The Benefits of Walking Training Program to Cardiovascular Health in Individuals with Chronic Spinal Cord Injury

By

Ramzi Abdu Alajam
DPT, Loma Linda University, 2014
MPT, Loma Linda University, 2013
BSPT, Kind Saud University, 2009

Submitted to the graduate degree program in Rehabilitation Science and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

____
Committee Chair: Wen Liu, Ph.D.

____
Hannes Devos, Ph.D., DRS

____
Irina V. Smirnova, Ph.D.

____
Linda D’Silva, PT, Ph.D., NCS

____
Milind Phadnis, Ph.D.

Date Defended: 03/10/2020

The dissertation committee for Ramzi Abdu Alajam certifies that
this is the approved version of the following dissertation:

The Benefits of Walking Training Program to Cardiovascular Health in Individuals with Chronic Spinal Cord Injury

____
Chair: Wen Liu, Ph.D.

____
Graduate Director: Irina V. Smirnova, Ph.D.

Date Approved: 4/20/2020

Abstract

Background: Spinal cord injury (SCI) is a serious medical condition that impacts sensorimotor function and quality of life. Following SCI, individuals are subjected to various secondary complications, including cardiovascular events. Evidence showed that cardiovascular disease (CVD) is the common leading cause of death among people living with SCI. Physical inactivity in people with chronic SCI due to impaired/loss of motor function reduces cardiovascular fitness and increases multiple risk factors of CVD, including lipid profile disorder, diabetes, and elevated level of pro-inflammatory markers. Those factors play important roles in the development of CVD. Using body weight-supported treadmill training (BWSTT) as a form of aerobic exercise in people with SCI has shown promising results in improving cardiovascular fitness, as indicated by decreased resting and exercise heart rate (HR). However, the underlying mechanisms of resting and exercise HR adaptation after training are still unknown. Changes in leg muscle activity, cardiac autonomic function, lung capacity, and leg muscle spasticity after training might contribute to the change in HR, but quantitative examinations are needed. In addition, limited evidence is currently available concerning how BWSTT affects risk factors of CVD, including lipid profile, glycemic control, and levels of pro-inflammatory markers. Therefore, the objectives of this pilot study were: 1) to examine feasibility of an 8-week walking training program using a novel assistive training device in patients with chronic SCI; 2) to examine the potential association between changes in resting and exercise HR and changes in four potential factors after 8-week walking training in individuals with chronic SCI; and 3) to collect pilot data before and after the 8-week walking training program for evaluating changes in risk markers of CVD in study participants. **Methods:** This study used a pilot, single-group, pretest-posttest study design. All participants received 3 sessions a week for 8 weeks of walking training, using a treadmill, body weight-supported system, and novel assistive gait training device. Feasibility measures of recruitment, retention, compliance, and participants' performance were collected throughout the study period. Participants' perception about the walking training program and assistive gait training device were evaluated at the end of the study. Measurements of resting and exercise HR, electromyographic (EMG) activity of leg muscles, frequency-domain and time-domain measures of heart rate variability (HRV), vital lung capacity, and spasticity of leg muscles were performed before and after training. In regard to changes in risk markers of CVD, levels of hemoglobin A1c (HbA1c), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), C-reactive protein (CRP), and interleukin-6 (IL-6) were assessed before and after training. **Results:** Among 55 participants who were screened for eligibility, 15 participants agreed to enroll in the study, and a total of 11 participants completed the study. However, 3 participants withdrew from the study, and one participant was excluded from the study. Throughout the period of training, all participants were able to progress in their walking performance in terms of treadmill speed, walking distance, walking duration of the training, and percentage of body weight support. Based on responses to the end intervention questionnaire, the majority of participants showed acceptance and satisfaction with the walking training program and the use of the assistive gait training device. The change in the EMG activity of leg muscles was the only significant contributing factor to the change in exercise HR among the four factors examined. The changes in low-frequency power, high-frequency power and root mean square of the successive differences of HRV, and the change in vital lung capacity showed significant correlations with the change in resting HR. Following the walking training, levels of HbA1c, LDL-C, IL-6 significantly decreased, while the levels of HDL-C significantly increased. **Conclusion:** The findings of this dissertation project demonstrated the feasibility of

the 8-week walking training program as well as using our novel assistive training device in individuals with chronic SCI. The significant contribution by the increase in activities of leg muscles to exercise HR adaptation supports the clinical application of walking training targeting at improvement in cardiovascular function in individuals with chronic SCI. The exercise induced beneficial effect in modulations of cardiac autonomic function and lung capacity, which were significant contributors to resting HR adaptation. Besides, positive changes in risk markers of CVD were observed in individuals with chronic SCI following training.

Acknowledgments

First of foremost, I would like to express my thankful to Allah, my God, who has been on my side at all times throughout this academic journey and for this accomplishment as well as everything happening in my life.

I would like to thank my PhD adviser, Dr. Wen Liu, for his patience, constructive advice and continuous support throughout these years. His guidance has helped me increase depth of my research skills and realize my potential for research. Thank you, Dr. Liu, for your guidance to accomplish my dream by obtaining my PhD. My thanks also go to my committee members, Dr. Irina Smirnova, Dr. Hannes Devos, Dr. Linda D'Silva, and Dr. Milind Phadnis for their valuable comments, support, and guidance. Without my adviser and committee members, it would not be possible to achieve this journey.

Besides that, I would like to thank all members of Neuromuscular Research Laboratory for contribution and help in my research project. Without my lab mates' help, I would not be able to complete this research project.

I would like to express my deepest grateful to my parents and my wife for all of their prayers and encouragements to achieve my goals. Special thanks to my wife for all of perpetual support, not only throughout these years but also in my whole life. Big thanks to my children, Albaraa and Danah, for making my life for making my life flourish and full of happiness.

Last but not least, I would like to express my gratitude to my sponsorship, Jazan University, for providing me an opportunity to pursue my higher education and their financial support through these years.

Table of Contents

Chapter 1: Introduction

1.1. Background

1.1.1. An Overview of Spinal Cord Injury (SCI)

The spinal cord is a bundle of nerves that run throughout spinal columns and has 31 segmental levels. It is responsible for carrying out neuronal signals back and forth between the brain and peripheral nerves. Damage to the spinal cord can lead to disability (loss of sensory or motor functions) and disruption in functions of some organs due to loss of connections between the brain and body parts.[1] SCI may be a consequence of traumatic injury (such as motor vehicle accident, falls, sports-related injuries, gunshot, or knife wound) or non-traumatic injury (such as inflammation, infection, ischemia, or spine degeneration). It is classified as quadriplegia (tetraplegia) or paraplegia.[2] Quadriplegia can result from an injury in the cervical region, which involves loss of sensory or motor function in all four limbs. Paraplegia can occur as the result of an injury at T1 or below, which involves loss of function in the trunk and legs [1]. SCI is often described as incomplete or complete injury in sensory or motor function. In the incomplete injury, there is some degree of sensory and/or motor function remaining below the site of lesion, whereas all sensory and motor functions are lost below the site of the lesion for the complete injury. The American Spinal Injury Association (ASIA) has established a scale for classification of SCI [3]: A= no sensory and motor function; B = sensory is preserved, but no motor function; C = motor function is preserved and more half of key muscles below the lesion site < grade 3; D = motor function is preserved and at least half of key muscles below the lesion site ≥ grade 3; E = normal sensory and motor function.

In the United States, the incidence of SCI has been estimated to be 17000 new cases per year. The number of people in the United States who were alive in 2016 and had SCI was estimated to be approximately 282,000. The frequency of neurological categories of SCI is incomplete quadriplegia (47.2%), incomplete paraplegia (20.4%), complete paraplegia (20.2%), and complete quadriplegia (11.5%).[4] About 80 % of SCI occurs in individuals aged between 18 and 30 years-old [5, 6]. It was reported that about 53-70% of individuals SCI are unable to walk independently at one-year post-injury [7, 8]. SCI is a serious medical condition that impacts sensorimotor function and quality of life. Following SCI, individuals are subjected to various secondary complications, including cardiovascular events [9, 10].

1.1.2. Cardiovascular Disease (CVD) in Patients after SCI

With advances in medical care, the life expectancy of people with SCI has increased significantly as compared to the past few decades [11]. However, cardiovascular disease (CVD) is the common leading cause of death among people living with SCI and occurs at an early age in people with SCI as compared to able-bodied people [12]. The findings are consistent in demonstrating a high prevalence of CVD among people with SCI [5, 13]. It has been estimated that the prevalence rate of symptomatic CVD in the SCI population (30-50%) is significantly higher compared to the able-bodied population (5-10%) [4]. The SCI population has 3 times higher risk of heart disease and 4 times higher risk of stroke compared to the general population [14]. In terms of mortality from CVD, a cohort study that followed individuals with SCI for five years after discharge from inpatient rehabilitation found that 37% of individuals died due to cardiovascular causes [15].

1.1.3. Risk Factors of CVD in SCI Population

Physical inactivity is a major risk factor for the development of CVD in the general population [16, 17]. Likewise, lack of physical activity and/or prolonged sitting in people with SCI is associated with the increased risk of CVD [18]. Previous studies reported low levels of dynamic physical activity among individuals with chronic SCI, and around 50 % of them did not meet recommended levels of physical activity for people with disabilities [19, 20]. Physical inactivity contributes to increase in multiple risk factors of CVD, including reduced cardiovascular fitness, abnormal lipid profile, diabetes mellitus, and elevated levels of pro-inflammatory markers [18, 21, 22]. It is well known that reduced cardiovascular fitness as indicated by increased resting and exercise heart rate (HR) is associated with increased risk of CVD [23], and cardiovascular risk factors, such as abnormal lipid profile, diabetes mellitus and elevated levels of pro-inflammatory markers, play important roles in damaging endothelium of blood vessels and formation of atherosclerosis, leading to developments of ischemic heart disease, stroke, and peripheral vascular disease [24-26]. A previous study found that approximately 76.9 % of the SCI population had two or more risk factors for CVD [27].

Elevated Heart Rate (HR):

Elevated HR is linked to the increased risk of CVD [28]. A study showed that an increase in resting HR by 10 beats/minute increases the risk of CVD by 15% [29]. In healthy individuals, studies have found that a sedentary lifestyle results in increased resting and exercise HR [30, 31]. Thus, physical deconditioning following SCI due to loss/impaired motor function can contribute to increased HR at resting and during exercise [32]. Individuals with SCI, particularly those with paraplegia, have higher HR at rest and during exercise as compared to healthy individuals [33-37]. Due to the disruption of sympathetic autonomic control in individuals with cervical SCI, elevated HR seems to be more common in individuals with thoracic or lumbar SCI compared with those with cervical SCI [33, 34].

Abnormal Lipid Profile:

Abnormal lipid profile has been proved as a major risk factor for the development of heart disease [38]. Following SCI, there is a trend toward increased total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), as well as decreased high-density lipoprotein cholesterol (HDL-C) [39]. Individuals with chronic SCI had higher levels of TC and LDL-C, and lower levels of HDL-C as compared to age-matched able-bodied individuals [40]. A meta-analysis found significantly lower levels of HDL-C and higher TC/HDL-C ratio in individuals with SCI as compared to able-bodied individuals [41]. Furthermore, longitudinal cohort studies that followed individuals with acute SCI over one year reported increases in levels of TC, LDL-C, as well as a decrease in levels of HDL-C after one year [42, 43]. Vichiansiri et al. [44] assessed the prevalence of abnormal lipid profile in 90 individuals with chronic SCI and found that about 77% of participants had an abnormality in lipid profile, and about 78% of them had abnormal lipid levels of more than one type of lipids. Furthermore, individuals with complete or higher-level SCI had lower levels of HDL-C as compared to those with incomplete or lower-level SCI [45]. Individuals with paraplegia had higher levels of TC and LDL-C than those with quadriplegia [46]. In terms of ambulatory status, it was found that individuals with SCI who could not be community ambulating had a higher TC/HDL ratio than those who were community ambulating [47].

Diabetes Mellitus:

Another risk factor for CVD is persistently high levels of blood glucose resulting from insulin resistance. Impaired glucose tolerance and abnormal glucose homeostasis, which are usually observed in people with chronic SCI, increase the risk of diabetes mellitus and CVD. People with diabetes have a 2- to 4-fold increase in the risk of CVD compared to those without diabetes [48]. A study reported that 22 % of individuals with SCI had diabetes mellitus compared to 6 % in able-bodied individuals [49]. Another study found that the odds of type 2 diabetes was 2 times greater among individuals with SCI compared to those without SCI [50]. Individuals with complete SCI had even higher levels of glucose and insulin in the blood and higher incidences of diabetes than individuals with incomplete SCI [51]. The high rate of diabetes after SCI can be attributed to the lack of physical activity and consequently, alterations in body composition and muscle characteristics [52-55].

Inflammatory Status:

SCI can lead to increased adipose tissue, and frequent pressure ulcers and urinary tract infections as well as decreased levels of physical activity. All those conditions may contribute to elevated levels of pro-inflammatory markers in the blood [56]. Physical inactivity leads to an increase in the amount of adipose tissue and abnormal lipid profiles among people with chronic SCI, which contribute to elevated levels of inflammatory markers and consequently increase risks of CVD [49, 57]. According to the American Heart Association, inflammatory markers, an emerging nontraditional risk factor, have been shown to predict CVD across populations [58-60]. The American Heart Association has established clinical guidelines for the levels of C-reactive protein (CRP) in the blood: association with high (> 3 mg/l), average (1.0-3.0 mg/l), and low (< 1 mg/l) risk of CVD [61]. Plasma concentrations of CRP above 3 mg/l are associated with 2 times increase in the risk of stroke, 3 times increase in the risk of ischemic heart disease, and 4 times increase in the risk of peripheral vascular disease [62]. In studies in individuals with SCI, Gibson et al. [21] and Mann's et al. [22] reported a mean CRP level of 3 to 3.37 mg/l, which fell into the high-risk category. Furthermore, individuals with chronic SCI exhibited higher serum concentrations of CRP and interleukin 6 (IL-6) compared with age-matched able-bodied individuals, which might explain the increased risk of atherosclerosis [22, 63, 64]. Physically active persons with SCI had lower CRP levels than those who were physically inactive [22, 64-66]. Based on mobility status, Morse et al. [56] and Goldstein et al. [67] reported that individuals with chronic SCI who were wheelchair-dependent had greater CRP concentrations than those who could walk with assistive devices or independently.

1.1.4. Beneficial Effects of Exercise on Risk Factors of CVD

Regular exercise is necessary to reduce or prevent secondary complications following SCI, including cardiovascular events and cardiometabolic syndrome. According to the Canadian physical activity guidelines for adults with SCI, individuals with SCI should engage in at least 20 minutes of moderate to vigorous intensity aerobic exercises twice a week and strength training exercises twice a week [68]. Similarly, the American College of Sports Medicine recommends people with SCI to participate in moderate to vigorous intensity aerobic exercises 2 to 3 times per week from 20 to 30 minutes [69]. For cardiometabolic health benefits, a recent guideline suggested that adults with SCI should engage in 30 minutes moderate to vigorous intensity aerobic exercises 3 times per week [70]. A variety of modalities such as arm cycling, functional electrical stimulation (FES) leg cycling, circuit resistance training, and walking training have been utilized to improve cardiovascular health in the SCI population [71].

Upper Extremity Exercises:

Upper limb exercise has been shown to improve cardiovascular fitness and reduce risk markers of CVD in patients after SCI. A previous single group study reported a decrease in submaximal exercise HR in 8 individuals with cervical SCI after 8 weeks of low-intensity arm ergometer training [72]. When comparing the effects of 8 weeks of arm cycling exercise at different training intensities in 11 individuals with chronic SCI (low-intensity training group: n = 6 and moderate-intensity training group: n = 5), moderate-intensity training significantly reduced submaximal exercise HR compared to baseline, and it also significantly increased HDL-C and decreased triglyceride (TG), LDL-C, and the TC/HDL-C ratio as compared to low-intensity training [73]. A randomized study with 8 weeks of arm cycling exercise in 6 individuals with acute SCI found high-intensity training (n = 3) significantly decreased TG and TC/HDL-C ratio and improved insulin sensitivity in individuals with acute SCI to more extent than low-intensity training (n=3) [74]. In a randomized controlled trial in 15 persons with chromic complete SCI, 6-week hand-bike exercise program 3 times weekly significantly reduced the fasting levels of insulin and insulin resistance and increased the levels of HDL-C in participants in intervention group (n = 8) compared to those in the control group (n = 7) [75]. Similar findings were reported in other a single group studies with 12-week of arm cycling exercise in 5 individuals with chronic paraplegia [76] or 3-month of arm cycling exercise in 5 individuals with chronic SCI [77]. A randomized controlled trial in 17 individuals with chronic paraplegia showed a significant reduction in the levels of IL-6 in the intervention group (n = 9) compared to the control group (n = 8) [78]. Positive changes in levels of CRP and IL-6 were reported after 16 weeks of arm cycling exercise in 10 individuals with chronic SCI [79].

However, many of the studies, mentioned above, also reported no significant changes in some of the measured risk markers of CVD. For instance, no changes were observed in levels of TC and TG after arm cycling exercise in 5 individuals with chronic paraplegia [76] or 5 individuals with chronic SCI [77]. Groot et al. [74] observed no changes in levels of TC, HDL-C, and LDL-C values relative to baseline measures after 8-weeks of low-intensity or high-intensity arm cycling exercise in individuals with acute SCI. Bakkum et al. [79] reported no significant changes in levels of HDL-C, TG, and glucose in reference to baseline measures following 16-weeks of arm cycling exercise in 10 sedentary individuals with chronic SCI. Furthermore, a randomized controlled trial with 16 weeks of upper extremity aerobic exercise in individuals with chronic SCI concluded no significant benefits of the intervention in risk markers of CVD in participants in the intervention group (n = 12) compared to those in the control group (n = 11) [80]. A recent cross-sectional study reported no differences in lipid profile and the level of blood glucose between physically active (n = 27) versus physically inactive (n = 107) wheelchair-dependent persons with chronic paraplegia [81]. Hjeltnes et al. [35] observed a significant decrease in submaximal exercise HR in 10 individuals with subacute complete cervical SCI during 3-7 months of primary rehabilitation including arm aerobic exercise, but exercise HR remained higher compared to able-bodied individuals. Putting information together, inconsistent findings in past studies can be due to different methodological approaches, participants' characteristics (i.e., level and severity of injury, duration of injury, level of physical activity) and training protocols. Also, we need to take in consideration that arm aerobic exercise activates only small muscles in the upper body that may not generate sufficient challenges to the cardiovascular system [82].

Lower Extremity Exercises:

FES-leg cycling, and resistance training have been utilized to exercise paralyzed muscles below the level of the lesion and to activate large muscle mass in order to increase the level of physical activity and prevent cardiovascular complications after SCI. Some of past studies have shown that FES-leg cycling exercise or resistance training helped to improve cardiovascular fitness (i.e., HR adaptation) and reduced the risk makers of CVD following SCI [83-87]. Faghri et al. [83] examined the effects of 12-week FES-leg cycling on the cardiopulmonary response at rest and submaximal exercise test in a single group of 13 individuals with chronic incomplete or complete SCI. They found that resting HR slightly decreased in individuals with paraplegia and significantly increased in individuals with quadriplegia following training, and submaximal exercise HR significantly decreased in both groups. In regard to changes in risk markers of CVD, 8-10 weeks of FES-leg cycling exercise in 7 to 10 participants with chronic incomplete or complete SCI significantly decreased levels of glucose and insulin [86, 88] and levels of pro-inflammatory markers, including CRP and IL-6. [88]. A number of studies have explored physiological mechanisms of improvements in glucose homeostasis following lower extremities exercise. Past studies showed that 8 weeks of FES-leg cycling significantly increased levels of GLUT-1 and GLUT-4, insulin-stimulated glucose transport, and insulin-mediated disposal in 5 to 6 individuals with complete SCI [89, 90].

On the other hand, other studies found no positive changes in cardiovascular fitness or risk makers of CVD after FES-leg cycling or resistance training [91-96]. Hooker et al. [91] studied the effects of 19 weeks of FES-leg cycling on the maximal and submaximal cardiorespiratory response in a single group of 8 individuals with chronic complete SCI and demonstrated no significant changes in maximal and submaximal exercise HR after training. Another single group study showed no significant change in resting HR in 9 individuals with chronic complete motor SCI following 6 weeks of FES-legs cycle exercise [92]. Related to changes in risk markers of CVD, Griffin et al.[88] found no significant decreases in levels of TC, LDL-C, and TG and no significant increase in levels of HDL-C in a single group of 8 individuals with chronic incomplete or complete SCI after 10 weeks of FES-leg cycling exercise. Ward et al. [93] reported similar findings in 4 persons with chronic complete cervical SCI following 12 months of FES-leg cycling exercise. Ryan et al. [95] and Mahoney et al. [96] reported no changes in levels of glucose and insulin and no improvement in insulin sensitivity in 5 to 14 individuals with complete chronic SCI after 12-16 weeks of electrical stimulation resistance training.

Inconsistent findings in past studies can be due to different methodologies and training protocols as well as differences in participants' injury level and severity and duration of injury. In addition, we need to take into consideration that many patients reported muscle fatigue and pain due to frequent electrical stimulation, and they could perform FES-leg cycling exercise only for a short period of time [82, 97, 98]. This may explain the mixed results in the literature of FES-leg cycling studies.

Walking Training:

Walking training using a treadmill and body weight support system was developed to help patients to relearn independent walking following neurological conditions such as SCI or stroke [99]. Current research in SCI patients regarding the use of body weight-supported treadmill training (BWSTT) has focused on restoring motor function and walking ability. The evidence supports the use of BWSTT as a rehabilitation intervention to improve functional ambulation in people with SCI [100-102], particularly in those with motor incomplete SCI. People with motor incomplete (ASIA grade C or D) have a higher potential to regain walking ability following training as compared to those with motor complete SCI (ASIA grade A or B). Even though walking training might not help the majority of people with motor complete SCI to regain independent walking over ground, it can provide great benefits on other health outcomes, including prevention of comorbidity or secondary health complications [103]. However, studies examining the benefits of BWSTT on preventing or reducing secondary health complications after SCI are limited.

BWSTT can be used as a form of aerobic exercise in individuals with SCI. Preliminary results from a number of past studies have shown that regular BWSTT can help to improve cardiovascular and pulmonary function and to reduce risk factors of CVD in the SCI population.

In individuals with incomplete or complete SCI, BWSTT training significantly improved resting HR and blood pressure variability, oxygen consumption, and dynamic oxygen cost with the duration of training ranging from 6 weeks to 6 months [104-106]. Furthermore, previous studies have shown that 6-10 weeks of robotic-assisted BWSTT significantly decreased resting and exercise HR [107] and significantly improved other measurements of cardiovascular function in individuals with incomplete SCI [108]. In a past study of individuals with complete motor SCI, a sub-group of participants showed significant improvement in HR and blood pressure variability and artery compliance after BWSTT [82]. In terms of the pulmonary function, two past studies demonstrated that 4-6 weeks of BWSTT significantly improved respiratory parameters, including forced vital capacity, forced expiratory volume in 1 second, forced expiratory flow rate, and vital lung capacity, in individuals with incomplete or complete SCI [104, 109]. In regard to change in risk markers of CVD, a previous study by Phillips et al. [110] reported that 6-month BWSTT, in 6 individuals with motor incomplete SCI, significantly reduced glucose and insulin concentrations during the 2-hour oral glucose tolerance test. Similarly, the same training load significantly decreased levels of TC, LDL-C, and TC/HDL-C ratio and significantly increased levels of HDL-C in 6 individuals with motor incomplete SCI [111]. However, past studies had various limitations including small sample size and inclusion of only incomplete SCI and therefore limited the generalization of the findings. In addition, prior studies have not considered the intensity of walking training.

1.1.5. Why Upright Walking Training

As mentioned above, findings of aerobic exercise using arm cycling or FES-leg cycling have been inconsistent regarding their effects on risk factors of CVD in SCI survivors. Limitations of those modalities have been discussed in the literature. Arm cycling targets only upper limb muscles, which may be insufficient to stress the cardiovascular system [104, 106]. In addition, many wheelchair users report shoulder pain as a result of overuse [112]. FES-leg cycling can be performed for a short duration as patients often report muscle fatigue and pain due to frequent stimulation [113]. FES-leg cycling may also aggravate the symptoms of autonomic dysreflexia, particularly in those with SCI above T6 because of electrical stimulation [114]. Furthermore, both arm cycling and leg cycling exercise are performed in the sitting position which might not provide sufficient challenges to the cardiovascular system.

Upright walking can activate larger muscles in the body, including leg and trunk muscles. Even with the absence of supraspinal control from the brain in case of complete SCI, locomotor training has been shown to elicit muscle activity of leg and trunk muscles through center pattern generator activity which is located within the spinal cord [115-117]. In an animal experiment, adult cats with complete spinalization showed activation pattern of hind limb and lumbar trunk muscles similar to activation pattern of intact cats during walking on a motorized treadmill, and the amplitude of muscle electromyography (EMG) activity of the spinalized cats was improved by prolonged locomotor training [118-120]. Similar muscle activations were observed in humans with SCI while walking on a treadmill. Individuals with incomplete SCI showed activation patterns of leg muscles similar to that observed in healthy individuals [121], and the timing and amplitude of EMG activity of leg muscles improved after a period of walking training [122, 123]. In individuals with complete SCI where there are no voluntary contractions, locomotor training on a treadmill induced EMG activity of leg muscles in a similar way to those seen in healthy individuals during walking [124]. The amplitude of EMG activity of leg muscles significantly increased after a period of walking training and worsened after stopping training [124, 125]. In addition, individuals with high thoracic complete SCI showed activation of trunk muscles (back and abdominal muscles) while walking on a treadmill or overground [126]. These findings suggest that walking training induces neuroplasticity of spinal cord circuits that can only be maintained by continuous walking training. It is suggested that limb loading and afferent inputs during a treadmill walking can trigger spinal neuronal circuits to activate lower limbs and trunk muscles [117, 127].

When comparing to arm cycling or leg cycling exercise, upright walking can provide greater challenges to the cardiovascular and respiratory system that might induce greater cardiovascular and respiratory adaptive changes [103]. In healthy individuals, it was found that the value of HR and systolic blood pressure was significantly higher during treadmill exercise than bicycle ergometer exercise [128]. In addition, peak values of oxygen uptake and HR were significantly higher during treadmill exercise than during arm ergometer exercise [129]. Similar benefits in cardiovascular and respiratory systems have been observed in individuals with SCI. For instance, under the equivalent workload, the peak value of HR and oxygen uptake was higher during treadmill walking than that during stationary cycling in individuals with motor incomplete SCI [130]. Carvalho et al. [131] reported similar observations in individuals with complete quadriplegia after walking on a treadmill compared to endurance exercise in sitting position. Therefore, upright walking using BWSTT can be used as an alternative form of aerobic exercise in individuals with SCI.

Unlike upper extremity exercise, lower extremity exercise such as walking training can enhance the circulation of blood in the legs and reduce peripheral vascular stiffness below the level of injury [132], which might reduce secondary complications such as thrombosis, pressure ulcers, and attenuated wound healing [133]. Leg muscle pump during locomotor training promotes venous return, leading to the increased blood returning to the heart. For instance, an acute bout of passive leg cycling significantly increased femoral artery blood flow and decreased velocity index (i.e., vascular resistance) in individuals with SCI [134]. Furthermore, a 4-month walking training program in individuals with motor complete SCI significantly improved femoral artery compliance and showed a trend towards an increase in femoral artery blood flow [82].

1.1.6. Heart Rate Adaptation to Walking Exercise in SCI

The benefits of walking exercise on cardiovascular fitness in people with SCI can be measured by examining adaptive changes in HR. A decrease in cardiovascular fitness, which increases the risk of CVD, can lead to increased HR due to reduced stroke volume [135]. Elevated HR in rest or during physical activities is more common in individuals with SCI as compared to healthy individuals, and in individuals with thoracic or lumbar SCI compared to those with cervical SCI [33, 34]. Previous studies in individuals with SCI reported improvement of cardiovascular fitness after walking training, as shown in reduced resting and exercise HR [82, 104, 106, 107]. Recent work by Steven et al. [136] demonstrated that 2 months of underwater treadmill training significantly reduced exercise heart rate in individuals with incomplete SCI. As has been suggested in the literature, regular walking training might induce resting and exercise HR adaptation through various possible mechanisms [137, 138], including increases in cardiopulmonary fitness, stroke volume, ventricular contraction and blood volume, decrease in peripheral arterial stiffness, modulations of the autonomic nervous system, and improvements in lung function and ventricular function. Also, changes in the neuromuscular system (i.e., changes in leg muscle activity and spasticity) after walking training might contribute to resting and exercise HR adaptation. However, the contributing factors of HR adaptation following walking training in individuals with SCI is still unknown. In the current project, we focused on examining some potential contributing factors, including changes in leg muscle activity, cardiac autonomic function, lung vital capacity, and leg muscle spasticity, to resting and exercise HR adaptation after walking training in individuals with SCI.

Leg Muscle Activity:

Repetitive walking training has been shown to increase the activity of leg muscles in individuals with SCI through the activation of central pattern generators [125, 139-141]. Rhythmical contraction of leg muscles (contraction and relaxation) during a locomotor activity such as walking can promote venous return from legs to the heart. Passive cycling contributed to an increase in stroke volume as a result of increased venous return [142, 143]. In addition, repetitive standing training resulted in increased leg muscle activity and consequently improved cardiovascular response to orthostatic stress test [144]. Generally, increased activity of leg muscles after a course of walking training can increase venous return and lead to cardiac adaptation. However, the extent of increased muscle activity of lower limbs to the improvement of HR in individuals with SCI has not been studied when compared to the contributions of other factors.

Cardiac Autonomic Function:

Injury to the spinal cord, especially above T6, is associated with alterations in autonomic control of the cardiovascular system [145]. HR variability (HRV) has been commonly utilized as a non-invasive method to quantify the cardiac autonomic function in the SCI population [146, 147]. Power spectral analysis of HRV provides information on a high-frequency (HF) power which reflects vagal (parasympathetic) outflow and a low-frequency (LF) power that is a combination of both sympathetic and parasympathetic outflows, At the same time, their ratio of LF/HF is defined as a marker for sympathovagal balance [148]. Past studies showed individuals with SCI had alterations in HRV, as shown by reduced total power as well as LF and HF powers, and an increased ratio of LF/HF, as compared to healthy individuals [149, 150]. Decreased HRV is associated with an increased risk of CVD [37, 151]. Despite that cardiac autonomic function is intact in individuals with injury at T6 or below, lack of physical activity after injury might alter cardiac autonomic function as shown by decreased HF (i.e. diminished parasympathetic activity) [150, 152]. Regular exercise can promote positive changes in HRV towards a balance between parasympathetic outflow and sympathetic outflow [151]. Previous studies by Ditor et al. showed that walking training for 4-6 months significantly decreased LF power and the ratio of LF/HF of HRV in individuals with incomplete cervical SCI [106] and a subgroup of individuals with complete SCI [82]. Similarly, another past study reported a significant improvement in HRV after 4-week walking training as, explained by a significant increase in HR complexity which is a nonlinear measure of HRV and trends toward an increase in HF and a decrease in LF [153]. Thus, it is possible that modulation of HRV after walking training in individuals with SCI can contribute to positive cardiac adaptation. However, the relationship between changes in HRV and HR after walking training has not been examined.

Lung Vital Capacity:

Lung vital capacity is the maximum amount of air that can be breathed in and out of the lung during a single respiratory cycle. It is an important parameter in predicting respiratory and cardiovascular health. In theory, a person who has high lung capacity can take more oxygen into his/her body which can make more oxygenated blood. Subsequently, the heart would work less hard because of the high amount of oxygen in the blood. Consequently, the heart rate would decrease. A study reported an inverse relationship between lung vital capacity and HR [154]. After SCI, lung capacity decreases as a result of respiratory muscle weakness as well as decreased physical activity [155]. Higher-level SCI is associated with the greatest reduction in lung capacity.[156] Hence, the decline in lung vital capacity after SCI might be associated with adverse cardiovascular outcomes, including increased HR. After a period of aerobic exercise, lung capacity to exchange oxygen and carbon dioxide would increase, and HR would decrease [157]. Previous studies have demonstrated that walking training significantly improved lung vital capacity in individuals with incomplete and complete SCI [104, 109, 158]. Thus, it is possible that increased lung vital capacity after a period of walking training might contribute to HR adaptation in individuals with SCI, but quantitative examination is needed

Leg Muscle Spasticity:

Muscle spasticity after SCI is associated with decreased arterial diameter and increased blood flow resistance [159], which might increase the load on the heart to deliver the blood to body tissues. It has been reported that increased muscle spasticity may contribute to increased HR in individuals with neurological diseases. In patients with multiple sclerosis, muscle spasticity was associated with increased HR [160]. A study in persons with cerebral palsy demonstrated those with high levels of muscle spasticity had high HR response as compared to those with low levels of spasticity or healthy subjects during walking on a treadmill under the same speed [161]. Furthermore, muscle spasticity negatively influenced HR variability in stroke patients [162]. In individuals with SCI, a greater HR response to walking training was observed in those who had higher levels of muscle spasticity [82]. Many studies in individuals with SCI demonstrated a significant reduction in muscle spasticity of the lower extremity after a period of walking training [163-165]. Thus, it is possible that changes in muscle spasticity after a course of walking training in individuals with SCI may influence HR adaptation, but quantitative examinations are needed.

1.1.7. Assistive Gait Training Device with Treadmill Walking

Body weight-supported treadmill training (BWSTT) is a tool that is used in rehabilitation to retrain patients with neurological conditions to improve or regain their walking ability. Patients are trained on a treadmill while fitted into a safety harness, which is attached to an overhead suspension system to provide body weight support and prevent the risk of falls during training. Leg assistance during training is provided either manually by therapists or by a robotic-assistive device. Assistance from a physical therapist during BWSTT can be effective in providing task-specific walking training to individuals with SCI. However, when physical therapists provide manual assistance during training, the number of gait cycle repetitions can be limited due to intensive efforts that physical therapists make to move the patient's impaired legs/feet during every forward step [166]. Part of the problem is that non-ergonomic body posture of physical therapists while providing manual assistance to patient's legs/feet during training can increase workload and put therapists under risk of developing low-back pain [167]. Therefore, these limitations can directly impact the length and intensity of training.

To overcome these limitations, physical therapists often use rehabilitation robotic-assistive devices such as Lokomat to help them when providing intensive gait training for people with SCI. Although the robotic-assistive gait training can provide intensive, task-specific stepping training for patients, the high cost of robotic-assistive gait devices cannot be afforded by most of the rehabilitation clinics [168, 169]. Passive assistance provided by robotic-assistive devices can limit the active involvement of the patients and hinder activity-related learning during walking training. In addition, standardized movement pattern provided by robotic-assisted devices does not allow walking variability and corrective action by the patient during walking training, which can impair motor learning [169].

A novel assistive gait training device using a pulling system was developed in our laboratory to provide stepping-assistance for patient's legs/feet and reduce the workload of therapist therapists during treadmill walking training (**Figure 1.1**) [170, 171]. The assistive device comprises of a brace and one pulling cable. The brace is placed on the patient's thigh and secured using Velcro straps. The first part of the pulling cable attaches to a handle and runs through a fixed pulley anchor. The other end of the pulling cable wraps a wheel fixed at the front of the thigh brace and then attaches to a foot strap that is tightened around the forefoot.

A physical therapist pulls the handle attached to the cable to assist a patient with hip flexion and ankle dorsiflexion during the swing phase of the gait cycle. The physical therapist can adjust the amount of leg assistance provided to the patient based on the needs of the patient and therapist's experience. The strategy of using this assistive device is to provide only minimal assistance, "assistance-as-needed" for completing the forward step movement of the leg. After the swing phase is completed, the physical therapist releases the pulling cable to allow the affected leg to lower on the treadmill track at the end of the swing phase. The treadmill belt moves the affected leg backward, and then through visual information of the foot position on the treadmill the physical therapist determines the cycle when he/she should repeat and apply the assistive force by the pulling cable. The assistive device can allow physical therapists to work from a more ergonomic body posture at a sitting position while providing the necessary stepping-assistance for the patient's legs/feet during walking on a treadmill. The workload is decreased for the physical therapist by the use of the pulling cable in comparison to traditional manual assistance. In addition, this assistive device is of low cost and lightweight compared to the robotic-assistive devices such as Lokomat. It allows the active involvement of the patient by providing minimal assistance when needed.

The feasibility of the novel assistive training device was examined in non-ambulatory patients with stroke [171]. In that feasibility study, leg-stepping assistance was provided in one leg since stroke patients have only one side that is affected. However, individuals with SCI usually have both legs/feet are affected and need stepping assistance for both legs/feet during treadmill walking. In such case, two thigh braces are affixed on both right and left thighs (**Figure 1.2**). Two physical therapists coordinate to operate the assistive device by pulling the cable at the beginning of the swing phase of the gait cycle. However, the use of this assistive gait training device has not been tested on individuals with SCI previously. Therefore, this study aimed to examine the feasibility of the assistive gait training device using two pulling cables in individuals with SCI.

1.2. Significance

Leg assistance provided manually by a physical therapist or by robotic-assistive gait training devices such as Lokomat during BWSTT can be effective in delivering task-specific training for patients with SCI. However, each method introduces some limitations. The number of gait cycle repetitions can be limited when the physical therapist provides manual assistance to move the affected leg during the training due to intensive efforts and non-ergonomic body position on the part of the physical therapist. Robotic-assistive gait training devices have developed to reduce the intensive workload of the physical therapist while providing manual assistance during treadmill walking training. However, current robotic-assistive gait devices are expensive and cannot be afforded by most of the rehabilitation clinics. Also, it can limit the active participation of the patient during walking training. Therefore, there is a need for designing a novel assistive gait device that is low in cost, can alleviate the intensive workload of physical therapists, and provides the necessary leg assistance for patients during treadmill walking training. A novel assistive gait training device using a pulley system was developed in our laboratory to aid physical therapists during treadmill walking training. Using this device, physical therapists can work at a low workload and more ergonomic body position. Also, this device has low cost and allows physical therapists to provide leg assistance to patients as needed. However, the use of this device has not be tested in persons with SCI.

