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RESEARCH PROTOCOL INVOLVING HUMAN SUBJECTS
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Study Title: Benefits of a Walk-Training Program on Cardiovascular Health in Individuals with Chronic Spinal Cord Injury

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I. Purpose, Background and Rationale

A. Aim and Hypotheses

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. Those factors play important roles in the development of ischemic heart disease, stroke, and peripheral vascular diseases. 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 to 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. Body-weight supported treadmill training (BWSTT) may be 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 heart rate) and increase leg muscle activities during walking, even in complete SCI. Such preliminary findings suggest that walking training may initiate central pattern generator that activates leg muscles and leads to improvement in the cardiovascular system. However, past studies reported also changes in cardiac autonomic function, leg muscle spasticity and/or lung capacity after walking exercise that may lead to changes in heart rate. There has been no study that examined the potential association between the improved heart rate and increased leg muscle activity, with a comparison to its association with changes in cardiac autonomic function, lung capacity or 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.

In this pilot study, we will enroll 19 individuals with chronic motor incomplete or SCI. The **primary objectives** are 1) to examine the feasibility of an 8-week walk-training program and 2) to examine the potential association between changes in resting and exercise heart rate and changes in three major factors after 8-week walk-training in individuals with chronic SCI. The **secondary objective** is 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 feasibility (recruitment, perception, compliance, retention, and walking performance) of an 8-week walk-training program 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 heart rate after the walk-training program in individuals with chronic SCI. *Primary hypothesis:* Decreases in resting and exercise heart rate after the

walk-training will show significant correlations with increased leg muscle activity, which will be stronger than its correlations with an changes in changes in cardiac autonomic function, leg muscle spasticity, or vital lung capacity.

Aim#3: To investigate the effects of an 8-week walk-training on lipid profile, the level of glycated hemoglobin (HbA1c), and the levels of pro-inflammatory markers in individuals with chronic SCI.
Secondary hypothesis: Compared to baseline measures, the 8-week walk-training program will decrease the levels of total cholesterol, low-density lipoprotein cholesterol and HbA1c, will 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 walk-training program on lower limbs muscle strength and spasticity, functional independence, the level of depression, anxiety and stress, and health-related quality of life in individuals with chronic SCI.

B. Background and Significance

B.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 can be caused by 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 result of an injury at T1 or below, which involves loss of function in the trunk and legs [1]. SCI is often described as complete or complete injury. In the incomplete injury, there is some degree of sensory and/or motor function below the site of lesion, whereas all sensory and motor functions are lost below the site of 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 have muscle strength grade < 3; D = motor function is preserved and at least half of key muscles below the lesion site muscle strength 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 State 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]. Around 52-70% of individuals with SCI are unable to walk independently at one post-injury [4, 7]. SCI is a serious medical condition that lead to loss/impairment of sensorimotor function which negatively impacts quality of life. Following SCI, individuals are subjected to various secondary complications, including cardiovascular events [8, 9].

B.2. Cardiovascular Disease in SCI Population

With advances in medical care, the life expectancy of people with SCI has increased significantly as compared to past few decades [10]. However, cardiovascular disease (CVD) is the common leading cause of death among people with SCI and occurs at the early age in people with SCI as compared to able-bodied people [11]. The findings are consistent in demonstrating a high prevalence of CVD among people with SCI [5, 12]. It has been estimated that the prevalence rate of symptomatic CVD in SCI population (30-50%) is higher compared to able-bodied population (5-10%) [3]. SCI population has 2.72 times higher risk of heart disease and 3.72 times higher risk of stroke compared to the general population [13]. In term of mortality

from CVD, a cohort study that followed individuals with SCI for five years after discharge from inpatient rehabilitation found that 37% individuals died due to cardiovascular causes [14].

B.3. Risk Factors of CVD in SCI population

Physical inactivity is a major risk factor for the development of CVD in general population [15, 16]. Likewise, lack of physical activity and/or prolonged sitting in people with SCI is associated with increased risk of CVD [17]. 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 [18, 19]. 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 [17, 20, 21]. It is well known that reduced cardiovascular fitness is associated with increased risk of CVD [22], and those risk factors, such as abnormal lipid profile, diabetes mellitus, 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 [23-25]. A previous study found that approximately 76.9 % of SCI population had two or more risk factors for CVD [26].

Elevated heart rate:

Physical deconditioning following SCI is associated with increased resting and exercise heart rate. In healthy individuals, studies have found that sedentary lifestyle results in increased resting and exercise heart rate [27, 28]. Likewise, inactive individuals with SCI have higher heart rate than those who are physically active. Elevated heart rate is common in individuals with lower-level SCI when compared with those with higher-level SCI [29, 30]. In addition, individuals with SCI have high heart rate at rest and during exercise as compared to healthy individuals [29-32].

