 months (48 total sessions) with manual assistance from therapists. Muscle biopsies of the vastus lateralis were taken prior to the beginning of the training program as well as following the completion of 4 months of training. Histochemical analysis was utilized to evaluate changes in muscle fiber size and type following training.

Results: At baseline, vastus lateralis muscle biopsies showed evidence of fiber atrophy and fiber type redistribution typical of persons with SCI, with mean fiber areas (and % distributions) of type I, type IIa and type IIx fibers being $3474\mu\text{m}^2$ (1.3%), $3146\mu\text{m}^2$ (30.8%) and $1284\mu\text{m}^2$ (68.0%), respectively. Following training, there were increases in treadmill walking speed (pre: 1.0km/h; post: 2.5km/h) and distance walked/session (pre: 500m; post: 1875m). Vastus lateralis mean fiber area increased by 27.1% and type I fiber % distribution increased to 24.6%, whereas type IIa and type IIx fiber % distributions both decreased following training.

Conclusion: These data indicate that 4 months of thrice-weekly BWSTT improved muscle morphology in an individual with chronic, motor-complete SCI.

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Key Words: Treadmill; Rehabilitation; Body weight support; Spinal cord injuries, incomplete; Muscle morphology

INTRODUCTION

It is well established that, following a spinal cord injury (SCI) in humans, affected skeletal muscle undergoes atrophy (1–5) and a fiber type shift toward fast-glycolytic, type IIx fibers (1–12). These early SCI-induced adapta-

tions are maintained, if not increased, into the chronic stage of injury (>1 year post-injury), resulting in a dramatic reduction in the proportion of type I (slow oxidative) fibers compared to the able-bodied population (2,6,7). Muscle atrophy and redistribution toward a less fatigue-resistant, type IIx fiber type may, in turn, contribute to the development of further complications. In particular, muscle atrophy may contribute to development of pressure sores (13) and reduced insulin-mediated glucose utilization (14,15), and the fiber type shift leads to early muscle fatigue (16). The ultimate result may be reduced quality of life for the individual (17).

The factors causing skeletal muscle atrophy and fiber-type shifting after SCI remain poorly understood (17).

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The dramatic reduction in neural input and mechanical loading following SCI have been hypothesized to be important factors determining the changes in muscle properties in humans (6). Therefore, exercise involving affected skeletal muscle is a potentially effective intervention to improve the injury-induced deconditioning of skeletal muscle in individuals with SCI (16); a reversal of the muscle atrophy and reduced oxidative fibers may, in turn, reduce the likelihood for development of related complications. However, due to the absence of voluntary control of skeletal muscle below the level of injury in individuals with motor-complete SCI, it is inherently impossible for this population to engage in regular exercise involving voluntary activation of the leg musculature (18). Numerous studies have demonstrated the ability of involuntary functional electrical stimulation (FES)-assisted cycling to cause a reversal of the complete SCI-induced adaptations in skeletal muscle properties (2,4,6,10). More recently, body-weight supported treadmill training (BWSTT) has emerged as a promising form of rehabilitation, allowing individuals who have deficits in independent walking ability to participate in upright treadmill ambulation. The participant is supported over the treadmill by a harness mechanism and an overhead pulley system using dynamic counterbalancing that can support a percentage of the participant's body weight. As required, a therapist is positioned at each of the legs of the participant to assist in the performance of a functional gait. In contrast to what occurs during FES, BWSTT does not involve 'external' stimulation of the musculature by electrodes. However, it has been shown that individuals with motor-complete SCI exhibit electromyographic (EMG) activity in lower extremity muscles during passive ambulation on a body weight-support (BWS) treadmill (19–21). Therefore, BWSTT may offer an adjunct or alternative therapy to FES in the motor-complete SCI population.

In a recent study performed at our center, 6 months of thrice-weekly BWSTT in individuals with chronic, motor-incomplete (American Spinal Injury Association (ASIA) C (22)) SCI was shown to: *i*) increase type I, type IIa and overall fiber cross-sectional area (CSA), *ii*) increase the percentage distribution and area of type IIa fibers, and *iii*) decrease the percentage area of type IIx fibers in the vastus lateralis (23). BWSTT is not commonly suggested as a form of therapy for individuals with chronic, motor-complete SCI, however, as the likelihood for improving independent ambulation (a common goal of BWSTT therapy) is poor within this population. This is unfortunate, because people with complete or motor-complete SCI could still benefit from the assisted pattern of locomotion inherent in BWSTT therapy despite their inability to voluntarily activate their leg musculature.