As mentioned previously, aerobic exercise using arm cycling or FES-leg cycling has shown inconsistent results on risk factors of CVD in SCI survivors. Arm cycling targets only upper limb muscles, which does not provide sufficient challenge to the cardiovascular system [104, 106]. FES-leg cycling is limited as patients often report muscle fatigue due to frequent stimulation and aggravate the symptoms of autonomic dysreflexia particularly in those with SCI above T6 [82, 97, 98]. Both arm cycling and leg cycling exercises are performed in sitting position which may not provide sufficient stress to the cardiovascular system. As an alternative, upright walking using BWSTT has shown preliminary results to improve cardiovascular function in individuals with incomplete or complete SCI [82, 104, 106-109]. BWSTT induced leg muscles activity in a similar way to those seen in healthy individuals during walking [124]. The amplitude of muscles activity significantly increased after repetitive walking training [122, 124, 125]. It is unknown how significantly the increased leg muscle activities after a period of walking training can help to improve cardiovascular health. Therefore, it is both clinically and scientifically important to determine the extent that the increased leg muscle activity after a period of walking training can contribute to the HR adaptation (i.e., decreased resting and exercise HR) in comparison to the contribution by other factors including changes in cardiac autonomic function, lung capacity and muscle spasticity. Such findings will help to guide the clinical practice and researchers to focus future effort in the right direction.

In addition, there is still limited evidence about the effects of upright walking training on risk markers of CVD in individuals with chronic SCI. Furthermore, previous studies have not investigated the effects of walking training on risk markers of CVD in individuals with motor complete SCI. The influence of walking training on the levels of pro-inflammatory markers has also not been studied in individuals with either incomplete or complete SCI. To explore those issues would further extend our knowledge to better understand the benefits of walking exercise in individuals with chronic SCI.

1.3. Innovation

This study was innovative as we tested the feasibility of a novel assistive gait training device and examined contributions of selected factors (leg muscle activity, cardiac autonomic function, lung vital capacity, and leg muscle spasticity) to resting and exercise HR adaptation after a course of walking training in individuals with chronic SCI.

This study would add to knowledge by 1) investigating the effect of walking training on lipid profile and glycemic control in individuals with chronic complete SCI and 2) exploring the effects of walking training on pro-inflammatory markers in individuals with chronic motor incomplete or complete SCI.

1.4. Specific Aims

Physical inactivity in people with chronic spinal cord injury (SCI) due to impaired/loss of motor function reduces cardiovascular fitness and increases multiple risk factors for the development of cardiovascular diseases (CVD), including lipid profile disorder, diabetes, and elevated level of pro-inflammatory markers [18, 21, 22]. Those factors play important roles in the development of ischemic heart disease, stroke, and peripheral vascular diseases [23-26]. For

SCI patients with impaired/loss of lower limb motor function, inconsistent findings have been reported in the literature regarding benefits of arm cycling or functional electrical stimulation (FES)-leg cycling exercise on risk factors of CVD. A major limitation of those exercises is the lack of sustainable activities of large leg muscles, leading to insufficient challenges to the cardiovascular system [104, 106]. Body weight supported treadmill training (BWSTT) can be used as an alternative form of aerobic exercise in individuals with SCI. Past preliminary studies have shown that BWSTT can improve cardiovascular fitness (e.g., decrease in resting and exercise HR) [82, 104, 106, 107, 136] and increase leg muscle activities during walking, even in complete SCI [122-125]. Such preliminary findings suggest that walking training may initiate central pattern generators that activate leg muscles and lead to improvements in the cardiovascular system. However, past studies reported also changes in cardiac autonomic function, leg muscle spasticity and/or lung vital capacity after walking exercise that may lead to change in heart rate. There has been no study that examined the potential association between the improved HR and increased leg muscle activity, with a comparison to its association with changes in cardiac autonomic function, lung vital capacity or leg muscle spasticity. It is an important question both scientifically and clinically. In addition, limited evidence is currently available concerning how BWSTT affects risk factors of CVD, including lipid profile, glycemic control, and pro-inflammatory markers.

The **primary objectives** were 1) to examine the feasibility of an 8-week walking training program and 2) to examine the potential association between changes in resting and exercise HR and changes in four major factors after 8-week walking training in individuals with chronic SCI. The **secondary objective** was to collect pilot data before and after the 8-week walking exercise program for evaluating changes in risk markers of CVD in study participants.

Aim#1: To examine the feasibility (recruitment, perception, compliance, retention, and walking performance) of an 8-week walking training program using a novel assistive gait training device in patients with chronic SCI.

Aim#2: To examine correlations between changes in four factors (muscle activity, autonomic function, spasticity, and lung capacity) and changes in resting and exercise HR after the walking training program in individuals with chronic SCI. *Primary hypothesis:* Decreases in resting and exercise HR after the walking training would show significant correlations with increased leg muscle activity, which would be stronger than its correlations with changes in cardiac autonomic function, leg muscle spasticity, or lung vital capacity.

Aim#3: To investigate the effects of an 8-week walking training on glycated hemoglobin (HbA1c), lipid profile, and the levels of pro-inflammatory markers in individuals with chronic SCI. *Secondary hypothesis:* Compared to baseline measures, the 8-week walking training program would decrease the levels of HbA1c and low-density lipoprotein cholesterol, increase the level of high-density lipoprotein cholesterol and reduce the levels of C-reactive protein and interleukin-6.

Exploratory aims*:* To investigate the effects of the walking training program on lower limbs muscle strength, functional independence, the level of depression, anxiety and stress, and health-related quality of life in individuals with chronic SCI.

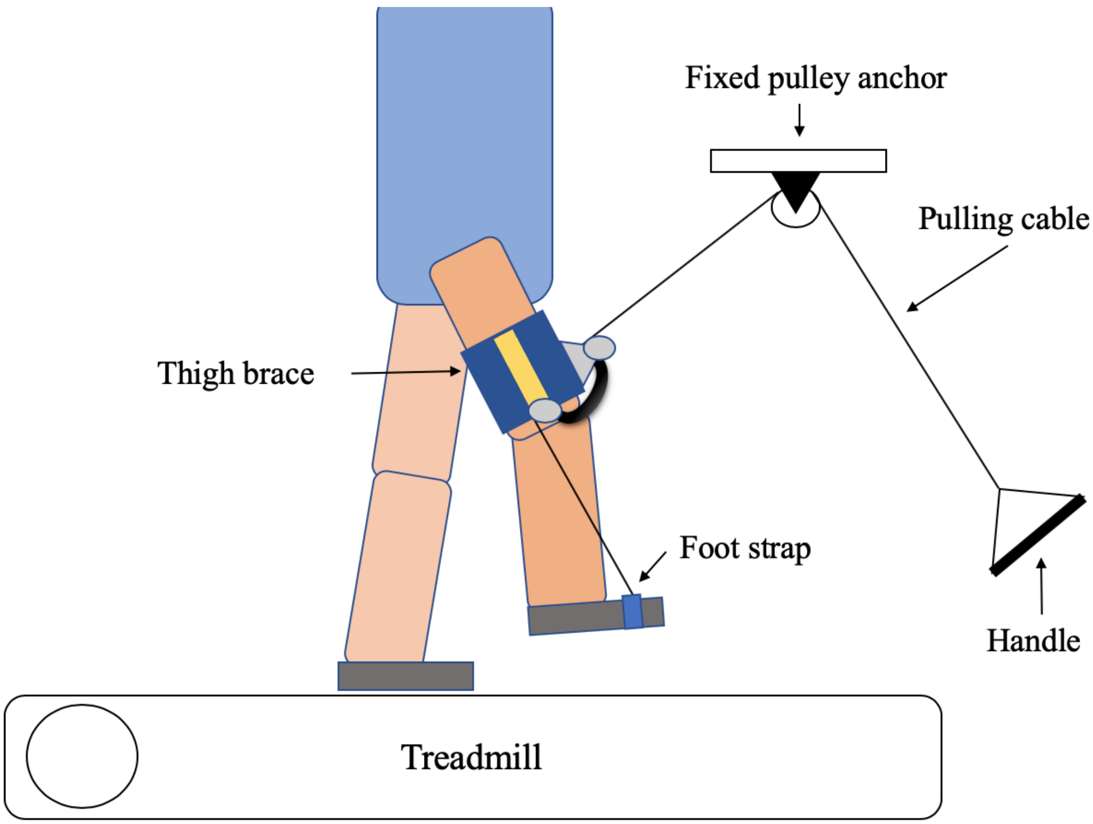


Figure 1.1: The components of the assistive gait training device

The assistive device comprises of a brace and one pulling cable. The brace is placed on the patient's thigh and secured using Velcro straps. The first part of the pulling cable attaches to a handle and runs through a fixed pulley anchor. The other end of the pulling cable wraps a wheel fixed at the front of the thigh brace and then attaches to a foot strap that is tightened around the forefoot.

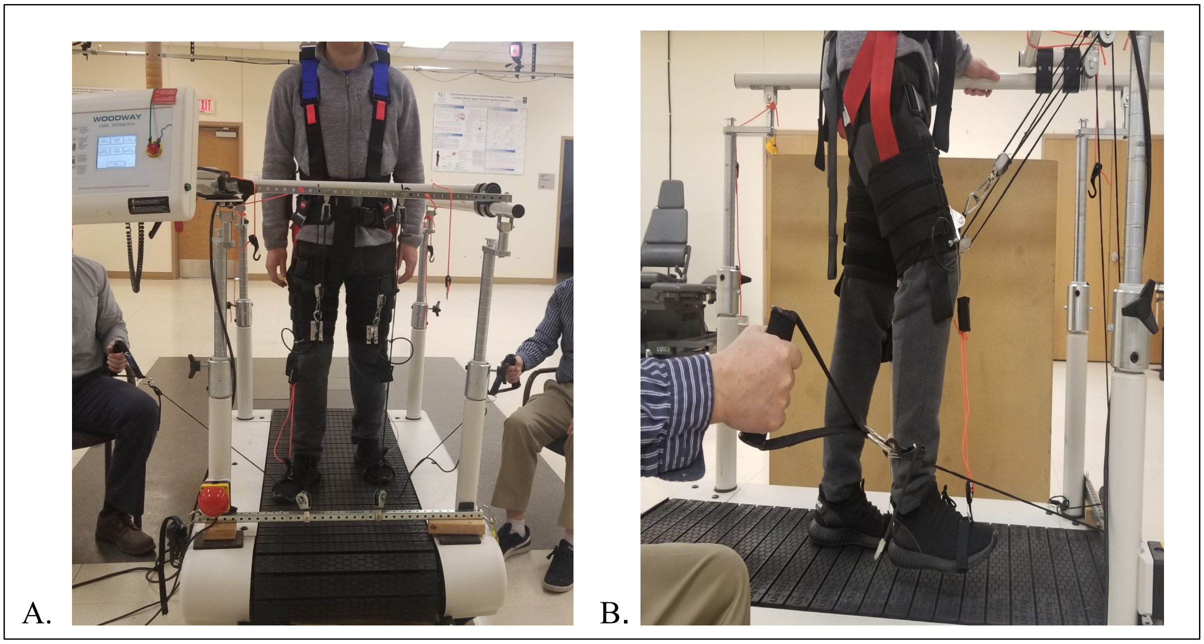


Figure 1.2: The assistive gait training device with two pulling cables operated by two therapists on either side of the treadmill

(A) Two thigh braces which attach to fixed pulleying system in the front of the treadmill via a cable are placed on both right and left thighs. Two physical therapists sit on the right and left side of the treadmill and coordinate to operate the assistive device by pulling the handle attached to the cable. (B) The physical therapists pull the handle attached to the cable to assist a patient with hip flexion and ankle dorsiflexion during the swing phase of the gait cycle

References

1. Field-Fote, E., *Spinal Cord Injury: An overview*, in *Spinal Cord Injury Rehabilitation*, E. Field-Fote, Editor. 2009, F. A. Davis, 2009: Philadelphia, PA. p. pp. 3-87.
2. Nas, K., et al., *Rehabilitation of spinal cord injuries.* World J Orthop, 2015. **6**(1): p. 8-16.
3. Kirshblum, S.C., et al., *International standards for neurological classification of spinal cord injury (Revised 2011).* J Spinal Cord Med, 2011. **34**(6): p. 535-46.
4. *Spinal Cord Injury (SCI) 2016 Facts and Figures at a Glance.* J Spinal Cord Med, 2016. **39**(4): p. 493-4.
5. Phillips, W.T., et al., *Effect of spinal cord injury on the heart and cardiovascular fitness.* Curr Probl Cardiol, 1998. **23**(11): p. 641-716.
6. Warburton, D.E., et al., *Cardiovascular Health and Exercise Rehabilitation in Spinal Cord Injury.* Top Spinal Cord Inj Rehabil, 2007. **13**(1): p. 98-122.
7. O'Sullivan, S., *Locomotor Training*, in *Rahabilitation*, G. Fulk and T. Schmitz, Editors., F.A. Davis Company: Philadelphia, PA 19103. p. 444-463.
8. *National Spinal Cord Injury Statistical Center (NSCIS): The 2010 Annual Report for the Spinal Cord Injury Model Systems*. 2010, NSCIS: Birmingham, AL.
9. Kostovski, E., P.O. Iversen, and N. Hjeltnes, *[Complications of chronic spinal cord injury].* Tidsskr Nor Laegeforen, 2010. **130**(12): p. 1242-5.
10. Sezer, N., S. Akkus, and F.G. Ugurlu, *Chronic complications of spinal cord injury.* World J Orthop, 2015. **6**(1): p. 24-33.
11. Strauss, D.J., et al., *Trends in life expectancy after spinal cord injury.* Arch Phys Med Rehabil, 2006. **87**(8): p. 1079-85.
12. DeVivo, M.J., K.J. Black, and S.L. Stover, *Causes of death during the first 12 years after spinal cord injury.* Arch Phys Med Rehabil, 1993. **74**(3): p. 248-54.
13. Garshick, E., et al., *A prospective assessment of mortality in chronic spinal cord injury.* Spinal Cord, 2005. **43**(7): p. 408-16.
14. Cragg, J.J., et al., *Cardiovascular disease and spinal cord injury: results from a national population health survey.* Neurology, 2013. **81**(8): p. 723-8.
15. Osterthun, R., et al., *Causes of death following spinal cord injury during inpatient rehabilitation and the first five years after discharge. A Dutch cohort study.* Spinal Cord, 2014. **52**(6): p. 483-8.
16. Go, A.S., et al., *Heart disease and stroke statistics--2014 update: a report from the American Heart Association.* Circulation, 2014. **129**(3): p. e28-e292.
17. Katzmarzyk, P.T., et al., *Sitting time and mortality from all causes, cardiovascular disease, and cancer.* Med Sci Sports Exerc, 2009. **41**(5): p. 998-1005.
18. Myers, J., M. Lee, and J. Kiratli, *Cardiovascular disease in spinal cord injury: an overview of prevalence, risk, evaluation, and management.* Am J Phys Med Rehabil, 2007. **86**(2): p. 142-52.

19. Buchholz, A.C., C.F. McGillivray, and P.B. Pencharz, *Physical activity levels are low in free-living adults with chronic paraplegia.* Obes Res, 2003. **11**(4): p. 563-70.

20. van den Berg-Emons, R.J., et al., *A prospective study on physical activity levels after spinal cord injury during inpatient rehabilitation and the year after discharge.* Arch Phys Med Rehabil, 2008. **89**(11): p. 2094-101.

21. Gibson, A.E., A.C. Buchholz, and K.A. Martin Ginis, *C-Reactive protein in adults with chronic spinal cord injury: increased chronic inflammation in tetraplegia vs paraplegia.* Spinal Cord, 2008. **46**(9): p. 616-21.

22. Manns, P.J., J.A. McCubbin, and D.P. Williams, *Fitness, inflammation, and the metabolic syndrome in men with paraplegia.* Arch Phys Med Rehabil, 2005. **86**(6): p. 1176-81.

23. LaMonte, M.J., et al., *Cardiorespiratory fitness and coronary heart disease risk factors: the LDS Hospital Fitness Institute cohort.* Circulation, 2000. **102**(14): p. 1623-8.

24. Chait, A. and K.E. Bornfeldt, *Diabetes and atherosclerosis: is there a role for hyperglycemia?* J Lipid Res, 2009. **50 Suppl**: p. S335-9.

25. Libby, P., *Inflammation and cardiovascular disease mechanisms.* Am J Clin Nutr, 2006. **83**(2): p. 456s-460s.

26. Tousoulis, D., et al., *Pathophysiology of atherosclerosis: the role of inflammation.* Curr Pharm Des, 2011. **17**(37): p. 4089-110.

27. Libin, A., et al., *Cardiometabolic risk clustering in spinal cord injury: results of exploratory factor analysis.* Top Spinal Cord Inj Rehabil, 2013. **19**(3): p. 183-94.

28. Zhang, D., W. Wang, and F. Li, *Association between resting heart rate and coronary artery disease, stroke, sudden death and noncardiovascular diseases: a meta-analysis.* Cmaj, 2016. **188**(15): p. E384-e392.

29. Aune, D., et al., *Resting heart rate and the risk of cardiovascular disease, total cancer, and all-cause mortality - A systematic review and dose-response meta-analysis of prospective studies.* Nutr Metab Cardiovasc Dis, 2017. **27**(6): p. 504-517.

30. Steinhaus, L.A., et al., *Cardio-respiratory fitness of young and older active and sedentary men.* Br J Sports Med, 1988. **22**(4): p. 163-6.

31. Sieverdes, J.C., X. Sui, and S.N. Blair, *Associations between Physical Activity and Submaximal Cardiorespiratory and Pulmonary Responses in Men.* J Sports Med Doping Stud, 2011. **1**(1).

32. Maher, J.L., D.W. McMillan, and M.S. Nash, *Exercise and Health-Related Risks of Physical Deconditioning After Spinal Cord Injury.* Top Spinal Cord Inj Rehabil, 2017. **23**(3): p. 175-187.

33. Lee, Y.H., et al., *Hemodynamic Adaptations to Regular Exercise in People With Spinal Cord Injury.* Ann Rehabil Med, 2017. **41**(1): p. 25-33.

34. Schmid, A., et al., *Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury.* J Appl Physiol (1985), 1998. **85**(2): p. 635-41.

35. Hjeltnes, N. and H. Wallberg-Henriksson, *Improved work capacity but unchanged peak oxygen uptake during primary rehabilitation in tetraplegic patients.* Spinal Cord, 1998. **36**(10): p. 691-8.

36. Yamasaki, M., et al., *Daily energy expenditure in active and inactive persons with spinal cord injury.* J Hum Ergol (Tokyo), 1992. **21**(2): p. 125-33.

37. Rosado-Rivera, D., et al., *Comparison of 24-hour cardiovascular and autonomic function in paraplegia, tetraplegia, and control groups: implications for cardiovascular risk.* J Spinal Cord Med, 2011. **34**(4): p. 395-403.

38. *Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III).* Jama, 2001. **285**(19): p. 2486-97.

39. Bauman, W.A. and A.M. Spungen, *Metabolic changes in persons after spinal cord injury.* Phys Med Rehabil Clin N Am, 2000. **11**(1): p. 109-40.

40. Demirel, S., et al., *Risk factors for coronary heart disease in patients with spinal cord injury in Turkey.* Spinal Cord, 2001. **39**(3): p. 134-8.

41. Gilbert, O., et al., *Serum lipid concentrations among persons with spinal cord injury - a systematic review and meta-analysis of the literature.* Atherosclerosis, 2014. **232**(2): p. 305-12.

42. de Groot, S., et al., *Prospective analysis of lipid profiles in persons with a spinal cord injury during and 1 year after inpatient rehabilitation.* Arch Phys Med Rehabil, 2008. **89**(3): p. 531-7.

43. Apstein, M.D. and B.C. George, *Serum lipids during the first year following acute spinal cord injury.* Metabolism, 1998. **47**(4): p. 367-70.

44. Vichiansiri, R., et al., *The prevalence of dyslipidemia in patients with spinal cord lesion in Thailand.* Cholesterol, 2012. **2012**: p. 847462.

45. Laclaustra, M., et al., *Serum lipid profile in subjects with traumatic spinal cord injury.* PLoS One, 2015. **10**(2): p. e0115522.

46. Sabour, H., et al., *Cardiometabolic risk factors in Iranians with spinal cord injury: analysis by injury-related variables.* J Rehabil Res Dev, 2013. **50**(5): p. 635-42.

47. Koyuncu, E., et al., *The analysis of serum lipid levels in patients with spinal cord injury.* J Spinal Cord Med, 2017. **40**(5): p. 567-572.

48. Fox, C.S., et al., *Trends in cardiovascular complications of diabetes.* Jama, 2004. **292**(20): p. 2495-9.

49. Bauman, W.A. and A.M. Spungen, *Disorders of carbohydrate and lipid metabolism in veterans with paraplegia or quadriplegia: a model of premature aging.* Metabolism, 1994. **43**(6): p. 749-56.

50. Cragg, J.J., et al., *Spinal cord injury and type 2 diabetes: results from a population health survey.* Neurology, 2013. **81**(21): p. 1864-8.

51. Bauman, W.A., et al., *The effect of residual neurological deficit on oral glucose tolerance in persons with chronic spinal cord injury.* Spinal Cord, 1999. **37**(11): p. 765-71.

52. Bjorntorp, P., *"Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes.* Arteriosclerosis, 1990. **10**(4): p. 493-6.

53. Castro, M.J., et al., *Influence of complete spinal cord injury on skeletal muscle within 6 mo of injury.* J Appl Physiol (1985), 1999. **86**(1): p. 350-8.

54. Burant, C.F., et al., *Insulin resistance of denervated rat muscle: a model for impaired receptor-function coupling.* Am J Physiol, 1984. **247**(5 Pt 1): p. E657-66.

55. Myllynen, P., V.A. Koivisto, and E.A. Nikkila, *Glucose intolerance and insulin resistance accompany immobilization.* Acta Med Scand, 1987. **222**(1): p. 75-81.

56. Morse, L.R., et al., *Association between mobility mode and C-reactive protein levels in men with chronic spinal cord injury.* Arch Phys Med Rehabil, 2008. **89**(4): p. 726-31.

57. da Silva Alves, E., et al., *Low-grade inflammation and spinal cord injury: exercise as therapy?* Mediators Inflamm, 2013. **2013**: p. 971841.

58. Koenig, W., et al., *C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992.* Circulation, 1999. **99**(2): p. 237-42.

59. Ridker, P.M., et al., *Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events.* N Engl J Med, 2002. **347**(20): p. 1557-65.

60. Folsom, A.R., et al., *C-reactive protein and incident coronary heart disease in the Atherosclerosis Risk In Communities (ARIC) study.* Am Heart J, 2002. **144**(2): p. 233-8.

61. Pearson, T.A., et al., *Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association.* Circulation, 2003. **107**(3): p. 499-511.

62. Nash, M. and A.J. Mendez, *Nonfasting lipemia and inflammation as cardiovascular disease risks after SCI.* Topics in Spinal Cord Injury Rehabilitation, 2009. **14**(3): p. 15-31.

63. Wang, T.D., et al., *Circulating levels of markers of inflammation and endothelial activation are increased in men with chronic spinal cord injury.* J Formos Med Assoc, 2007. **106**(11): p. 919-28.

64. Liang, H., et al., *Elevated C-reactive protein associated with decreased high-density lipoprotein cholesterol in men with spinal cord injury.* Arch Phys Med Rehabil, 2008. **89**(1): p. 36-41.

65. Buchholz, A.C., et al., *Greater daily leisure time physical activity is associated with lower chronic disease risk in adults with spinal cord injury.* Appl Physiol Nutr Metab, 2009. **34**(4): p. 640-7.

66. Koury, J.C., et al., *Time of physical exercise practice after injury in cervical spinal cord-injured men is related to the increase in insulin sensitivity.* Spinal Cord, 2013. **51**(2): p. 116-9.

67. Goldstein, R.L., et al., *Clinical factors associated with C-reactive protein in chronic spinal cord injury.* Spinal Cord, 2017. **55**(12): p. 1088-1095.

68. Ginis, K.A., et al., *The development of evidence-informed physical activity guidelines for adults with spinal cord injury.* Spinal Cord, 2011. **49**(11): p. 1088-96.

69. Riebe, D., et al., *ACSM's guidelines for exercise testing and prescription*. Tenth edition ed. 2018, Philadelphia, PA: Wolters Kluwer. 480.

70. Martin Ginis, K.A., et al., *Evidence-based scientific exercise guidelines for adults with spinal cord injury: an update and a new guideline.* Spinal Cord, 2017.

71. Evans, N., et al., *Exercise Recommendations and Considerations for Persons With Spinal Cord Injury.* Arch Phys Med Rehabil, 2015. **96**(9): p. 1749-50.

72. DiCarlo, S.E., *Effect of arm ergometry training on wheelchair propulsion endurance of individuals with quadriplegia.* Phys Ther, 1988. **68**(1): p. 40-4.

73. Hooker, S.P. and C.L. Wells, *Effects of low- and moderate-intensity training in spinal cord-injured persons.* Med Sci Sports Exerc, 1989. **21**(1): p. 18-22.

74. de Groot, P.C., et al., *Effect of training intensity on physical capacity, lipid profile and insulin sensitivity in early rehabilitation of spinal cord injured individuals.* Spinal Cord, 2003. **41**(12): p. 673-9.

75. Kim, D.I., et al., *Effects of a 6-Week Indoor Hand-Bike Exercise Program on Health and Fitness Levels in People With Spinal Cord Injury: A Randomized Controlled Trial Study.* Arch Phys Med Rehabil, 2015. **96**(11): p. 2033-40.e1.

76. Nash, M.S., et al., *Circuit resistance training improves the atherogenic lipid profiles of persons with chronic paraplegia.* J Spinal Cord Med, 2001. **24**(1): p. 2-9.

77. El-Sayed, M.S. and A. Younesian, *Lipid profiles are influenced by arm cranking exercise and training in individuals with spinal cord injury.* Spinal Cord, 2005. **43**(5): p. 299-305.

78. Rosety-Rodriguez, M., et al., *Low-grade systemic inflammation and leptin levels were improved by arm cranking exercise in adults with chronic spinal cord injury.* Arch Phys Med Rehabil, 2014. **95**(2): p. 297-302.

79. Bakkum, A.J., et al., *Effects of hybrid cycle and handcycle exercise on cardiovascular disease risk factors in people with spinal cord injury: A randomized controlled trial.* J Rehabil Med, 2015. **47**(6): p. 523-30.

80. Totosy de Zepetnek, J.O., et al., *Following the Physical Activity Guidelines for Adults With Spinal Cord Injury for 16 Weeks Does Not Improve Vascular Health: A Randomized Controlled Trial.* Arch Phys Med Rehabil, 2015. **96**(9): p. 1566-75.

81. Flank, P., et al., *Self-reported physical activity and risk markers for cardiovascular disease after spinal cord injury.* J Rehabil Med, 2014. **46**(9): p. 886-90.

82. Ditor, D.S., et al., *The effects of body-weight supported treadmill training on cardiovascular regulation in individuals with motor-complete SCI.* Spinal Cord, 2005. **43**(11): p. 664-73.

83. Faghri, P.D., R.M. Glaser, and S.F. Figoni, *Functional electrical stimulation leg cycle ergometer exercise: training effects on cardiorespiratory responses of spinal cord injured subjects at rest and during submaximal exercise.* Arch Phys Med Rehabil, 1992. **73**(11): p. 1085-93.

84. Barstow, T.J., et al., *Changes in gas exchange kinetics with training in patients with spinal cord injury.* Med Sci Sports Exerc, 1996. **28**(10): p. 1221-8.

85. Petrofsky, J.S. and R. Stacy, *The effect of training on endurance and the cardiovascular responses of individuals with paraplegia during dynamic exercise induced by functional electrical stimulation.* Eur J Appl Physiol Occup Physiol, 1992. **64**(6): p. 487-92.

86. Jeon, J.Y., et al., *Improved glucose tolerance and insulin sensitivity after electrical stimulation-assisted cycling in people with spinal cord injury.* Spinal Cord, 2002. **40**(3): p. 110-7.

87. Mohr, T., et al., *Insulin action and long-term electrically induced training in individuals with spinal cord injuries.* Med Sci Sports Exerc, 2001. **33**(8): p. 1247-52.

88. Griffin, L., et al., *Functional electrical stimulation cycling improves body composition, metabolic and neural factors in persons with spinal cord injury.* J Electromyogr Kinesiol, 2009. **19**(4): p. 614-22.

89. Chilibeck, P.D., et al., *Histochemical changes in muscle of individuals with spinal cord injury following functional electrical stimulated exercise training.* Spinal Cord, 1999. **37**(4): p. 264-8.

90. Hjeltnes, N., et al., *Exercise-induced overexpression of key regulatory proteins involved in glucose uptake and metabolism in tetraplegic persons: molecular mechanism for improved glucose homeostasis.* Faseb j, 1998. **12**(15): p. 1701-12.

91. Hooker, S.P., et al., *Peak and submaximal physiologic responses following electrical stimulation leg cycle ergometer training.* J Rehabil Res Dev, 1995. **32**(4): p. 361-6.

92. Gerrits, H.L., et al., *Peripheral vascular changes after electrically stimulated cycle training in people with spinal cord injury.* Arch Phys Med Rehabil, 2001. **82**(6): p. 832-9.

93. Ward, E., et al., *Lipid levels and body composition in individuals with spinal cord injury participating in activity-based rehabilitation: 1080.* . Medicine and Science in Sports and Exercise, 2010. **42**(5): p. 155.

94. Gorgey, A.S., et al., *A report of anticipated benefits of functional electrical stimulation after spinal cord injury.* J Spinal Cord Med, 2012. **35**(2): p. 107-12.

95. Ryan, T.E., et al., *Electrically induced resistance training in individuals with motor complete spinal cord injury.* Arch Phys Med Rehabil, 2013. **94**(11): p. 2166-73.

96. Mahoney, E.T., et al., *Changes in skeletal muscle size and glucose tolerance with electrically stimulated resistance training in subjects with chronic spinal cord injury.* Arch Phys Med Rehabil, 2005. **86**(7): p. 1502-4.

97. Jacobs, P.L. and M.S. Nash, *Modes, benefits, and risks of voluntary an delectrically induced exercise in persons with spinal cord injury.* J Spinal Cord Med, 2001. **24**(1): p. 10-8.

98. Olive, J.L., et al., *Blood flow and muscle fatigue in SCI individuals during electrical stimulation.* J Appl Physiol (1985), 2003. **94**(2): p. 701-8.

99. Barbeau, H., M. Wainberg, and L. Finch, *Description and application of a system for locomotor rehabilitation.* Med Biol Eng Comput, 1987. **25**(3): p. 341-4.

100. Dobkin, B., et al., *Weight-supported treadmill vs over-ground training for walking after acute incomplete SCI.* Neurology, 2006. **66**(4): p. 484-93.

101. Wessels, M., et al., *Body weight-supported gait training for restoration of walking in people with an incomplete spinal cord injury: a systematic review.* J Rehabil Med, 2010. **42**(6): p. 513-9.

102. Morawietz, C. and F. Moffat, *Effects of locomotor training after incomplete spinal cord injury: a systematic review.* Arch Phys Med Rehabil, 2013. **94**(11): p. 2297-308.

103. Hicks, A.L. and K.A. Ginis, *Treadmill training after spinal cord injury: it's not just about the walking.* J Rehabil Res Dev, 2008. **45**(2): p. 241-8.

104. Soyupek, F., et al., *Effects of body weight supported treadmill training on cardiac and pulmonary functions in the patients with incomplete spinal cord injury.* J Back Musculoskelet Rehabil, 2009. **22**(4): p. 213-8.

105. Jack, L.P., D.B. Allan, and K.J. Hunt, *Cardiopulmonary exercise testing during body weight supported treadmill exercise in incomplete spinal cord injury: a feasibility study.* Technol Health Care, 2009. **17**(1): p. 13-23.

106. Ditor, D.S., et al., *Effects of body weight-supported treadmill training on heart rate variability and blood pressure variability in individuals with spinal cord injury.* J Appl Physiol (1985), 2005. **98**(4): p. 1519-25.

107. Hoekstra, F., et al., *Effect of robotic gait training on cardiorespiratory system in incomplete spinal cord injury.* J Rehabil Res Dev, 2013. **50**(10): p. 1411-22.

108. Turiel, M., et al., *Robotic treadmill training improves cardiovascular function in spinal cord injury patients.* Int J Cardiol, 2011. **149**(3): p. 323-9.

109. Tiftik, T., et al., *Does locomotor training improve pulmonary function in patients with spinal cord injury?* Spinal Cord, 2015. **53**(6): p. 467-70.

110. Phillips, S.M., et al., *Body-weight-support treadmill training improves blood glucose regulation in persons with incomplete spinal cord injury.* J Appl Physiol (1985), 2004. **97**(2): p. 716-24.

111. Stewart, B.G., et al., *Treadmill training-induced adaptations in muscle phenotype in persons with incomplete spinal cord injury.* Muscle Nerve, 2004. **30**(1): p. 61-8.

112. Alm, M., H. Saraste, and C. Norrbrink, *Shoulder pain in persons with thoracic spinal cord injury: prevalence and characteristics.* J Rehabil Med, 2008. **40**(4): p. 277-83.

113. Ailey, S.H., et al., *Factors related to complications among adult patients with intellectual disabilities hospitalized at an academic medical center.* Intellect Dev Disabil, 2015. **53**(2): p. 114-9.

114. Ashley, E.A., et al., *Evidence of autonomic dysreflexia during functional electrical stimulation in individuals with spinal cord injuries.* Paraplegia, 1993. **31**(9): p. 593-605.

115. Pinter, M.M. and M.R. Dimitrijevic, *Gait after spinal cord injury and the central pattern generator for locomotion.* Spinal Cord, 1999. **37**(8): p. 531-7.

116. Minassian, K., et al., *The Human Central Pattern Generator for Locomotion.* Neuroscientist, 2017: p. 1073858417699790.

117. MacKay-Lyons, M., *Central Pattern Generation of Locomotion: A Review of the Evidence.* Physical Therapy, 2002. **82**(1): p. 69-83.

118. Barriere, G., et al., *Prominent role of the spinal central pattern generator in the recovery of locomotion after partial spinal cord injuries.* J Neurosci, 2008. **28**(15): p. 3976-87.

119. Zomlefer, M.R., et al., *Electromyographic study of lumbar back muscles during locomotion in acute high decerebrate and in low spinal cats.* Brain Res, 1984. **290**(2): p. 249-60.

120. Barbeau, H. and S. Rossignol, *Recovery of locomotion after chronic spinalization in the adult cat.* Brain Res, 1987. **412**(1): p. 84-95.

121. Domingo, A., G.S. Sawicki, and D.P. Ferris, *Kinematics and muscle activity of individuals with incomplete spinal cord injury during treadmill stepping with and without manual assistance.* J Neuroeng Rehabil, 2007. **4**: p. 32.

122. Knikou, M., *Functional reorganization of soleus H-reflex modulation during stepping after robotic-assisted step training in people with complete and incomplete spinal cord injury.* Exp Brain Res, 2013. **228**(3): p. 279-96.

123. Gorassini, M.A., et al., *Changes in locomotor muscle activity after treadmill training in subjects with incomplete spinal cord injury.* J Neurophysiol, 2009. **101**(2): p. 969-79.

124. Dobkin, B.H., et al., *Modulation of locomotor-like EMG activity in subjects with complete and incomplete spinal cord injury.* J Neurol Rehabil, 1995. **9**(4): p. 183-90.

125. Dietz, V., et al., *Locomotor capacity of spinal cord in paraplegic patients.* Ann Neurol, 1995. **37**(5): p. 574-82.

126. Alamro, R., *Trunk muscle activation patterns during walking with robotic exoskeletons in people with high thoracic motor-complete spinal cord injury*, in *Education, Faculty of Kinesiology*. 2017, University of British Columbia.

127. Maegele, M., et al., *Recruitment of spinal motor pools during voluntary movements versus stepping after human spinal cord injury.* J Neurotrauma, 2002. **19**(10): p. 1217-29.

128. Kisan, R., et al., *Treadmill and Bicycle Ergometer Exercise: Cardiovascular Response comparison.* Global Journal of Medical research, 2012. **12**(5): p. 23-25.

129. Schrieks, I.C., M.J. Barnes, and L.D. Hodges, *Comparison study of treadmill versus arm ergometry.* Clin Physiol Funct Imaging, 2011. **31**(4): p. 326-31.

130. Wouda, M.F., et al., *Energetic and cardiovascular responses to treadmill walking and stationary cycling in subjects with incomplete spinal cord injury.* Spinal Cord, 2016. **54**(1): p. 51-6.

131. Carvalho, D.C., et al., *Metabolic and cardiorespiratory responses of tetraplegic subjects during treadmill walking using neuromuscular electrical stimulation and partial body weight support.* Spinal Cord, 2005. **43**(7): p. 400-5.

132. Phillips, A.A., A.T. Cote, and D.E. Warburton, *A systematic review of exercise as a therapeutic intervention to improve arterial function in persons living with spinal cord injury.* Spinal Cord, 2011. **49**(6): p. 702-14.

133. Burns, K.J., et al., *Passive limb movement intervals results in repeated hyperemic responses in those with paraplegia.* Spinal Cord, 2018. **56**(10): p. 940-948.

134. Ballaz, L., et al., *Acute peripheral blood flow response induced by passive leg cycle exercise in people with spinal cord injury.* Arch Phys Med Rehabil, 2007. **88**(4): p. 471-6.