Abnormal Lipid profile:

Abnormal lipid profile has been proved as a major risk factor for the development of heart disease [33]. 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) [34]. 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 [35]. A meta-analysis found significant lower levels of HDL-C and higher TC/HDL-C ratio in individuals with SCI as compared to able-bodied individuals [36]. 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 [37, 38]. Vichiansiri et al.[39] 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 [40]. Individuals with paraplegia had higher levels of TC and LDL-C than those with quadriplegia [41]. In term of ambulatory status, it was found that individuals with SCI who could not be community ambulating had higher TC/HDL ratio than those who were community ambulating [42].

Diabetes Mellitus:

Another risk factor for CVD is persistent high blood glucose levels resulted 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 two- to four-fold increase in the risk of CVD compared to those without diabetes [43]. A study reported that 22 % of individuals with SCI had diabetes mellitus compared to 6 % in able-bodied individuals [44]. Another study found that the odds of type 2 diabetes was 2 times greater among individuals with SCI compared to those without SCI [45].

Individuals with complete SCI had even higher levels of glucose and insulin in blood and higher incidences of diabetes than individuals with incomplete SCI [46]. The high incidence of diabetes after SCI can be attributed to lack of physical activity and consequently alterations in body composition and muscle characteristics [47-50].

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.[51] 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. [52, 53] According to the American Heart Association, inflammatory markers, an emerging nontraditional risk factor, have been shown to predict CVD across populations.[54-56] 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 [57]. 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 [58]. In studies in individuals with SCI, Gibson et al.[20] and Mann's et al.[21] 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 [21, 59, 60]. Physically inactive persons with SCI had higher CRP levels than those who were physically active [21, 60-62]. Based on mobility status, Morse et al.[51] and Goldstein et al.[63] reported that individuals with chronic SCI who were wheelchair-dependent had greater CRP concentrations than those who could walk with assistive devices or independently.

B.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 cardio-metabolic 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 in strength training exercises twice a week[64]. Similarly, American College of Sports Medicine recommends people with SCI to participate in moderate to vigorous intensity aerobic exercises two to three times per week from 20 to 30 minutes [65]. For cardio-metabolic health benefits, a recent guideline suggested that adults with SCI should engage in 30 minutes moderate to vigorous intensity aerobic exercises three time per weeks [66]. A variety of modalities such as arm cycling, functional electrical stimulation (FES) leg cycling, circuit resistance training, and walk-training have been utilized to improve cardiovascular health in SCI population [67]

Upper Extremity Exercises

Upper limbs exercise has been shown to improve cardiovascular fitness and reduce risk markers of CVD in patients after SCI. A previous study reported a decrease in submaximal exercise heart rate in individuals with cervical SCI after 8 weeks of low-intensity arm ergometer training [68]. When comparing the effect of different intensities of arm cycling in individuals with chronic SCI, moderate-intensity training significantly reduced submaximal exercise heart rate 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 [69]. A similar study found that high-intensity arm cycling significantly decreased TG and TC/HDL-C ratio and improved insulin sensitivity in individuals with SCI as compared to low-intensity arm cycling [70]. In a randomized control study, 6-week hand-bike exercise program three times weekly in persons with complete SCI significantly reduced the fasting levels of insulin and insulin resistance and increased the levels of HDL-C compared to the control group [71]. Similar findings were reported in other studies with 12-week or 3-

month of arm cycling exercise [72, 73]. Positive changes in levels of inflammatory markers including CRP and IL-6, were also reported after 8-12 weeks of arm cycling in individuals with chronic SCI [74, 75].

However, many of above-mentioned studies 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 [72, 73]. Groot et al. [70] observed no changes in levels of TC, HDL-C, and LDL-C values relative to baseline measures after low- or high-intensity arm cycling exercise. Bakkum et al.[75] reported no significant changes in levels of HDL-C, TG, and glucose in reference to baseline measures in both arm cycling and hybrid cycling groups. Furthermore, some studies of aerobic exercise of upper extremities for 10 or 16 weeks concluded no significant benefits of the intervention in risk markers of CVD in individuals with SCI [76, 77]. A recent cross-sectional study reported no differences in lipid profile and the level of blood glucose between physically active versus inactive wheelchair-dependent persons with chronic paraplegia [78]. Hjeltnes et al.[31] observed a significant decrease in submaximal exercise heart rate in individuals with subacute complete SCI during 3-7 months of primary rehabilitation including arm aerobic exercise, but exercise heart rate remained higher compared to able-bodied individuals. Putting together, arm aerobic exercise may improve cardiovascular fitness in individuals with SCI, but the improvement is limited and not comparable to cardiovascular fitness of able-bodied individuals because arm aerobic exercise activates only small muscles in the upper body that may not generate sufficient challenges to the cardiovascular system [79].