To our knowledge, there have been no studies to date that have examined the ability of BWSTT to improve muscle morphology in individuals with motor-complete SCI. Therefore, the purpose of this case study was to

investigate the effects of 4 months of thrice-weekly BWSTT on functional walking ability and skeletal muscle fiber size and type in an individual with chronic, motor-complete SCI.

METHODS

Participant

As it is not yet common practice to include individuals with motor-complete SCI in studies investigating the effects of BWSTT and because the muscle biopsy procedure is invasive, a case study was conducted. The participant for this study was recruited from an on-going 4-month BWSTT study in our laboratory. Volunteers for the 4-month BWSTT study had to be at least 18 years of age and diagnosed with a medically stable, chronic (>1 year since injury) motor-complete (ASIA A or B) traumatic SCI. Medical clearance was required to confirm no evidence or past history of ischemic heart disease, unstable angina, dysrhythmia, recent osteoporotic fracture, or tracheostomy.

Almost 5 years prior to beginning BWSTT, the participant for this case study (female; age 27; height: 170 cm; weight: 54.5kg) had a fall that resulted in a motor-complete SCI at C4 (ASIA B). Prior to this study, the exercise involvement of the participant included some arm ergometry and passive standing on a tilt table. This study was approved by the McMaster Research Ethics Board (MREB) and the participant provided written informed consent in accordance with MREB guidelines.

BWSTT Exercise Protocol

BWSTT sessions were conducted as described previously (24). Upright walking was completed on a motor-driven treadmill (Woodway USA Inc., Foster, CT) with a harness and overhead pulley system capable of supporting a percentage of the participant's body weight. Initial BWS and speed of the treadmill were chosen to enable appropriate gait with full knee extension during stance; the first week of the program (3 sessions) was a habituation period (59% BWS; 0.5 km/h; 3 × 5 min bouts). Leg movement was assisted by a therapist positioned at each of the legs of the participant; assistance was particularly important in extension of the knee and hip joints during the stance phase of the gait cycle. The participant was instructed to place her entire body weight over her fully extended leg during the stance phase of the walking cycle and to swing her arms and/or use the parallel supports for balance only. Our BWSTT protocol included an increase in speed and duration of walking and/or a decrease in the amount of BWS provided if and when possible; decisions to change training parameters were made according to our training protocol, outlined previously (24). The aim of BWSTT was to maximize independent walking, if possible. However, manual assistance of leg movements by therapists and provision of BWS were required throughout the training period for this participant with motor-complete SCI.

Treadmill Performance and Ambulatory Capacity

External BWS, walking speed, duration of the training sessions, and functional ambulation capacity were assessed pre-training and following 4 months of BWSTT (post-training). Baseline treadmill walking parameters were recorded for the session immediately following the habituation week (session #4). Functional ambulation capacity was assessed by the use of a scale developed by Wernig et al. (25) and modified for use in our testing center. The Wernig Walking Scale is a 6-item (0–5) classification scheme that delineates between independent and dependent walking with or without ambulatory aids. However, this scale is limited in its sensitivity because of the requirement for large changes in walking ability for a corresponding change in score on the Wernig scale. In effort to increase the sensitivity, we added to this scale so that it included separate levels for number of steps walked: 0, no voluntary walking capability, even with the help of two therapists; 1, can walk <5 steps with the help of two therapists or along parallel bars; 3, can walk >1 length of parallel bars (3 m), requiring assistance to turn; 4, can walk >1 length of parallel bars (3 m), turning independently; 5, able to walk along railing (<5 steps) with the help of one therapist; 6, can walk along railing (>5 steps) with the help of one therapist; 7, capable of walking with a rolling walking frame (“walker”) >5 steps; 8, can walk with canes (regular or four point) >5 steps; and 9, capable of walking without devices >5 steps.

Muscle Needle Biopsy

Prior to initiating the training program and within 3 days of completing the final training session, the participant had a muscle biopsy taken from the vastus lateralis of the right leg. A needle (5-mm Bergstrom) biopsy sample (100–150 mg) was obtained under local anesthesia (2% lidocaine) using a needle custom-modified for manual suction. The biopsy was taken from the middle portion of the vastus lateralis (15 ± 2 cm proximal to the lateral knee joint space) before and after the BWSTT program (within 2 cm of each other). The biopsy sample was placed in optimal cutting temperature (OCT) embedding medium (Tissue Tek, Sakura Finetechnical, Tokyo, Japan) with its fibers oriented perpendicular to the plane in which it was to be cut. The muscle portion placed in the OCT medium was then quickly frozen in 2-methylbutane cooled by liquid nitrogen and stored at -70°C until subsequent analysis.