135. Hoffman, M.D., *Cardiorespiratory fitness and training in quadriplegics and paraplegics.* Sports Med, 1986. **3**(5): p. 312-30.

136. Stevens, S.L. and D.W. Morgan, *Heart rate response during underwater treadmill training in adults with incomplete spinal cord injury.* Top Spinal Cord Inj Rehabil, 2015. **21**(1): p. 40-8.

137. Evans, D.L., *Cardiovascular adaptations to exercise and training.* Vet Clin North Am Equine Pract, 1985. **1**(3): p. 513-31.

138. Hellsten, Y. and M. Nyberg, *Cardiovascular Adaptations to Exercise Training.* Compr Physiol, 2015. **6**(1): p. 1-32.

139. Meyns, P., et al., *Locomotor training with body weight support in SCI: EMG improvement is more optimally expressed at a low testing speed.* Spinal Cord, 2014. **52**(12): p. 887-93.

140. Mazzoleni, S., et al., *Changes on EMG activation in healthy subjects and incomplete SCI patients following a robot-assisted locomotor training.* IEEE Int Conf Rehabil Robot, 2011. **2011**: p. 5975467.

141. Wirz, M., G. Colombo, and V. Dietz, *Long term effects of locomotor training in spinal humans.* J Neurol Neurosurg Psychiatry, 2001. **71**(1): p. 93-6.

142. Muraki, S., et al., *Cardiovascular and respiratory responses to passive leg cycle exercise in people with spinal cord injuries.* Eur J Appl Physiol Occup Physiol, 1996. **74**(1-2): p. 23-8.

143. Muraki, S., Y. Ehara, and M. Yamasaki, *Cardiovascular responses at the onset of passive leg cycle exercise in paraplegics with spinal cord injury.* Eur J Appl Physiol, 2000. **81**(4): p. 271-4.

144. Harkema, S.J., et al., *Improvements in orthostatic instability with stand locomotor training in individuals with spinal cord injury.* J Neurotrauma, 2008. **25**(12): p. 1467-75.

145. Teasell, R.W., et al., *Cardiovascular consequences of loss of supraspinal control of the sympathetic nervous system after spinal cord injury.* Arch Phys Med Rehabil, 2000. **81**(4): p. 506-16.

146. Malmqvist, L., et al., *Assessment of autonomic function after acute spinal cord injury using heart rate variability analyses.* Spinal Cord, 2015. **53**(1): p. 54-8.

147. Ditor, D.S., et al., *Reproducibility of heart rate variability and blood pressure variability in individuals with spinal cord injury.* Clin Auton Res, 2005. **15**(6): p. 387-93.

148. *Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.* Eur Heart J, 1996. **17**(3): p. 354-81.

149. de Carvalho Abreu, E.M., et al., *Cardiovascular autonomic control in paraplegic and quadriplegic.* Clin Auton Res, 2016. **26**(2): p. 117-26.

150. Kyriakides, A., et al., *The effect of level of injury and physical activity on heart rate variability following spinal cord injury.* J Spinal Cord Med, 2019. **42**(2): p. 212-219.

151. Buker, D.B., C.C. Oyarce, and R.S. Plaza, *Effects of Spinal Cord Injury in Heart Rate Variability After Acute and Chronic Exercise: A Systematic Review.* Top Spinal Cord Inj Rehabil, 2018. **24**(2): p. 167-176.

152. Serra-Ano, P., et al., *Heart rate variability in individuals with thoracic spinal cord injury.* Spinal Cord, 2015. **53**(1): p. 59-63.

153. Millar, P.J., et al., *Effects of short-term training on heart rate dynamics in individuals with spinal cord injury.* Auton Neurosci, 2009. **150**(1-2): p. 116-21.

154. Fujiura, Y., et al., *Heart rate and mortality in a Japanese general population: an 18-year follow-up study.* J Clin Epidemiol, 2001. **54**(5): p. 495-500.

155. Berlowitz, D.J., B. Wadsworth, and J. Ross, *Respiratory problems and management in people with spinal cord injury.* Breathe (Sheff), 2016. **12**(4): p. 328-340.

156. Galeiras Vazquez, R., et al., *Respiratory management in the patient with spinal cord injury.* Biomed Res Int, 2013. **2013**: p. 168757.

157. Higgins, M., *Therapeutic Exercise: From Theory to Practice*. 2011, Philadelphia. PA 19103: F. A. Davis Company. 800.

158. Terson de Paleville, D., et al., *Locomotor step training with body weight support improves respiratory motor function in individuals with chronic spinal cord injury.* Respir Physiol Neurobiol, 2013. **189**(3): p. 491-7.

159. Dhindsa, M.S., et al., *Muscle spasticity associated with reduced whole-leg perfusion in persons with spinal cord injury.* J Spinal Cord Med, 2011. **34**(6): p. 594-9.

160. eHealthMe. *Muscle spasticity and Heart rate increased - from FDA reports*. n.d. [cited 2017 Dec 2]; Available from: https://www.ehealthme.com/cs/muscle-spasticity/heart-rate-increased/.

161. Suzuki, N., et al., *Exercise intensity based on heart rate while walking in spastic cerebral palsy.* Bull Hosp Jt Dis, 2001. **60**(1): p. 18-22.

162. Hung, C.Y., et al., *Cardiac autonomic status is associated with spasticity in post-stroke patients.* NeuroRehabilitation, 2014. **34**(2): p. 227-33.

163. Ghiasi, F., et al., *Change of Spasticity Following Robotic-Assisted Gait Training in Patients with Chronic Incomplete Spinal Cord Injury: Preliminary Results.* American Journal of Neuroprotection and Neuroregeneration, 2012. **4**(1): p. 53-57(5).

164. Stampacchia, G., et al., *Walking with a powered robotic exoskeleton: Subjective experience, spasticity and pain in spinal cord injured persons.* NeuroRehabilitation, 2016. **39**(2): p. 277-83.

165. Mirbagheri, M.M., et al., *The effects of Robotic-Assisted Locomotor training on spasticity and volitional control.* IEEE Int Conf Rehabil Robot, 2011. **2011**: p. 5975443.

166. Tefertiller, C., et al., *Efficacy of rehabilitation robotics for walking training in neurological disorders: a review.* J Rehabil Res Dev, 2011. **48**(4): p. 387-416.

167. Colombo, G., et al., *Treadmill training of paraplegic patients using a robotic orthosis.* J Rehabil Res Dev, 2000. **37**(6): p. 693-700.

168. Schwartz, I. and Z. Meiner, *Robotic-assisted gait training in neurological patients: who may benefit?* Ann Biomed Eng, 2015. **43**(5): p. 1260-9.

169. Nam, K.Y., et al., *Robot-assisted gait training (Lokomat) improves walking function and activity in people with spinal cord injury: a systematic review.* J Neuroeng Rehabil, 2017. **14**(1): p. 24.

170. Liu, W., *A narrative review of gait training after stroke and a proposal for developing a novel gait training device that provides minimal assistance.* Top Stroke Rehabil, 2018. **25**(5): p. 375-383.

171. Alqahtani, A.S., et al., *Feasibility and trend of pulmonary function in a pilot trial of aerobic walking exercise in non-ambulatory stroke survivors.* Top Stroke Rehabil, 2019: p. 1-9.

Chapter 2: Effect of Body Weight–Supported Treadmill Training on Cardiovascular and Pulmonary Function in People with Spinal Cord Injury: A Systematic Review

This chapter has previously been published in whole without any adaptations since publication and is reprinted here with permission. Alajam, R., Alqahtani, A.S., Liu, W. *Effect of Body Weight–Supported Treadmill Training on Cardiovascular and Pulmonary Function in People with Spinal Cord Injury: A Systematic Review.* Topics in Spinal Cord Injury Rehabilitation, 2019. **25**(4): p. 355-369.

Abstract

Objective: To assess the current evidence with regard to the effects of body weight–supported treadmill training (BWSTT) on cardiovascular and pulmonary function in people with spinal cord injury (SCI) with a focus on outcomes of heart rate (HR), blood pressure (BP), and respiratory parameters.

Methods: A systematic literature search was conducted through MEDLINE/PubMed, the Cumulative Index to Nursing and Allied Health Literature, and Physiotherapy Evidence Database. Clinical trials involving adults with SCI and focusing on the effects of BWSTT on HR, BP, and respiratory measurements were included. The quality of included studies was assessed using the Downs and Black scale. The level of evidence of each study was identified using the Spinal Cord Injury Rehabilitation Evidence system.

Results: Nine studies that met inclusion criteria were evaluated and included in this review. Overall, the quality index of all included studies was low. All studies scored less than 21 out of 28 on the Downs and Black scale. The level of evidence varied from level 2 to level 4. Level 4 evidence supports the use of BWSTT to decrease resting and exercise HR and improve heart rate variability. The use of BWSTT to improve respiratory parameters after SCI is supported by one study with level 2 evidence. The evidence that supports the use of BWSTT to improve resting BP is inconclusive.

Conclusion: There has been low to moderate evidence to support the use of BWSTT in individuals with SCI to improve cardiovascular and pulmonary health. Future randomized controlled trials are needed to investigate the effect of BWSTT on cardiovascular and pulmonary function in people with SCI and compare BWSTT to other physical rehabilitation interventions.

Keywords: *body weight–supported treadmill training; blood pressure; heart rate; respiratory parameters; spinal cord injury; locomotion training; walking training*

2.1. Introduction

Spinal cord injury (SCI) is a serious medical condition that leads to loss of or impairment in sensorimotor function, which negatively impacts the quality of life. Individuals with SCI are susceptible to various secondary complications, including deterioration in cardiovascular and respiratory health [1, 2]. It has been estimated that the prevalence rate of cardiovascular disease (CVD) in the SCI population (30%-50%) is significantly higher compared to the able-bodied population (5%-10 %) [3]. A study reported that the SCI population has a 2.72 times higher risk of heart disease and a 3.72 times higher risk of stroke compared to the general population [4]. In terms of morality after CVD, a cohort study that followed individuals with SCI for 5 years after discharge from inpatient rehabilitation found that 37% of individuals died due to cardiovascular causes [5].

Physical inactivity is associated with a decrease in cardiovascular fitness (e.g., elevated heart rate [HR]) and consequently an increased risk of CVD [6-8]. Lack of physical activity or prolonged sitting observed in many SCI survivors due to limited functional mobility can contribute to elevated HR and other risk factors for CVD [3, 6]. Elevated resting HR is more common in individuals with SCI at T1 or below (paraplegia) as compared to those with cervical SCI (quadriplegia) [8, 9]. Studies report that people with SCI, especially those with paraplegia, have higher resting HR compared to able-bodied people [7-10]. Furthermore, the cardiac autonomic system can be altered after SCI due to the sedentary lifestyle and lack of mobility resulting from the injury [11]. Heart rate variability (HRV) can be used as a sensitive measure to determine cardiac autonomic function. Reduced HRV after the injury is associated with an increased risk of heart diseases [12, 13]. Past studies show reduced HRV in individuals with

SCI in comparison with able-bodied individuals [11, 14]. Physically inactive individuals with SCI have less HRV than those who are physically active [11].

High blood pressure (BP; i.e., hypertension) is another factor that can increase the risk of cardiovascular disease [15]. Hypertension is common in people living with long-term SCI, and it is more prevalent in people with paraplegia compared to quadriplegia [16-18]. Previous studies report a high prevalence of hypertension among people with SCI, especially those with paraplegia, as compared to able-bodied people [16, 19]. Sedentary lifestyle after SCI plays an important role in the development of hypertension. Other risk factors such as obesity, hypercholesterolemia, diabetes, smoking, and age can also contribute to an increased risk of hypertension after SCI [16].

The paralysis of the respiratory musculature following SCI can result in an alteration of the mechanical properties of the lung and chest wall [20]. Pulmonary function may decline after SCI because of decreased muscle strength of the diaphragm, respiratory, and abdominal muscles as well as a lack of sufficient mobility [21]. Reduction in pulmonary function after SCI is reflected by decreases in spirometric and lung volume parameters and static mouth pressures [22]. The weakness of respiratory muscles can result in an ineffective cough and a tendency toward mucus retention, which might increase susceptibility to respiratory diseases [22]. Respiratory impairments are more severe in people with cervical injury compared to those with thoracic or lumbar injury due to a disruption in the function of the majority of the respiratory and abdominal muscles [22]. Respiratory deficiency can lead to secondary problems such as respiratory infectious diseases, which are the leading causes of death in people with chronic SCI [23].

Participating in regular exercise is necessary to prevent or reduce secondary complications after SCI. Aerobic exercise using arm cycling or functional electrical stimulation (FES) leg cycling has been utilized to improve cardiovascular and pulmonary health in the SCI population. However, exercise using these modalities has shown inconsistent results regarding its effects on cardiovascular and pulmonary health in the SCI population [24-27]. Limitations of these modalities have been discussed in the literature [28-31]. A major limitation of arm cycling or FES leg cycling exercise is the lack of sustainable activities of large leg muscles, leading to insufficient challenges to the cardiopulmonary system [32, 33]. As an alternative, body weight–supported treadmill training (BWSTT) is a tool that is widely used in the rehabilitation program for people with neurological conditions. Even though most studies have focused on investigating the effectiveness of BWSTT on motor function and concluded that it might be an appropriate intervention to improve walking ability, promising findings from previous studies have shown that regular walking training using BWSTT can help to improve cardiovascular and pulmonary health in the SCI population [34-42]. Therefore, this systematic review aimed to qualitatively assess the current evidence with regard to the effects of walking training using BWSTT on cardiovascular and pulmonary health among people with SCI. The focus of the study is on the effects of BWSTT on HR and BP measurements and respiratory parameters.

2.2. Methods

2.2.1. Search Strategy

A systematic literature search was conducted to examine the effect of BWSTT on measures of cardiovascular and pulmonary health in people with SCI. MEDLINE (PubMed), the Cumulative Index to Nursing and Allied Health Literature (CINAHL), and the Physiotherapy Evidence Database (PEDro) were searched. The search was limited to clinical trial studies in humans, with adult patients (≥18 years), and written in English. It was conducted for the time period of January 2004 until May 2018.

The search strategy included the terms for target population (spinal cord injury, paraplegia, quadriplegia or tetraplegia), outcome measures (heart rate, blood pressure, pulmonary function, respiratory function, and respiratory parameters), and intervention (locomotor training, walking training, gait training, or body weight support treadmill training). Different combinations of the terms were made using "AND" and "OR" in order to achieve a specific selection of literature (**Table 2.1**).

2.2.2. Study Eligibility Criteria

Inclusion criteria were the following:

1. Studies with adult patients (≥18 years) with traumatic or nontraumatic SCI (cervical, thoracic, and lumbar), complete or incomplete lesions, and American Spinal Injury Association Impairment Scale (AIS) of A, B, C, or D.
2. Treadmill walking training, including manual-assisted BWSTT, robotic-assisted BWSTT, and treadmill walking training in water.
3. Studies focused on measurements of HR and BP and respiratory parameters after a course of treadmill walking training.
4. Clinical trial studies, including randomized controlled (RCTs), quasi-experimental or pre-experimental trials.
5. Published in English.

Exclusion criteria were (a) overground walking training and (b) animal studies or studies on children.

2.2.3. Study Selection

After removing duplicated studies, two independent researchers (R.A and A.A) screened the articles by reading titles and abstracts. Then, the full text of relevant articles to study objectives was read in detail to determine eligibility (**Figure 2.1**). Disagreements on study selection were resolved by discussions between two researchers (R.A and A.A)

2.2.4. Data Extraction

The study information was extracted by two reviewers (R.A. and A.A) independently. The following data were extracted from included studies: author's name, year, study design, sample size, participants' characteristics (gender, age, injury level on the SCI, grade based on AIS, and time since injury), intervention program (length and frequency), and outcomes related to measurements of HR, BP, and respiratory parameters. There were no disagreements between the reviewers regarding data extraction.

2.2.5. Assessment of Quality Study and Level of Evidence

Included studies were assessed using the modified Downs and Black scale to determine their methodological quality. The Downs and Black scale is composed of 27 questions to assess the quality of a study across five categories, including reporting (10 questions), external validity (3 questions), internal validity (bias and confounding; 13 questions), and power (one question) (see *Appendix I*) [43]. The total score of the scale ranges from 0 to 28. A higher score indicates a better quality of methodology. From the percentage of derived total score, studies were classified as high (>75%), moderate (50%-74%), or low quality (<50%). The level of evidence of each study was identified using the Spinal Cord Injury Rehabilitation Evidence (SCIRE) system, a five-level system that differentiates between studies of differing quality and incorporates the types of research designs commonly used in rehabilitation research (**Table 2.2**) [13]. This scale has been used in published systematic review studies of exercise training in people with SCI [44, 45]. Two researchers evaluated and scored studies independently. In case of disagreement between two evaluators, a consensus was made through discussion.

2.3. Results

An overview of the results of the literature search and screening process is provided in Figure 1. The electronic database search retrieved 264 articles. Removal of duplicates within and between the individual databases left 79 articles for further examination. Of the 79 retrieved articles, 53 articles were excluded after screening for titles and abstracts. The remaining 26 articles were screened by reading full text to determine eligibility. Specific reasons for article exclusion are presented in **Figure 2.1**. Nine articles were evaluated in detail and included in this review study.

A summary of the study designs, participants' characteristics, interventions, and outcome measures for each of the nine studies reviewed is provided in **Table 2.3**. The number of participants in the included studies ranged from 6 to 52 participants; a total of 121 participants took part in those studies. They included individuals with incomplete SCI, complete SCI, or both. The duration of interventions ranged from 2 to 5 times per week for 4 weeks to 6 months. Seven studies examined the effects of walking training on HR measurements [34-40]. Four studies examined the effects of walking training on BP measurements [34-36, 40]. Three studies examined the effects of walking training on respiratory parameters [36, 41, 42].

2.3.1. Quality Assessment for Included Studies

The results of the quality review are presented in **Table 2.4**. Overall, the quality index of all included studies was low. All studies scored less than 21 out of 28 on the Downs and Black scale. All studies fulfilled most of the criteria for reporting. The objectives of the study, the main outcomes, the characteristics of the patients, the interventions of interest, and the main findings were clearly described in these studies [34-42]. However, all studies [34-37, 39-42] except one [38] were rated poorly on the measurements of the internal validity (bias and confounding) because of lack of control group and randomization. Two studies were scored poorly on the measurement of the external validity because only men were included, and this might have an influence on the generalizability of findings [38, 40]. All studies did not report estimation of sample size; thus, they were rated poorly on the measurements of the power [34-42].

2.3.2. Levels of Evidences for Included Studies

The level of evidence varied from level 2 to level 4 (**Table 2.3**). Two studies included a control group [38, 42]. One of the studies was a randomized cross-over study design with small sample size and was classified as level 2 [38]. The other study was a prospective study with a control group (i.e., quasi-experimental study design) and was also categorized as level 2 [42]. The remaining seven studies used a single group, pre- and post-test study design and were placed at level 4 [34-37, 39-41]. The sample sizes of all studies [34-41] were small (range, 6 -12) except one study [42] that included 52 participants.

2.4. Discussion

To the best of our knowledge, this review is the first to systematically synthesize the evidence regarding the effects of BWSTT program on measurements of HR, BP, and respiratory parameters in people with SCI. An exhaustive search found nine studies that met inclusion/exclusion criteria. Given the heterogeneous nature of the SCI population and low research quality, there is weak to moderate evidence to support the effects of BWSTT on improving HR measures and respiratory parameters; however, the evidence regarding BP measures is still lacking or needed.

2.4.1. Heart Rate Measurements

Changes in HR were measured pre- and post-BWSTT in seven single group studies [34-40]. One study [40] included only individuals with motor complete cervical SCI (i.e., quadriplegia) and found no changes in resting HR after BWSTT (2 sessions per week for 3 months). On the contrary, another study [34] that included both motor incomplete and complete cervical SCI reported a significant decrease in resting HR after 6 months of BWSTT with 3 sessions per week. Two studies with mixed SCI lesion levels (i.e., quadriplegia and paraplegia) [36, 37] also reported a significant decrease in resting HR after 6 weeks or 24 sessions over 10 to 16 weeks of BWSTT. Two other studies with mixed SCI lesion levels [35, 38] reported a nonsignificant decrease in resting HR after 4 months or 4 weeks of BWSTT. Exercise HR was measured in 2 studies [37, 39] with incomplete and mixed SCI lesion levels and was significantly decreased after 24 sessions of BWSTT.

The intensity and duration of BWSTT may influence HR adaptation. One study [38] found no significant decrease in resting HR, but the duration of this study was very short (4 weeks of BWSTT) as compared to the other studies [34, 36, 37] (8 weeks to 6 months of BWSTT). Authors of this study did not provide information about the intensity of training. Also, it might be challenging to find a significant change in HR after 4 weeks of exercise. It has been shown that at least 6 to 8 weeks of walking training would be ideal for producing HR adaptation

[36, 37]. Furthermore, moderate to high intensity of arm aerobic exercise for 8 weeks has been shown to improve cardiopulmonary fitness in individuals with SCI [46]. Future studies in walking training should focus on the impact of the training intensity on HR outcomes.

Completeness of injury (incomplete or complete SCI) might also have an influence on HR response to walking training. In two studies [35, 40] that included only individuals with motor complete injuries, BWSTT did not produce a significant decrease in resting HR. In Ditor's study,[35] only participants who had HR response (i.e., increased HR) during walking training showed a decrease in resting HR. It seems that HR response and voluntary contraction of leg muscles during BWSTT are essential factors for inducing HR adaptation [35]. A repetitive and intensive locomotor training has been shown to improve the activity of leg muscles even in persons with motor complete SCI [47, 48]. It is still unknown how significantly increased activity of leg muscles after a course of walking training can contribute to HR adaptation.

Compared to arm cycling exercise, walking training can trigger the activity of larger muscles in the body (i.e., leg and trunk muscles) through activation of central pattern generators located within the spinal cord [49-51]. In addition, being in upright posture during walking training can provide greater stress and challenge to the cardiovascular system. It has been shown that in individuals with SCI, HR and oxygen uptake were significantly higher during BWSTT in comparison to exercise in a sitting position [32, 33]. Consequently, walking exercise might induce a greater positive adaptation in HR than conventional exercise approaches such as cycling or FES leg cycling exercise [52]. However, the efficacy of walking training compared to other exercise approaches has not been studied.

In relation to cardiac autonomic function, one study [34] showed an improvement in HRV, as reflected by a significant reduction in the ratio of low-frequency power to high-frequency power, in individuals with incomplete cervical SCI after 6 months of BWSTT. Two studies [35, 38] reported a trend toward an improvement in HRV after 4 weeks or 4 months of BWSTT. The duration of one of these studies that found nonsignificant improvement in HRV after training was very short (i.e., 4 weeks) [38]. It might be difficult to find a significant improvement in HRV within a short term of training. However, this study has noted a significant improvement in HR complexity after training, which is an indirect measurement of cardiac autonomic function [38]. The other study included only individuals with motor complete SCI, and only participants who showed HR response (i.e., increased HR during training) to BWSTT improved their HRV after 4 months of training [35].

The findings of these studies [34, 36, 37, 39] suggest that BWSTT might result in decreased resting and exercise HR and improved cardiac autonomic function in individuals with SCI. These studies that support the use of BWSTT in improving HR measurements used a single group, pre- and post-test study design. Therefore, level 4 evidence is given for the use of BWSTT to improve HR measurements following SCI.

2.4.2. Blood Pressure Measurements

Four studies [34-36, 40] investigated the effects of BWSTT on changes in BP measurements in individuals with SCI. Two studies [35, 36] included individuals with cervical, thoracic, or lumbar SCI, and the other two studies [34, 40] targeted only individuals with cervical SCI. In the studies with mixed SCI levels, no significant changes in resting BP were observed in individuals with motor incomplete SCI after 6 weeks of BWSTT [36] or in motor complete SCI after 4 months of BWSTT [35]. In the studies of cervical SCI, one study observed no significant change in resting BP in motor incomplete SCI after 6 months of BWSTT [34]. Another study with motor complete cervical SCI reported a significant increase in resting BP after 3 months of

BWSTT combined with neuromuscular electrical stimulation [40]. The authors of this study suggested that the increase in resting BP might be due to improvement in sympathetic activity after the BWSTT [40].

It is well known that regular exercise at moderate/high intensity can lead to a decrease in resting BP [53, 54]. Past studies indicated that regular exercise might decrease resting BP in people with paraplegia (SCI at T1 or below) for whom the prevalence rate of hypertension is high [6, 55]. However, people with cervical SCI (quadriplegia) tend to have abnormally lower resting BP compared to able-bodied individuals and those with thoracic or lumbar SCI (paraplegia) due to impaired cardiovascular autonomic function after SCI [16-18]. Additional reduction in BP after a period of exercise in people with quadriplegia might provoke symptoms of hypotension [40]. A physical exercise program of FES leg cycling for several months increased resting BP in people with quadriplegia [56]. The use of BWSTT in people with quadriplegia may or may not lead to an increase in resting BP, as shown in two reviewed studies [34, 40]. Studies that included participants with quadriplegia and paraplegia [35, 36] were therefore inconclusive in terms of changes in BP due to the fact that resting BP may change in opposite directions in these populations.

As discussed previously, the four studies [34-36, 40] that reported outcomes in resting BP after BWSTT were inconclusive due to their heterogeneity in terms of study participants and the response of the participants. In addition, all four studies had small sample sizes, ranging from 6 to 12 participants. This limited number of studies, small sample sizes, different levels of SCI, and variable responses to walking exercise allow no conclusions to be drawn regarding the effects of BWSTT on resting BP. Future clinical trials need to include large sample sizes and focus on either paraplegia or quadriplegia.

Two studies [34, 35] also measured BP variability pre- and post-BWSTT training. One study [34] reported a significant decrease in low-frequency systolic BP (SBP) in individuals with incomplete cervical SCI after 6 months of BWSTT. The other study from the same investigation team found no significant changes in measurements of BP variability in individuals with motor complete cervical or thoracic SCI after 4 months of BWSTT [35]. No conclusion can be made at present.

2.4.3. Respiratory Parameters

Only 3 out of 9 studies that were included in this review measured pulmonary function as an outcome [36, 41, 42]. In all three studies, there were improvements in some of the respiratory parameters after a course of walking training. Soyupek et al. [36] found significant increases in forced vital capacity (FVC) and inspiratory capacity (IC) in individuals with motor incomplete SCI after 6 weeks of BWSTT. Another prospective study with a control group showed that vital capacity (VC), VC%, FVC, forced expiratory volume in one second (FEV 1), and forced expiratory flow rate 25% to 75% were significantly increased in participants who received 4 weeks of BWSTT (the experimental group) but not in those who received standard rehabilitation program (the control group) [42]. Both groups showed a significant increase in maximum voluntary ventilation [42]. However, this study did not use a random procedure in assigning the participants into one of two groups.

In addition to measuring pulmonary function, a study explored the underlying mechanisms of improvement in pulmonary function after walking training [41]. They reported significant increases in FVC, FEV 1, and maximum expiratory pressure after 62±10 sessions of BWSTT. Also, the amplitude and motor unit recruitment of all respiratory muscles during respiratory tasks significantly increased after training as compared to baseline measures. The findings of the study suggest that BWSTT could induce neuroplasticity in spinal neural circuitry that is responsible for the activation of respiratory muscles.

In comparison to exercises in sitting position, upright posture during walking training can trigger the activity of trunk muscles including abdominal muscles, which play an important role in respiration [49-51]. It is also a highly effective stressor of the cardiopulmonary system since the lungs need to work harder to deliver more oxygen to larger working muscles [57]. Furthermore, it has been shown that walking training increases the connectivity of neural spinal circuity between motor cortex and leg muscles [58]. The increased muscle activity of inspiratory and expiratory muscles after BWSTT may be explained by neuroplastic changes within the neural spinal circuity that controls respiration [41]. All of those factors might induce greater adaptive changes in pulmonary function. Based on this information mentioned, the use of BWSTT to improve pulmonary function after SCI is supported by one study [42] with level 2 evidence.

2.5. Conclusion

To maintain cardiovascular and pulmonary health after SCI, it is crucial for people with SCI to engage in regular physical activity. There is some evidence that the use of BWSTT as an exercise in individuals with SCI has positive effects on cardiovascular and pulmonary health by improving resting and exercise HR and respiratory parameters. No clear evidence is currently available about which type of physical rehabilitation produces the best results. In addition, it is not clear whether BWSTT leads to a better outcome in cardiovascular and pulmonary function compared to conventional physical rehabilitation methods, such as arm cycling or FES leg cycling exercise. Furthermore, current studies have not provided information about the effects of the intensity level of walking training on these outcome measurements. Because of limited studies, further investigations are necessary. Future randomized-controlled trial studies are needed to investigate the effects of BWSTT on cardiovascular and pulmonary health compared to other physical rehabilitation interventions. Further studies also should investigate the influence of walking training intensity (i.e., walking speed or amount of body weight support) on cardiovascular or pulmonary health.

Table 2.1: Key words and combination of key word used in the search

Population	Outcomes	Intervention
- Spinal cord injury - Paraplegia, - Quadriplegia/tetraplegia	- Heart rate - Blood pressure - Pulmonary function - Respiratory function - Respiratory parameters	- Locomotor training - Walking training - Gait training - Body weight support treadmill training

Note: "AND" was used between terms in population, outcomes, and intervention columns. The terms in the columns are allied with "OR."

Table 2.2: Level of evidence and criteria based on the Spinal Cord Injury Rehabilitation Evidence (SCIRE) system

Level of evidence	Criteria
Level 1	• **RCT:** PEDro score ≥ 6. Includes crossover design with randomized experimental conditions and within-subjects comparison.
Level 2 (*n* = 2)	• **RCT:** PEDro score ≤ 6. • **Prospective controlled trial: N**onrandomized. • **Cohort: L**ongitudinal study using at least two similar groups with one group being exposed to a condition.
Level 3	• **Case-control studies: R**etrospective study comparing control conditions.
Level 4 (*n* = 7)	• **Pre-post:** Trial with a baseline measure, intervention, and a post-est using a single group of subjects. • **Posttest:** Posttest with two or more groups using a single group (intervention followed by a posttest with no retest or baseline assessment).
Level 5	• **Observational:** Study using cross-sectional analysis to interpret relations. • **Case report:** Pre-post or case series involving one subject.

Note: The PEDro scale is developed by the Physiotherapy Evidence Database to determine the quality of clinical trials (high quality = score 6-10, fair quality = 4-5, poor quality = score ≤ 3). RCT = randomized controlled trail.

.

Table 2.3: Summary of the studies, participant characteristics, interventions, outcome measures, and main findings (N = 9)

Author	Study design	Evidence level	Participants	Intervention	Outcome measures	Main findings
Ditor et al, 2004 [34]	Single group, pre- and posttest	Level 4	8 individuals with chronic cervical SCI [6 men, 2 women; mean age, 27.6 y; SCI level: C4-C5; AIS: B-C; mean postinjury, 9.6 y]	BWSTT with manual assistance [3x/wk for 6 mo]	• Heart rate (HR) • Blood Pressure (BP) • Heart rate variability (HRV) • Blood pressure variability (BPV)	• Significant reduction in the resting HR • No significant change in resting BP • Significant reduction in the resting low-frequency power (LF) to high-frequency power (HF) ratio of HRV • Significant decrease in resting $LF_{Systolic\ BP}$ of BPV
Ditor et al, 2005 [35]	Single group, pre- and posttest	Level 4	6 individuals with chronic SCI [4 men, 2 women; mean age, 37.77±15.4 y; SCI level: C4-T12; AIS: A-B; mean postinjury, 7.67±9.4 y]	BWSTT with manual assistance [3x/wk for 4 mo]	• HR response measures • HRV and BPV • Resting measures of arterial dimension and function	• No significant changes in resting HRV and BPV • However, subgroup of individuals who had HR response to training showed improvements in HRV (i.e., ↓ LF to HF ratio due to ↑HF and ↓LF) and reductions of BPV (i.e., ↓LF_{SBP}) • Significant increase in femoral artery compliance
Soyupek et al, 2009 [36]	Single group, pre- and posttest	Level 4	8 individuals with SCI [6 men, 2 women; mean age, 40.75±13.93 y; SCI level: C6-L1; AIS: B-D]	BWSTT with manual assistance [5x/wk for 6 wk]	• Cardiovascular parameters (resting HR and BP) • Pulmonary function test • Depression	• Significant decrease in resting HR • No significant change in resting systolic BP • Significant improvement only in FVC and IC
Hoekstra et al, 2013 [37]	Single group, pre- and posttest	Level 4	10 individuals with chronic SCI [4 men, 6 women; mean age, 49±14 y; SCI level: C3-L2; AIS: C-D; mean postinjury, 9±10 y]	BWSTT with robotic assistance [2-3x/wk; total of 24 sessions]	Cardiopulmonary fitness, including: • Resting, submaximal, and peak VO_2 and O_2 pulse • Resting, submaximal, and peak HR Walking training intensity: • %HR reserve • %VO_2 reserve • METs	• Significant decrease in resting and submaximal HR after training • Three subjects met the recommended guidelines of exercise intensity based on % VO_2 reserve • Two subjects met the recommended guidelines of exercise intensity based on % HR reserve • Two subjects met the recommended guidelines of exercise intensity based on METs • Rest of subject's exercise at low intensity
Millar et al, 2009 [38]	Randomized cross-over	Level 2	6 individuals with chronic SCI (6 men; mean age, 37.1±7.7 y; SCI level: C5-T10; AIS: A-C; mean postinjury, 5.0±4.4 y)	Group 1: BWSTT with manual assistance Group 2: Passive head-uptilt training [3x/wk for 4 wk]	• Resting HR • HRV • HR complexity • Detrended fluctuation analysis of HR behavior	• Decrease in resting HR after BWSTT and HUTT, but was not significant • A trend toward improvement of HRV, as reflected by reduced LF/HF ratio, after BWSTT • Significant increase HR complexity and reduction in the fractal scaling distance score

Table 2.3: Summary of the studies, participant characteristics, interventions, outcome measures, and main findings (*N* = 9) (CONT.)