Lower Extremity Exercises

FES-leg cycling, and resistance training have been utilized to exercise paralyzed muscles below the level of 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, or resistance training helped to improve cardiovascular fitness (i.e. heart rate adaptation) and reduced the risk makers of CVD following SCI [80-84]. Faghri et al.[80] examined the effects of 12-week FES-leg cycling on cardiopulmonary response at rest and submaximal exercise test in individuals with incomplete and complete SCI. They found that resting heart rate slightly decreased in individuals with paraplegia and significantly increased in individuals with quadriplegia following training, and submaximal exercise heart rate significantly decreased in both groups. In regard to changes in risk markers of CVD, 8-10 weeks of FES-leg cycling exercise significantly decreased levels of glucose and insulin [83, 85] and levels of pro-inflammatory markers, including CRP and IL-6. [85]. 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 individuals with complete SCI [86, 87].

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 [88-94]. Hooker et al.[88] studied the effects of 19 weeks of FES-leg cycling on maximal and submaximal cardiorespiratory response in individuals with SCI and demonstrated no significant changes in maximal and submaximal exercise heart rate after training. Another study showed no a significant change in resting heart rate in individuals with complete motor SCI following 6 weeks of FES-legs cycle exercise [89]. Related to changes in risk markers of CVD, Ward et al.[90] and Griffin et al.[85] found no significant decreases in levels of TC, LDL-C, and TG and no significant increase in levels of HDL-C in individuals with chronic SCI after 10 weeks or 12 months of the FES-leg cycling exercise. Ryan et al. [92] and Mahoney et al.[93] reported no changes in levels of glucose and insulin and no improvement in insulin sensitivity in individuals with chronic complete SCI after 12-16 weeks of electrical stimulation resistance training.

Inconsistent findings in past studies can be due to different methodologies and training protocols. In addition, we need to take into consideration that many patients reported muscles fatigue and pain from frequent

electrical stimulation, and they could perform FES-leg cycling exercise only for a short period of time [79, 95, 96]. Therefore, positive changes in fitness level and risk factors for CVD after FES-leg cycling might not be observed in a short duration of training session.

Walk-training:

As an alternative, body weight support treadmill training (BWSTT) can be used as a form of aerobic exercise in individuals with SCI. Although most of the previous studies have focused on studying the effects of BWSTT on motor function, promising 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 SCI population. In individuals with incomplete SCI, BWSTT training significantly improved resting heart rate and blood pressure variability, oxygen consumption, and dynamic oxygen cost with duration of training ranging from six weeks to six months [97-99]. Furthermore, previous studies have shown that 6-10 weeks of robotic-assisted BWSTT significantly decreased resting and exercise heart rate [100] and significantly improved other measurements of cardiovascular function in individuals with incomplete SCI [101]. In a past study of individuals with complete motor SCI, a sub-group of participants showed significant improvement in heart rate and blood pressure variability and artery compliance after BWSTT [79]. In term 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 capacity, in individuals with incomplete and complete SCI [97, 102]. In regard to change in risk markers of CVD, a previous study by Phillips et al.[103] reported that 6-month BWSTT, in individuals with 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 individuals with incomplete SCI [104]. 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, past studies have not considered the intensity of walk-training.

B.5. Why Upright Walk-training

As above-mentioned, findings of aerobic exercise using arm cycling or FES-leg cycling have been shown inconsistent regarding its 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 [97, 99]. In addition, many wheelchair users report shoulder pain as result of overuse [105]. FES-leg cycling can be performed for a short duration as patients often report muscle fatigue and pain due to frequent stimulation [79, 95, 96]. FES-leg cycling may also aggravate the symptoms of autonomic dysreflexia particularly in those with SCI above T6 because of electrical stimulation [106]. 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 [107-109]. 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 [110-112]. Similar muscle activations were observed in human with SCI while walking on a treadmill. Individuals with incomplete SCI showed activation pattern of leg muscles similar to that observed in healthy individuals [113], and the timing and amplitude of EMG activity of leg muscles improved after a period of walk-training [114, 115]. 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 [116]. The amplitude of EMG activity of leg muscles significantly increased after a period of walk-training and worsened after stopping training [116, 117]. In addition, individuals with high thoracic complete SCI showed activation of trunk muscles (back and abdominal muscles) while walking on a treadmill or overground [118]. These findings suggest that walk-training induces neuroplasticity of spinal cord circuits that can only be maintained by continuous walk-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 [109, 119].

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 [120]. In healthy individuals, it was found that the value of heart rate and systolic blood pressure was significantly higher during treadmill exercise than bicycle ergometer exercise [121]. In addition, peak values of oxygen uptake and heart rate were significantly higher during treadmill exercise than during arm ergometer exercise [122]. Similar benefits in cardiovascular and respiratory systems have been observed in individuals with SCI. For instance, under the equivalent workload, the peak value of heart rate and oxygen uptake was higher during treadmill walking than that during stationary cycling in individuals with motor incomplete SCI [123]. Carvalho et al.[124] 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.