Fiber Size and Type Analysis

Muscle fibers were characterized manually according to their adenosine triphosphatase (ATPase) activity following acidic pH (4.60) pre-incubation, as done previously (23). The embedded muscle sample was sectioned to 10- μm thickness at -20°C (Microm HM500OM, Walldorf, Germany) and the sections were transferred to microscope slides. The slides were incubated in an acid pre-incubation solution (pH 4.60). They were then rinsed in

distilled, deionized water (ddH_2O), after which they were bathed in a second incubation solution containing 0.003 M ATP and 0.06 M calcium chloride at pH 9.4 at 37°C . After 45 minutes, the tissue section was removed from the ATP solution, placed in 2% (w/v) cobalt chloride (CoCl_2) solution, and then placed in a solution of 1% (w/v) ammonium sulfide $[(\text{NH}_4)_2\text{S}]$ for 30 seconds.

Once stained, pictures of the muscle were collected at $\times 200$ magnification using a microscope (Olympus BX60, Melville, NY). Images were analyzed for fiber number, size, and area by using ImagePro Plus (version 4.1, Media Cybernetics, Silver Spring, MD). Muscle fibers were classified as type I, IIa, or IIx based on staining intensity. A total of 556 and 288 fibers were analyzed from baseline and post-training samples, respectively. The percentage distribution of each fiber type (I, IIa, and IIx) at baseline and post-training was calculated as the total number of fibers of each type divided by the total number of fibers. The total CSA of each fiber type was summed using SPOT software (Diagnostic Instruments Inc., Houston, TX). The relative percentage area of each fiber type (I, IIa, and IIx) was then calculated as the sum of that fiber's CSA divided by the sum of the total fiber CSA (I + IIa + IIx).

RESULTS

Treadmill Performance and Ambulatory Capacity

It required 55 scheduled sessions for our participant to complete the full 48 BWSTT workouts (with 7 cancellations), yielding a compliance rate of 87.3%. Medical complications unrelated to BWSTT, transportation difficulties, and personal commitments were reasons given for being unable to attend a BWSTT session. BWSTT walking speed (1.0 km/h pre; 2.5 km/h post) and distance walked/session (500 m pre; 1875 m post) increased considerably following BWSTT. The amount of body weight supported externally (59%) and functional locomotor capacity (scale score = 0) did not change throughout the BWSTT period, however.

Muscle Fiber Characteristics

Prior to training, the CSAs of type I, type IIa and type IIx fibers were $3474\mu\text{m}^2$, $3146\mu\text{m}^2$ and $1284\mu\text{m}^2$, respectively. Following 4 months of BWSTT, type I mean fiber area decreased by 18.6%, type IIa mean fiber area increased by 17.2%, and type IIx mean fiber area increased by 32.8%. The overall mean fiber area increased by 27.1% following 4 months of training. Fiber distribution was altered with BWSTT to an increased representation of type I fibers; prior to training, 1.3% of fibers were identified as being type I, but following training, 24.6% of fibers were classified as type I. The increase in type I fiber percentage distribution was accompanied by a decrease in both type IIa and IIx fibers percentage distributions. Similar changes occurred in the percentage area occupied by each fiber type after training. All of the muscle fiber characteristics are depicted in Table 1.

Table 1. Muscle morphology pre- and post-BWSTT

	Mean	CSA (μm^2)			% Distribution			% Area		
		I	Ila	IIx	I	Ila	IIx	I	Ila	IIx
Baseline	1884	3474	3146	1284	1.3	30.8	68.0	2.3	51.4	46.3
Post-training	2395	2828	3686	1705	24.6	20.8	54.5	29.1	32.1	38.8

BWSTT, body weight-supported treadmill training

DISCUSSION

Skeletal muscle atrophy and fiber type shift in the direction of fast fibers occur rapidly secondary to SCI, becoming relatively stable at 1 to 2 years post-injury (1–12). These negative muscular adaptations, in turn, may lead to a decreased resistance to fatigue, decreased insulin-mediated glucose tolerance, and increased risk for pressure ulcers, all which could contribute to reduced quality of life (14–17). To date, the only option for individuals with motor-complete SCI to train the muscles in their legs has been through FES (cycling or walking), an intervention which has been shown to cause a reversal of these negative skeletal muscle adaptations (2,4,6,10). This case study has shown that passive BWSTT, without the use of extraneous electrical input, was an adequate intervention to promote muscle hypertrophy and fiber-type shift in an individual with chronic, motor-complete SCI. This novel finding, despite no observed improvements in independent walking ability, provides support for further exploration into the possible benefits of BWSTT for the complete SCI population.