Author	Study design	Evidence level	Participants	Intervention	Outcome measures	Main findings
Stevens et al, 2015 [39]	Single group, pre- and posttest	Level 4	11 individuals with chronic incomplete SCI (7 men and 4 women; mean age, 48±12 y; SCI level: C2-L2; AIS: C-D; mean postinjury, 5±8 y)	Underwater treadmill training [3x/wk for 8 wk]	• Exercise HR during training period 1 (wk 2-3), 2 (wk 4-5), and 3 (wk 6-7)	• Significant decrease in mean exercise HR during each training period 1, 2 and 3 • All participants experience a decrease in daily walking HR for each 2-week block period
Carvalho et al, 2005 [40]	Single group, pre- and posttest	Level 4	12 individuals with complete cervical SCI (12 men; mean age, 33.8±33.73 y; SCI level: C4-C7; AIS: A; median postinjury, 77.58 months	BWSTT with manual assistance and NES [2x/wk for 3 months]	• HR and BP at rest, during treadmill walking and during the recovery phase pre- and post-training	• Significant increase in HR and systolic BP from rest to walking training • After 3 months walking training, significant increase in systolic BP at rest and during walking training, but in recovery phase • No significant changes in HR at rest, during walking training, in recovery phase after 3 months walking training
Terson de Paleville et al, 2013 [41]	Single group, pre- and posttest	Level 4	8 individuals with chronic SCI (7 men, 1 woman; mean age, 37±18 y; SCI level: C3-T12; AIS: A-D; mean postinjury, 25±12 months	BWSTT with manual assistance [5x/wk; total of 62±10 sessions]	• Pulmonary function test • EMG signal of respiratory muscles during respiratory tasks as follows: • MIPT • MEPT • cough	• Significant increase in FVC, FEV1 • Significant increase maximum inspiratory pressure (PI max) and maximum expiratory pressure (PE max) • Significant increase EMG activity and motor unit recruitment rate of all respiratory muscles during respiratory tasks after training
Tiftik et al, 2015 [42]	Quasi-experimental study	Level 2	52 individuals with SCI (Group A: 19 men and 7 women; mean age, 31.2±12.7 y; SCI level: 7 C1-C8, 6 T1-T12, 13 L1-S4/5; mean postinjury, 10.6±13.5 months) (Group B: 21 men and 5 women; mean age, 35.6±15.0 y; SCI level: 10 C1-C8, 9 T1-T12, 7 L1-S4/5; mean postinjury, 14.5±12.5 months	Divided into two groups: Group A: BWSTT + rehabilitation program Group B: rehabilitation program alone [3 sessions/wk for 4 wk]	• Pulmonary function test	• Significant increase FVC, FEV1, vital capacity, % vital capacity, peak expiratory flow rate, and maximum voluntary ventilation

Note: AIS = American Spinal Injury Association Impairment Scale; BP = blood pressure; BPV = blood pressure variability, BWSTT = body weight–supported treadmill training; EMF = electromyography; FEV1 = forced expiratory volume 1 second; FVC = forced vital capacity; HR = heart rate; HRV = heart rate variability; HUTT = head-uptilt training; IC = inspiratory capacity; METs = metabolic equivalents; MEPT = maximum expiratory pressure task; NES = neuromuscular electrical stimulation

Table 2.4: The quality of included studies based on the modified Down and Black scale

Aspects	Reporting										External validity-bias			Internal validity-bias							Internal validity-confounding						Power	
Question	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Total score
Ditor et al, 2004 (34)	1	1	1	1	0	1	1	1	0	1	0	0	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0	13
Ditor et al, 2005 (35)	1	1	1	1	0	1	1	1	0	1	0	1	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0	14
Soyupek et al, 2009 (36)	1	1	1	1	0	1	1	0	0	0	0	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	11
Hoekstra et al, 2013 (37)	1	1	1	1	0	1	1	0	0	1	0	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	12
Millar et al, 2009 (38)	1	1	1	1	0	1	1	0	0	1	0	0	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	15
Stevens et al, 2015 (39)	1	1	1	1	0	1	1	1	0	1	0	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	13
Carvalho et al, 2005 (40)	1	1	1	1	0	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	10
Terson de Paleville et al, 2013 (41)	1	1	1	1	0	1	1	0	0	0	0	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	11
Tiftik et al, 2015 (42)	1	1	1	1	0	1	1	0	0	1	0	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	12

Note: 1 = " yes" ; 0 = " no" or " unable to determine"

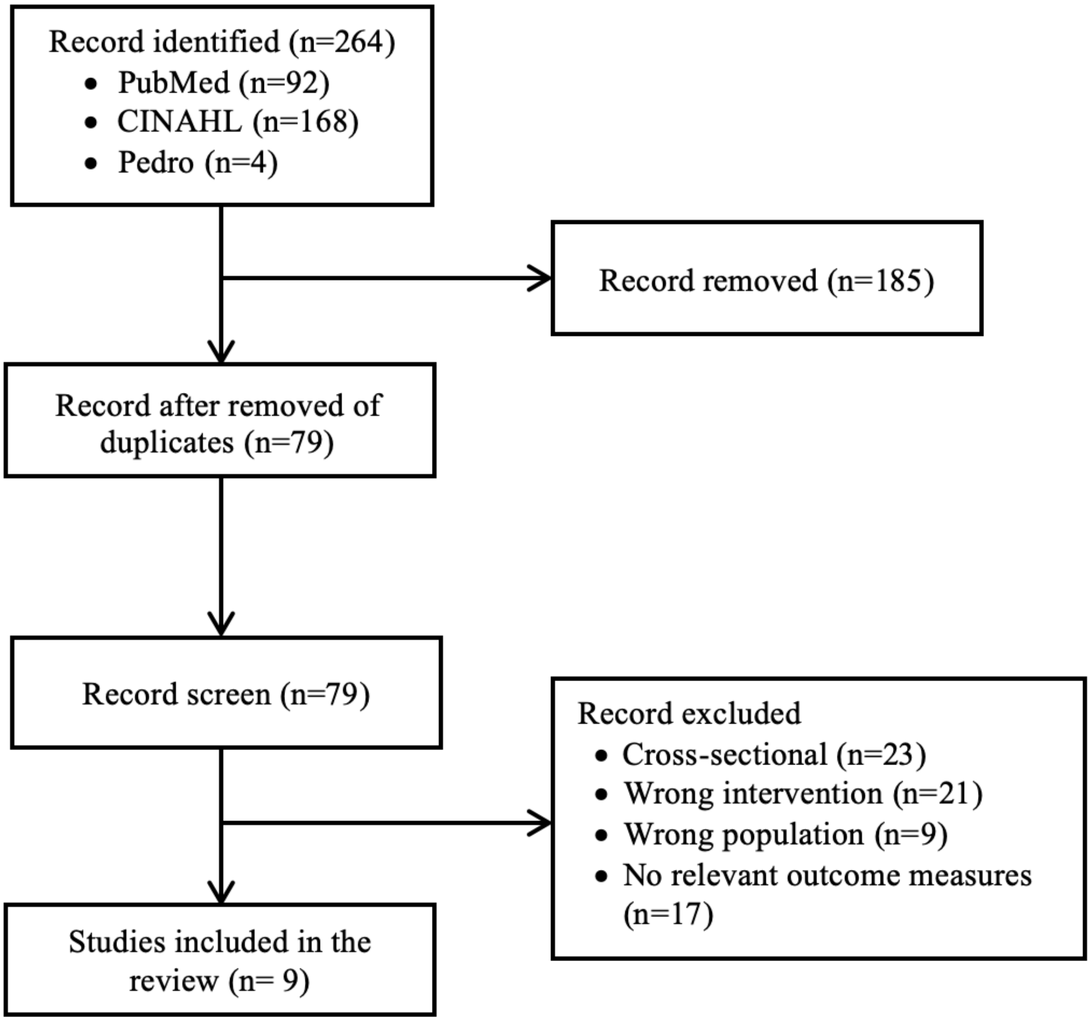


Figure 2.1: The flowchart of the literature search and selection process

References

1. Kostovski, E., P.O. Iversen, and N. Hjeltnes, *[Complications of chronic spinal cord injury].* Tidsskr Nor Laegeforen, 2010. **130**(12): p. 1242-5.
2. Sezer, N., S. Akkus, and F.G. Ugurlu, *Chronic complications of spinal cord injury.* World J Orthop, 2015. **6**(1): p. 24-33.
3. Myers, J., M. Lee, and J. Kiratli, *Cardiovascular disease in spinal cord injury: an overview of prevalence, risk, evaluation, and management.* Am J Phys Med Rehabil, 2007. **86**(2): p. 142-52.
4. Cragg, J.J., et al., *Cardiovascular disease and spinal cord injury: results from a national population health survey.* Neurology, 2013. **81**(8): p. 723-8.
5. Osterthun, R., et al., *Causes of death following spinal cord injury during inpatient rehabilitation and the first five years after discharge. A Dutch cohort study.* Spinal Cord, 2014. **52**(6): p. 483-8.
6. Flank, P., et al., *Self-reported physical activity and risk markers for cardiovascular disease after spinal cord injury.* J Rehabil Med, 2014. **46**(9): p. 886-90.
7. Yamasaki, M., et al., *Daily energy expenditure in active and inactive persons with spinal cord injury.* J Hum Ergol (Tokyo), 1992. **21**(2): p. 125-33.
8. Lee, Y.H., et al., *Hemodynamic Adaptations to Regular Exercise in People With Spinal Cord Injury.* Ann Rehabil Med, 2017. **41**(1): p. 25-33.
9. Schmid, A., et al., *Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury.* J Appl Physiol (1985), 1998. **85**(2): p. 635-41.
10. Hjeltnes, N. and H. Wallberg-Henriksson, *Improved work capacity but unchanged peak oxygen uptake during primary rehabilitation in tetraplegic patients.* Spinal Cord, 1998. **36**(10): p. 691-8.
11. Serra-Ano, P., et al., *Heart rate variability in individuals with thoracic spinal cord injury.* Spinal Cord, 2015. **53**(1): p. 59-63.
12. Liao, D., et al., *Cardiac autonomic function and incident coronary heart disease: a population-based case-cohort study. The ARIC Study. Atherosclerosis Risk in Communities Study.* Am J Epidemiol, 1997. **145**(8): p. 696-706.
13. Tsuji, H., et al., *Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study.* Circulation, 1996. **94**(11): p. 2850-5.
14. Bunten, D.C., et al., *Heart rate variability is altered following spinal cord injury.* Clin Auton Res, 1998. **8**(6): p. 329-34.
15. Alwan, A., et al., *Raised blood pressure (hypertension): a major risk factor for CVDs.*, in *Global atlas on cardiovascular disease prevention and control*, S. Mendis, P. Puska, and B. Norrving, Editors. 2011, World Health Organization: Geneva, Switzerland p. 38–39.
16. Adriaansen, J.J., et al., *Prevalence of hypertension and associated risk factors in people with long-term spinal cord injury living in the Netherlands.* Disabil Rehabil, 2017. **39**(9): p. 919-927.

17. Wecht, J.M., et al., *A prospective report on the prevalence of heart rate and blood pressure abnormalities in veterans with spinal cord injuries.* J Spinal Cord Med, 2013. **36**(5): p. 454-62.

18. Zhu, C., et al., *A retrospective chart review of heart rate and blood pressure abnormalities in veterans with spinal cord injury.* J Spinal Cord Med, 2013. **36**(5): p. 463-75.

19. Wahman, K., et al., *Increased cardiovascular disease risk in Swedish persons with paraplegia: The Stockholm spinal cord injury study.* J Rehabil Med, 2010. **42**(5): p. 489-92.

20. Fugl-Meyer, A.R., *Effects of respiratory muscle paralysis in tetraplegic and paraplegic patients.* Scand J Rehabil Med, 1971. **3**(4): p. 141-50.

21. Halar, E.M. and K.R. Bell, *In mobility and inactivity: Physiological and functional changes, prevention and treatment*, in *Physical Medicine and Rehabilitation*, J.A. Delisa, Editor. 2005, Lippincott Williams and Wilkins: Phidelphia. p. 1447–1467.

22. Schilero, G.J., et al., *Pulmonary function and spinal cord injury.* Respiratory Physiology & Neurobiology, 2009. **166**(3): p. 129-141.

23. Garshick, E., et al., *A prospective assessment of mortality in chronic spinal cord injury.* Spinal Cord, 2005. **43**(7): p. 408-16.

24. Warburton, D.E., et al., *Cardiovascular Health and Exercise Rehabilitation in Spinal Cord Injury.* Top Spinal Cord Inj Rehabil, 2007. **13**(1): p. 98-122.

25. Carlson, K.F., et al., *Effect of exercise on disorders of carbohydrate and lipid metabolism in adults with traumatic spinal cord injury: systematic review of the evidence.* J Spinal Cord Med, 2009. **32**(4): p. 361-78.

26. Akkurt, H., et al., *The effects of upper extremity aerobic exercise in patients with spinal cord injury: a randomized controlled study.* Eur J Phys Rehabil Med, 2017. **53**(2): p. 219-227.

27. Sheel, A.W., et al., *Effects of exercise training and inspiratory muscle training in spinal cord injury: a systematic review.* J Spinal Cord Med, 2008. **31**(5): p. 500-8.

28. Alm, M., H. Saraste, and C. Norrbrink, *Shoulder pain in persons with thoracic spinal cord injury: prevalence and characteristics.* J Rehabil Med, 2008. **40**(4): p. 277-83.

29. Jacobs, P.L. and M.S. Nash, *Modes, benefits, and risks of voluntary an delectrically induced exercise in persons with spinal cord injury.* J Spinal Cord Med, 2001. **24**(1): p. 10-8.

30. Olive, J.L., et al., *Blood flow and muscle fatigue in SCI individuals during electrical stimulation.* J Appl Physiol (1985), 2003. **94**(2): p. 701-8.

31. Ashley, E.A., et al., *Evidence of autonomic dysreflexia during functional electrical stimulation in individuals with spinal cord injuries.* Paraplegia, 1993. **31**(9): p. 593-605.

32. Wouda, M.F., et al., *Energetic and cardiovascular responses to treadmill walking and stationary cycling in subjects with incomplete spinal cord injury.* Spinal Cord, 2016. **54**(1): p. 51-6.

33. Carvalho, D.C., et al., *Metabolic and cardiorespiratory responses of tetraplegic subjects during treadmill walking using neuromuscular electrical stimulation and partial body weight support.* Spinal Cord, 2005. **43**(7): p. 400-5.

34. Ditor, D.S., et al., *Effects of body weight-supported treadmill training on heart rate variability and blood pressure variability in individuals with spinal cord injury.* J Appl Physiol (1985), 2005. **98**(4): p. 1519-25.

35. Ditor, D.S., et al., *The effects of body-weight supported treadmill training on cardiovascular regulation in individuals with motor-complete SCI.* Spinal Cord, 2005. **43**(11): p. 664-73.

36. Soyupek, F., et al., *Effects of body weight supported treadmill training on cardiac and pulmonary functions in the patients with incomplete spinal cord injury.* J Back Musculoskelet Rehabil, 2009. **22**(4): p. 213-8.

37. Hoekstra, F., et al., *Effect of robotic gait training on cardiorespiratory system in incomplete spinal cord injury.* J Rehabil Res Dev, 2013. **50**(10): p. 1411-22.

38. Millar, P.J., et al., *Effects of short-term training on heart rate dynamics in individuals with spinal cord injury.* Auton Neurosci, 2009. **150**(1-2): p. 116-21.

39. Stevens, S.L. and D.W. Morgan, *Heart rate response during underwater treadmill training in adults with incomplete spinal cord injury.* Top Spinal Cord Inj Rehabil, 2015. **21**(1): p. 40-8.

40. Carvalho, D.C. and A. Cliquet, Jr., *Response of the arterial blood pressure of quadriplegic patients to treadmill gait training.* Braz J Med Biol Res, 2005. **38**(9): p. 1367-73.

41. Terson de Paleville, D., et al., *Locomotor step training with body weight support improves respiratory motor function in individuals with chronic spinal cord injury.* Respir Physiol Neurobiol, 2013. **189**(3): p. 491-7.

42. Tiftik, T., et al., *Does locomotor training improve pulmonary function in patients with spinal cord injury?* Spinal Cord, 2015. **53**(6): p. 467-70.

43. Downs, S.H. and N. Black, *The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions.* J Epidemiol Community Health, 1998. **52**(6): p. 377-84.

44. van der Scheer, J.W., et al., *Effects of exercise on fitness and health of adults with spinal cord injury: A systematic review.* Neurology, 2017. **89**(7): p. 736-745.

45. Eng, J.J., et al., *Spinal Cord Injury Rehabilitation Evidence: Methods of the SCIRE Systematic Review.* Top Spinal Cord Inj Rehabil, 2007. **13**(1): p. 1-10.

46. de Groot, P.C., et al., *Effect of training intensity on physical capacity, lipid profile and insulin sensitivity in early rehabilitation of spinal cord injured individuals.* Spinal Cord, 2003. **41**(12): p. 673-9.

47. Wirz, M., G. Colombo, and V. Dietz, *Long term effects of locomotor training in spinal humans.* J Neurol Neurosurg Psychiatry, 2001. **71**(1): p. 93-6.

48. Knikou, M., *Functional reorganization of soleus H-reflex modulation during stepping after robotic-assisted step training in people with complete and incomplete spinal cord injury.* Exp Brain Res, 2013. **228**(3): p. 279-96.

49. Pinter, M.M. and M.R. Dimitrijevic, *Gait after spinal cord injury and the central pattern generator for locomotion.* Spinal Cord, 1999. **37**(8): p. 531-7.

50. Minassian, K., et al., *The Human Central Pattern Generator for Locomotion: Does It Exist and Contribute to Walking?* Neuroscientist, 2017. **23**(6): p. 649-663.

51. MacKay-Lyons, M., *Central pattern generation of locomotion: a review of the evidence.* Phys Ther, 2002. **82**(1): p. 69-83.

52. Hicks, A.L. and K.A. Ginis, *Treadmill training after spinal cord injury: it's not just about the walking.* J Rehabil Res Dev, 2008. **45**(2): p. 241-8.

53. Cornelissen, V.A., et al., *Effects of aerobic training intensity on resting, exercise and post-exercise blood pressure, heart rate and heart-rate variability.* J Hum Hypertens, 2010. **24**(3): p. 175-82.

54. Pescatello, L.S., et al., *American College of Sports Medicine position stand. Exercise and hypertension.* Med Sci Sports Exerc, 2004. **36**(3): p. 533-53.

55. Haddad, S., et al., *[The effects of aerobic physical training of short duration using upper limbs in paraplegic persons with mild hypertension].* Arq Bras Cardiol, 1997. **69**(3): p. 169-73.

56. Faghri, P.D., R.M. Glaser, and S.F. Figoni, *Functional electrical stimulation leg cycle ergometer exercise: training effects on cardiorespiratory responses of spinal cord injured subjects at rest and during submaximal exercise.* Arch Phys Med Rehabil, 1992. **73**(11): p. 1085-93.

57. Jack, L.P., et al., *Comparison of peak cardiopulmonary performance parameters during robotics-assisted treadmill exercise and arm crank ergometry in incomplete spinal cord injury.* Technol Health Care, 2010. **18**(4-5): p. 285-96.

58. Thomas, S.L. and M.A. Gorassini, *Increases in corticospinal tract function by treadmill training after incomplete spinal cord injury.* J Neurophysiol, 2005. **94**(4): p. 2844-55.

Chapter 3: The Feasibility of an 8-Week Walking Training Program Using a Novel Assistive Gait Training Device in Individuals with Spinal Cord Injury

Abstract

Background: A body weight-supported treadmill is widely used in rehabilitation interventions to improve or restore walking ability after spinal cord injury [1]. During walking training, leg assistance is usually provided through manual assistance by physical therapists or robotic-assisted devices. A novel assistive gait training device using a pulling cable was developed in our laboratory to aid physical therapists with leg assistance during treadmill gait training.

Purpose: The primary goal of this study was to examine the feasibility (recruitment, retention, compliance, walking performance, and participant's perception,) of an 8-week (3 sessions per week, 30 min per session) walking training program using a novel assistive gait training device in patients with chronic SCI.

Participants: A total of 11 individuals with either motor incomplete or complete chronic SCI (8 males, 3 females, age: 38±10 years old, injury levels T2-L1; injury onset 8±10 years) completed the study.

Methods: All participants received walking training, 3 sessions a week for 8 weeks (a total of 24 sessions). Participants were trained on a treadmill with a body weight support system. Stepping assistance of the legs during training was provided through a novel assistive gait training device. Feasibility measures of recruitment, retention, compliance, and participants' performance were collected throughout the study period. In addition, participants' perception about the walking training program and assistive gait training device were evaluated at the end of the study using a questionnaire.

Results: Out of 55 participants who were screened for eligibility, 36 participants met the eligibility criteria of the study. A total of 15 participants agreed to enroll in the study among those who met the eligibility criteria. The recruitment rate (27.2%) was acceptable. At the end, 11 participants completed the study. However, 3 participants withdrew from the study, and one participant was excluded from the study. Throughout the period of training, all participants were able to progress in their walking performance with respect to treadmill speed (start: 0.76±0.1 miles/hour vs. end: 1.28±0.14 miles/hour), distance (start: 0.21±0.07 miles vs. end: 0.62±0.08 miles), duration of training (start: 16.27±4.33 minutes vs. end: 29.36±0.92 minutes), and percentage of body weight support (start: 54.55±10.59% vs. end: 35.91±11.36%). The overall compliance rate of participants with training sessions was 96.2±6.5%. From responses to the end of intervention questionnaire, the majority of participants showed acceptance and satisfaction with the walking training program as well as the assistive gait training. No serious issues or concerns were raised regarding the walking training program and assistive gait training device.

Conclusion: The 8-weeks walking training program was feasible in individuals with chronic SCI. The assistive gait training device was feasible and safe to use with individuals with paraplegia.

Keyword: *spinal cord injury; assistive gait training device; body weight-supported treadmill training; feasibility measures*

3.1. Introduction

In the United States, the incidence of spinal cord injury (SCI) has been estimated to be 17000 new cases per year. The number of people in the United States who were alive in 2016 and had SCI was estimated to be approximately 282,000. About 80 % of SCI occurs in individuals aged between 18 and 30 years-old [2, 3]. It was reported that 53% of individuals with SCI are unable to walk independently at one-year post-injury [1].

SCI is a serious medical condition that impacts sensorimotor function leading to various secondary complications and, consequently, influences the quality of life. The extent of sensorimotor impairment is determined by the level and completeness of injury to the spinal cord. After SCI, patients usually undergo intensive rehabilitation therapy in order to restore motor function and improve the functional ability of daily living activity. One of the primary goals for people with SCI is to be able to regain walking ability [4].

Body weight-supported treadmill training (BWSTT) is a tool that is used in rehabilitation to retrain patients with neurological conditions to improve or regain their walking ability. Patients train on a treadmill while fitted into a harness, which is attached to an overhead suspension system to provide body weight support and prevent the risk of falls during training. Leg assistance during training is provided either manually by therapists or by robotic-assistive devices such as a Lokomat. However, when physical therapists provide manual assistance during training, the number of gait cycle repetitions can be limited due to the exhaustion of the physical therapists who provide manual assistance [5]. Part of the problem is that a non-ergonomic body posture of physical therapists while providing manual assistance to patient's legs/feet during training, which can increase workload and put therapists under risk of developing low-back pain [6]. Although the robotic-assistive gait training can provide intensive, task-specific stepping training for patients, the cost of robotic-assistive gait devices cannot be afforded by most of the rehabilitation clinics [7, 8]. In addition, it limits normal walking patterns and does not allow walking variability during training [8].

A novel assistive gait training device using a pulling cable was developed in our laboratory to aid physical therapists during treadmill gait training (**Figure 3.1**) [9, 10]. The therapists pull a cable to assist patients with hip and knee flexion and ankle dorsiflexion during the swing phase of the gait cycle. This device is low cost and allows therapists to work in a more ergonomic body posture at a sitting position. The feasibility of this novel assistive gait training device was examined previously in non-ambulatory patients with stroke in our laboratory [11]. From responses to the questionnaire, patients showed that the device was helpful and comfortable, and they did not raise any concerns or issues regarding the use of the device. In our previous feasibility study, stepping assistance was provided in one leg since stroke patients have only one side that is affected. However, individuals with SCI usually have both legs affected and need stepping-assistance for both legs during treadmill walking training. The assistive gait training device using two pulling cables has not been tested previously.

Therefore, the primary purpose of the current study was to examine the feasibility (recruitment, retention, compliance, walking performance, and participant's perception) of an 8-week (3 sessions per week, 30 min per session) walking training program using a novel assistive gait training device in patients with chronic SCI. As an exploratory purpose, we investigated the effects of the walking training program on lower extremity muscle strength, functional independence, the level of depression, anxiety and stress, and health-related quality of life (HRQOL) in individuals with chronic SCI.

3.2. Methods

3.2.1. Participant

A total of 15 participants with chronic paraplegia were enrolled in the study. Participants were mainly recruited as a convenience sample from the Spine Center Clinic at the University of Kansas Hospital. In addition, the Healthcare Enterprise Repository for Ontological Narration database at the University of Kansas Medical Center was utilized to obtain the contact information of patients with SCI who were admitted to the University of Kansas Hospital and agreed to be contacted for participating in relevant research studies. Study flyers were also distributed at out-patient rehabilitation centers and SCI support groups in the Kansas City area. A member of the study teams contacted potential participants by phone calls or emails to determine their eligibility. Inclusion criteria for participants were chronic (≥ 1 year) incomplete or complete paraplegia (T1-L2), age between 18-60 years-old, relying on a wheelchair as the primary mode of mobility, and not receiving any similar form of walking training activity. In addition, participants needed to have approval from their physicians to participate in walking training. Participants were excluded from the study if they had a history of cardiovascular disease such as heart diseases or peripheral vascular diseases; other neurological diseases such as stroke, Parkinson's, multiple sclerosis, etc.; severe orthopedic issues such as joint stiffness or recent fractures; severe muscle spasticity (> 3 according to Ashworth scale); severe osteoporosis; current inflammatory diseases or infections; or uncontrolled autonomic dysreflexia. In addition, pregnant women were excluded from participating in the study. After a detail explanation of study purpose and protocol, all participants signed written informed consent. The study received approval from the Institutional Review Boards at the University of Kansas Medical Center.

3.2.2. Study Design

This study was a pilot, single group, pretest-posttest study design. Eligible participants received 3 sessions a week of walking training for 8 weeks. Feasibility measures were taken throughout the study. Clinical assessments and subjective reporting through questionnaires were conducted at baseline (pre-training) and after completion of 8-week walking training (post-training).

3.2.3. Walking Training Protocol

Participants underwent 3 sessions per week of walking training for 8 weeks, a total of 24 sessions. Walking training was performed on a treadmill with an overhead motorized lift system that was placed above a treadmill. The overall duration of the training session was one hour, including set up time. The actual time for walking training was 30 minutes (2 bouts of 15 minutes). The participant was allowed to take rest from 3-5 minutes between bouts or at any time during training. First, the participant fitted on a harness attached to an overhead motorized lift to provide body weight support (BWS) and prevent the risk of falls. At the beginning of the training sessions, approximately 60-40 % of the participant's body weight was supported as tolerated without knee buckling or feet drag. The amount of BWS was reduced by 5% during each week as tolerated by the participant. A digital scale attached to a motorized lift was used to determine the amount of BWS. A novel assistive gait training device developed in our laboratory was used to assist the participant with forward leg stepping during walking on a treadmill [9]. Two thigh braces were placed onto the participant's right and left thighs and secured using straps. These braces were attached to a pulley system via pulling cords (**Figure 3.1**). The assistive gait training device was operated by two therapists to assist the participant with hip and knee flexion and ankle dorsiflexion of the legs during the swing phase of the gait cycle. The participant was encouraged to actively move his/her legs throughout training. At the beginning of training, the initial speed of the treadmill was set at 0.5 miles/hour. After two minutes of warm-up, the speed of the treadmill was gradually increased until the participant's heart rate (HR) reached 40-59% of his/her HR reserve ([maximum HR – resting HR]×target intensity+ resting HR) as recommended by American College of Sports Medicine guidelines for moderate intensity [12]. The maximum heart rate was determined through the age-predicted maximum heart rate (220 – participant's age) [13]. In addition, the participant rated his/her level of exertion between each training bout and by the end of the training session, using the Borg Rating of Perceived Exertion Scale (RPE: 6-20). At the end of the training session, treadmill speed was decreased to 0.5 miles/hour, and the participant ambulated at this speed for 2 minutes to cool down. For safety reasons, the participant's HR was monitored throughout the training session using a polar OH1 optical HR sensor (Polar Electro Inc., New York), and the participant's blood pressure at rest was recorded before, in the middle, and at the end of the training session. The training session was stopped if the participant's heart rate exceeded 85% of age-predicted maximum HR, or participant's systolic or diastolic blood pressure reached 220 mmHg or 110 mmHg, respectively. A walking training log was utilized to record training duration, treadmill speed, walking distance, BWS, HR, blood pressure, and RPE during each training session.

3.2.4. Feasibility Outcome Measures

Throughout the study, we collected data on feasibility measures of recruitment, retention, compliance, walking performance, and participant's perception. The recruitment rate was assessed by recording the number of participants who were screened for eligibility, those who were excluded because of eligibility criteria, and those who declined or enrolled to participate in the study. Retention data was obtained by recording the number of participants who dropped out during the study, along with their reasons. The compliance rate was documented through recording the total number of completed sessions and incomplete sessions along with reasons for absence. Walking performance data was obtained by recording walking duration and distance, treadmill walking speed, and the amount of BWS during each session. Information on participant's perception was acquired through a questionnaire that was administered to the participant at the end of the walking training program. The questionnaire, which was derived from previous studies, evaluated participant's acceptance of and satisfaction with the walking training program and the assistive gait training device *(see Appendix II)* [14-17].

3.2.5. Muscle Strength of Lower Extremity

Muscle strength was evaluated using lower extremity motor score (LEMS), according to guidelines of the American Spinal Injury Association (ASIA) exam [18]. LEMS is a manual muscle testing that assesses the strength of five key muscle groups of the lower extremity bilaterally: hip flexors, knee extensors, ankle dorsiflexors, great toe extensors, and ankle plantarflexors. Each muscle group was graded from 0 (absence of muscle contraction) to 5 (active movement with the full range of motion against full resistance). The total score of LEMS ranges from 0 to 50.

3.2.6. Level of Functional Independence

The Spinal Cord Independence Measure (SCIM) self-report version (SCIM-SR) was utilized to evaluate the level of functional independence. The SCIM-SR is a valid self-reported questionnaire that is designed to measure the functional ability of daily living activities for persons with SCI [19]. It has an excellent agreement with the clinician-administered SCIM version-III [19]. This questionnaire contains 17 items, divided into three subscales: self-care (items 1-4), respiration and sphincter management (items 5-8), and mobility ability (items 9-17).

The total score of SCIM-SR ranges from 0 to100, which indicates the level of functional independence. The higher score represents a higher level of functional independence.

3.2.7. Level of Depression, Anxiety, and Stress

Depression Anxiety Stress Scales-21 (DASS-21) was used to evaluate the level of depression, anxiety, and stress [20]. DASS-21 is a self-administered questionnaire that is designed to assess the negative emotional states of depression, anxiety, and stress over the past few weeks. It consists of 3 subscales with seven items in each subscale (total of 21 items). The response to each question is given on a 4-point Likert scale ranging from 0 = "Did not apply to me at all" to 3 = "Applied to me very much or most of the time." The score for each subscale is calculated by summing the scores for the relevant items. The low score indicates a normal level, whereas the high score indicates an extremely severe level of depression, anxiety, and stress.

3.2.8. Health-Related Quality of Life (HRQOL)

The change in HRQOL was assessed via the Short form-36 Health Survey (SF-36). SF-36 is a self-reported, 36-items questionnaire that assesses eight domains of perceived HRQOL during the previous 4 weeks [21, 22]. The eight domains include physical functioning, role-physical functioning, bodily pain, general health, vitality, social functioning, role-emotional functioning, and mental health. The higher score represents better HRQOL. The overall score of SF-36 was calculated by taking the average score of eight domains.

3.2.9. Statistical Analysis

Descriptive statistics of frequency, mean, and standard deviation were generated on the feasibility data collected during the study. The Wilcoxon signed-rank test was conducted to assess differences in outcome variables between the pre-training and post-training assessment due to the small sample size and because data of independent variables might not be normally distributed. The level of significance was set at 0.1 (one-tailed test) due to the small sample size of the study.

3.3. Results

3.3.1. Feasibility Outcomes

A summary of the recruitment processes is shown in **Figure 3.2**. A total of 65 potential participants with SCI were contacted via phone or email between November 2018 and August 2019. Among those who were contacted, 55 individuals were screened for eligibility. Upon completing a phone screening, 16 individuals were excluded due to various reasons based on answers provided to screening questions, and 3 individuals were not interested in participating in the study. The reasons for exclusion include cervical injury (n = 6), injury onset less than a year (n = 2), ability to walking independently (n = 4), having other medical complications (n = 3), and over the age limit (n = 1). Among 36 potential participants who met eligibility criteria, 21 participants declined to participate in the study due to different reasons, including transportation limitations (n = 6), conflict with work schedule (n = 4), living far away from our laboratory (n = 3), or lost interest (n = 3). Besides, 5 participants, who met eligibility and agreed to enroll in the study via phone, did not show for the baseline assessment due to transportation limitations. Out of those who met the eligibility criteria of the study, 15 participants agreed to enroll in the study. However, 3 participants withdrew from the study after completing 3-4 weeks of training due to demands of work or family schedule (n = 2) or an adverse event not related to the study (n = 1). One participant was excluded from the study after the first session because he had a colostomy, which might have interfered with the wearing of a safety harness. In the end, a total of 11 participants successfully completed the study, and their demographic and clinical characteristics are illustrated in **Table 3.1**. The average age of participants was 38.09±10.31 years. 8 participants were males, and 3 participants were females. 8 participants had complete SCI (ASIA grade A or B), and 3 participants had incomplete SCI (ASIA C or D). 5 participants had upper paraplegia (T2-T5), and 6 participants had lower paraplegia (T6-L1). The average time of injury onset for study participants was 8.72±10.40 years.

In respect to walking performance, the mean treadmill speed, distance, and walking duration that participants walked in the first session were 0.76±0.1 miles/hour, 0.21±0.07 miles, and 16.27±4.33 minutes, respectively. All participants were able to gradually increase their treadmill speed, walking distance, duration of training during the 8-week walking training program. The mean treadmill speed, distance, and walking duration during the last training session were 1.28±0.14 miles/hour, 0.62±0.08 miles, and 29.36±0.92 minutes, respectively. The mean percentage of BWS provided during treadmill walking training was 54.55±10.59% in the first week of training sessions and 35.91±11.36% in the last week of training sessions. Out of 11 participants, 8 participants completed all training sessions, which included 24 sessions. The number average of completed sessions was 23.09±1.5, and the overall compliance rate was 96.2±6.5%. Details about participant's walking performance and their attendance are presented in **Table 3.2**.

After completing 8 weeks of walking training, participant's acceptance of and satisfaction with the walking training program and the assistive gait training device was evaluated by a questionnaire. A total of 11 participants completed the end intervention questionnaires. As shown in **Figure 3.3-A**, all participants were satisfied with the time length and procedures of walking training, and they felt walking training was safe and secure. Out of 11 participants, 9 participants felt walking training was comfortable regarding harness. All participants enjoyed the walking training program and would participate in walking training again. Ten participants reported that walking training improved their life. The improvement of walking ability after the training program was reported from 6 participants. Participants' acceptance of and satisfaction with the assistive gait training device was rated high, see **Figure 3.3-B**. All participants agreed to strongly agreed that the device was safe, secure, and comfortable and provided sufficient assistance during walking. All participants were satisfied with the time needed to set up the assistive device and would recommend using this assistive device by others.

3.3.2. Muscle Strength of Lower Extremity

The overall results of the LEMS are shown in **Table 3.3**. Following the training, motor score significantly improved from 3.64±2.05 to 4.82±2.42 at the right leg ($p < 0.05$) and from 3.09±1.76 to 4.27±7.07 at the left legs ($p = 0.05$). The total score of LEMS significantly increased from 6.73±3.91 to 9.09±4.55 after the training ($p<0.05$). By conducting subgroup analysis based on injury severity, a significant change in the total score of LEMS was found in participants with motor incomplete SCI (ASIA grade C or D) compared to those with motor complete SCI (ASIA grade A or B). Specifically, the total score of LEMS increased on average by 6.67 points in participants with motor incomplete SCI. In contrast, participants with motor complete SCI showed a slight mean increase of 0.75 points in the total score of LEMS.

3.3.3. Questionnaire Outcomes

SCIM-SR score of each subscale showed significant improvements after training (**Table 3.4**). The total score of SCIM-SR significantly increased from 61.36±12.43 to 71±9.09 after training (p <0.01). There were significant decreases in the mean score of depression (pre: 4.09±2.70 vs. post: 1.73±1.19, $p < 0.01$), anxiety (pre: 3.82±4.60 vs. post: 2.36±3.98, $p < 0.05$) and stress (pre: 4±2.93 vs. post: 1.91±1.22, $p < 0.01$) after training. The overall score of SF-36 significantly increased after training as compared to before training (pre: 57.51±12.18 vs. post: 71.04±6.97, $p < 0.01$).

3.4. Discussion

The primary findings of this study provide insights into the feasibility of the 8-week walking program regarding participants' recruitment, retention, and compliance with training as well as feasibility information about using a novel assistive gait training device in individuals with SCI. In addition, the findings of this study suggested that 8-week of walking training might improve levels of lower extremity muscle strength and functional independence of daily living activity as well as have positive impacts on psychological and health status in individuals with chronic SCI.

The recruitment rate (27.2%) of participants with chronic paraplegia reached in this study was acceptable, although it remains relatively low, considering the number of potential participants (n=55) who were screened for eligibility. Our recruitment rate was higher than other feasibility studies investigating locomotor training in individuals with chronic SCI [23, 24]. Similar to our findings, Gagnon et al. [25] reported a recruitment rate of 28.6% in 6 to 8 weeks of locomotor training. Of participants who were screened for eligibility, around 29% of them were excluded, and 5% of them were not interested. The reasons for exclusion were having a cervical injury, injury onset less than a year, ability to walking independently, or having other medical complications. About 58% of participants who met eligibility criteria did not agree to participate in the study. In reviewing the reasons for the decline, lack of transportation, schedule conflict, and living far away from the location of the study were the major challenges. Future studies may consider providing transportation assistance; offering training sessions at various locations such as other rehabilitation centers and community recreation activity centers;

adjusting the schedule of training sessions to meet participant's availability such as providing training sessions in evenings or weekend days; and providing temporary housing for eligible and interested candidates who live further away. The dropout rate of this study was 26% (n=4/15). The reasons for withdrawing from the study included demands of work or family schedule, an adverse event not related to the study, or a medical reason. The dropout rate in our study was lower than in the study that involved 10 weeks of walking training activity (20 sessions) in individuals with chronic paraplegia, showing a dropout rate of 50% [23]. Another study involving 6 months of walking training in individuals with chronic SCI reported dropout rate of 30% [26]. The compliance rate (96.21%) of this study was excellent. The majority of participants were able to complete 24 sessions within 8 weeks. The high rate of compliance with the schedule of training sessions attests the commitment of the participants. Throughout 8 weeks of walking training, all participants showed the ability to progress in their walking performance in related to treadmill speed, training duration, walking distance, and the amount of body weight support. Out of 11 participants, 9 participants were able to exercise at the target HR zone (40%-59% of HR reserve) in the majority of training sessions. However, two participants (SCI03 and SCI06) were not able to achieve the target HR zone in the majority of training sessions, and they were exercising at 30%-39% of their HR reserve most of the time. The participant SCI03 was on a beta-blocker medication and was not trying to engage in the training actively on many occasions. The participant SCI06 did not actively engage in the majority of training sessions.

The findings of this study showed that the 8-week walking training program using the novel assistive gait training device was feasible to perform and well-tolerated. Subjectively, all participants reported positive perceptions about time length, procedures, and safety of training. All participants enjoyed the 8-weeks walking training program. Some participants commented that they wanted to continue training sessions for a longer time because they observed some benefits of walking training. Participants were asked to report any adverse events (e.g., pain, fatigue, or dizziness) during training sessions and to check their skin for breakdown or sores after every training session. No adverse events were reported during the walking training program. All participants' perceptions were positive toward the use of the assistive gait training device. No concerns or safety issues were raised regarding the walking training and assistive device.

Only participants with motor incomplete SCI showed a significant increase in their scores of lower extremity muscle strength. Unlike individuals with motor complete SCI (ASIA grade B or A), those with motor incomplete SCI (ASIA grade D or C) are expected to gain improvements in functional motor ability with an intervention that provides mobility training [27]. Thus, it could be that walking training in participants with incomplete SCI strengthened spared neural pathways in the spinal cord, which resulted in improved muscle strength [28]. Interestingly, we observed that 2 out of 8 participants with motor complete SCI showed the potential to improve their motor scores of hip flexor and knee extensors muscle strength after training and ability to take some steps without assistance during walking training on a treadmill. Case studies, in individuals with chronic motor complete SCI (ASIA grade A or B), reported potential improvements in scores of lower extremity muscle strength and walking ability on a treadmill or over-ground following 7 months or 85 sessions of intensive walking training [29, 30]. The duration and number of training sessions in our study were much shorter compared to those case studies. It may be possible that long-term intensive gait training using our training device may further improve leg muscle strength and lead to the recovery of independent walking in some patients with motor complete SCI.