B.6. Heart Rate Adaptation to Walking Exercise in People with SCI

The benefits of walking exercise on cardiovascular fitness in people with SCI can be measured by examining adaptive changes in heart rate. A decrease in cardiovascular fitness, which increases the risk of CVD, can lead to increased heart rate due to reduced stroke volume [125]. Elevated heart rate in rest or during physical activities is more common in individuals with SCI as compared to healthy individuals, and in individuals with lower-level SCI compared to those with higher-level SCI [29, 30]. Previous studies in individuals with SCI reported improvement of cardiovascular fitness after walk-training as shown in reduced resting and exercise heart rate [79, 97, 99, 100]. Recent work by Steven et al.[126] demonstrated that 2 months of underwater treadmill training significantly reduced exercise heart rate in individuals with incomplete SCI. The underlying mechanisms of heart rate adaptation following walk-training in individuals with SCI is still unknown. Several factors including changes in leg muscle activity, cardiac autonomic function, leg muscle spasticity, and lung capacity may contribute to heart rate adaptation after walk-training in patients with SCI.

Leg muscle activity:

Repetitive walk-training has been shown to increase EMG activity of leg muscles in individuals with SCI through activation of the central pattern generator [117, 127-129]. Rhythmical contraction of leg muscles (contraction and relaxation) during 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 [130, 131]. In addition, repetitive standing training resulted in increased leg muscle activity and consequently improved cardiovascular response to orthostatic stress test [132]. Generally, increased activity of leg muscles after a course of walk-training can increase venous return and lead to cardiac adaptation. However, the extent of increased muscle activity of lower limbs to the improvement of heart rate in individuals with SCI has not been studied when compared to contributions of other factors.

Cardiac Autonomic function:

Injury to spinal cord, especially above T6, is associated with alterations in autonomic control of cardiovascular system [133]. HR variability (HRV) has been commonly utilized as a non-invasive method to quantify the cardiac autonomic function in SCI population [134, 135]. 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, while their ratio of LF/HF is defined as a marker for sympathovagal balance [136]. Past studies showed individuals with SCI had alterations in HRV, as shown by reduced total power as well as LF and HF powers, and increased ratio of LF/HF, as compared healthy individuals [137, 138]. Decreased HRV is associated with increased the risk CVD [139, 140]. 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)[138, 141]. Regular exercise can promote a positive changes in HRV towards a balance between parasympathetic outflow and sympathetic outflow [140]. Previous studies by Ditor et al. showed that walk-training for 4-6 months significantly increased LF power and decreased ratio of LF/HF of HRV in individuals with incomplete cervical SCI [99] and subgroup of individuals with complete SCI [79]. Similarly, another past study reported a significant improvement in HRV after 4-week walk-training as explained by significant increase in HR complexity which is a nonlinear measure of HRV and trends toward an increase in HF and a decrease in LF.[142] Thus, it is possible that modulation of HRV after walk-training in individuals with SCI can contribute to positive cardiac adaptation. However, relationship between changes in HRV and HR after walk-training has not been examined.

Leg Muscle Spasticity:

It has been reported that muscle spasticity may be associated with increased heart rate in individuals with neurological diseases. Muscle spasticity was associated with increased heart rate in patients with multiple sclerosis [143]. A study in subjects with cerebral palsy demonstrated those with high levels of leg muscle spasticity had high heart rate response as compared to those with low levels of leg muscle spasticity or healthy subjects during walking on a treadmill under the same speed [144]. Furthermore, muscle spasticity negatively influenced heart rate variability in stroke patients [145]. In individuals with SCI, greater heart rate response to walk-training was observed in those who had higher levels of muscle spasticity [79]. A number of studies in individuals with SCI demonstrated a significant reduction in muscle spasticity of the lower extremity after a period of walk-training [146-148]. Thus, it is possible that changes in muscle spasticity after a course of walk-training in individuals with SCI may influence heart rate adaptation, but quantitative examinations are needed.

Lung capacity:

Lung capacity is the total amount of air that an individual can maximally inhale and hold in the lungs. 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. After SCI, lung capacity decreases as a result of respiratory muscle weakness as well as decreased physical activity [149]. Higher-level SCI is associated with the greatest reduction in lung capacity.[150] After a period of aerobic exercise, lung capacity to exchange oxygen and carbon dioxide would increase, and heart rate would decrease [151]. Previous studies have demonstrated that walk-training significantly improved lung capacity in individuals with incomplete and complete SCI [97, 102, 152]. Thus, increased lung capacity after a period of walk-training might contribute to heart rate adaptation in individuals with SCI.

B.7. Assistive Training Device with Treadmill Walking

Assistance from a physical therapist during BWSTT can be effective in providing task-specific walk-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 exhaustion of the physical therapists providing manual assistance.[153] Part of problem is that non-ergonomic body posture of physical therapists while providing manual assistance to patient's legs during training can increase workload and put therapist under risk of

developing low-back pain[154]. Therefore, these limitations can directly impact length and intensity of training. To overcome these limitations, physical therapists often use rehabilitation assistive device to help them providing intensive gait training for people with SCI.