At baseline, the vastus lateralis of our participant demonstrated the muscle fiber atrophy typical of chronic SCI (1–5). The type I, type IIa and type IIx CSA values for our participant were 15%, 19%, and 59% below those of age-matched able-bodied control values from the literature (26). Following training, there was an increase of ~27% in mean fiber area. Individual fiber type analyses revealed that most of this increase was due to hypertrophy of the type IIa and IIx fibers. The observed decrease in mean type I fiber CSA may be partially attributable to the difference in number of type I fibers available for CSA calculation in pre-training and post-training muscle samples (7 and 71, respectively). However, the increase in total type I % area suggests an overall increase in the representation of slow, oxidative fibers. Previous research incorporating either BWSTT in people with incomplete SCI (23) or FES-assisted cycling in the motor-complete population (27) also has demonstrated significant training-induced increases in fiber area. Therefore, BWSTT may provide an adjunct or alternative therapy to FES.

The most widely reported change in muscle morphology after SCI is the dramatic reduction in slow, oxidative (type I) fibers, together with an increase in the representation of fast-fatigable (type IIx) fibers (1–12). Indeed, the fiber type representation of our participant at baseline was characteristic of the SCI population, with percentage areas

of 2.3%, 51.4%, and 46.3% in type I, type IIa, and type IIx fibers, respectively. In comparison, the percentage fiber type area in healthy, untrained able-bodied women has been found to be $44.0 \pm 11.6\%$, $33.6 \pm 8.7\%$, and $22.4 \pm 10.3\%$ for type I, type IIa, and type IIx fibers respectively (26). Following the 4 months of thrice-weekly BWSTT, there was an increased representation of type I fibers and a decreased representation of both type IIa and type IIx fibers in our participant, despite the fact that she only completed 48 (out of a possible 55) training sessions. Although previous studies have shown FES-assisted exercise to cause a similar shift toward slower fibers in the vastus lateralis of individuals with motor-complete SCI (2,4,6,10), our findings are novel in the identification of an increased percentage of type I fibers following training.

We can only speculate on the possible mechanisms behind an increased representation of type I fibers following BWSTT, as the signalling mechanisms involved with the exercise-induced regulation of muscle properties remain elusive, with multiple control pathways likely being implicated in the role of neuromuscular activity (28). It is possible that the combination of *i*) very low initial type I fiber representation and *ii*) the loading and neural activity provided by BWSTT despite no extraneous electrical stimulation was sufficient and required for the observed increase in type I fiber representation in our participant. The role of muscle spasticity as a potential contributor to training-induced changes in muscle morphology should also be explored. It has been hypothesized that muscle spasticity can serve to maintain or even increase the type I fiber representation in individuals with SCI (29). Although not measured, our participant did demonstrate some BWSTT-induced extensor reflexes of her legs during stance and resistance to manual knee extension during swing with no voluntary motor control of the active muscles.

BWSTT is a relatively novel form of therapy that allows individuals with compromised independent walking ability to undergo 'functional ambulation' training. Although this form of exercise is financially costly and physically demanding to the therapists, and could carry some risks to the participant (autonomic dysreflexia, pressure sores, orthostatic intolerance, fracture), our participant was free from complications. Our data would suggest that the potential benefits of BWSTT in individuals with motor-complete SCI outweigh the associated risks and labour-costs, but future research is needed to validate this claim.

CONCLUSIONS

This case study has provided preliminary data indicating the potential ability of BWSTT to improve skeletal muscle fiber size and fiber composition in individuals with chronic motor-complete SCI despite no improvements in independent walking ability. To our knowledge, these data are the first to document changes in muscle morphology after BWSTT in a person with motor-complete SCI. This is a promising finding that, if replicated, could lead to a wider use of BWSTT as a therapy for the SCI population.

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