Furthermore, 5 participants reported significant improvements in their functional independence of daily living activity after training. The total score of SCIM increased on average by 9 points, with considerable increases in the respiration and sphincter management and mobility subdomains. This finding was similar to that of other studies that reported an improvement of functional independence after walking training, using the clinical version of SCIM-III [31, 32]. In this study, we utilized the self-report version of SCIM because it does not require additional resources, provides fast data collection, and can be administrated in any setting. It has an excellent agreement with the clinician-administered SCIM-III [19]. To our knowledge, this study was the first to investigate the influence of walking training on the self-reported version of SCIM in SCI patients.

Due to neurological loss or impairment, patients with SCI deal with different stresses of coping with environmental barriers, high economic costs, occupational limitations, and strains on personal and social interaction on a daily basis [33]. Such intense situations might lead to tremendous psychological distress and impact on the quality of life. The current study showed significant changes in depression, anxiety, and stress components of the DASS 21 after 8 weeks of walking training. Also, significant changes by the participants in their perception regarding health-related quality of life were reported. Positive changes seen in these components might be due to the benefits of regular exercise on mood and quality of life. A previous study showed that a single bout of BWSTT has small to medium positive effects on mood in individuals with SCI [34]. However, few studies to date have studied the cumulative effects of walking training on psychological health among people with SCI. Thus, the findings of this study showed that the 8-week walking training program could have considerable impacts on psychological status in participants with SCI. Regular participation in exercise could improve participants' functional independence and prevent any secondary complication, which might positively influence their quality of life [35]. Another possibility is that walking training might help to reduce pain as reported by some participants, resulting in improvements in psychological status and quality of life. Providing the opportunity to stand upright during walking training could improve the participant's motivation and engagement in physical activity. Also, participating in physical activity in which they see themselves walking again could have positive effects on emotional thrill [36]. Taken all together, walking training using BWSTT can provide powerful improvements in aspects of psychological status and quality of life even though some people with SCI might not regain their walking ability after training.

Limitations of this study include the small sample size of participants recruited from a single site as well as the lack of a control group. This feasibility study tested only participants' perceptions to the use of the assistive gait training device. However, the study failed to include an evaluation of therapists' perceptions on the use of the training device. Only individuals with thoracic or lumbar SCI (T1-L2) were included in the study. Thus, the findings cannot be generalized to all SCI populations, such as those with a cervical injury.

3.5. Conclusion

The findings of the current study suggest that the 8-week walking training program was feasible and tolerable for individuals with chronic SCI. Our novel assistive gait training device was feasible and safe to use in individuals with SCI. Furthermore, regular walking training improved functional independence, psychological health, and quality of life in people with SCI. A future study is needed to evaluate physical therapists' perceptions and satisfaction with the use of an assistive gait training device in comparison to manual assistance provided by therapists during treadmill walking training. The feasibility of using the assistive gait training device in persons with cervical SCI as well as in clinical settings warrants further study.

Table 3.1: Demographic and clinical characteristics of participants who completed the study (n = 11)

Participant ID	Gender, (Male/Female)	Age, (yrs) Mean±SD	Body weight (kg) Mean±SD		Height (cm) Mean±SD	Injury level	ASIA grade	Injury duration (yrs.) Mean±SD
			Pre-training	Post-training				
SCI 02	Male	40	71	68	173	T9	A	7
SCI 03	Male	47	130	131	196	T8	B	3
SCI 04	Male	37	89	89	189	T3	B	1
SCI 06	Male	40	118	120	189	T5	B	19
SCI 07	Male	19	117	117	206	T3	B	2
SCI 08	Male	48	78	78	198	T9	B	3
SCI 09	Male	35	108	107	155	T6	C	35
SCI 10	Female	32	107	105	178	L1	C	14
SCI 11	Female	25	58	58	168	T7	B	7
SCI 13	Male	41	71	71	180	T5	A	4
SCI 15	Female	55	122	125	178	T2	D	1
	8/3	38.09±10.31	97.18±24.64	97.18±25.48	182.73±14.70			8.72±10.40

ASIA: American Spinal Injury Association

Table 3.2: Walking performance and attendance of participants throughout the study

Participant ID	**Treadmill speed (miles/hour.)**		**Walking duration (minute)**		**Distance (mile)**		**BWS (%)**		**No. sessions with exercise at HR zone**	**No. attended sessions**	**Compliance (%)**
	Start	**End**	**Start**	**End**	**Start**	**End**	**Start**	**End**			
SCI 02	0.7	1.2	14	30	0.16	0.6	70	45	22	24	100
SCI 03	0.6	1.2	12	29	0.12	0.58	60	45	12	24	100
SCI 04	0.7	1.2	14	30	0.16	0.6	60	40	24	24	100
SCI 06	0.7	1.2	12	30	0.12	0.6	60	40	10	24	100
SCI 07	0.8	1.2	14	28	0.18	0.56	60	45	23	21	87.5
SCI 08	0.8	1.4	20	30	0.26	0.7	60	35	24	24	100
SCI 09	0.7	1.1	12	28	0.14	0.51	45	25	22	24	100
SCI 10	0.8	1.2	16	28	0.21	0.56	35	25	24	20	83.33
SCI 11	0.8	1.4	20	30	0.26	0.7	60	40	24	24	100
SCI 13	1	1.4	20	30	0.33	0.7	50	45	23	21	87.5
SCI 14	0.8	1.6	25	30	0.33	0.8	40	10	24	24	100
Total Mean±SD	0.76±0.10	1.28±0.14	16.27±4.33	29.36±0.92	0.21±0.07	0.62±0.08	54.4±10.59	35.9±11.36	21.09±5.06	23.09±1.57	96.21± 6.57

BWS: Body weight support; HR: Heart rate

Table 3.3: Lower extremity motor score (LEMS) pre- and post-training

	Pre-training	**Post-training**	**Mean change**	***p*-value**
Right LEMS	3.64±2.05	4.82±2.42	1.18±0.46	0.02
Left LEMS	3.09±1.76	4.27±7.07	1.18±0.46	0.05
Total LEMS	6.73±3.91	9.09±4.55	2.36±0.91	0.02

Values are presented in mean ± standard error of mean.

LRMS: Lower Extremity Motor Score.

Table 3.4: The results of functional independence, physiological heath, and quality of life pre- and post-training

	Pre-training	**Post-training**	**Mean change (post-pre)**	***p*-value**
Spinal cord independence measure-self report (SCIM-SR)				
Self-care	16±4.26	18±2.32	2±2.19	< 0.05
Respiration and sphincter management	28.55±6.29	33.09±4.72	4.54±3.93	< 0.01
Mobility	16.82±3.31	19.91±3.61	3.09±2.21	< 0.001
Total score of SCIM	61.36±12.43	71±9.09	9.64±5.46	< 0.001
Depression, Anxiety, Stress Scale-21				
Depression	4.09±2.7	1.73±1.19	2.36±2.01	< 0.01
Anxiety	3.82±4.60	2.36±3.98	1.45±1.57	< 0.05
Stress	4±2.93	1.91±1.22	2.09±1.81	< 0.01
Health-related quality of life				
Total score of SF-36	57.51±12.18	71.04±6.97	13.53±7.67	< 0.001

Values are presented in mean ± standard Deviation.

SF-36: Short form-36 Health Survey

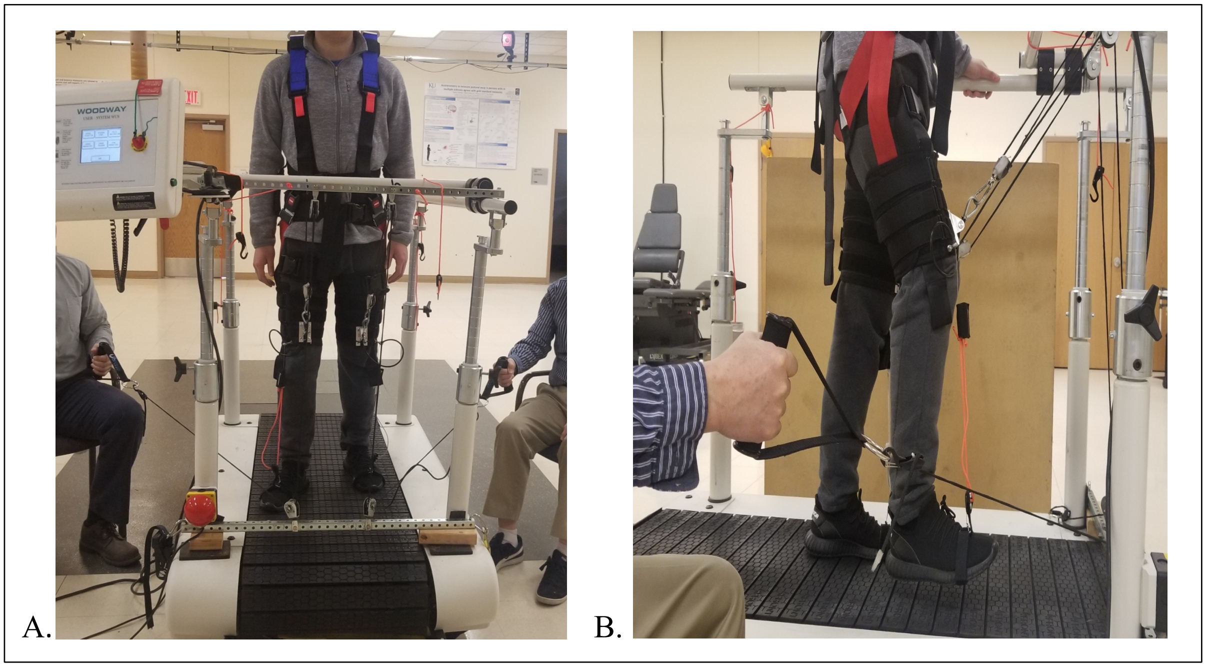


Figure 3.1: The assistive gait training device with two pulling cables operated by two therapists on either side of the treadmill

(A) Two thigh braces which attach to fixed pulleying system in the front of the treadmill via a cable are placed on both right and left thighs. Two physical therapists sit on the right and left side of the treadmill and coordinate to operate the assistive device by pulling the handle attached to the cable. (B) The physical therapists pull the handle attached to the cable to assist a patient with hip flexion and ankle dorsiflexion during the swing phase of the gait cycle

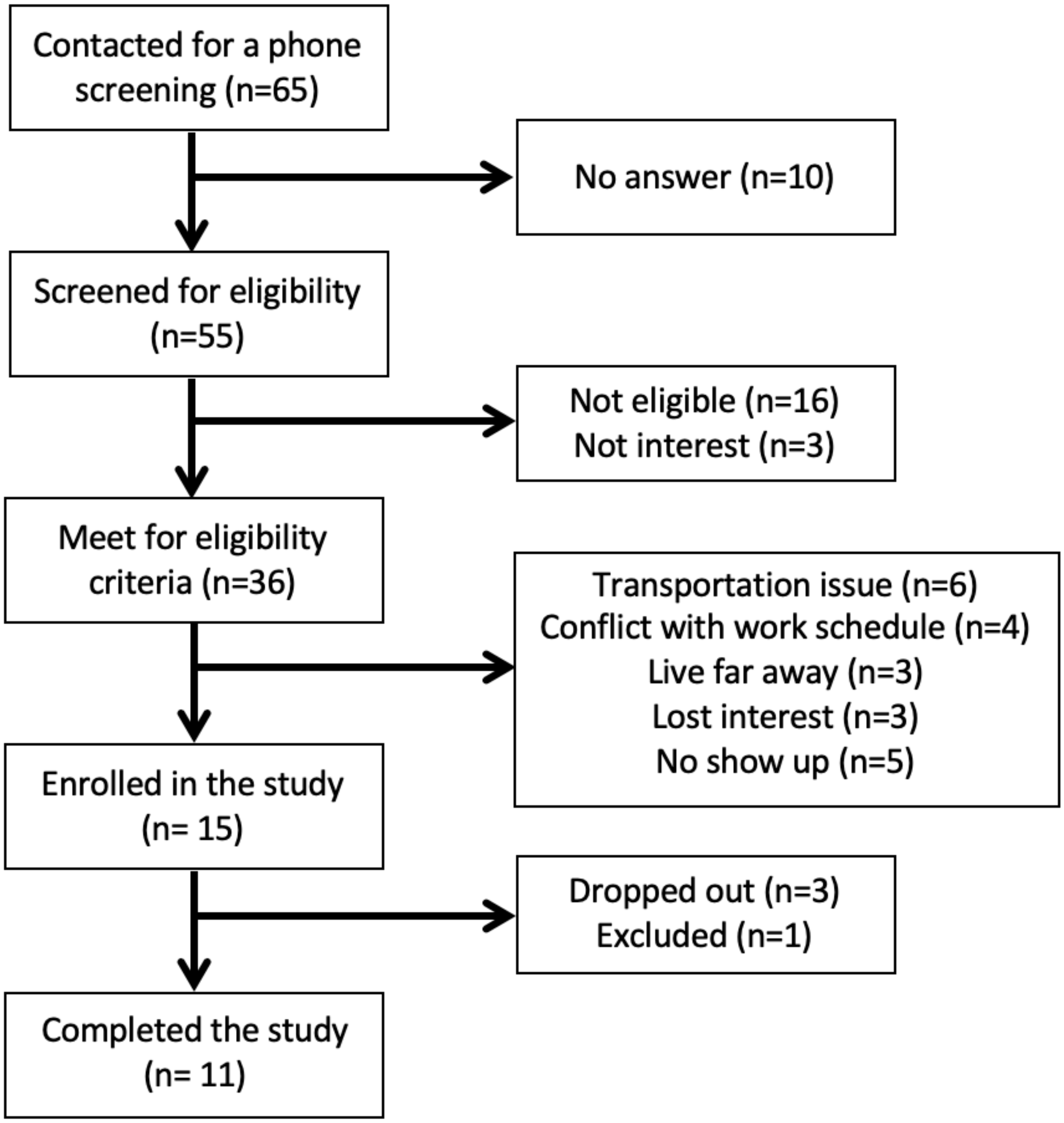
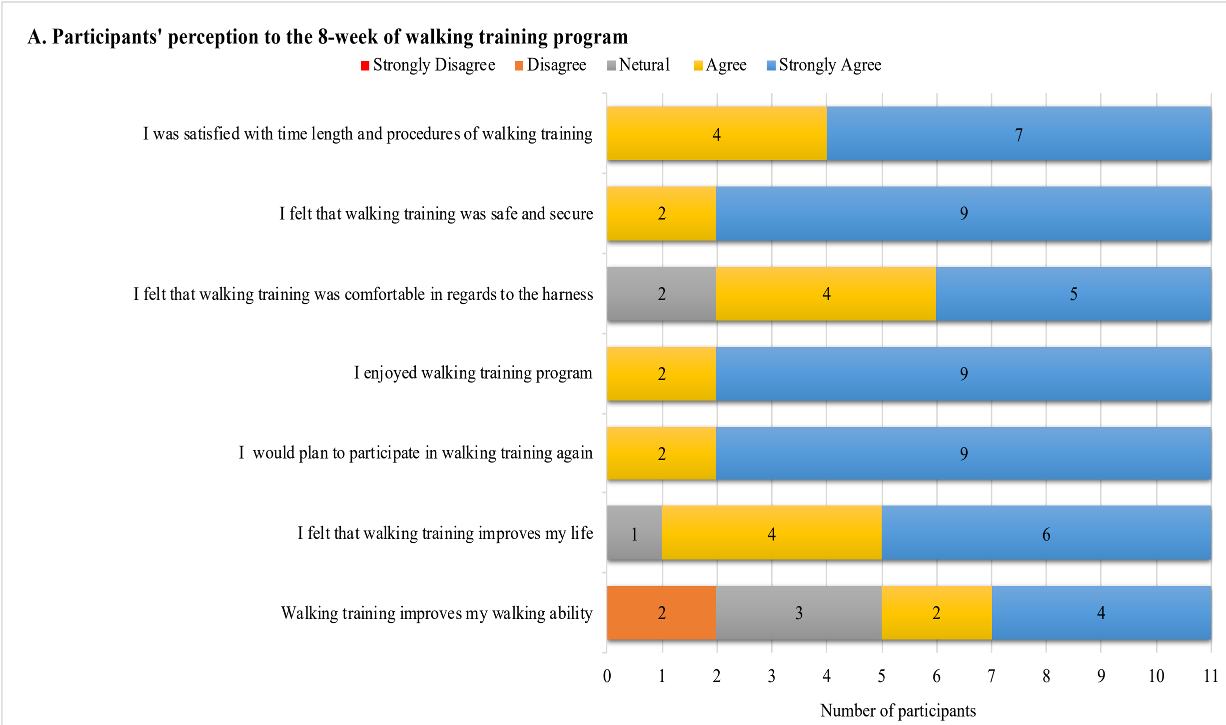


Figure 3.2: Processes of participants recruitment



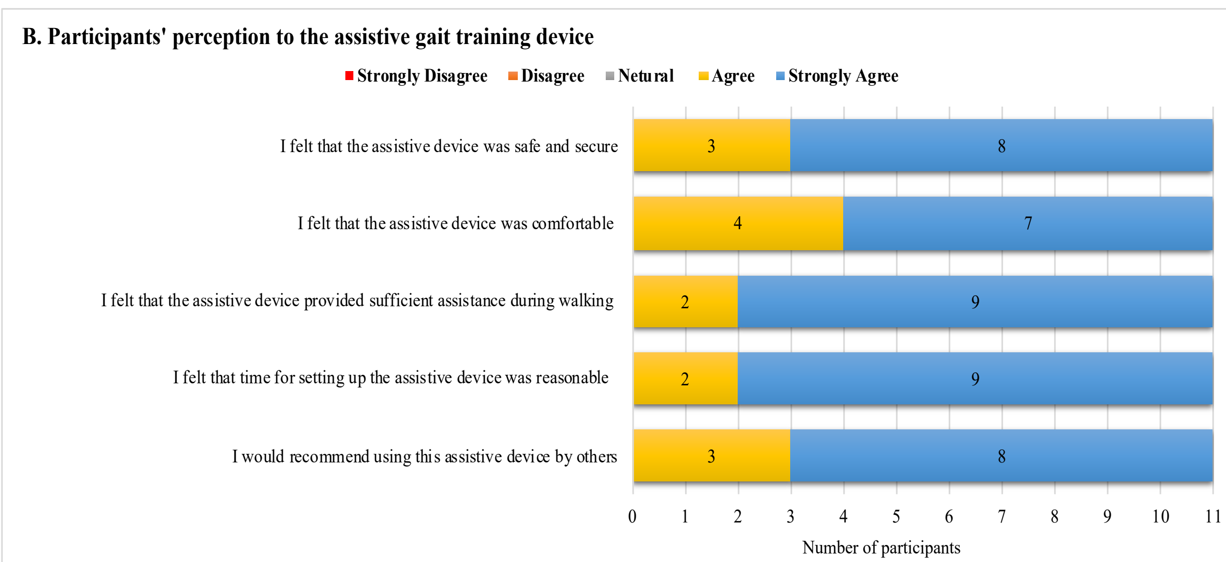


Figure 3.3: Participants' perception to the 8-week walking training program and the assistive gait training device

References

1. *Spinal Cord Injury (SCI) 2016 Facts and Figures at a Glance.* J Spinal Cord Med, 2016. **39**(4): p. 493-4.
2. Phillips, W.T., et al., *Effect of spinal cord injury on the heart and cardiovascular fitness.* Curr Probl Cardiol, 1998. **23**(11): p. 641-716.
3. Warburton, D.E., et al., *Cardiovascular Health and Exercise Rehabilitation in Spinal Cord Injury.* Top Spinal Cord Inj Rehabil, 2007. **13**(1): p. 98-122.
4. Ditunno, P.L., et al., *Who wants to walk? Preferences for recovery after SCI: a longitudinal and cross-sectional study.* Spinal Cord, 2008. **46**(7): p. 500-6.
5. Tefertiller, C., et al., *Efficacy of rehabilitation robotics for walking training in neurological disorders: a review.* J Rehabil Res Dev, 2011. **48**(4): p. 387-416.
6. Colombo, G., et al., *Treadmill training of paraplegic patients using a robotic orthosis.* J Rehabil Res Dev, 2000. **37**(6): p. 693-700.
7. Schwartz, I. and Z. Meiner, *Robotic-assisted gait training in neurological patients: who may benefit?* Ann Biomed Eng, 2015. **43**(5): p. 1260-9.
8. Nam, K.Y., et al., *Robot-assisted gait training (Lokomat) improves walking function and activity in people with spinal cord injury: a systematic review.* J Neuroeng Rehabil, 2017. **14**(1): p. 24.
9. Liu, W., *A narrative review of gait training after stroke and a proposal for developing a novel gait training device that provides minimal assistance.* Top Stroke Rehabil, 2018. **25**(5): p. 375-383.
10. Liu, W. *Developing a Novel Gait Training Device Using a Minimally-Required-Assistance Principle*. [PROC] 2008; Available from: http://dx.doi.org/10.1115/DMD2018-6947.
11. Alqahtani, A.S., et al., *Feasibility and trend of pulmonary function in a pilot trial of aerobic walking exercise in non-ambulatory stroke survivors.* Top Stroke Rehabil, 2019: p. 1-9.
12. Riebe, D., et al., *ACSM's guidelines for exercise testing and prescription*. Tenth edition ed. 2018, Philadelphia, PA: Wolters Kluwer. 480.
13. Sporis, G., et al., *How reliable are the equations for predicting maximal heart rate values in military personnel?* Mil Med, 2011. **176**(3): p. 347-51.
14. Gagnon, D.H., et al., *Satisfaction and perceptions of long-term manual wheelchair users with a spinal cord injury upon completion of a locomotor training program with an overground robotic exoskeleton.* Disabil Rehabil Assist Technol, 2017: p. 1-8.
15. Esquenazi, A., et al., *The ReWalk powered exoskeleton to restore ambulatory function to individuals with thoracic-level motor-complete spinal cord injury.* Am J Phys Med Rehabil, 2012. **91**(11): p. 911-21.

16. Demers, L., R. Weiss-Lambrou, and B. Ska, *The Quebec User Evaluation of Satisfaction with Assistive Technology (QUEST 20): An overview of recent progress.* Techology and disbaility, 2002. **14**: p. 101-105.

17. Peruzzi, A., et al., *Feasibility and Acceptance of a Virtual Reality System for Gait Training of Individuals with Multiple Sclerosis.* European International Journal of Science and Technology, 2013. **2**(6): p. 171-181.

18. Kirshblum, S.C., et al., *International standards for neurological classification of spinal cord injury (Revised 2011).* J Spinal Cord Med, 2011. **34**(6): p. 535-46.

19. Fekete, C., et al., *Development and validation of a self-report version of the Spinal Cord Independence Measure (SCIM III).* Spinal Cord, 2013. **51**(1): p. 40-7.

20. Mitchell, M.C., N.R. Burns, and D.S. Dorstyn, *Screening for depression and anxiety in spinal cord injury with DASS-21.* Spinal Cord, 2008. **46**(8): p. 547-51.

21. Westgren, N. and R. Levi, *Quality of life and traumatic spinal cord injury.* Arch Phys Med Rehabil, 1998. **79**(11): p. 1433-9.

22. Tate, D.G., C.Z. Kalpakjian, and M.B. Forchheimer, *Quality of life issues in individuals with spinal cord injury.* Arch Phys Med Rehabil, 2002. **83**(12 Suppl 2): p. S18-25.

23. Benson, I., et al., *Lower-limb exoskeletons for individuals with chronic spinal cord injury: findings from a feasibility study.* Clin Rehabil, 2016. **30**(1): p. 73-84.

24. Platz, T., et al., *Device-Training for Individuals with Thoracic and Lumbar Spinal Cord Injury Using a Powered Exoskeleton for Technically Assisted Mobility: Achievements and User Satisfaction.* Biomed Res Int, 2016. **2016**: p. 8459018.

25. Gagnon, D.H., et al., *Locomotor training using an overground robotic exoskeleton in long-term manual wheelchair users with a chronic spinal cord injury living in the community: Lessons learned from a feasibility study in terms of recruitment, attendance, learnability, performance and safety.* J Neuroeng Rehabil, 2018. **15**(1): p. 12.

26. Ditor, D.S., et al., *The effects of body-weight supported treadmill training on cardiovascular regulation in individuals with motor-complete SCI.* Spinal Cord, 2005. **43**(11): p. 664-73.

27. Dobkin, B., et al., *The evolution of walking-related outcomes over the first 12 weeks of rehabilitation for incomplete traumatic spinal cord injury: the multicenter randomized Spinal Cord Injury Locomotor Trial.* Neurorehabil Neural Repair, 2007. **21**(1): p. 25-35.

28. Anwer, S., et al., *Effect of locomotor training on motor recovery and walking ability in patients with incomplete spinal cord injury: a case series.* J Phys Ther Sci, 2014. **26**(6): p. 951-3.

29. Manella, K.J., J. Torres, and E.C. Field-Fote, *Restoration of walking function in an individual with chronic complete (AIS A) spinal cord injury.* J Rehabil Med, 2010. **42**(8): p. 795-8.

30. Behrman, A.L. and S.J. Harkema, *Locomotor training after human spinal cord injury: a series of case studies.* Phys Ther, 2000. **80**(7): p. 688-700.

31. Schwartz, I., et al., *Locomotor training using a robotic device in patients with subacute spinal cord injury.* Spinal Cord, 2011. **49**(10): p. 1062-7.

32. Shin, J.C., et al., *Effect of robotic-assisted gait training in patients with incomplete spinal cord injury.* Ann Rehabil Med, 2014. **38**(6): p. 719-25.

33. Hoffman, J.M., et al., *A longitudinal study of depression from 1 to 5 years after spinal cord injury.* Arch Phys Med Rehabil, 2011. **92**(3): p. 411-8.

34. Martin Ginis, K.A. and A.E. Latimer, *The effects of single bouts of body-weight supported treadmill training on the feeling states of people with spinal cord injury.* Spinal Cord, 2007. **45**(1): p. 112-5.

35. Semerjian, T., et al., *Enhancement of Quality of Life and Body Satisfaction Through the Use of Adapted Exercise Devices for Individuals with Spinal Cord Injuries.* Topics in Spinal Cord Injury Rehabilitation, 2005. **11**: p. 95-108.

36. Hicks, A.L. and K.A. Ginis, *Treadmill training after spinal cord injury: it's not just about the walking.* J Rehabil Res Dev, 2008. **45**(2): p. 241-8.

Chapter 4: Potential Factors of Heart Rate Adaptation after 8 Weeks of Walking Training in Individuals with Chronic Spinal Cord Injury – A Preliminary Study

Abstract

Objective: Previous studies, in individuals with spinal cord injury [1], reported a significant decrease in resting and exercise heart rate (HR) after a course of walking training. The potential underlying mechanisms of resting and exercise HR adaptation after a course of walking training in people with SCI is still unknow. The purpose of this study was to investigate the underlying mechanisms of HR adaptation by examining correlations between changes in four factors (leg muscle activity, cardiac autonomic function, lung vital capacity, and leg muscle spasticity) and changes in resting and exercise HR after walking training program in individuals with chronic SCI.

Design: This was a pilot, single group, before-and-after study design.

Participants: A total of 11 participants with chronic SCI completed pre- and post-training assessments and walking training sessions.

Interventions: Participants received a walking training program using a treadmill, a body weight supported system, and an assistive gait training device, 3 sessions per week for 8 weeks, a total of 24 training sessions.

Outcome Measures: Resting and exercise HR, electromyographic activity of leg muscles, frequency-and time-domain measures of heart rate variability (HRV), lung vital capacity, and spasticity of leg muscles were assessed before and after completing 8-week of walking training.

Main Results: Strong correlations were found between the change in low-frequency power of HRV and the change in resting HR ($r = -0.737$, $p < 0.01$), between the change in root mean square of the successive differences (RMSSD) of HRV and the change in resting HR ($r = -0.6$, $p < 0.05$), and the change in lung vital capacity and the change in resting HR ($r = -0.624$, $p <$

0.05). There was a moderate correlation between the change in high-frequency power of HRV and the change in resting HR (r = - 0.56, $p < 0.05$). The change in neuromuscular activity of leg muscles was strongly correlated with the change in exercise HR (r = - 0.622, $p < 0.05$).

Conclusion: The primary findings of this study suggest that the modulations of cardiac autonomic function and improvement of lung function after training were significant contributors to resting HR adaptation, while increased neuromuscular activity of leg muscles was a significant contributor to exercise HR adaptation.

Keywords: *spinal cord injury; heart rate, leg muscle activity, cardiac autonomic function* lung vital capacity*; leg muscle spasticity; walking training*

4.1. Introduction

With an increased life expectancy of people with spinal cord injury (SCI), cardiovascular disease (CVD) has become the common leading cause of death [2-4]. A persistently elevated heart rate (HR) is linked to increased risks of CVD [5]. The prevalence of elevated resting HR in people with SCI has estimated to be 27- 41%, and it is more prevalent in people with paraplegia [6, 7]. Lack of physical activity after injury plays an important role in decreased cardiovascular fitness, as reflected by increased resting and exercise HR. Previous studies reported low levels of physical activity among people living with SCI, and about 50% of them did not achieve the recommended level of physical activity for people with disabilities [8, 9]. This could explain the decreased cardiovascular fitness among the SCI population. Elevated HR in rest or during physical activities is more common in individuals with SCI as compared to healthy individuals and in individuals with paraplegia compared to those with quadriplegia [10, 11].

Previous studies in individuals with SCI reported improvement of cardiovascular fitness after walking training, as shown by reduced resting and exercise HR [12-16]. However, the underlying mechanisms of HR adaptation following walking training in individuals with SCI is still unknown. After SCI, the reduced leg muscle pump, dysregulation of the cardiac autonomic nervous system, declined lung function, and increased muscle spasticity may negatively influence the cardiovascular system. Rhythmic contraction of leg muscles (contraction and relaxation) during locomotion plays an important role in promoting venous return from legs to the heart. The absence of leg muscle contraction due to loss or impairment of sensorimotor function can lead to a significant decrease in venous return and stroke volume and consequently increases HR as a compensatory reflex [17]. Furthermore, the cardiac autonomic nervous system is an important modulator of HR. The function of the cardiac autonomic nervous system can be non-invasively quantified by measuring heart rate variability (HRV) [18, 19]. Past studies showed that individuals with SCI, particularly those with paraplegia, had alterations in HRV as demonstrated by increased low frequency (LF) powers and decreased high-frequency (HF) powers and root mean square of the successive differences (RMSSD), as compared to healthy individuals [20, 21]. Lack of physical activity plays an important role in the alteration of cardiac autonomic function, as shown by decreased HF and RMSSD (i.e., diminished parasympathetic activity), which results in an increased HR [21, 22]. Besides, lung vital capacity is an important parameter in predicting respiratory and cardiovascular health. After SCI, lung vital capacity decreases as a result of respiratory muscle weakness as well as reduced physical activity [23]. It has been shown that the decline in lung vital capacity is associated with adverse cardiovascular outcomes, including increased HR [24]. In addition, muscle spasticity after SCI is associated with decreased arterial diameter and increased blood flow resistance [25]. Thus, the workload on the heart would increase in order to deliver blood to body tissues. It has been reported that increased muscle spasticity may be associated with increased HR in individuals with neurological diseases [13, 26, 27]. Repetitive locomotor training in individuals with SCI has been shown to increase the rhythmic neuromuscular activity of leg muscles [28-31], regulate cardiac autonomic function [12, 13], improve lung vital capacity [14, 32, 33], and reduce spasticity of leg muscles [34-36]. Thus, it is possible that changes in those factors after a course of walking training might contribute to an improvement in HR, but quantitative examinations are needed.

Therefore, the purpose of this study was to examine correlations between changes in four factors (leg muscle activity, cardiac autonomic function, lung vital capacity, and leg muscle spasticity) and changes in resting and exercise HR after a walking training program in individuals with chronic SCI. The hypothesis was that decreases in resting and exercise HR after the walking training would show significant correlations with increased leg muscle activity, which would be stronger than its correlations with changes in cardiac autonomic function, lung vital capacity, or leg muscle spasticity.

4.2. Methods

4.2.1. Participants

We primarily recruited a convivence sampling of individuals with chronic SCI from the Spine Center Clinical at the University of Kansas Hospital. In addition, we used the Healthcare Enterprise Repository for Ontological Narration database at the University of Kansas Medical Center to obtain the contact information of patients with SCI who were admitted to the University of Kansas Hospital and agreed to be contacted for participating in a research study. We also visited and distributed study flyers in outpatient rehabilitation centers and SCI supported groups in Kansas City area. Individuals were included in the study if they had chronic SCI ($\geq$12 months) and paraplegia (T1-L2) and were between age 18-60 years-old, unable to walking independently (scored < 5 in Functional Independence Measure, Locomotion: Walk), and not participating in any similar walking training activity. In addition, individuals should have medical approval from their physician to participate in walking training. Participants were excluded if they had one of the following conditions: major CVD such as heart diseases; any other neurological diseases such as stroke, multiple sclerosis, and Parkinson diseases, etc.; severe muscle spasticity (> 3 according to Ashworth scale); severe orthopedic issues such as joint stiffness and fractures; severe osteoporosis (bone mineral density T-score less than – 2.5); current inflammatory diseases or infections; pregnant women; or uncontrolled autonomic dysreflexia. Prior to participating in the study, participants signed a written informed consent.

The study protocol was approved by the Institutional Review Boards at the University of Kansas Medical Center.

4.2.2. Study Design

This study used a pilot, single group, pretest-posttest study design. Eligible participants received 3 sessions per week for 8 weeks of walking training using a treadmill, body weight supported system, and assistive gait training device. The outcome measures listed below were performed at baseline (pre-training) and within 3 days after completing the 8-week walking training (post-training). Participants were instructed to restrain from caffeine, cigarette smoking, and physical activity for at least 12 hours, and empty their bladder (or urine bad) before a testing session. Participants were also asked not to take any medication 9 hours before measurements unless it is necessary.

4.2.3. Walking Training Protocol

The walking training session was scheduled 3 times per week for 8 weeks. Participants exercised on a treadmill with a body-weight support (BWS) system and an assistive gait training device (Figure 4.1). The participant wore a safety harness, which was attached to an overhead motorized lift to provide BWS and to prevent the risk of falling. The amount of BWS was chosen based on the participant's tolerance without knee buckling or toe dragging. At the initial sessions, 60 to 40 % of the participant's body weight was supported. In the following sessions, the amount of BWS was decreased by 5% each week as tolerated. However, if the participant was unable to tolerate new BWS or showed knee buckling or toe dragging, the BWS was increased by 5%. The amount of BWS was determined using a digital scale attached to a motorized lift. A novel assistive gait training device developed in our laboratory was used to assist the participant with leg stepping during walking on a treadmill [37]. Two thigh braces were placed onto the participant's left and right thighs and secured using straps. These braces were attached to a pulley system via pulling cords (**Figure 4.1**). The assistive gait training was operated by two therapists to assist the participant with hip flexion and ankle dorsiflexion at the beginning and during the swing phase of a gait cycle. The participant was encouraged to actively engage in moving his/her legs throughout training. The duration of each training session was chosen based on the participant's tolerance. The goal was to have the participant ambulated for a total of 30 min (2 bouts of 15 minutes). The participant was allowed to take a rest at any time. At the beginning of training, the initial treadmill speed was set at 0.5 miles/hour. The participant ambulated at 0.5 miles/hour for 2 minutes to warm up. Then, the treadmill speed was gradually increased until the participant's HR reached 40-59% of his/her HR reserve ([maximum HR – resting HR] * target intensity% + resting HR) as recommended by American College of Sports Medicine guidelines [38]. Maximum HR was determined using the age-predicted maximum HR (220 – participant's age) [39]. In addition, the Borg Rating of Perceived Exertion Scale 6-20 was used to rate participant's level of exertion during training between bouts and by the end of the training session. At the end of the training, participants walked at slow speed (0.5 miles/hour) for 2 minutes to cool down. During each training session, training duration, treadmill speed, BWS, HR, blood pressure, and rate of perceived exertion were recorded using a walking training log. To ensure the safety of training, HR was monitored throughout training using a Polar OH1 optical HR sensor (Polar Electro Inc., New York), and blood pressure was recorded before the training, between each training bout, and at the end of the training session. The participant's HR should not have exceeded 85% of the age-predicted maximum HR, and the participant's blood pressure should have been maintained below 220/110 mm Hg.

4.2.4. Resting and Exercise Heart rate (HR)

Resting HR was measured during a sitting position after 10 minutes rest using an automated oscillometric device (Omron 7 Series®-BP760N, Omron Healthcare Co., Muko, Kyoto, Japan). This device was used to measure resting HR and blood pressure simultaneously. Three readings were obtained, and the average of three readings was calculated to determine resting HR. Exercise HR was measured during a graded treadmill walking test with the same testing conditions in terms of treadmill speed and percentage of body weight support (BWS) pre- and post-training assessment. Exercise HR was obtained in the second session of week 1 and the last session of week 8. Participants walked on a treadmill with a starting warm-up speed of 0.5 miles/hour. The treadmill speed was increased by 0.2 miles/hour every two minutes. Exercise HR was monitored by a Polar OH1 optical HR sensor (Polar Electro Inc., New York) placed in the participant's upper part of the left forearm. This HR sensor has an excellent agreement with ECG measure and can be utilized as a valid measure of HR during moderate to high intensity physical activities [40]. Exercise HR was recorded at the beginning and every 2 minutes (**Figure 4.2**). Exercise HR recorded at the end of the 6 minutes at a treadmill speed of 0.9 miles/hour was included in data analysis. Leg assistance was provided to participants as needed.