A novel assistive training device using pulley system was developed in our laboratory to provide stepping-assistance for patient's leg and reduce workload of therapist therapists during treadmill walk-training (*Figure1*). This device assists patients with hip and knee flexion and ankle dorsiflexion during swing phase of gait cycle. In addition, the device can allow physical therapists to work from a more ergonomic position while providing the necessary stepping-assistance for patient's leg during walking on a treadmill.

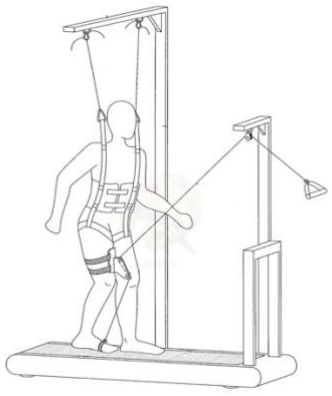


Figure 1: BWSTT with a novel assistive training device

The feasibility of the novel assistive training device was examined in non-ambulatory patients with stroke. In that 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 are affected and need stepping-assistance with both legs during treadmill walking. Therefore, this proposed study will examine feasibility of the assistive training device using two pulley systems in individuals with SCI.

C. Rationale

We propose a pilot study to examine whether the increased leg muscle activities after a walk-training program contribute significantly to the improved cardiovascular health as indicated by heart rate adaptation. Cardiovascular disease (CVD) has a high prevalence rate (30%-50%) and is a leading cause of death among people with SCI [5, 11, 12][3]. For instance, a cohort study that followed individuals with SCI for five years after discharge from inpatient rehabilitation found that 37% individuals died due to cardiovascular causes [14]. Lack of physical activity/exercise and prolonged sitting in people with SCI is significantly associated with increased risk of CVD [17]. However, 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 [97, 99]. FES-leg cycling is limited as patients often report muscle fatigue due to frequent stimulation [79, 95, 96], and aggravate the symptoms of autonomic dysreflexia particularly in those with SCI above T6 [106]. 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 using BWSTT has shown preliminary results of improving cardiovascular function in individuals with incomplete or complete SCI [79, 97, 99-102]. BWSTT induced leg muscle activities in a similar way to those seen in healthy individuals during walking [116]. However, the amplitude of the induced leg muscle EMG activities in persons with complete SCI was much lower compared to that in healthy individuals [117]. It is unknown how significantly the increased leg muscle activities after a period of walk-training can help to improve cardiovascular health. It is important to determine the extent that the increased leg muscle activities after a walk-training program can contribute to the heart rate adaptation, a reliable indication of improvement in cardiovascular fitness, in comparison to the contribution by other factors including changes in autonomic function, muscle spasticity, and lung capacity. The findings of the proposed study will help us to better understand the underlying mechanism of walk-training program on cardiovascular health. Clinically, such findings will help to guide clinical practice in rehabilitation for patients with chronic SCI on issues such as setting up priorities in patient's training.

In addition, there is still limited evidence about the effects of upright walk-training on risk markers of CVD in individuals with chronic SCI. Furthermore, previous studies have not investigated the effects of walk-training on risk markers of CVD in individuals with motor complete SCI. The influence of walk-training on

the pro-inflammatory markers has also not been studied in both incomplete and complete SCI. To explore those issues would further extend our knowledge to better understand the benefits of walking exercise in individuals with chronic SCI.

II. Research Plan and Design

A. Study Objectives:

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

B. Study Type and Design:

The proposed study is a pilot, single group, pre- and post-clinical study design for gathering feasibility and preliminary data for preparing a subsequent future clinical trial. At the initial contact with a study candidate, a phone screening will be conducted by a member of our research team to determine whether an individual is qualifying for the study. An eligible participant will receive BWSTT with assistive training device three sessions a week for eight weeks (a total of 24 sessions). Outcome measures will be taken at baseline and after completion of the walk-training program.

C. Sample size, statistical methods, and power calculation

In this pilot study, we focus more on feasibility issues. We will analyze the data of the study to find trends and to gather pilot data for preparing a future trial. Thus, we will recruit a total of 19 participants.

We have calculated sample size for one factor based on specific aim#2. With power of 80% and significance level of 0.1, we need 15 participants to find a significant correlation of (e = 0.6) between the one factor and the outcome variable. Considering drop out of 20 %, we will recruit a total of 19 participants.

D. Subject Criteria (See Vulnerable Populations appendix, if applicable):

a. *Inclusion criteria:*

We will include spinal injured individuals between 18 and 60 years old, who have paraplegia (T1-L2) SCI, and who are scored less than 5 in Functional Independence Measure, Locomotion: Walk. The onset of SCI must be one year or more at the beginning of the study. Participants must not be participating in any other similar gait training activities. Participants must have medical approval from their physician to participate in walk-training. We will encourage women and all minority group to participate in this study. Children will be not included in the study because we are recruiting individuals who are 18 or older.

b. *Exclusion criteria:*

We will exclude individuals who have one of following conditions:

1) Major Cardiovascular diseases
2) Other neurological diseases
3) Muscle spasticity (greater than 3 according to Ashworth scale)
4) Severe orthopedic issues such as joint stiffness and fractures
5) Osteoporosis (bone mineral density T-score less than – 2.5)[155]
6) Inflammatory diseases or infections
7) Open wound and pressure ulcer

8) Pregnant women
9) Cognitive or psychiatric disorders
10) Uncontrolled autonomic dysreflexia; sudden increase in blood pressure

Participants who take anti-spasticity medications will not be excluded from the study as long as they meet eligibility criteria for the study. They will be instructed to maintain the same medications and dosage throughout the study period.

c. *Withdrawal/Termination criteria:*

1) Insufficient compliance with exercise program, 2) Withdrawal of consent form for any reason, 3) Physician discretion, and 4) Death.

d. We will not recruit a participant who is participating in other research studies.

E. Specific methods and techniques used throughout the study

E.1. outcome measures:

Aim#1: To examine feasibility (recruitment, perception, compliance, retention, and walking performance) of an 8-week (3 sessions per week, 30 min per session) walk-training program in patients with chronic SCI.

Throughout the study, we will collect data on feasibility measures of recruitment, perception, compliance, retention and walking performance. The recruitment rate will be assessed by recording number of participants who are screened for eligibility, those who are excluded because of eligibility criteria, and those who decline to participate in the study. Information on perception will be acquired through a questionnaire that will be administered to participants at the end of walk-training program. The questionnaire, which is derived from previous studies, will evaluate participant's acceptance of and satisfaction with the walk-training program *(Appendix I)* [156-159]. Data on compliance rate will be documented during a period of training. The total number of completed sessions and incomplete sessions along with reasons for absence will be recorded throughout the study. Data on retention will be obtained by recording the number of participants who drop out during the study along with their reasons. Data on walking performance will be obtained by recording walking time, treadmill walking speed, and number of stepping during each session.

Aim#2: To examine correlations between changes in four factors (muscle activity, autonomic function, spasticity, and lung capacity) and changes in heart rate after the walk-training program in individuals with chronic SCI.

The outcome measures listed below will be performed at baseline (pre-measurement) and within 72 hours after completing the 8-week walk-training (post-measurement). Participants will be instructed to restrain from caffeine and cigarette smoking, physical activity for at least 12 hours before testing and will be asked to empty their bladder (or urine bag) prior to the measurements.

The resting heart rate will be measured during sitting position after 5 minutes rest. Exercise heart rate will be measured during a graded treadmill walking test with the same testing conditions (i.e., treadmill speed and body weight support) pre- and post-training as described in past studies [160-162]. Participants will walk on a treadmill with starting warm-up speed of 0.5 miles/hour. The treadmill speed will be increased by 0.2 miles/hour every two minutes. The heart rate will be monitored by a polar sport T31 transmitter (Polar Electro Inc., New York) and will be recorded at the beginning and every two minutes. Leg assistance will be provided to participants as needed (*Figure 2*).

Figure 2: Measure of HR response during a graded treadmill walking test

Electromyography (EMG) system will be utilized to measure the activity of four muscles in the lower limbs: biceps femoris, rectus femoris, gastrocnemius medialis, and tibialis anterior. Prior to the test, surface electrodes will be placed on these muscles bilaterally according to the SENIAM guidelines [163]. The EMG recording will be obtained during walking on a treadmill. At least 10 steps cycle will be recorded. Foot switches will be placed on participant's insoles, and it will detect the time each foot was on the ground. The level of BWS (50%) and treadmill speed (0.9 miles/hour) will be kept constant for each participant during the measurement at baseline and after completing the study, as described in a previous study [127]. The average root-mean-square EMG for each muscle during gait cycle will be calculated and summarized.

Cardiac autonomic function will be determined through power spectral of HRV using electrocardiography system. As descripted in previous studies [137, 141], HRV will be recorded in a sitting position after at least 5 min rest. Data acquisition will last 10 min and will performed in a room with temperature between 20 and 22 C, with no noise atmosphere. During the recording, participants will be asked to breathe normally and remain silent, without speaking or moving. Recording of HRV will be performed around the same time and conditions pre- and post-measurement. HRV frequency domain, including total power, LF and HF powers, and ratio of LF/HF, will be calculated and analyzed using a computer program made in matlab.

A previous study reported consistent muscle spasticity in individuals with paraplegia during the day and from day to day [164]. However, to control for fluctuation in muscle spasticity during the day and the time of day, conducting the assessment will be consistent for each participant during pre- and post-testing [165]. In addition, the assessment will be performed by the same therapist in order to minimize interrater variability of examiners [166]. Muscle spasticity for the lower extremities (hip, knee and ankle flexors and extensors) will be assessed through Modified Tardieu Scale (MTS). MTS is a reliable clinical assessment for measuring muscle tone and joint angle reaction in response to passive stretch at specified velocities (as slow as possible (V1), falling under gravity (V2) or as fast as possible (V3)) [167]. The patients will be tested in the supine position, and the quality of muscle reaction and angle of muscle reaction of the lower limbs will be assessed at specified speeds (V1, V2, V3). The level of muscle reaction is measured on a 6-point scale (0 indicated "No resistance throughout passive movement" and 5 indicates "Joint immobile"). The angle at which the muscle reaction occurs will be determined via a goniometer. The angle of the full range of motion during slow passive movement is defined as R2, while the angle of muscle reaction during fast passive movement is defined R1. The difference between R2 and R1 (R2-R1) represents the dynamic tone component of the muscle spasticity. The large spasticity angle indicates the more spastic muscle *(Appendix II)*.