4.2.5. Electromyography Activity of Lower Limbs Muscles

The neuromuscular activity of four muscles in both lower limbs (biceps femoris, rectus femoris, gastrocnemius medialis, and tibialis anterior) was measured using an electromyography (EMG) system (Trigno™ Wireless EMG, Delsys incorporated, Natick, MA, USA). Prior to the test, skin areas on where EMG sensors will be placed were cleaned using alcohol wipes and shaved if needed. Then, wireless EMG sensors were placed on these muscles bilaterally according to the SENIAM guidelines [41]. The signal recording was obtained during walking on a treadmill. The amount of BWS (50%) and treadmill speed (0.9 miles/hour) was kept constant for each participant during the measurement at baseline and after completing the study, as described in a previous study [28]. EMG signals were recorded from at least 10 step cycles. Manual assistance for both legs was provided by two therapists when it was needed.

EMG signals were recorded at a sample rate of 1800 Hz and processed using a lab-made program in MATLAB. Briefly, the raw EMG signals first were digitally filtered using Butterworth filters (band-pass 30–300 Hz) and then rectified. Root mean square (RMS) EMG signals were calculated using a time-window of 0.2 seconds. Peak RMS amplitudes for each muscle during 10 step cycles were averaged. In some testing data, the averaged data were obtained from less than 10 step cycles due to movement artifact in the recorded EMG signals. The peak RMS values for the same muscle on the right and left lower limbs were averaged, and then the total EMG activity was calculated by summing peak RMS values of all four muscles.

4.2.6. Measurement of Cardiac Autonomic Function

An electrocardiogram (ECG) acquisition system (MP160, BIOPAC Systems Inc., Camino Goleta, California) was used to assess cardiac autonomic function through frequency and time analysis of HRV. The ECG signals were recorded using three disposable electrodes placed on the participant's chest in a standard lead II configuration. The sampling rate of signal acquisition was set at 2000 Hz, and the signal was amplified with a BIOPAC ECG100C amplifier with a band-pass filter between 1 Hz and 35 Hz. As described in previous studies [20, 22], the ECG acquisition was conducted in a sitting position after at least 10 minutes of rest and lasted for 5 minutes. The measurement was conducted in a quiet room with a temperature between 22 and 24 C. During data acquisition, participants were asked to breathe normally and remain silent, without speaking or moving. Data collection was performed around the same time (between 9 and 11 am) and conditions pre- and post-training. The frequency-domain and time-domain parameters of HRV were calculated using BIOPAC *AcqKnowledge* 5.0 software. The frequency-domain parameters consisted of LF power (0.04–0.15 Hz) and HF power (0.15–0.4 Hz) and LF/HF ratio. The time-domain parameters included RMSSD.

4.2.7. Lung Vital Capacity

Lung vital capacity was measured using a spirometer (Spirotel® Spirometer, MIR USA INC.) The measurement was conducted according to the guidelines from the American Thoracic Society/European Respiratory Society [42]. The participant sat upright on a chair with back and arm support and received instruction on how to perform the test. The nose clips were placed on the participant's nose to close his/her nose. The participant was asked to insert the mouthpiece of the spirometer inside his/her mouth and seal his/her lips around it to prevent air leakage. To perform the test, the participant was instructed to breathe normally three times, and then inhale and exhale maximally (i.e., to fully fill and empty their lungs during inspiration and expiration, respectively). In addition, the participant was instructed not to flex or extend his/her neck or trunk during the test. Three readings from three repeated tests were obtained, and the largest reading was recorded. There was a 30-second rest between each test.

4.2.8. Muscle Spasticity for The Lower Extremities

Modified Tardieu Scale (MTS) was utilized to assess muscle spasticity for the lower extremity (hip extensors, knee flexors and extensors, and ankle planterflexors). MTS is a reliable clinical assessment for measuring muscle tone and joint reaction in response to passive stretch at specified velocities, including as slow as possible (V1), falling under gravity (V2), or as fast as possible (V3) [43]. The participants were tested in the supine position, and the level of muscle reaction of the lower limbs was assessed at specified speeds (V1, V3). The level of muscle reaction was measured on a 6-point scale (0 indicated "No resistance throughout the passive movement," and 5 indicates "Joint immobile"). The level of muscle reactions during fast passive movement (V3) was included in the analysis. The total score of MTS was calculated by summing the overall score of the right and left legs.

4.2.9. Statistical Analysis

Statistical analysis was conducted using SPSS statistical software version 25 (IBM Corporation; Armonk, New York). Due to the small number of participants and independent variables might not be normally distributed, the Wilcoxon signed-rank test was used to examine differences in independent variables between pre-training and post-training assessment. The level of significance was set at 0.1 (one-tailed test). In addition, the Mann Whitney U test was used to compare differences in changes in outcome measures between upper paraplegia (T1-T5) and lower paraplegia (T6-L2).

The normality of residual for each factor was checked using the Shapiro-Wilk test. Since residuals for the factors were normally distributed, the Pearson's correlation analysis was conducted to determine whether there was a significant correlation between the change in each factor and the change in primary outcome measure (resting and exercise HR). The level of significance at 0.1 (one-tailed test) due to the small sample size of this pilot study.

4.3. Results

4.3.1. Participants

A total of 15 individuals with chronic SCI volunteered to participate in the study, and 11 participants completed the study. Three participants dropped out of the study due to schedule conflict with work or family members (n = 2) or an adverse event not related to the study (n = 1). One participant was excluded after the first week because he had a colostomy which might have interfered with a harness. The characteristics of participants who completed the study are summarized in **Table 4.1**.

4.3.2. Change in Resting and Exercise Heart Rate

After training, the resting and exercise HR significantly decreased from 83±10.76 to 74±8.42 beats/min ($p < 0.01$) and from 119.45±15.26 to 112.18±14.93 beats/min ($p < 0.01$), respectively (**Figure 4.3**). The individual data for value in resting and exercise HR before and after training are presented in **Table 4.2**. There were no significant differences in changes in resting and exercise HR after training between participants with upper paraplegia and those with lower paraplegia.

4.3.3. Changes in Relevant Factors

The changes in the EMG activity of leg muscles for 10 participants who completed pre- and post-test are presented in **Figure 4.4**. The peak EMG activity of all four tested muscles significantly increased after training. Overall, the total EMG activity of leg muscles significantly increased from 69.42±13.15 μV to 118.36±21.38 μV after training ($p < 0.01$). The raw EMG signal of one muscle in a participant with motor complete SCI (ASIA grade B) before and after training is presented in **Figure 4.5**.

As shown in **Table 4.3**, LF power significantly decreased (pre: 0.82±0.15 vs. post: 0.44±0.11; $p < 0.05$) and HF power of HRV significantly increased (pre: 80.73±53.86 ms^2 vs. post: 100.15±57.56 ms^2; $p < 0.01$) after training. There was a significant reduction in LF to HF ratio (pre: 0.82±0.15 vs. post: 0.44±0.11; $p < 0.01$). RMSSD significantly increased from 26.05±9.36 msec to 36.03±9.73 msec ($p < 0.01$).

There was a significant increase in lung vital capacity after training (pre: 2.92±1.18 L vs. post: 3.08±1.17 L; $p < 0.01$) (**Figure 4.6**). MTS scores of left hip extensors and right and left knee flexors significantly decreased ($p < 0.05$) after gait training (**Table 4.4**). There was a significant decrease in total MTS scores of both legs after training (pre: 5.36±1.88 vs. post: 3.27 ± 1.83; $p < 0.01$).

No significant differences in changes in factors after training were observed between participants with upper paraplegia and those with lower paraplegia (**Table 4.2**).

4.3.4. Association Between Changes in Relevant Factors and Change in Heart Rate

Table 4.5 shows the correlations between changes in four factors and changes in resting and exercise HR, calculated as the difference between post-training and pre-training measurements. Strong correlations were found between the change in LF power of HRV and the change in resting HR (r = - 0.737, $p < 0.01$), between the change in RMSSD of HRV and the change in resting HR (r = - 0.6, $p < 0.05$), and between the change in lung vital capacity and the change in resting HR (r = - 0.624, $p < 0.05$). There was a moderate correlation between the change in HF power of HRV and the change in resting HR (r = - 0.56, $p < 0.05$). The correlation between the change in the ratio of LF to HF and the change in resting HR showed a trend towards significance (r = 0.401, $p = 0.11$). Only the change in EMG activity of leg muscles was strongly correlated with the change in exercise HR (r = - 0.622, $p < 0.05$).

4.4. Discussion

The results of the current study showed that 8 weeks of walking training in individuals with chronic paraplegia decreased resting and exercise HR. This finding suggests the beneficial effects of walking training on inducing HR adaptation in individuals with either upper paraplegia or lower paraplegia. We observed an increase in the EMG activity of leg muscles during training. Our finding of changes in EMG activity of leg muscles generally agreed with findings from previous studies that showed the increased EMG activity of leg muscles in individuals with SCI after repetitive locomotor training [28-31]. The same changes were reported even in those with the absence of supraspinal input in the case of motor complete SCI [44], which was confirmed by our findings since the majority of our participants (8/11) had motor complete SCI. It is suggested that sensory inputs during leg loading on a treadmill can trigger the neuromuscular activity of leg muscles through activation of central pattern generators located within the lumbar segment of the spinal cord [45, 46]. In the current study, we utilized a novel assistive gait training device to help participants with forward leg stepping during treadmill walking. By providing a minimal amount of assistance during leg stepping, this device can promote active involvement of participants and minimize sensory disturbance [37], which may enhance the afferent sensory input to stimulate spinal neural circuits (i.e. center pattern generators) and promote changes in neuroplasticity of neural circuits. Consequently, the use of the training device may improve the neuromuscular activity of leg muscles.

Our finding of the significant correlation between the increase in EMG activity of leg muscles and decrease in exercise HR may indicate an important functionality of the increased activity of leg muscles after the walking training in individuals with SCI. The absence or reduction of leg muscle pump in SCI survivors due to impaired motor control and loss of muscle mass can lead to a decline in venous return and stroke volume. Consequently, HR increases as a compensatory reflex mechanism to a decrease in stroke volume [17]. A few past studies indicated that elevated HR in individuals after SCI might be reversible through exercise interventions. More relevant studies in individuals with motor incomplete SCI examined changes in exercise HR after 8 weeks of robotic walking training [15] or during 8 weeks of underwater treadmill training [16]. In the study by Hoekstra et al. [15], exercise HR decreased by 7 beats/minute at the constant workload, which was similar to the decrease in exercise HR of 7.27 beats/minute observed in the current study. Stevens et al. [16] reported a significant decrease in exercise HR over the course of 3 biweekly periods in which walking speed remained constant, with a larger decrease in HR occurred at the biweekly period with faster walking speed. The investigators suggested several contributing factors for the decrease in exercise HR, including a shift in the balance of autonomic activity toward parasympathetic tone, improved aerobic performance, and enhanced activity of leg muscle pump. A previous study also reported that repetitive standing resulted in increased EMG activity of leg muscles and consequently improved cardiovascular response to orthostatic stress test due to increased blood venous return to the heart [47]. However, past studies have not explored possible correlations between the changes in exercise HR and some of the potential contributing factors. Whether the increased EMG activity of leg muscles during walking training can contribute significantly to exercise HR adaptation in individuals with SCI is still unknown. The current study is the first to examine contributions of four potential factors to HR adaptation after a walking training program in individuals with SCI, including EMG activity of leg muscles, cardiac autonomic function, lung capacity, and spasticity of leg muscles. We observed that the only significant correlation existed between change in exercise HR and change in EMG activity of leg muscles. These results provide a clear indication that the increased neuromuscular activity of leg muscles significantly contributed to enhanced venous return that leads to an increase in stroke volume and a decrease in exercise HR. Our findings are clinically important in promoting the effort to improve the activity of leg muscles during exercise for cardiovascular benefits in individuals with SCI, even those with motor complete injury.

HR adaptation to walking exercise in individuals with SCI may present unique features different from that in the healthy population. In able-bodied individuals, HR normally increases during physical activity to meet metabolic requirements of the active skeletal muscle in an intensity-dependent manner, through withdrawal of vagal tone and activation of the sympathetic nervous system centrally, as well as peripheral mechanism involving group III and IV afference nerves from the active skeletal muscles to deliver mechanical and metabolic feedback signals that further increases sympathetic activity [48, 49]. After continued practice of a task such as an endurance training, human movement becomes more skilled as shown by decreased amplitude and duration of muscle activity [50-53], and exercise HR decreases under the same exercise intensity [54-56]. However, in the current study we observed a significant decrease in exercise HR and increase in leg muscle activity, as well as a significant correlation between the two variables after the 8-week walking training. Such a unique association was most likely because the increased activity of leg muscles enhanced leg muscle pump, venous return, and stroke volume, and subsequently led to a decrease in exercise HR [17]. Conversely, the normal processes that lead to the increase in sympathetic nerve activity and exercise HR due to increases in leg muscle activities are significantly interrupted in persons with SCI. First, the disruption of descending neural pathways impairs its regulation of sympathetic pathways to the heart, which is often the case in SCI above the level of sympathetic outflow (i.e., T5 and above) [57, 58]. In fact, the increased activity of leg muscles in the current study was the result of neuroplasticity of central pattern generators in the spinal cord, not came from supraspinal control centers. Second, the damage to ascending neural pathways after SCI interrupts feedback signals of muscle metaboreflex to sympathetic nerve system from group III and IV afference nerves of the activated muscles and consequently diminish the increase in HR [59]. The same reasons may also explain why no correlation was observed in the current study between the change in exercise HR and changes in cardiac autonomic function as shown in HR variability measurements.

Although the modulations of cardiac autonomic function did not contribute to the change in exercise HR, it significantly contributed to the resting HR adaptation post walking training. Regular walking training might induce resting HR adaptation through various possible mechanisms, including increases in cardiopulmonary fitness, stroke volume and blood volume, decrease in peripheral arterial stiffness, modulations of the autonomic nervous system, and improvements in lung function and cardiac ventricular function [60, 61]. Metabolic and molecular changes in cardiac structure might also be involved in HR adaptation after exercise [62, 63]. However, SCI above T6 can alter autonomic control of the cardiovascular system due to loss or impairment of sympathetic supraspinal innervation [64]. Cardiac autonomic control can be also impaired in those with injury levels at T6 or below because of a lack of mobility and a sedentary lifestyle after injury [22]. Dysfunction of cardiac autonomic control might play a role in a persistent elevation in HR, which is observed in many people with SCI below T5 as well as some individuals with SCI between T1and T5 [6, 7]. Participating in regular exercise could be a feasible option in the modulations of cardiac autonomic function in individuals with injury above or below T5 [65]. The function of the cardiac autonomic nervous system can be non-invasively examined through HRV in frequency-domain and time-domain analysis [66, 67]. The frequency-domain analysis of HRV provides information on HF power reflecting parasympathetic outflow and LF power, which is a combination of both sympathetic and parasympathetic outflows. The ratio of LF to HF is defined as a marker for sympathovagal balance [67, 68]. The time-domain analysis of HRV provides information on R-R interval (beat-to-beat) variances or differences. The RMSSD, which reflects the activity of parasympathetic tone, is the most commonly used parameter with a short-term recording [67]. Measurements of HRV indices can provide valuable information about the mechanisms of HR adaptation to training. In the current study, we observed significant increases in HF power and RMSSD and a significant decrease in the ratio of LF to HF. LF power showed a signification reduction after walking training. Both participants with upper paraplegia and those with lower paraplegia experienced similar improvements in the parameters of HRV after training. These findings agreed with findings from a systematic review study that reported beneficial effects of regular exercise on cardiac autonomic function in persons with either upper or lower SCI [65]. We also observed that the changes in LF power, HF power, and RMSSD after walking training were significantly correlated with resting HR adaptation. The increased parasympathetic outflow after the walking training, as shown by increases in HF power and RMSSD of HRV, was important because it was associated with a decrease in resting HR and, therefore, a reduction in the risk of CVD. It is still unclear in regard to the connection between changes in LF power of HRV and cardiac sympathetic modulation since conflicting results have been reported in the literature [69, 70]. Evidence indicated that LF power of HRV reflects the baroreflex function, which is responsible for the regulation of blood pressure [70, 71].

The result of the current study indicated that improved lung function might be another important factor for the decrease in resting HR. After SCI, individuals are prone to a significant reduction in lung function due to paralysis of respiratory muscles as well as a sedentary lifestyle [23]. A study showed an increase in resting HR in people with impaired lung function [72]. A longitudinal study in middle-aged adults showed an inverse relation between resting HR and lung vital capacity [24]. A decline in lung vital capacity with aging was associated with increased resting HR [24]. In the current study, we found an increase in lung vital capacity by 5% following the 8-week walking training in participants with either upper or lower paraplegia. The improvement of lung capacity after walking training in individuals with SCI can be attributed to increased strength or activity of respiratory muscles [33]. The current study also showed that the increased lung vital capacity after walking training was correlated with decreased resting HR. It might be that increased strength of respiratory muscles after walking training increased the expansion of the chest wall which could improve the lung's capacity to take in more oxygen into the body, resulting in more oxygenated blood [73, 74]. Thus, the workload on the heart to deliver oxygenated blood to body tissues would decrease and therefore resting HR decreased. Besides, it has been shown that HR is linked to respiratory rate. Decreased respiratory rate is associated with reduced HR through its contribution to the modulations of the autonomic nervous system [75, 76]. Although the respiratory rate was not directly measured in the current study, it could be that the improvement in lung vital capacity after walking training might lead to decreased respiratory rate, which resulted in increased activity of the parasympathetic system and reduced activation of the sympathetic system and consequently decreased resting HR [75, 77].

Results of the current study implied that changes in leg muscle spasticity did not contribute to changes in HR after the walking training. Muscle spasticity after SCI is associated with decreased arterial diameter and increased blood flow resistance [25], which might increase the load on the heart to deliver the blood to body tissues. Previous studies in individuals with SCI or cerebral palsy showed higher HR response during gait training in individuals with a high degree of muscle spasticity compared to those who had a low degree of muscle spasticity [13, 27]. We speculated that a decrease in spasticity of leg muscles after a course of walking training might contribute to HR adaptation. The results of the current study, however, showed that the change in spasticity of leg muscles after the walking training was not associated with the decrease in resting or exercise HR. This result may be related to a fact that the majority of participants of the study used anti-spasticity medications and showed no muscle spasticity in most of the tested leg muscles at pre-training assessment. Thus, no benefit from walking training could be added to reduce the degree of muscle spasticity in those participants. This may justify why we did not notice any potential association between the change in leg muscle spasticity and HR adaptation after training.

The current study has some limitations that need to be considered. The sample size is relatively small. The statistical powers were less than 80% for factors that did not show significant corrections with the primary outcome. More importantly, the small number of participants can significantly increase the sampling bias compared to a large sample size. The sample mean might deviate significantly from the population mean due to sampling bias, leading to overstated conclusion. Therefore, results of this preliminary study should be only used to help researchers in finding trends and need to be interpreted with caution. Second, we examined only four potential contributing factors of HR adaptation to training. There might be other important factors that may contribute to HR adaptation after walking exercise, such as changes in physical fitness level, stroke volume, or cardiac ventricle function, etc., which were not included in the model of the current study. Third, only individuals with paraplegia (T1-L2) were included in the present study. The findings may not be generalized to people with cervical injury. Finally, we did not control for medications, such as the use of anti-spasticity medications, that might influence the measurement of muscle spasticity.

4.5. Conclusion

The findings of the present study suggest the benefits of walking training on cardiovascular health in individuals with chronic paraplegia, as shown by decreased resting and exercise HR. More importantly, this study was the first step to explore potential underlying mechanisms of improvement in the cardiovascular system, specifically the HR adaptation in response to walking training in individuals with SCI. The preliminary results of the current study seem to support a hypothesis that adaptive change in central pattern generators in the spinal cord may contribute significantly to exercise HR adaptation after the walking training. In parallel or maybe with a short time delay, improvement in cardiac autonomic function and the pulmonary system might occur, and consequently, the resting HR adaptation takes place. In order to draw definitive conclusion, future studies with a large sample size should confirm findings of the current study and further examine the contributions to HR adaptation by changes in other factors, such as physical fitness level, stroke volume, cardiac ventricular size and contractility, and peripheral arterial stiffness, etc.

Table 4.1: Demographic and clinical characteristics of participants who completed the study (n=11)

Participant ID	Gender, (Male/Female)	Age, (yrs) Mean±SD	Body weight (kg) Mean±SD		Height (cm) Mean±SD	Injury level	ASIA grade	Injury duration (yrs) Mean±SD	Medications (Yes/No)		
			Pre-training	Post-training					Anti-hypertension	Beta-blockers	Anti-spasticity
SCI 02	Male	40	71	68	173	T9	A	7	No	No	No
SCI 03	Male	47	130	131	196	T8	B	3	Yes	Yes	Yes
SCI 04	Male	37	89	89	189	T3	B	1	No	No	Yes
SCI 06	Male	40	118	120	189	T5	B	19	No	No	Yes
SCI 07	Male	19	117	117	206	T3	B	2	No	No	Yes
SCI 08	Male	48	78	78	198	T9	B	3	No	No	Yes
SCI 09	Male	35	108	107	155	T6	C	35	Yes	Yes	No
SCI 10	Female	32	107	105	178	L1	C	14	No	No	Yes
SCI 11	Female	25	58	58	168	T7	B	7	No	No	Yes
SCI 13	Male	41	71	71	180	T5	A	4	No	No	Yes
SCI 15	Female	55	122	125	178	T2	D	1	No	No	No
	8/3	38.09±10.31	97.18±24.64	97.18±25.48	182.73±14.70			8.72±10.40	2/9	2/9	9/2

ASIA: American Spinal Injury Association

Table 4.2: Individual data of resting and exercise heart rate, EMG activity of leg muscles, HRV parameters, lung vital capacity, and spasticity of leg muscles pre-training and post-training

Participant ID	Resting HR (beats/min)		Exercise HR (beats/min)		Total leg EMG (µV)		LF (ms^2)		HF (ms^2)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
SCI 02	89	83	118	109	24.96	72.81	12.13	7.33	52.62	54.61
SCI 03	68	63	106	94	34.79	149.305	49.46	37.98	39.16	52.41
SCI 04	86	62	130	123	32.605	65.66	5.63	36.02	5.25	112.59
SCI 06	93	80	103	98	91.655	102.815	21.9	14.91	20.84	35.69
SCI 07	65	61	123	121	N/A	N/A	74.74	69.614	613.35	665.94
SCI 08	89	80	116	109	108.93	230.28	8.89	4.83	11.72	21.71
SCI 09	82	77	97	93	79.05	87.81	5.15	4.81	2.61	3.87
SCI 10	79	77	149	140	95.045	118.135	57.56	34.51	94.52	100.92
SCI 11	102	82	133	126	20.12	33.58	6.45	4.39	11.23	12.57
SCI 13	76	70	110	102	61.84	88.495	24.67	22.71	23.2	25.81
SCI 15	84	79	129	119	145.23	234.79	5.32	3.78	13.61	15.54

Table 4.2 (continued): Individual data of resting and exercise heart rate, EMG activity of leg muscles, HRV parameters, lung vital capacity, and spasticity of leg muscles pre-training and post-training

Participant ID	HF/LF ratio		RMSSD (msecs)		LVC (L)		MTS score total	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
SCI 02	0.23	0.14	19.34	21.66	3.39	3.56	5	0
SCI 03	1.26	0.72	28.11	28.28	3.02	3.2	2	0
SCI 04	1.04	0.32	9.22	43.63	1.97	2.13	6	4
SCI 06	1.05	0.38	16.6	40.71	2.75	2.96	14	12
SCI 07	0.12	0.1	114.56	120.29	5.47	5.52	20	18
SCI 08	0.75	0.22	11.79	17.41	4.35	4.47	5	0
SCI 09	1.96	1.24	9.32	10.39	1.24	1.32	0	0
SCI 10	0.61	0.34	42	64.11	3.15	3.27	1	0
SCI 11	0.57	0.34	7.32	15.59	2.73	3.17	2	0
SCI 13	1.06	0.88	17.98	22.14	1.96	2.03	4	2
SCI 15	0.39	0.24	10.33	12.22	2.17	2.31	0	0

EMG: Electromyography; VC: Vital capacity; LF: Low-frequency; HF: High-frequency; RMSSD: Root means square of successive difference; MTS: Modified Tardieu Scale.

Table 4.3: Frequency-domain and time-domain parameters of heart rate variability (n=11)

	Pre-training	Post-training	Mean change (post - pre)	*p*-value
LF (ms^2)	24.71±7.42	21.89±6.31	- 2.81±3.84	0.02
HF (ms^2)	80.73±53.86	100.15±57.56	19.41±9.86	0.001
LF/HF ratio	0.82±0.15	0.44±0.11	- 0.37±0.07	0.001
RMSSD (msec)	26.05±9.36	36.03±9.73	9.98±3.46	0.001

Values are presented in mean ± standard error. LF: Low-frequency; HF: High-frequency; RMSSD: Root mean square of successive difference.

Table 4.4: Modified Tardieu Scale (MTS) scores at fast speed passive movement (V3) per- and post-training (n=11)

	Pre-training		**Post-training**	
	Right	*Left*	*Right*	*Left*
Hip extensors	0.55 ± 0.28	1.09 ± 0.28	0.27 ± 0.19	**0.64 ± 0.27** *
Knee extensors	0.18 ± 0.18	0.00 ± 0.00	0.09 ± 0.09	0.00 ± 0.00
Knee flexors	0.73 ± 0.27	1 ± 0.27	**0.18 ± 0.18** *	**0.27 ± 0.19** *
Ankle dorsiflexsors	0.91 ± 0.61	0.91 ± 0.61	0.91 ± 0.61	0.91 ± 0.61
Total of each side	2.36 ± 1.01	3 ± 1.82	**1.45 ± 0.96** **	**1.82 ± 0.92** **
Total of both sides	5.36 ±1.88		**3.27±1.83** **	

Values are presented in mean ± standard error.
**significant difference compared to pre-training, p-value < 0.05 (one-tailed test)*
***significant difference compared to pre-training, p-value < 0.01 (one-tailed test)*

Table 4.5: Correlations coefficient (r) between changes in resting and exercise HR and changes in contributed factors after training

	Δ Leg EMG	**Δ LF**	**Δ HF**	**Δ LF/ HF**	**Δ RMSSD**	**Δ LVC**	**Δ MTS scores**
Δ Resting HR	- 0.280	**- 0.737** **	**- 0.56** *	0.404	**- 0.6** *	**- 0.624** *	0.12
Δ Exercise HR	**- 0.622** *	0.195	- 0.284	0.095	- 0.149	- 0.211	0.080

EMG: Electromyography; LVC: Lung vital capacity; LF: Low-frequency power; HF: high-frequency power; RMSSD: Root means square of successive difference; MTS: Modified Tardieu Scale.
** significant correlation, p-value < 0.1 (one-tailed test)*
*** significant correlation, p-value < 0.05 (one-tailed test)*

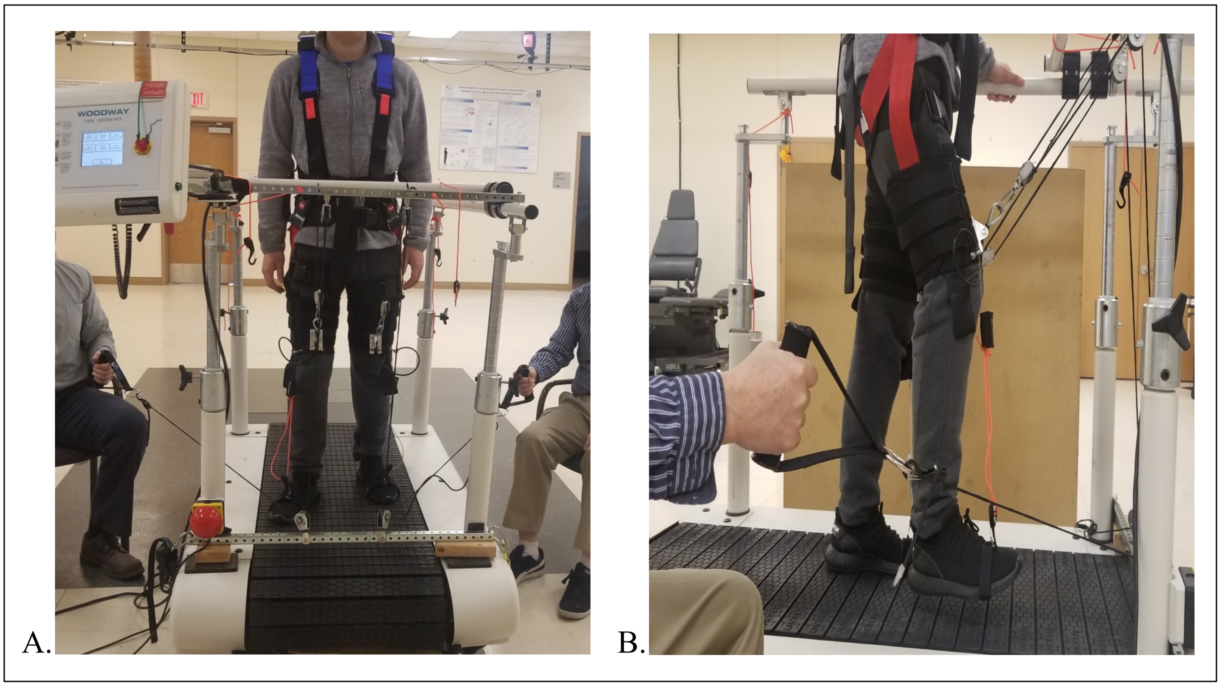


Figure 4.1: The assistive gait training device with two pulling cables operated by two therapists on either side of the treadmill

(A) Two thigh braces which attach to fixed pulleying system in the front of the treadmill via a cable are placed on both right and left thighs. Two physical therapists sit on the right and left side of the treadmill and coordinate to operate the assistive device by pulling the handle attached to the cable. (B) The physical therapists pull the handle attached to the cable to assist a patient with hip flexion and ankle dorsiflexion during the swing phase of the gait cycle

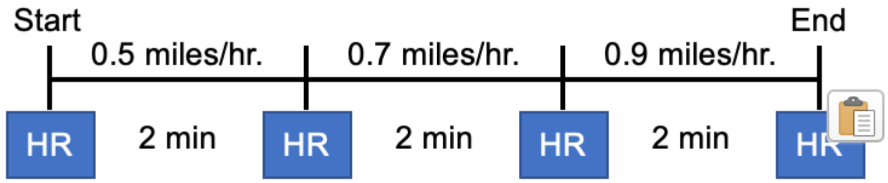
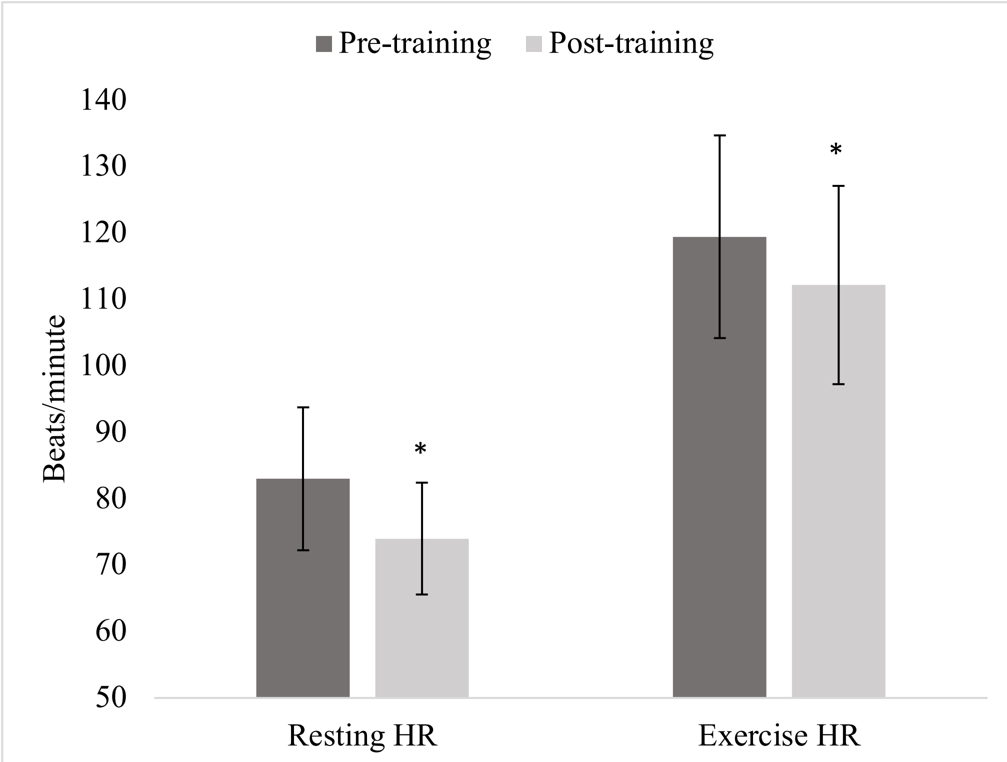
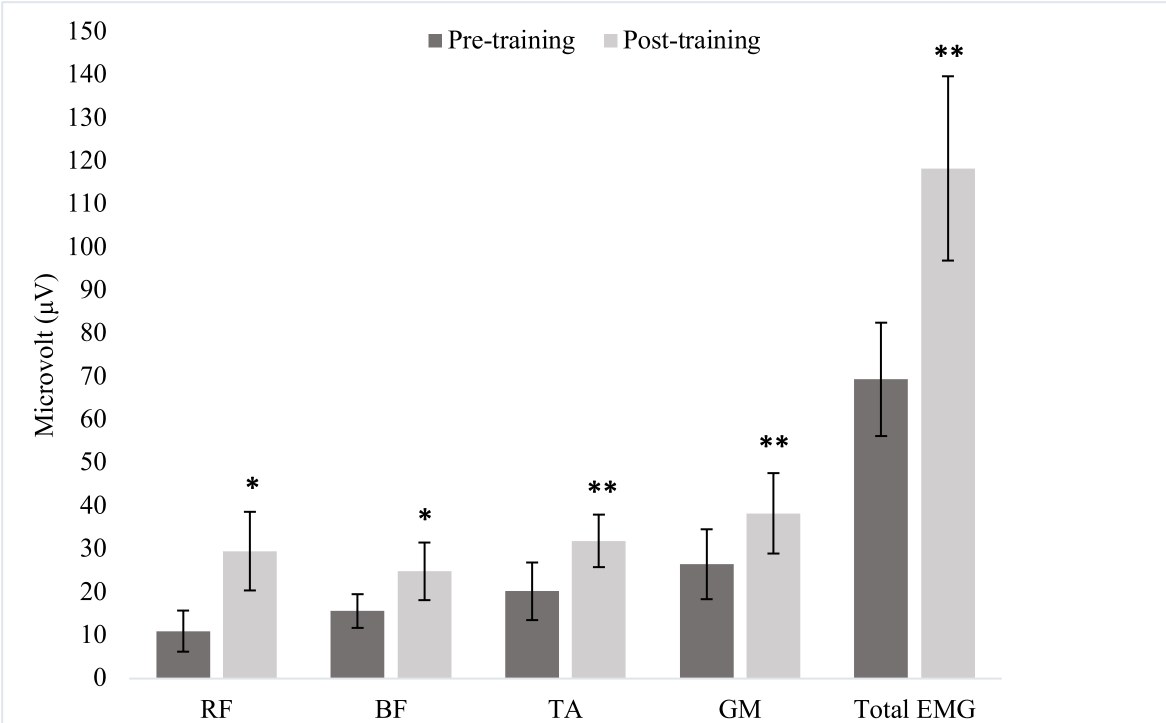


Figure 4.2: Measure of exercise heart rate (HR) response during a treadmill walking



Values are presented in mean ± standard deviation. HR: heart rate
** significant difference compared to pre-training, p-value <0.01 (one-tailed test)*

Figure 4.3: Resting and exercise heart rate (HR) at pre- and post-training (n=11)



Values are presented in mean ± standard error. RF: Rectus femoris; BF: Biceps femoris; TA: Tibialis anterior; GM: gastrocnemius medialis; EMG: Electromyography
**significant difference compared to pre-training, p-value <0.05 (one-tailed test)*
*** significant difference compared to pre-training, p-value <0.01 (one-tailed test)*

Figure 4.4: Average peak root mean square electromyography (EMG) activity of four leg muscles and total EMG activity of four leg muscles pre- and post-training (n=10)