Vital lung capacity will be measured through a spirometer (Spirotel® Spirometer, MIR USA INC.) The procedures for measuring vital capacity will be conducted according to the guidelines from the American Thoracic Society/European Respiratory Society [168]. Participants will sit upright on a chair with back and arm support. First, participants will be instructed how to perform the test. Then, nose clips will be placed on participant's nose, and participant's lips will be sealed around the mouthpiece of the spirometer to prevent air leakage. To perform the test, a participant will be informed to breathe normally three times, and then they will be instructed to maximally inhale and exhale. The participant will be instructed to fill and empty their lung during inspiration and expiration, respectively. In addition, the participant will be instructed to not flex or extend his/her neck or trunk during the test. Three readings will be obtained, and the largest reading will be recorded. There will be 30 second rest between each reading.

Aim#3*:* To investigate the effect of an 8-week walk-training program on lipid profile, the level of glycated hemoglobin (HbA1c), and the level of pro-inflammatory markers in individuals with chronic SCI.

Blood samples will be collected by a registered nurse or a certified technician at the KU Hospital and then will be transferred to the Diabetes Research Laboratory at the KUMC for analysis. We will collect blood samples from each participant at baseline and within 72 hours after completing training.

Blood samples will be collected in the morning (between 8 and 11 am) from each participant after12-hour overnight fasting; i.e., no eating, drinking (except water), or smoking 12 hours prior to measurement. About 20 ml of blood samples will be collected from an antecubital vein into a tube containing heparin as an anticoagulant. Immediately after drawing, the blood sample will be centrifuged at 1,800 g for 10 min at room temperature. Subsequently, serum be will be separated from the blood cells, and then serum specimens will be transferred to plastic tubes which will be stored at −80°C until analysis. We will assess serum concentrations of TC, LDL-C and HDL-C using an enzymatic colorimetric assay (abcam, Cambridge, MA, USA; catalog no. ab65390; Crystal Chem, USA, catalog no 80069). We will analyze HDL-C after separating HDL-C from LDL-C and very low-density lipoprotein by deposition with magnesium chloride in aqueous dextran sulfate 500. For inflammatory markers, Serum concentrations of CRP (abcam, Cambridge, MA; catalog no ab108826) and IL-6 (abcam, Cambridge, MA; catalog no ab46042) will be determined using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' protocols.

According to 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 2 to 3 months).[169] We will collect a small drop of blood (5 microliters) from the middle finger of the right hand. Immediately, blood samples will be placed on a blood fingerstick and then analyzed using a device A1CNow+ System (Test Medical Symptoms at Home, Inc., Maria Stein, OH, USA). Studies showed that A1CNow+ System test is strongly correlated with reference methods using venous blood sample test [170]

Exploratory aims: To investigate effects of an 8-week walk-training program on lower limbs muscles strength and spasticity, functional independence, the level of depression, anxiety and stress, and health-related quality of life (HRQOL) in individuals with chronic SCI.

Muscle strength will be evaluated using lower extremity motor score (LEMS), according to guidelines of ASIA [3]. LEMS is a manual muscle testing that assesses the strength of five key muscle groups of the lower extremities bilaterally: the hip flexors, knee extensors, ankle dorsiflexors, great toe extensors, and ankle plantarflexors. Each muscle group will be 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 *(Appendix III)*.

As mentioned previously, muscle spasticity for each participant will be assessed at about the same time of day during pre- and post-testing in order to control for fluctuation in muscle spasticity during the day [165]. In addition, the assessment will be performed by the same therapist during pre- and post-testing in order to minimize variability between examiners [166]. Muscle spasticity will be assessed through Modified Ashworth Scale (MAS) and Penn Spasm Frequency Scale. MAS is a subjective scale for clinical assessment of involuntary resistance to passive movement and, hence, muscle tone [165, 167]. An examiner will move the patient's limb through its full range of movement and will rate the amount of resistance felt. Resistance is rated based on a 6-point scale (grades 0, 1, 1+, 2, 3, 4), with lower scores indicating no spasticity and higher scores representing increasing resistance to passive movement. The MAS will be used to assess passive movements of the ankle and knee flexors and extensors *(Appendix IV)*. The Penn Spasm Frequency Scale is a two components self-report questionnaire that assesses an individual's perception of spasticity frequency and severity [171]. The first component is a 5-point scale assessing the frequency with which spasms occur ranging from "0 = No spasms" to "4 = Spontaneous spasms occurring more than ten times per hour. The second component is a 3-point scale assessing the severity of spasms ranging from "1 = Mild" to "3 = Severe". If an individual indicates that he/she has no spasms in the first component, the second component is not evaluated *(Appendix V)*.