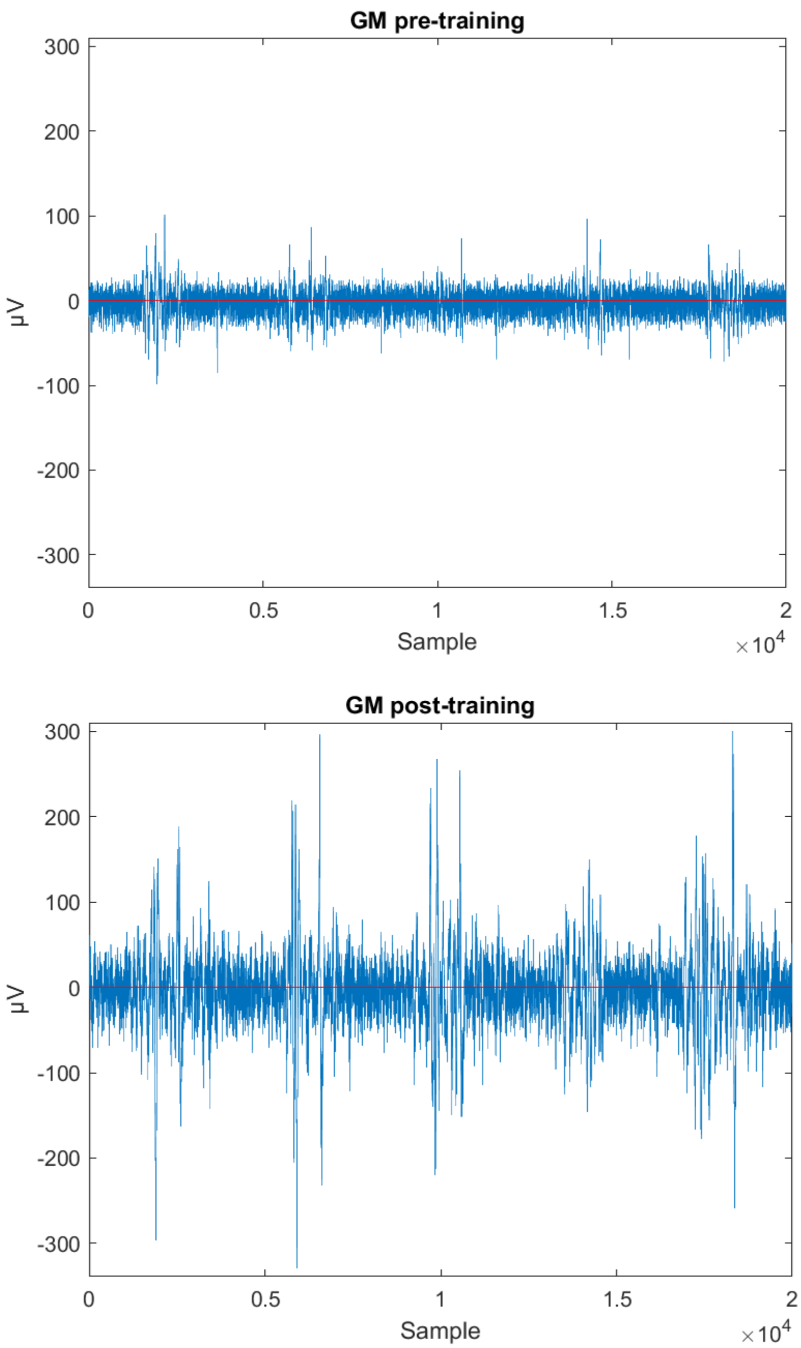
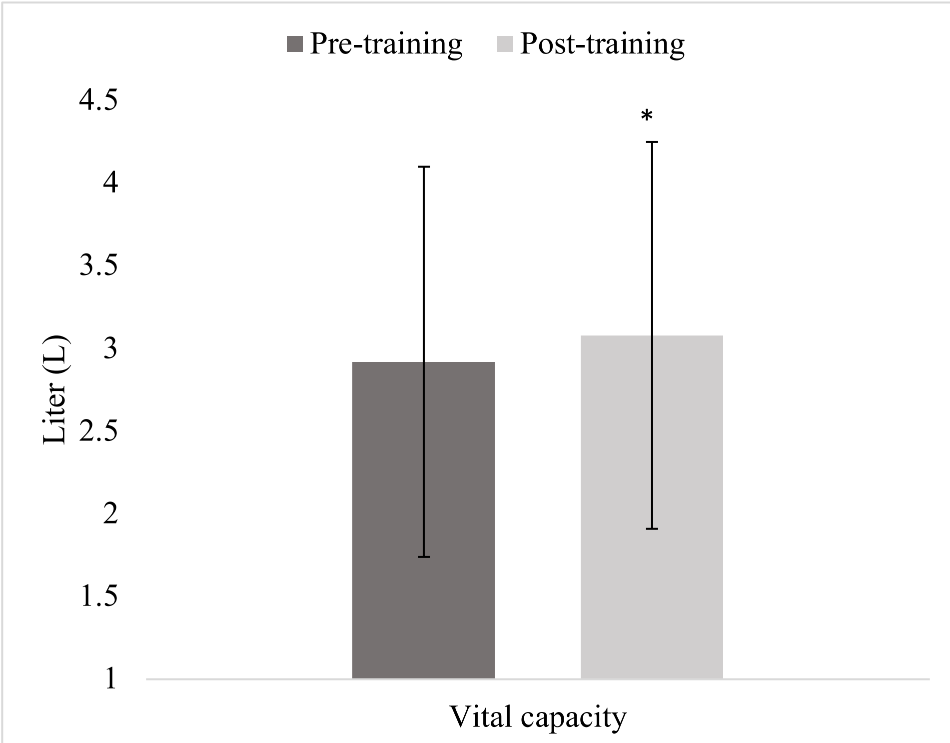


Figure 4.5: The raw electromyography (EMG) signal of right gastrocnemius medialis (GM) over 5 steps in participant with motor complete SCI (ASIA grade B) before (A) and after (B) 8-week of walking training



Values are presented in mean ± standard deviation.
** significant difference compared to pre-training, p-value <0.01 (one-tailed test)*

Figure 4.6: The mean and SD of lung vital capacity pre- and post-training (n=11)

References

1. *Spinal Cord Injury (SCI) 2016 Facts and Figures at a Glance.* J Spinal Cord Med, 2016. **39**(4): p. 493-4.
2. Frankel, H.L., et al., *Long-term survival in spinal cord injury: a fifty year investigation.* Spinal Cord, 1998. **36**(4): p. 266-74.
3. DeVivo, M.J., K.J. Black, and S.L. Stover, *Causes of death during the first 12 years after spinal cord injury.* Arch Phys Med Rehabil, 1993. **74**(3): p. 248-54.
4. Osterthun, R., et al., *Causes of death following spinal cord injury during inpatient rehabilitation and the first five years after discharge. A Dutch cohort study.* Spinal Cord, 2014. **52**(6): p. 483-8.
5. Zhang, D., W. Wang, and F. Li, *Association between resting heart rate and coronary artery disease, stroke, sudden death and noncardiovascular diseases: a meta-analysis.* Cmaj, 2016. **188**(15): p. E384-e392.
6. Wecht, J.M., et al., *A prospective report on the prevalence of heart rate and blood pressure abnormalities in veterans with spinal cord injuries.* J Spinal Cord Med, 2013. **36**(5): p. 454-62.
7. Zhu, C., et al., *A retrospective chart review of heart rate and blood pressure abnormalities in veterans with spinal cord injury.* J Spinal Cord Med, 2013. **36**(5): p. 463-75.
8. Buchholz, A.C., C.F. McGillivray, and P.B. Pencharz, *Physical activity levels are low in free-living adults with chronic paraplegia.* Obes Res, 2003. **11**(4): p. 563-70.
9. van den Berg-Emons, R.J., et al., *A prospective study on physical activity levels after spinal cord injury during inpatient rehabilitation and the year after discharge.* Arch Phys Med Rehabil, 2008. **89**(11): p. 2094-101.
10. Lee, Y.H., et al., *Hemodynamic Adaptations to Regular Exercise in People With Spinal Cord Injury.* Ann Rehabil Med, 2017. **41**(1): p. 25-33.
11. Schmid, A., et al., *Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury.* J Appl Physiol (1985), 1998. **85**(2): p. 635-41.
12. Ditor, D.S., et al., *Effects of body weight-supported treadmill training on heart rate variability and blood pressure variability in individuals with spinal cord injury.* J Appl Physiol (1985), 2005. **98**(4): p. 1519-25.
13. Ditor, D.S., et al., *The effects of body-weight supported treadmill training on cardiovascular regulation in individuals with motor-complete SCI.* Spinal Cord, 2005. **43**(11): p. 664-73.
14. Soyupek, F., et al., *Effects of body weight supported treadmill training on cardiac and pulmonary functions in the patients with incomplete spinal cord injury.* J Back Musculoskelet Rehabil, 2009. **22**(4): p. 213-8.
15. Hoekstra, F., et al., *Effect of robotic gait training on cardiorespiratory system in incomplete spinal cord injury.* J Rehabil Res Dev, 2013. **50**(10): p. 1411-22.

16. Stevens, S.L. and D.W. Morgan, *Heart rate response during underwater treadmill training in adults with incomplete spinal cord injury.* Top Spinal Cord Inj Rehabil, 2015. **21**(1): p. 40-8.

17. Barton, T.J., D.A. Low, and D.H.J. Thijssen, *Cardiovascular Responses to Exercise in Spinal Cord Injury*, in *The Physiology of Exercise in Spinal Cord Injury*, J.A. Taylor, Editor. 2016, Springer US: Boston, MA. p. 105-126.

18. Ditor, D.S., et al., *Reproducibility of heart rate variability and blood pressure variability in individuals with spinal cord injury.* Clin Auton Res, 2005. **15**(6): p. 387-93.

19. Malmqvist, L., et al., *Assessment of autonomic function after acute spinal cord injury using heart rate variability analyses.* Spinal Cord, 2015. **53**(1): p. 54-8.

20. de Carvalho Abreu, E.M., et al., *Cardiovascular autonomic control in paraplegic and quadriplegic.* Clin Auton Res, 2016. **26**(2): p. 117-26.

21. Kyriakides, A., et al., *The effect of level of injury and physical activity on heart rate variability following spinal cord injury.* J Spinal Cord Med, 2019. **42**(2): p. 212-219.

22. Serra-Ano, P., et al., *Heart rate variability in individuals with thoracic spinal cord injury.* Spinal Cord, 2015. **53**(1): p. 59-63.

23. Berlowitz, D.J., B. Wadsworth, and J. Ross, *Respiratory problems and management in people with spinal cord injury.* Breathe (Sheff), 2016. **12**(4): p. 328-340.

24. Fujiura, Y., et al., *Heart rate and mortality in a Japanese general population: an 18-year follow-up study.* J Clin Epidemiol, 2001. **54**(5): p. 495-500.

25. Dhindsa, M.S., et al., *Muscle spasticity associated with reduced whole-leg perfusion in persons with spinal cord injury.* J Spinal Cord Med, 2011. **34**(6): p. 594-9.

26. eHealthMe. *Muscle spasticity and Heart rate increased - from FDA reports*. n.d. [cited 2017 Dec 2]; Available from: https://www.ehealthme.com/cs/muscle-spasticity/heart-rate-increased/.

27. Suzuki, N., et al., *Exercise intensity based on heart rate while walking in spastic cerebral palsy.* Bull Hosp Jt Dis, 2001. **60**(1): p. 18-22.

28. Meyns, P., et al., *Locomotor training with body weight support in SCI: EMG improvement is more optimally expressed at a low testing speed.* Spinal Cord, 2014. **52**(12): p. 887-93.

29. Mazzoleni, S., et al., *Changes on EMG activation in healthy subjects and incomplete SCI patients following a robot-assisted locomotor training.* IEEE Int Conf Rehabil Robot, 2011. **2011**: p. 5975467.

30. Dietz, V., et al., *Locomotor capacity of spinal cord in paraplegic patients.* Ann Neurol, 1995. **37**(5): p. 574-82.

31. Wirz, M., G. Colombo, and V. Dietz, *Long term effects of locomotor training in spinal humans.* J Neurol Neurosurg Psychiatry, 2001. **71**(1): p. 93-6.

32. Tiftik, T., et al., *Does locomotor training improve pulmonary function in patients with spinal cord injury?* Spinal Cord, 2015. **53**(6): p. 467-70.

33. Terson de Paleville, D., et al., *Locomotor step training with body weight support improves respiratory motor function in individuals with chronic spinal cord injury.* Respir Physiol Neurobiol, 2013. **189**(3): p. 491-7.

34. Ghiasi, F., et al., *Change of Spasticity Following Robotic-Assisted Gait Training in Patients with Chronic Incomplete Spinal Cord Injury: Preliminary Results.* American Journal of Neuroprotection and Neuroregeneration, 2012. **4**(1): p. 53-57(5).

35. Stampacchia, G., et al., *Walking with a powered robotic exoskeleton: Subjective experience, spasticity and pain in spinal cord injured persons.* NeuroRehabilitation, 2016. **39**(2): p. 277-83.

36. Mirbagheri, M.M., et al., *The effects of Robotic-Assisted Locomotor training on spasticity and volitional control.* IEEE Int Conf Rehabil Robot, 2011. **2011**: p. 5975443.

37. Liu, W., *A narrative review of gait training after stroke and a proposal for developing a novel gait training device that provides minimal assistance.* Top Stroke Rehabil, 2018. **25**(5): p. 375-383.

38. Riebe, D., et al., *ACSM's guidelines for exercise testing and prescription*. Tenth edition ed. 2018, Philadelphia, PA: Wolters Kluwer. 480.

39. Sporis, G., et al., *How reliable are the equations for predicting maximal heart rate values in military personnel?* Mil Med, 2011. **176**(3): p. 347-51.

40. Hettiarachchi, I.T., et al., *Validation of Polar OH1 optical heart rate sensor for moderate and high intensity physical activities.* PLoS One, 2019. **14**(5): p. e0217288.

41. J. Hermens, H., et al., *European Recommendations for Surface ElectroMyoGraphy: Results of the SENIAM project*. 9 ed. 1999, Enschede, the Netherlands: Roessingh Research and Development.

42. Miller, M.R., et al., *Standardisation of spirometry.* Eur Respir J, 2005. **26**(2): p. 319-38.

43. Akpinar, P., et al., *Reliability of the Modified Ashworth Scale and Modified Tardieu Scale in patients with spinal cord injuries.* Spinal Cord, 2017. **55**(10): p. 944-949.

44. Dobkin, B.H., et al., *Modulation of locomotor-like EMG activity in subjects with complete and incomplete spinal cord injury.* J Neurol Rehabil, 1995. **9**(4): p. 183-90.

45. MacKay-Lyons, M., *Central pattern generation of locomotion: a review of the evidence.* Phys Ther, 2002. **82**(1): p. 69-83.

46. Maegele, M., et al., *Recruitment of spinal motor pools during voluntary movements versus stepping after human spinal cord injury.* J Neurotrauma, 2002. **19**(10): p. 1217-29.

47. Harkema, S.J., et al., *Improvements in orthostatic instability with stand locomotor training in individuals with spinal cord injury.* J Neurotrauma, 2008. **25**(12): p. 1467-75.

48. Fisher, J.P., et al., *Autonomic control of heart rate by metabolically sensitive skeletal muscle afferents in humans.* J Physiol, 2010. **588**(Pt 7): p. 1117-27.

49. Freeman, J.V., et al., *Autonomic nervous system interaction with the cardiovascular system during exercise.* Prog Cardiovasc Dis, 2006. **48**(5): p. 342-62.

50. Burdet, E., et al., *The central nervous system stabilizes unstable dynamics by learning optimal impedance.* Nature, 2001. **414**(6862): p. 446-9.

51. Osu, R., et al., *Short- and long-term changes in joint co-contraction associated with motor learning as revealed from surface EMG.* J Neurophysiol, 2002. **88**(2): p. 991-1004.

52. Chapman, A.R., et al., *Leg muscle recruitment during cycling is less developed in triathletes than cyclists despite matched cycling training loads.* Exp Brain Res, 2007. **181**(3): p. 503-18.

53. Chapman, A.R., et al., *Is running less skilled in triathletes than runners matched for running training history?* Med Sci Sports Exerc, 2008. **40**(3): p. 557-65.

54. Tao, L., et al., *Exercise for the heart: signaling pathways.* Oncotarget, 2015. **6**(25): p. 20773-84.

55. Bahrainy, S., et al., *Exercise training bradycardia is largely explained by reduced intrinsic heart rate.* Int J Cardiol, 2016. **222**: p. 213-216.

56. Reimers, A.K., G. Knapp, and C.D. Reimers, *Effects of Exercise on the Resting Heart Rate: A Systematic Review and Meta-Analysis of Interventional Studies.* J Clin Med, 2018. **7**(12).

57. Teasell, R.W., et al., *Cardiovascular consequences of loss of supraspinal control of the sympathetic nervous system after spinal cord injury.* Arch Phys Med Rehabil, 2000. **81**(4): p. 506-16.

58. Bravo, G., et al., *Cardiovascular alterations after spinal cord injury: an overview.* Curr Med Chem Cardiovasc Hematol Agents, 2004. **2**(2): p. 133-48.

59. Fisher, J.P., et al., *Muscle metaboreflex and autonomic regulation of heart rate in humans.* J Physiol, 2013. **591**(15): p. 3777-88.

60. Evans, D.L., *Cardiovascular adaptations to exercise and training.* Vet Clin North Am Equine Pract, 1985. **1**(3): p. 513-31.

61. Hellsten, Y. and M. Nyberg, *Cardiovascular Adaptations to Exercise Training.* Compr Physiol, 2015. **6**(1): p. 1-32.

62. Vega, R.B., et al., *Molecular Mechanisms Underlying Cardiac Adaptation to Exercise.* Cell Metab, 2017. **25**(5): p. 1012-1026.

63. Fulghum, K. and B.G. Hill, *Metabolic Mechanisms of Exercise-Induced Cardiac Remodeling.* Front Cardiovasc Med, 2018. **5**: p. 127.

64. Biering-Sorensen, F., et al., *Alterations in cardiac autonomic control in spinal cord injury.* Auton Neurosci, 2018. **209**: p. 4-18.

65. Buker, D.B., C.C. Oyarce, and R.S. Plaza, *Effects of Spinal Cord Injury in Heart Rate Variability After Acute and Chronic Exercise: A Systematic Review.* Top Spinal Cord Inj Rehabil, 2018. **24**(2): p. 167-176.

66. Sztajzel, J., *Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system.* Swiss Med Wkly, 2004. **134**(35-36): p. 514-22.

67. Shaffer, F. and J.P. Ginsberg, *An Overview of Heart Rate Variability Metrics and Norms.* Front Public Health, 2017. **5**: p. 258.

68. *Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.* Eur Heart J, 1996. **17**(3): p. 354-81.

69. Hopf, H.B., et al., *Low-frequency spectral power of heart rate variability is not a specific marker of cardiac sympathetic modulation.* Anesthesiology, 1995. **82**(3): p. 609-19.

70. Goldstein, D.S., et al., *Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes.* Exp Physiol, 2011. **96**(12): p. 1255-61.

71. Rahman, F., et al., *Low frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation.* Clin Auton Res, 2011. **21**(3): p. 133-41.

72. Jensen, M.T., et al., *Resting heart rate is a predictor of mortality in COPD.* Eur Respir J, 2013. **42**(2): p. 341-9.

73. Lanza Fde, C., et al., *Chest wall mobility is related to respiratory muscle strength and lung volumes in healthy subjects.* Respir Care, 2013. **58**(12): p. 2107-12.

74. Lampe, R., et al., *Lung vital capacity and oxygen saturation in adults with cerebral palsy.* Patient Prefer Adherence, 2014. **8**: p. 1691-7.

75. Gasior, J.S., et al., *Heart Rate and Respiratory Rate Influence on Heart Rate Variability Repeatability: Effects of the Correction for the Prevailing Heart Rate.* Front Physiol, 2016. **7**: p. 356.

76. Song, H.S. and P.M. Lehrer, *The effects of specific respiratory rates on heart rate and heart rate variability.* Appl Psychophysiol Biofeedback, 2003. **28**(1): p. 13-23.

77. Li, C., et al., *Effects of slow breathing rate on heart rate variability and arterial baroreflex sensitivity in essential hypertension.* Medicine (Baltimore), 2018. **97**(18): p. e0639.

Chapter 5: The Effects of Walking Training on Cardiovascular Risk Markers in People with Chronic Spinal Cord Injury

Abstract

Background: Due to loss or impairment of motor function, individuals with spinal cord injury (SCI) are usually inactive. Reduced physical activity after SCI plays a major role in increased risk factors of cardiovascular disease (CVD) such as diabetes, dyslipidemia, and elevated levels of inflammatory markers which consequently increases the risk of CVD. Although past studies have focused on examining the effects of walking training on motor function, promising results showed regular walking training helped to improve cardiovascular health in SC survivors. However, limited studies have examined the effect of walking training on cardiovascular risk markers among people with SCI.

Purpose: This study aimed to investigate the effects of an 8-week walking training program on glycemic control, lipid profile, and pro-inflammatory markers in individuals with chronic SCI.

Participants: A total of 11 individuals (age 38.09±10.31 years, 8 males, 3 females, 3 motor incomplete, 8 motor complete, injury level T2-L1, injury duration 8.72±10.40 years) with chronic SCI completed training sessions and pre-training and post-training assessments.

Methods: All participants went through a walking training program using a treadmill, a body weight-supported system, and an assistive gait training device for 3 sessions per week for 8 weeks, a total of 24 training sessions. We assessed concentrations of hemoglobin A1c (HbA1c), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), C-reactive protein (CRP), and interleukin-6 (IL-6) before and after training.

Results: Following training, there was a significant decrease in HbA1c levels from 5.83 to 5.45% ($p < 0.01$). Lipid profile improved after training, as shown by a significant increase in HDL-C concentrations (pre: 47.49±19.27 mg/dl vs. post: 64.51±14.81 mg/dl; $p < 0.01$) and a significant decrease in LDL-C concentrations (pre: 81.35±8.61 mg/dl vs. post: 76.84±13.14 mg/dl; $p < 0.1$). The ratio of LDL-C to HDL-C was significantly reduced by 0.93 units ($p < 0.01$). In regard to pro-inflammatory markers, concentrations of IL-6 showed a significant reduction from 1.71±0.76 to 1.50±0.70 pg/ml after training ($p = 0.05$), while concentrations of CPR showed a trend to decrease from 5.51±3.83 to 4.71±3.59 mg/l ($p = 0.13$).

Conclusion: The findings of this pilot study suggest that the 8-week walking training program produced positive changes in cardiovascular risk markers in individuals with chronic SCI by reducing levels of Hb1Ac, LDL-C, IL-6 as well as increasing levels of HDL-C. A future randomized controlled trial is needed to fully examine the effects of walking training on those outcome measures.

Keywords: *Spinal cord injury; lipid profile; glycemic control; inflammatory markers; walking training*

5.1. Introduction

Cardiovascular disease (CVD) is the primary cause of mortality among people living with spinal cord injury (SCI) [1, 2]. Previous studies reported a high prevalence of CVD and death from CVD among the SCI population compared to the able-bodied population [2-4]. It has been estimated that the prevalence rate of CVD is significantly higher in people with SCI (30-50%) than able-bodied people (5-10%) [3]. Another study showed that individuals with SCI have a 3-fold increased risk of heart disease and a 4-fold increased risk of stroke compared to those without SCI [4]. In terms of death from CVD, a cohort study that followed individuals with SCI for five years after discharge from inpatient rehabilitation found that 37% of individuals died due to cardiovascular causes [2].

Physical inactivity is one of the major factors for increased cardiovascular risk factors in the general population and consequently increases the risk of CVD [5-7]. Due to loss or limitation of motor function after injury, people with SCI are usually physically inactive [3], and around 50% of them do not meet recommended levels of physical activity for people with disabilities [8, 9]. Previous studies reported high rates of dyslipidemia, abnormal glycemic control, and elevated levels of pro-inflammatory markers in people with SCI [10, 11]. A study showed that individuals with chronic SCI had higher levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and lower levels of high-density lipoprotein cholesterol (HDL-C) compared to age-matched able-bodied individuals [10]. A meta-analysis found lower levels of HDL-C and a higher ratio of TC/HDL-C in individuals with SCI as compared to able-bodied individuals [11]. The rate of type 2 diabetes has been estimated to be 2 to 3 times higher in individuals with SCI than in able-bodied individuals, with a prevalence of 13-22% in individuals with SCI compared to 6% in able-bodied individuals [12, 13]. In regard to pro-inflammatory markers, studies also showed higher concentrations of C-reactive protein (CRP) and interleukin 6 (IL-6) in individuals with chronic SCI in comparison with age-matched able-bodied individuals [14-16]. Studies in individuals with chronic SCI reported a mean CRP level of 3 to 3.37 mg/l, which falls into the high-risk category of CVD [14, 17]. Chronic low-grade inflammation, defined as CRP concentrations within 3-10 mg/l [18], is associated with an increased risk of CVD [19-21]. Alterations in body composition as well as lack of physical activity after injury could lead to an increase in the risk factors of CVD. Thus, high rates of CVD risk factors among SCI survivors may contribute to the increased development of CVD.

Participating in regular exercise is necessary to prevent or reduce risk factors of CVD after SCI. For cardiometabolic health benefits, a recent guideline suggested that adults with SCI should engage in 30 minutes of moderate to vigorous intensity aerobic exercises 3 times per week [22]. Exercise using arm cycling or functional electrical stimulation (FES)-leg cycling has been utilized to improve cardiovascular health in the SCI population. Systematic review studies reported that exercise using these exercise modalities showed inconsistent and inconclusive results on carbohydrate and lipid metabolism in SCI survivors [23, 24]. In addition, several limitations have been reported in the literature regarding the use of those exercise modalities. Arm cycling targets only upper limb muscles, which does not provide sufficient challenge to the cardiovascular system [25, 26]. FES-leg cycling is limited as patients often report muscle fatigue due to frequent stimulation [27-29] and might aggravate the symptoms of autonomic dysreflexia, particularly in those with SCI above T6 [30]. Both arm cycling and FES-leg cycling exercises are performed in a sitting position, which may not provide sufficient stress to the cardiovascular system.

As an alternative, upright walking training, using body weight-supported treadmill training (BWSTT), is widely used as a rehabilitation intervention for people with SCI. Although most studies have focused on examining the effects of walking training on measurements of motor function, some preliminary results showed that BWSTT helped to improve cardiovascular function and reduced risk factors of CVD in individuals with incomplete or complete SCI [25, 27, 31-34]. Compared to exercise in a sitting position, upright walking training can trigger the activity of larger muscles in the body, including leg and trunk muscles, through activation of center pattern generators located within the spinal cord [35-37] and provide a greater challenge to the cardiovascular system which can induce significant adaptive changes in the central and peripheral cardiovascular system [38, 39]. In regard to changes in risk markers of CVD, a study reported that a course of BWSTT, in individuals with motor incomplete SCI, significantly reduced concentrations of glucose and insulin [33]. Similarly, the same training load significantly decreased levels of TC and LDL-C, and TC/HDL-C ratio and significantly increased levels of HDL-C in individuals with motor incomplete SCI [34]. However, these studies had some limitations, including small sample size and inclusion of only motor incomplete SCI. Therefore, the generalization of the findings is limited to individuals with motor incomplete SCI. Furthermore, two recent studies found that individuals with chronic SCI who were wheelchair users had a higher level of pro-inflammatory markers (i.e., CRP) than those who could walk with assistive devices or independently [40, 41]. To the best of our knowledge, limited studies have examined the effects of walking training on glycemic control, lipid profile and levels of pro-inflammatory markers in individuals with SCI, particularly in those with motor complete SCI.

Therefore, the purpose of this study was to investigate the effects of an 8-week walking training program on glycemic control, lipid profile, and the levels of pro-inflammatory markers in individuals with chronic SCI, including those with motor complete SCI. We hypothesized that the 8-week walking training program would 1) decrease the levels of glycated hemoglobin (HbA1c) and LDL-C, 2) increase the levels of HDL-C, and 3) reduce the levels of CRP and IL-6.

5.2. Methods

5.2.1. Participants

Participants were primarily recruited from the Spine Center Clinical at the University of Kansas Hospital. In addition, the contact information of patients with SCI who were admitted to the University of Kansas Hospital and agreed to be contacted for research studies was obtained through the Healthcare Enterprise Repository for the Ontological Narration database at the University of Kansas Medical Center. Study flyers were also distributed in outpatient rehabilitation centers and SCI support groups in the Kansas City area. A member of the study teams conducted potential participants to determine their eligibility to participate in the study. Inclusion criteria for participants were as follows: 1) age between 18 and 60 years; 2) had paraplegia (T1-L2); 3) onset of SCI at least one year prior to starting the study; 4) scored less than 5 in Functional Independence Measure, Locomotion: Walk; 5) not participating in any other similar gait training activities; and 6) had medical approval from their physician to participate in walking training. Participants were excluded from participating in the study if they had one of following conditions: 1) major CVD such as heart disease; 2) any other neurological diseases such as stroke, multiple sclerosis or Parkinson's disease, etc.; 3) severe orthopedic issues such as joint stiffness and fractures; 4) muscle spasticity greater than 3 according to Ashworth scale; 5) severe osteoporosis, 5) current inflammatory diseases or infections; 6) recent open wound or pressure ulcer; 7) pregnant women; 8) cognitive or psychiatric disorders; or 9) uncontrolled autonomic dysreflexia. Eligible participants were given written, informed consent prior to participating in the study. This study was approved by the institutional review board of the University of Kansas Medical Center, Kansas City. The participant's level, severity, and classification of injury were obtained through his/her medical records.

5.2.2. Study Design

This study was a pilot, single group, pretest-posttest study design. All participants went through an 8-week walking training using a body weight support system, a treadmill, and an assistive gait device. Outcome measures listed below were assessed at baseline (pre-training) and after completing the 8weeks of training (post-training).

5.2.3. Walking Training Protocol

Participants completed 3 sessions a week of BWSTT implemented with an assistive gait training device for 8 weeks, a total of 24 sessions. The duration of each training session lasted 30 minutes (2 bouts of 15 minutes). During walking training, the participant wore a harness that was attached to an overhead motorized lift to provide body weight support (BWS) and to prevent the risk of falling. At the initial sessions, 60-40% of the participant's body weight was supported based on his/her tolerance. Later, the BWS was decreased by 5% at each week as tolerated by the participant without knee buckling. However, if the participant was unable to tolerate new BWS or showed knee buckling or toes dragging, the BWS was increased by 5%. The amount of BWS was determined using a digital scale attached to the overhead motorized lift. This training protocol has been used and described in past studies [42-44]. A novel assistive gait training device developed in our laboratory was utilized to assist the participant with forward leg stepping during walking on the treadmill [45]. Two thigh braces were placed onto the right and left thighs of the participant and secured using straps. These braces were attached to a pulley system via a pulling cable (**Figure 5.1**). Two therapists coordinately operated the assistive gait training device by pulling the cable to assist the participant with hip flexion and ankle dorsiflexion during the swing phase of a gait cycle. The participant was encouraged to actively move his/her legs throughout training. At the beginning of training, the initial treadmill speed was set at 0.5 miles/hour. The participant walked at 0.5 miles/hour for 2 minutes to warm up. Subsequently, the treadmill speed was gradually increased until participant's heart rate (HR) reached 40-59% of his/her HR reserve as recommended by the American College of Sports Medicine guidelines [46]. The target zone of HR reserve was determined using the Karvonen formula = (maximum HR – resting HR) X target intensity % + resting HR. Maximum HR was determined using the age-predicted maximal HR (220 – participant's age) [47]. In addition, the participant rated his/her level of exertion in the middle of training and by the end of training using the Borg Rating of Perceived Exertion Scale (RPE) 6-20. The participant was allowed to take 3 to 5 minutes rest after 15±5 minutes of training. The participant was able to request rest at any time when needed. At the end of the training, participants walked at slow speed (0.5 miles/hour) for 2 minutes to cool down. To ensure the safety during training, we monitored participant's HR throughout the training using a polar OH1 optical HR sensor (Polar Electro Inc., New York) and recorded blood pressure before the training, between each training bout, and at the end of the training session. Participant's HR was monitored to ensure that it did not exceed 85% of the age-predicated maximum HR. Participant's systolic and diastolic blood pressure was monitored to ensure that it was maintained below 220 mm Hg and 110 mm Hg, respectively. A walking training log for each participant was used to record the duration of a training session, treadmill speed, percentage of BWS, HR, blood pressure, and PRE during each training session.

5.2.4. Glycated Hemoglobin A1c (HbA1c)

According to the American Diabetes Association and World Health Organization recommendation, HbA1c has been used as a tool to diagnose diabetes because it reflects the average glucose levels over the life of the red blood cell (around 1 to 3 months) [48, 49]. We collected a small drop of blood (5 microliters) from the middle finger of the right hand and immediately analyzed it using an A1CNow+ System kit (Test Medical Symptoms at Home, Inc., Maria Stein, OH, USA). Studies showed that the A1CNow+ System test is strongly correlated with reference methods using a venous blood sample test [50].

5.2.5. Lipid Profile and Pro-inflammatory Markers

Blood samples were collected in the morning (between 8-11 am) from each participant after 12-hour overnight fasting (i.e., no eating, drinking except water, and smoking for at least 12 hours before blood drawing). Participants were also instructed to restrain from physical activity for at least 12 hours before the blood draw. Also, participants were asked not to take any medication 9 hours before measurements unless it is necessary. About 20 ml of blood samples was collected from an antecubital vein into a tube. Blood samples were allowed to clot for 30 minutes and then centrifuged at 1,800 g for 10 min at room temperature. Subsequently, serum was separated from the blood cells, and then serum specimens were transferred to plastic tubes which were stored in a freezer at −80°C until analysis. We assessed serum concentrations of LDL-C and HDL-C using an LDL-C Assay kit (Crystal Chem, USA, catalog no 80069) and HDL-C Assay kit (Crystal Chem, USA, catalog no. 80059), respectively. For pro-inflammatory markers, serum concentrations of CRP were assessed using a high sensitivity *enzyme*-linked immunosorbent assay kit (Crystal Chem, USA, catalog no. 80955). To determine serum concentrations of IL-6, the analysis was performed at Eve Technologies using the Bio-Plex™ 200 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and a Milliplex Human High Sensitivity T-Cell panel (Millipore, St. Charles, MO, USA) according to their protocol [51].

5.2.6. Statistical Analysis

All statistical analyses were conducted using SPSS version 25 (IBM Corporation; Armonk, New York). The Wilcoxon signed-rank test (one-tailed test) was conducted to examine differences in outcome variables between pre- and post-training because the sample size of the study was small, and data of independent variables was not normally distributed. With the exploratory purpose, we used the Mann-Whitney U test to examine differences in changes in outcome variable after training between two subgroups based on level of injury (upper paraplegia vs. lower paraplegia) and its severity (motor incomplete vs. motor complete). In all cases, a *p*-value of less than 0.1 was considered significant due to the small sample size of this pilot study

5.3. Results

5.3.1. Participants

A total of 15 participants enrolled in the study. Out of those, 11 participants (age 38.09±10.31 years, 8 males, 3 females, 3 motor incomplete, 8 motor complete, injury level T2-L1, injury duration 8.72±10.40 years) completed the training sessions and pre-training and post-training assessments. All participants utilized a wheelchair as a primary mode of mobility. The participants' demographics and clinical characteristics are presented in **Table 5.1**. Three participants dropped from the study. Of these, one participant dropped out after 4 weeks of training because of an adverse event not related to the study. The other two participants dropped after 3 weeks of training due to demands of work or family schedule. One participant was excluded in the first week of training because he had a colostomy tube which interfered with the harness.

5.3.2. Changes in The Levels of Cardiovascular Risk Markers

Table 5.2 shows the levels of HbA1c, HDL-C, LDL-C, CRP, and IL-6 based on individual data and group mean (SD) before and after training. The overall mean levels of HbA1c, HDL-C, LDL-C, CRP, and IL-6 before and after training are summarized in **Table 5.3**. After the training, HbA1c significantly decreased from 5.83 to 5.45% ($p < 0.01$). The levels of HDL-C significantly increased from 47.49 mg/dl to 64.51 mg/dl ($p < 0.01$) while the levels of LDL-C significantly decreased from 81.35 mg/dl to 76.84 mg/dl ($p < 0.1$). The ratio of LDL-C to HDL-C was significantly decreased from 2.16 to 1.23 after training ($p < 0.01$). In regard to changes in levels of pro-inflammatory markers following the training, there were significant reductions in the levels of IL-6 (pre: 1.71 pg/ml vs. post: 1.50 pg/ml; p =0.05), but the levels of CRP showed a trend toward a significant reduction (pre: 5.51 mg/l vs. 4.71 mg/l; p = 0.12).

Based on the injury level sub-analysis, there were no significant differences in changes in the levels of cardiovascular risk markers after training between participants with upper paraplegia (T1-T5) and those with lower paraplegia (T6-L2), except for levels of LDL-C. The change in levels of LDL-C was significantly greater in participants with upper paraplegia than those with lower paraplegia (p <0.04). In respect to injury severity sub-analysis, no significant differences were observed in changes in levels of cardiovascular risk markers after training between participants with motor incomplete SCI (grade C and D) and those with motor complete (grade A and B).

5.4. Discussion

The findings of this study demonstrated that a walking training program, 3 times per week for 8 weeks, significantly decreased levels of HbA1c, LDL-C, and IL-6 and significantly increased levels of HDL-C in wheelchair-dependent individuals with chronic motor incomplete or motor complete SCI. These improvements in glycemic control and lipid profile agree well with previous walking training studies involving persons with motor incomplete SCI [33, 34]. In addition, the findings of the study showed that both participants with upper paraplegia and those with lower paraplegia experienced similar improvements in the risk markers of CVD after training. Similar improvements were observed in participants with incomplete SCI and those with complete SCI. No significant differences in changes in outcome measures after training were observed between two groups after training. These findings suggest that regular walking training could induce cardiovascular benefits in individuals with either upper paraplegia or lower paraplegia and those with either incomplete or complete SCI.

In this study, we observed a significant decrease in concentrations of blood glucose as determined by the HbA1c test, which presents the average levels of blood glucose for the past 1-3 months [48]. Levels of HbA1c decreased by 6.67% after training. Our finding is in accordance with a study by Philips et al. [33] who reported a significant decrease in concentrations of blood glucose, as tested by oral glucose tolerance test, in individuals with motor incomplete SCI following walking training. Past studies also showed a course of walking training significantly increased leg lean mass and the activity of leg muscles in individuals with motor incomplete or motor complete SCI [52-55]. The increased lean mass and activity of muscles after walking training might improve glucose utilization by muscle tissues and reduce the risk of type 2 diabetes. Although underlying mechanisms involved in the decreased levels of Hb1Ac as a result of walking training were not examined in the current study, several possible explanations have been proposed. Past evidence has suggested that increases in glucose storage capacity, glucose utilization by muscle tissues (including increases in levels of glucose phosphorylating and oxidizing enzyme, glucose transporter proteins (GLUT-4)), leg lean mass, and the activity of leg muscles as well as the shifts of muscle fibers toward more oxidative muscle fiber type (type I and IIa) after walking training in individuals with SCI might be involved in the regulation of blood glucose [56]. Similar mechanisms might be responsible for the decreased levels of HbA1c observed in the current study.

Abnormal lipid profile is observed in a large number of individuals with chronic SCI, mainly as a consequence of a sedentary lifestyle after injury [57-59]. In particular, the low level of HDL-C was found to be more common among SCI survivors [11]. Evidence demonstrated that physically active individuals with SCI had favorable lipid profiles compared to those physically inactive [14, 60-62], indicating the importance of the regular physical activity. In the current study, we found an increase in levels of HDL-C by 35% and a decrease in levels of LDL-C by 7% as a result of the 8 weeks of walking training. The ratio of LDL-C to HDL-C reduced by 0.93, which is associated with a decrease in the risk of CVD [63]. Similar to our findings, Stewart et al. [34] reported an increase in levels of HDL-C by 11% and a reduction in levels of LDL-C by 13% in persons with motor incomplete SCI (ASIA grade C) following 6-month of walking training. The results of the current study and the study by Stewart et al. indicate that regular walking training can be an effective stimulus in improving lipid profile in individuals with either motor incomplete or motor complete SCI. The underlying mechanisms of improvement in lipid profile in individuals with SCI after exercise are still unknown. It might be related to increased cholesterol transport, vascular wall lipoprotein lipase activity, and cholesterol metabolism in the liver as a result of training [64].

Chronic low-grade inflammation is associated with an increase in the risk of CVD.[19-21] Elevated levels of inflammatory markers are common in individuals with chronic SCI due to a sedentary lifestyle and changes in body composition (i.e., increased fat mass and decreased lean mass) after injury [14, 15, 17]. Higher levels of CRP and IL6 were observed in physically inactive individuals with SCI compared to those who were physically active [65]. Around 50% of the participants in the current study showed, at baseline assessment, elevated concentrations of resting inflammatory markers (CRP > 3 mg/l and IL-6 > 2 pg/ml). The walking training significantly decreased the levels of IL-6 and showed a trend to reduce levels of CRP in the study participants as the result of the walking training. The current study is the first to report an improvement in levels of pro-inflammatory markers in individuals with SCI following a walking training program. Our findings are in agreement with past studies that reported improvements in the levels of pro-inflammatory markers following a course of exercise in people with SCI. Griffin et al.[66] reported that following a 10-week FES leg cycling exercise, in individuals with motor incomplete or motor complete SCI, the levels of CRP and IL-6 decreased by 18% and 22%, respectively. Bakkum et al. [67] showed that a 16-week arm cycling or hybrid cycling exercise, in individuals with motor incomplete or motor complete SCI, reduced the levels of CRP and IL-6 by about 16% and 25%, respectively. In addition, past studies showed that individuals with SCI who were able to walk independently or with assistive devices had lower CRP levels compared to those who were a wheelchair user [40]. The findings of the present study indicated that walking training in wheelchair-dependent participants with SCI might reduce the levels of pro-inflammatory markers. Furthermore, increased deposits of adipose tissues after SCI contribute to increased secretions of pro-inflammatory cytokines such as IL-6, resulting in inducing productions of CRP in the liver [41, 68]. In the current study, we observed a significant positive correlation (r = 0.604, $p < 0.05$) between the change in the levels of IL-6 and the change in the levels CRP after the walking training, which may imply that the decreased levels of IL-6 lead to reduce the levels CRP. However, the underlying mechanisms of changes in concentrations of pro-inflammatory markers after exercise in people with SCI are still unclear and warranted further investigations.

The current study had some limitations need to be considered. First, the small sample size and absence of a control group may limit the generalization of the results [33, 34]. As the result, the findings of the study are inconclusive and need to be interpreted with caution. The current study should be considered a pilot study. Second, we included only individuals with paraplegia (T1-L2). Thus, the findings of this study might not be generalized to the SCI population, particularly those with a cervical SCI. Third, a lack of follow-up assessment was another limitation in the study. We did not include determining how long these positive changes induced by walking training would be maintained. Finally, we did not have control over covariates, such as physical activity, diet, medications, and health conditions (e.g., urinary tract or respiratory infections), that might have influenced the levels of blood markers. However, participants filled out daily activity log during the study period and reported no changes in their physical activity. Also, participants reported no changes in their medication lists, diet habits, and health conditions throughout the study periods.

5.5. Conclusion

The findings of this study suggested that an 8-week walking training program might improve cardiovascular risk markers in individuals with chronic SCI, as shown by decreases in the levels of Hb1Ac, LDL-C, and IL-6 and an increase in the levels of HDL-C. These improvements in cardiovascular risk markers observed after walking training are important in reducing the risk of CVD. A future randomized clinical trial is needed to determine which exercise modalities (upper extremity vs. walking exercise) will be more effective in changing the risk markers of CVD in SCI survivors.

Table 5.1: The demographic and clinical characteristics of participants who completed the study (n=11)

Participant ID	Gender, (Male/Female)	Age, (yrs) Mean±SD	Body weight (kg) Mean±SD		Height (cm) Mean±SD	Injury level	ASIA grade	Injury duration (yrs.) Mean±SD	Medications (Yes/No)		
			Pre-training	Post-training					Cholesterol	Diabetes	Anti-inflammatory
SCI 02	Male	40	71	68	173	T9	A	7	No	No	No
SCI 03	Male	47	130	131	196	T8	B	3	Yes	Yes	Yes
SCI 04	Male	37	89	89	189	T3	B	1	No	No	No
SCI 06	Male	40	118	120	189	T5	B	19	No	No	No
SCI 07	Male	19	117	117	206	T3	B	2	No	No	No
SCI 08	Male	48	78	78	198	T9	B	3	No	No	Yes
SCI 09	Male	35	108	107	155	T6	C	35	No	No	No
SCI 10	Female	32	107	105	178	L1	C	14	No	No	No
SCI 11	Female	25	58	58	168	T7	B	7	No	No	Yes
SCI 13	Male	41	71	71	180	T5	A	4	Yes	Yes	No
SCI 15	Female	55	122	125	178	T2	D	1	No	No	No
	8/3	38.09±10.31	97.18±24.64	97.18±25.48	182.73±14.70			8.72±10.40	2/9	2/9	3/8

ASIA: American Spinal Injury Association

Table 5.2: The individual data of levels of cardiovascular risk markers before (pre) and after (post) completing walking training (n=11)

Participant ID	**HbA1c** (%)		**HDL-C** (mg/dl)		**LDL-C** (mg/dl)		**LDL/HDL**		**CRP** (mg/l)		**IL-6** (pg/ml)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
SCI 02	5.6	4.9	64.75	70.48	80.86	83.71	1.25	1.19	0.86	0.41	0.93	1.17
SCI 03	6.5	5.8	60.35	64.31	74.22	75.16	1.23	1.17	8.95	7.28	2.72	2.16
SCI 04	5.6	5.3	36.56	68.72	70.58	72.55	1.93	1.06	6.29	9.72	1.60	1.76
SCI 06	5.7	5.5	21.58	49.33	80.35	72.83	3.72	1.48	7.4	1.87	2.45	1.31
SCI 07	4.5	4.5	40.08	39.64	73.44	45.69	1.83	1.15	1.63	1.03	2.50	2.42
SCI 08	6.7	6.4	51.09	69.16	73.55	72.61	1.44	1.05	4.05	3.18	0.81	0.74
SCI 09	5.7	5.3	33.47	72.24	87.51	85.52	2.61	1.18	9.72	9.72	1.83	1.64
SCI 10	5.9	5.5	53.74	72.68	87.76	85.80	1.63	1.18	1.63	1.31	2.00	1.76
SCI 11	5.2	4.9	74.01	83.25	91.34	96.17	1.23	1.16	9.72	6.88	2.41	2.50
SCI 13	7.1	6.7	70.04	79.29	77.84	69.72	1.11	0.88	0.63	2.69	1.04	0.71
SCI 15	5.7	5.2	16.73	40.52	97.44	85.51	5.82	2.11	9.72	7.78	0.61	0.42

HbA1c: Hemoglobin A1c; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; CRP: C-reactive protein; IL-6: Interleukin

Table 5.3: The overall mean levels of cardiovascular risk markers before and after walking training (n=11)

	Pre-training	Post-training	Change (Post-Pre)	*p*-value
HbA1c (%)	5.83±0.73	5.45±0.64	- 0.38±0.2	0.002
HDL-C (mg/dl)	47.49±19.27	64.51±14.81	17.02±12.61	0.002
LDL-C (mg/dl)	81.35±8.61	76.84±13.14	- 4.51±9.25	0.09
LDL/HDL	2.16±1.43	1.23±0.32	0.93±1.14	0.001
CRP (mg/l)	5.51±3.83	4.71±3.59	- 0.8±2.36	0.13
IL-6 (pg/ml)	1.71±0.76	1.50±0.70	- 0.21±0.38	0.05

Values are presented in mean ± standard deviation.
HbA1c: Hemoglobin A1c; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; CRP: C-reactive protein; IL-6: Interleukin-6

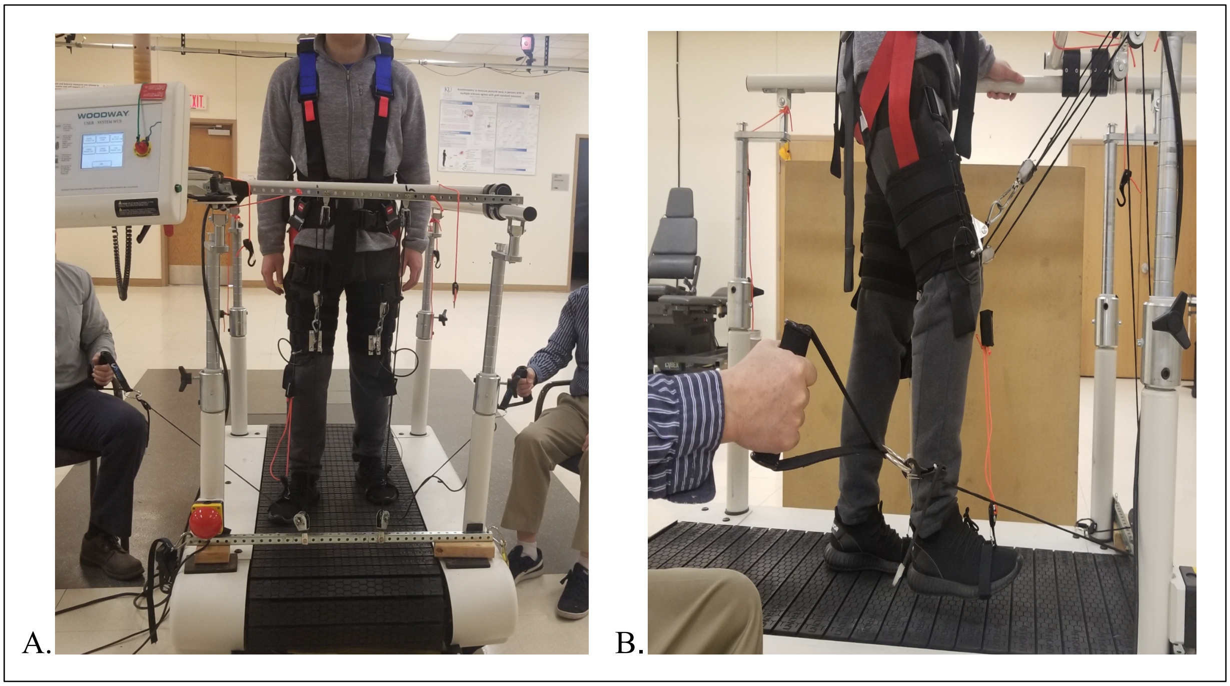


Figure 5.1: The assistive gait training device with two pulling cables operated by two therapists on either side of the treadmill

(A) Two thigh braces which attach to fixed pulleying system in the front of the treadmill via a cable are placed on both right and left thighs. Two physical therapists sit on the right and left side of the treadmill and coordinate to operate the assistive device by pulling the handle attached to the cable. (B) The physical therapists pull the handle attached to the cable to assist a patient with hip flexion and ankle dorsiflexion during the swing phase of the gait cycle

References

1. DeVivo, M.J., K.J. Black, and S.L. Stover, *Causes of death during the first 12 years after spinal cord injury.* Arch Phys Med Rehabil, 1993. **74**(3): p. 248-54.
2. Osterthun, R., et al., *Causes of death following spinal cord injury during inpatient rehabilitation and the first five years after discharge. A Dutch cohort study.* Spinal Cord, 2014. **52**(6): p. 483-8.
3. Myers, J., M. Lee, and J. Kiratli, *Cardiovascular disease in spinal cord injury: an overview of prevalence, risk, evaluation, and management.* Am J Phys Med Rehabil, 2007. **86**(2): p. 142-52.
4. Cragg, J.J., et al., *Cardiovascular disease and spinal cord injury: results from a national population health survey.* Neurology, 2013. **81**(8): p. 723-8.
5. Wahid, A., et al., *Quantifying the Association Between Physical Activity and Cardiovascular Disease and Diabetes: A Systematic Review and Meta-Analysis.* J Am Heart Assoc, 2016. **5**(9).
6. Go, A.S., et al., *Heart disease and stroke statistics--2014 update: a report from the American Heart Association.* Circulation, 2014. **129**(3): p. e28-e292.
7. Katzmarzyk, P.T., et al., *Sitting time and mortality from all causes, cardiovascular disease, and cancer.* Med Sci Sports Exerc, 2009. **41**(5): p. 998-1005.
8. Buchholz, A.C., C.F. McGillivray, and P.B. Pencharz, *Physical activity levels are low in free-living adults with chronic paraplegia.* Obes Res, 2003. **11**(4): p. 563-70.
9. van den Berg-Emons, R.J., et al., *A prospective study on physical activity levels after spinal cord injury during inpatient rehabilitation and the year after discharge.* Arch Phys Med Rehabil, 2008. **89**(11): p. 2094-101.
10. Demirel, S., et al., *Risk factors for coronary heart disease in patients with spinal cord injury in Turkey.* Spinal Cord, 2001. **39**(3): p. 134-8.
11. Gilbert, O., et al., *Serum lipid concentrations among persons with spinal cord injury - a systematic review and meta-analysis of the literature.* Atherosclerosis, 2014. **232**(2): p. 305-12.
12. Cragg, J.J., et al., *Spinal cord injury and type 2 diabetes: results from a population health survey.* Neurology, 2013. **81**(21): p. 1864-8.
13. Bauman, W.A. and A.M. Spungen, *Disorders of carbohydrate and lipid metabolism in veterans with paraplegia or quadriplegia: a model of premature aging.* Metabolism, 1994. **43**(6): p. 749-56.
14. Manns, P.J., J.A. McCubbin, and D.P. Williams, *Fitness, inflammation, and the metabolic syndrome in men with paraplegia.* Arch Phys Med Rehabil, 2005. **86**(6): p. 1176-81.
15. Wang, T.D., et al., *Circulating levels of markers of inflammation and endothelial activation are increased in men with chronic spinal cord injury.* J Formos Med Assoc, 2007. **106**(11): p. 919-28.

16. Liang, H., et al., *Elevated C-reactive protein associated with decreased high-density lipoprotein cholesterol in men with spinal cord injury.* Arch Phys Med Rehabil, 2008. **89**(1): p. 36-41.

17. Gibson, A.E., A.C. Buchholz, and K.A. Martin Ginis, *C-Reactive protein in adults with chronic spinal cord injury: increased chronic inflammation in tetraplegia vs paraplegia.* Spinal Cord, 2008. **46**(9): p. 616-21.

18. Biasucci, L.M., *CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: clinical use of inflammatory markers in patients with cardiovascular diseases: a background paper.* Circulation, 2004. **110**(25): p. e560-7.

19. Koenig, W., et al., *C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992.* Circulation, 1999. **99**(2): p. 237-42.

20. Ridker, P.M., et al., *Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events.* N Engl J Med, 2002. **347**(20): p. 1557-65.

21. Folsom, A.R., et al., *C-reactive protein and incident coronary heart disease in the Atherosclerosis Risk In Communities (ARIC) study.* Am Heart J, 2002. **144**(2): p. 233-8.

22. Martin Ginis, K.A., et al., *Evidence-based scientific exercise guidelines for adults with spinal cord injury: an update and a new guideline.* Spinal Cord, 2018. **56**(4): p. 308-321.

23. Carlson, K.F., et al., *Effect of exercise on disorders of carbohydrate and lipid metabolism in adults with traumatic spinal cord injury: systematic review of the evidence.* J Spinal Cord Med, 2009. **32**(4): p. 361-78.

24. Gorgey, A.S., et al., *The effects of electrical stimulation on body composition and metabolic profile after spinal cord injury--Part II.* J Spinal Cord Med, 2015. **38**(1): p. 23-37.

25. Soyupek, F., et al., *Effects of body weight supported treadmill training on cardiac and pulmonary functions in the patients with incomplete spinal cord injury.* J Back Musculoskelet Rehabil, 2009. **22**(4): p. 213-8.

26. Ditor, D.S., et al., *Effects of body weight-supported treadmill training on heart rate variability and blood pressure variability in individuals with spinal cord injury.* J Appl Physiol (1985), 2005. **98**(4): p. 1519-25.

27. Ditor, D.S., et al., *The effects of body-weight supported treadmill training on cardiovascular regulation in individuals with motor-complete SCI.* Spinal Cord, 2005. **43**(11): p. 664-73.

28. Jacobs, P.L. and M.S. Nash, *Modes, benefits, and risks of voluntary an delectrically induced exercise in persons with spinal cord injury.* J Spinal Cord Med, 2001. **24**(1): p. 10-8.

29. Olive, J.L., et al., *Blood flow and muscle fatigue in SCI individuals during electrical stimulation.* J Appl Physiol (1985), 2003. **94**(2): p. 701-8.

30. Ashley, E.A., et al., *Evidence of autonomic dysreflexia during functional electrical stimulation in individuals with spinal cord injuries.* Paraplegia, 1993. **31**(9): p. 593-605.

31. Hoekstra, F., et al., *Effect of robotic gait training on cardiorespiratory system in incomplete spinal cord injury.* J Rehabil Res Dev, 2013. **50**(10): p. 1411-22.

32. Turiel, M., et al., *Robotic treadmill training improves cardiovascular function in spinal cord injury patients.* Int J Cardiol, 2011. **149**(3): p. 323-9.

33. Phillips, S.M., et al., *Body-weight-support treadmill training improves blood glucose regulation in persons with incomplete spinal cord injury.* J Appl Physiol (1985), 2004. **97**(2): p. 716-24.

34. Stewart, B.G., et al., *Treadmill training-induced adaptations in muscle phenotype in persons with incomplete spinal cord injury.* Muscle Nerve, 2004. **30**(1): p. 61-8.

35. Pinter, M.M. and M.R. Dimitrijevic, *Gait after spinal cord injury and the central pattern generator for locomotion.* Spinal Cord, 1999. **37**(8): p. 531-7.

36. Minassian, K., et al., *The Human Central Pattern Generator for Locomotion: Does It Exist and Contribute to Walking?* Neuroscientist, 2017. **23**(6): p. 649-663.

37. MacKay-Lyons, M., *Central pattern generation of locomotion: a review of the evidence.* Phys Ther, 2002. **82**(1): p. 69-83.

38. Wouda, M.F., et al., *Energetic and cardiovascular responses to treadmill walking and stationary cycling in subjects with incomplete spinal cord injury.* Spinal Cord, 2016. **54**(1): p. 51-6.

39. Carvalho, D.C., et al., *Metabolic and cardiorespiratory responses of tetraplegic subjects during treadmill walking using neuromuscular electrical stimulation and partial body weight support.* Spinal Cord, 2005. **43**(7): p. 400-5.

40. Morse, L.R., et al., *Association between mobility mode and C-reactive protein levels in men with chronic spinal cord injury.* Arch Phys Med Rehabil, 2008. **89**(4): p. 726-31.

41. Goldstein, R.L., et al., *Clinical factors associated with C-reactive protein in chronic spinal cord injury.* Spinal Cord, 2017. **55**(12): p. 1088-1095.

42. Giangregorio, L.M., et al., *Body weight supported treadmill training in acute spinal cord injury: impact on muscle and bone.* Spinal Cord, 2005. **43**(11): p. 649-57.

43. Hicks, A.L., et al., *Long-term body-weight-supported treadmill training and subsequent follow-up in persons with chronic SCI: effects on functional walking ability and measures of subjective well-being.* Spinal Cord, 2005. **43**(5): p. 291-8.

44. Knikou, M., *Functional reorganization of soleus H-reflex modulation during stepping after robotic-assisted step training in people with complete and incomplete spinal cord injury.* Exp Brain Res, 2013. **228**(3): p. 279-96.

45. Liu, W., *A narrative review of gait training after stroke and a proposal for developing a novel gait training device that provides minimal assistance.* Top Stroke Rehabil, 2018. **25**(5): p. 375-383.

46. Riebe, D., et al., *ACSM's guidelines for exercise testing and prescription*. Tenth edition ed. 2018, Philadelphia, PA: Wolters Kluwer. 480.

47. Sporis, G., et al., *How reliable are the equations for predicting maximal heart rate values in military personnel?* Mil Med, 2011. **176**(3): p. 347-51.

48. Florkowski, C., *HbA1c as a Diagnostic Test for Diabetes Mellitus - Reviewing the Evidence.* Clin Biochem Rev, 2013. **34**(2): p. 75-83.

49. Tahara, Y. and K. Shima, *Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level.* Diabetes Care, 1995. **18**(4): p. 440-7.

50. Jiang, F., et al., *Assessment of the performance of A1CNow(+) and development of an error grid analysis graph for comparative hemoglobin A1c measurements.* Diabetes Technol Ther, 2014. **16**(6): p. 363-9.

51. Technologies, E. *Human High Sensitivity T-Cell Discovery Array 14-plex (HDHSTC14)*. Oct 18,2019]; Available from: https://www.evetechnologies.com/product/human-high-sensitivity-t-cell-discovery-array-14-plex/.

52. Jayaraman, A., et al., *Locomotor training and muscle function after incomplete spinal cord injury: case series.* J Spinal Cord Med, 2008. **31**(2): p. 185-93.

53. Forrest, G.F., et al., *Neuromotor and musculoskeletal responses to locomotor training for an individual with chronic motor complete AIS-B spinal cord injury.* J Spinal Cord Med, 2008. **31**(5): p. 509-21.

54. Coupaud, S., et al., *Muscle and bone adaptations after treadmill training in incomplete Spinal Cord Injury: a case study using peripheral Quantitative Computed Tomography.* J Musculoskelet Neuronal Interact, 2009. **9**(4): p. 288-97.

55. Giangregorio, L.M., et al., *Can body weight supported treadmill training increase bone mass and reverse muscle atrophy in individuals with chronic incomplete spinal cord injury?* Appl Physiol Nutr Metab, 2006. **31**(3): p. 283-91.

56. Chilibeck, P.D. and P.A. Guertin, *Locomotor Training and Factors Associated with Blood Glucose Regulation After Spinal Cord Injury.* Curr Pharm Des, 2017. **23**(12): p. 1834-1844.

57. Koyuncu, E., et al., *The analysis of serum lipid levels in patients with spinal cord injury.* J Spinal Cord Med, 2017. **40**(5): p. 567-572.

58. Wahman, K., et al., *Cardiovascular disease risk factors in persons with paraplegia: the Stockholm spinal cord injury study.* J Rehabil Med, 2010. **42**(3): p. 272-8.

59. Vichiansiri, R., et al., *The prevalence of dyslipidemia in patients with spinal cord lesion in Thailand.* Cholesterol, 2012. **2012**: p. 847462.

60. Jones, L.M., M. Legge, and A. Goulding, *Factor analysis of the metabolic syndrome in spinal cord-injured men.* Metabolism, 2004. **53**(10): p. 1372-7.

61. Janssen, T.W., et al., *Coronary heart disease risk indicators, aerobic power, and physical activity in men with spinal cord injuries.* Arch Phys Med Rehabil, 1997. **78**(7): p. 697-705.

62. Dallmeijer, A.J., M.T. Hopman, and L.H. van der Woude, *Lipid, lipoprotein, and apolipoprotein profiles in active and sedentary men with tetraplegia.* Arch Phys Med Rehabil, 1997. **78**(11): p. 1173-6.

63. Fernandez, M.L. and D. Webb, *The LDL to HDL cholesterol ratio as a valuable tool to evaluate coronary heart disease risk.* J Am Coll Nutr, 2008. **27**(1): p. 1-5.

64. Wang, Y. and D. Xu, *Effects of aerobic exercise on lipids and lipoproteins.* Lipids Health Dis, 2017. **16**(1): p. 132.

65. Neefkes-Zonneveld, C.R., et al., *Effect of long-term physical activity and acute exercise on markers of systemic inflammation in persons with chronic spinal cord injury: a systematic review.* Arch Phys Med Rehabil, 2015. **96**(1): p. 30-42.

66. Griffin, L., et al., *Functional electrical stimulation cycling improves body composition, metabolic and neural factors in persons with spinal cord injury.* J Electromyogr Kinesiol, 2009. **19**(4): p. 614-22.

67. Bakkum, A.J., et al., *Effects of hybrid cycle and handcycle exercise on cardiovascular disease risk factors in people with spinal cord injury: A randomized controlled trial.* J Rehabil Med, 2015. **47**(6): p. 523-30.

68. Bastard, J.P., et al., *Evidence for a link between adipose tissue interleukin-6 content and serum C-reactive protein concentrations in obese subjects.* Circulation, 1999. **99**(16): p. 2221-2.

Chapter 6: Summary of Findings and Conclusion

6.1. Summary of Findings

The current dissertation project investigated the feasibility of an 8-week walking training program using a novel assistive gait training device and its benefits on cardiovascular health in individuals with chronic spinal cord injury (SCI). The design of this project was a pilot, single group, pretest-posttest trial. All participants who were enrolled in the study received 3 sessions a week of walking training for 8 weeks. A total of 11 participants completed the training program and the pre-training and post-training assessments. The findings of the project are described in the following. The results of this project are not conclusive due to several limitations, which are discussed in the next section.

In the study for specific aim#1, we examined the feasibility (recruitment, retention, compliance, walking performance, and participants' perception) of an 8-week walking training program in individuals with chronic SCI. We screened 55 participants for eligibility and identified 36 candidates who met the eligibility criteria of the study. A total of 15 participants agreed to enroll in the study among those who met the eligibility criteria of the study. The recruitment rate (27.2%) was acceptable. At the end of the study, 11 participants completed the training program, and the pre-training and post-training assessment. However, 3 participants withdrew from the study due to demands of work or family schedule (n = 2) and an adverse event not related to the study (n = 1). One participant was excluded from the study because he had a colostomy, which might have interfered with wearing the safety harness. The overall compliance rate of the participants who completed the study was 96.2±6.5%. Throughout the period of training, all participants were able to progress in their walking performance in term of the increased treadmill speed from 0.76±0.10 to 1.28±0.14 miles/hour, walking distance from 0.21±0.07 to 0.62±0.08 miles, duration of training from 16.27±4.33 to 29.36±0.92 minutes as well as the reduced percentage of body weight support from 54.55±10.59% to 35.91±11.36%. Based on the responses of the participants to the end intervention questionnaire, the majority of participants showed acceptance and satisfaction with the walking training program as well as the assistive gait training device. No serious issues or concerns were raised from participants regarding the walking training program as well as the assistive gait training device used during the training.

In the study for specific aim#2, we examined correlations between changes in four potential factors (muscle activity, autonomic function, lung capacity, and muscle spasticity) and changes in resting and exercise heart rate (HR) after walking training program in individuals with chronic SCI. We hypothesized that decreases in resting and exercise HR after the walking training program would show significant correlations with increased leg muscle activity, which would be stronger than their correlations with changes in cardiac autonomic function, leg muscle spasticity, or vital lung capacity. The results of the current study showed that the change in exercise HR was significantly correlated with only the change in activities of leg muscles ($r = -0.622$, $p < 0.05$). The change in resting HR was significantly correlated with the change in low-frequency (LF) power of heart rate variability (HRV) ($r = -0.737$, $p < 0.01$), change in root mean square of the successive differences (RMSSD) of HRV ($r = -0.6$, $p < 0.05$), and change in vital lung capacity ($r = -0.624$, $p < 0.05$). There were moderate correlations between the change in high-frequency power of HRV and change in resting HR ($r = -0.56$, $p < 0.05$). A novel finding of the current study is that the increase in activities of leg muscles after our walking training may contribute significantly to exercise HR adaptation, which is a clear indication of an improved heart stroke volume due to the increased venous return. This finding is clinically important because it points to a great potential for improvement in cardiovascular function by walking training that can increase leg muscle activity during training in individuals with SCI. In addition, the increase in cardiac parasympathetic activity (i.e., HF and RMSSD) and improvement in lung capacity were observed after the walking training. Changes in those factors following training contributed significantly to resting HR adaptation. Taken together, the walking training led to HR adaptation in the participants of the current study, which is an important indication of a decrease in risks of CVD.

In the study for specific aim#3, we investigated the effects of the 8-week walking training program on glycemic control, lipid profile, and the levels of pro-inflammatory markers in individuals with chronic SCI. We hypothesized that the 8-week walking training would decrease the levels of glycated hemoglobin (HbA1c) and low-density lipoprotein cholesterol (LDL-C), increase the levels of high-density lipoprotein cholesterol (HDL-C) and reduce the levels of C-reactive protein (CRP) and interleukin-6 (IL-6). Following training, there was a significant decrease in the levels of HbA1c from 5.83 to 5.45 % ($p < 0.01$). Lipid profile improved after training, as shown by a significant increase in levels of HDL-C (pre: 47.49±19.27 mg/dl vs. post: 64.51±14.81 mg/dl; $p < 0.01$) and a significant decrease in levels of LDL-C (pre: 81.35±8.61 mg/dl vs. post: 76.84±13.14 mg/dl; $p < 0.1$). Also, the ratio of LDL-C to HDL-C was significantly reduced by 0.93 ($p < 0.01$). In regard to changes in levels of pro-inflammatory markers, the levels of IL-6 showed a significant reduction from 1.71±0.76 to 1.50±0.70 pg/ml after training ($p = 0.05$), while levels of CPR showed a trend toward a decrease from 5.51±3.83 to 4.71±3.59 mg/l ($p = 0.13$). The improvements of cardiovascular risk markers following the walking training, as mentioned above, are important in reducing the risk of CVD and potentially lowering the cost of healthcare for treating secondary complications, such as cardiovascular events, after SCI.

6.2. Limitations

This dissertation project has some limitations that need to be considered. First, the results of this study need to be interpreted with caution because of a small sample size, lack of a control group and long-term follow up assessments, and participants with mixed levels of SCI lesion (T1-T5 vs. below T5) or severity (motor incomplete, motor compete, or sensory complete). The small sample size might influence the study power to detect significant correlations between changes in some factors and changes in exercise or resting HR (aim#2) and to detect significant changes in some variables (aim#3). Also, the small sample size could increase the risk of sampling bias and influence the results of the study. This study included a single-arm group without a control group which might threaten internal validity and increase the risk of bias. However, this dissertation was a pilot project to gather data for preparing a future trial. We hope that the data from this dissertation project can be used in designing a future clinical trial. Second, we studied the contributions of four potential factors to resting and exercise HR after training (aim#3). There are many other contributing factors for HR adaptation, such as changes in physical fitness level, stroke volume, or cardiac ventricle function, etc., that were not included in the model of this dissertation project. In addition, causality relationships between changes in factors and changes in the primary outcome cannot be determined due to the nature of the correlational study. Third, we focused on individuals with paraplegia T1-L2. Thus, the findings of this study might not be generalized to all SCI population, such as those with a cervical injury (i.e., quadriplegia). Fourth, we did not perform follow-up assessments to determine the long-term effects of walking training on outcome measures in individuals with chronic SCI. We did not know how long these positive changes induced by walking training could be maintained. Finally, we did not have control over some potential covariates, including physical activity, diet, and medications. However, participants reported no changes in those variables throughout the study period.

6.3. Future Directions

The current study tested only participants' perceptions to the use of the assistive gait training device. It failed to include an evaluation of physical therapists' perception to the use of the training device. A future study is needed to evaluate physical therapists' satisfaction with the use of the assistive gait training device in comparison to manual assistance provided by therapists during treadmill walking training.

Although the findings of the current study showed significant correlations between the change in the outcome (resting and exercise HR) and changes in some potential factors following walking training, the interpretation of the results needs to be cautious due to the small sample size. Future trials with a large sample size should confirm findings of the current study and further examine the contribution of changes in other factors, such as stroke volume, cardiac ventricle function, peripheral arterial stiffness, and cardiopulmonary fitness, etc. to resting and exercise HR adaptation after walking training.

The current study used a single group design without a control group or follow-up assessment. A future randomized-clinical trial with an additional control arm (e.g. a standard care group without any additional intervention) and follow-up assessment is needed to investigate the effects of walking training on cardiovascular risk factors in individuals with SCI. Also, future research might consider determining the effects of walking training on cardiovascular risk factors in comparison to other exercise modalities, such as arm ergometer cycling exercise, in SCI survivors.

The current study was conducted at one site. A multi-site study might need to be considered for a future trial in order to overcome the difficulty in recruiting study participants with SCI.

6.4. Potential Clinical Relevance

The findings of this dissertation project might help clinicians to understand potential factors that contribute to HR adaptation in individuals with SCI after a course of walking training and might also guide their efforts to focus on improving those factors in order to reduce elevated HR among SCI survivors. In addition, the findings of this dissertation project would help to guide clinical practice in rehabilitation for patients with SCI to prevent or reduce secondary complications following injury, including cardiovascular risk factors.

6.5. Conclusion

The findings of this dissertation project provide insight about the feasibility of an 8-week walking training program as well as using our novel assistive training device in individuals with chronic SCI. In addition, resting and exercise HR decreased in individuals with chronic SCI after 8-week of walking training. As potential factors contributed to HR adaptation after training, the increased neuromuscular activity of leg muscles was a significant contributor to exercise HR adaptation, while the modulations of cardiac autonomic function and improvement of lung function after training were significant contributors to resting HR adaptation. Besides, positive changes in risk markers of cardiovascular disease were observed in individuals with chronic SCI following 8-week of walking training.

Appendices

Appendix I

Modified Downs and Black checklist for the assessment of the methodological quality of studies

Item	Criteria	Possible answers
Reporting		
1	Is the hypothesis/aim/objective of the study clearly described?	Yes = 1 No = 0
2	Are the main outcomes to be measured clearly described in the Introduction or Methods section?	Yes = 1 No = 0
3	Are the characteristics of the patients included in the study clearly described?	Yes = 1 No = 0
4	Are the interventions of interest clearly described?	Yes = 1 No = 0
5	Are the distributions of principal confounders in each group of subjects to be compared clearly described?	Yes = 2 Partially = 1 No = 0
6	Are the main findings of the study clearly described?	Yes = 1 No = 0
7	Does the study provide estimates of the random variability in the data for the main outcomes?	Yes = 1 No = 0
8	Have all important adverse events that may be a consequence of the intervention been reported?	Yes = 1 No = 0
9	Have the characteristics of patients lost to follow-up been described?	Yes = 1 No = 0
10	Have actual probability values been reported (eg, 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	Yes = 1 No = 0
External validity		
11	Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	Yes = 1 No = 0 Unable to determine = 0
12	Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	Yes = 1 No = 0 Unable to determine = 0
13	Were the staff, places, and facilities where the patients were treated representative of the treatment the majority of patients receive?	Yes = 1 No = 0 Unable to determine = 0
Internal validity - bias		
14	Was an attempt made to blind study subjects to the intervention they have received?	Yes = 1 No = 0 Unable to determine = 0
15	Was an attempt made to blind those measuring the main outcomes of the intervention?	Yes = 1 No = 0 Unable to determine = 0
16	If any of the results of the study were based on "data dredging" was this made clear?	Yes = 1 No = 0 Unable to determine = 0
17	In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	Yes = 1 No = 0 Unable to determine = 0
18	Were the statistical tests used to assess the main outcomes appropriate?	Yes = 1 No = 0

		Unable to determine = 0
19	Was compliance with the intervention(s) reliable?	Yes = 1 No = 0 Unable to determine = 0
20	Were the main outcome measures used accurate (valid and reliable)?	Yes = 1 No = 0 Unable to determine = 0
Internal validity - confounding		
21	Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	Yes = 1 No = 0 Unable to determine = 0
22	Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	Yes = 1 No = 0 Unable to determine = 0
23	Were study subjects randomized to intervention groups?	Yes = 1 No = 0 Unable to determine = 0
24	Was the randomized intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	Yes = 1 No = 0 Unable to determine = 0
25	Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	Yes = 1 No = 0 Unable to determine = 0
26	Were losses of patients to follow-up taken into account?	Yes = 1 No = 0 Unable to determine = 0
Power		
27	Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?	Yes = 1 No = 0 Unable to determine = 0

Appendix II

The End of Walking Training Questionnaire

The following statements ask you about your acceptability to and satisfaction with the walking training and the assistive device

1= Strongly disagree; 2 = Disagree; 3 = Neutral; 4 = Agree; 5 = Strongly agree

Walking training program	**1**	**2**	**3**	**4**	**5**
1- I was satisfied with time length and procedures of walking training Comments:					
2- I felt that walking training was safe and secure Comments:					
3- I felt that walking training was comfortable in regard to the harness Comments:					
4- I enjoyed walking training program Comments:					
5- I would plan to participate in walking training again Comments:					
6- I felt that walking training improves my life Comments:					
7- Walking training improves my walking ability					

Assistive gait training device	**1**	**2**	**3**	**4**	**5**
1- I felt that the assistive device was safe and secure Comments:					
2- I felt that the assistive device was comfortable Comments:					
3- I felt that the assistive device provided sufficient assistance during walking Comments:					
4- I felt that time for setting up the assistive device was reasonable Comments:					
5- I would recommend using this assistive device by others Comments:					