In addition, we will quantitatively measure spasticity of the knee joint (quadriceps and hamstring muscle) of the right leg using an isokinetic dynamometer (Biodex Medical System, Inc., Shirley, New York), as

described in the previous protocols with some modifications [172-175]. A participant will be seated on a dynamometer chair with back tilted 85˚ and hip joint positioned at 90˚. The trunk and legs will be stabilized by using straps across the chest, waist, and upper thigh. The axis of the dynamometer will be aligned with the lateral femoral condyle of the knee joint. The distal attachment of the lower limb to the lever of dynamometer will be made approximately 3 cm above the lateral malleolus and will be secured with the distal of the leg by straps. In the beginning, the knee joint will be positioned at 85° of knee flexion and then will be passively moved five times at low velocity of 5 degrees/second from 85° knee flexion to 25° knee flexion and back to 85°knee flexion. Following that, one set of five continuous passive movements from 85° of knee flexion to 25° of knee flexion will be performed at different velocity 15, 30, 60, 90, 120 degrees/second with return velocity of 5 degrees/second to assess knee flexors spasticity. One set of five continuous passive movements from 25° of knee flexion to 85° of knee flexion will be performed at different velocity 15, 30, 60, 90, 120 degrees/second with return velocity of 5 degrees/second to assess knee extensors spasticity. The order of velocity of movement will be randomized. There will be 60 seconds rest break between each movement velocity.

The Spinal Cord Independence Measure (SCIM) self-report version (SCIM-SR) will be utilized to evaluate the level of functional independence. The SCIM-SR is a valid self-reported questionnaire that is designed to measure functional ability of daily activities for persons with SCI.[176] It has an excellent agreement with the clinician administered SCIM version-III.[176] 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 SCM-SR ranges from 0 to100, which indicates the level of functional independence. The higher score represents higher level of functional independence *(Appendix VI)*.

The level of depression, anxiety, and stress will be measured using Depression Anxiety Stress Scales-21 (DASS-21).[177] 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 three subscales with seven items in each subscale (total of 21 items). The response to each item 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 normal level, whereas the high score indicates extremely severe level of depression, anxiety, and stress *(Appendix VII)*.

Change in HRQOL will be assessed via the Short form-36 (SF-36). SF-36 is a self-reported, 36-items questionnaire that assesses eight domains of perceived HRQOL during the previous 4 weeks.[178, 179] 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 *(Appendix VIII)*.

E.2. Intervention protocol

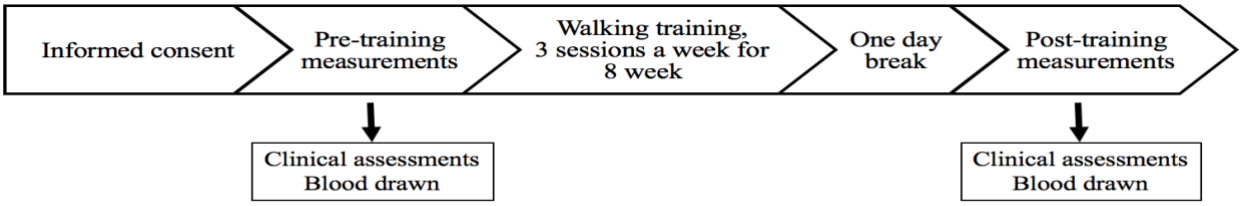
We will perform gait training at Neuromuscular Research Laboratory in the Department of Physical Therapy and Rehabilitation Science at the KUMC. Participants will receive three session a week of BWSTT with assistive training device for 8 weeks; total of 24 sessions. The duration of each training session will last 30 minutes (three bouts of ten-minute walk-training). Previous studies showed that 6 to 8 weeks (three times a week) of walk-training could have positive impacts on the cardiovascular and pulmonary system in individuals with SCI [97, 100-102]. During walk-training, the participant will wear a harness which is attached to an overhead motorized lift to provide body-weight support (BWS) and to prevent the risk of falling (*Figure 3*). At the initial sessions, 60% of participant’s body weight will be supported. Later, BWS will be decreased by 5% at each session as tolerated without knee buckling. However, if the participant is not able to tolerate new BWS or shows knee buckling, BWS will be increased by 5 %. This training protocol has been used and described in past studies.[114, 180, 181] In addition, two thigh braces which are attached

to a pulley system via pulling cords will be attached onto participant's thigh and will be secured using straps (*Figure 1*). Two therapists will operate the training device to assist the participants with hip and knee flexion and ankle dorsiflexion of the legs during the swing phase of a gait cycle. Another therapist will stand behind the participant and will help with balancing and supporting the participant if it is needed. The participant will be encouraged to actively move the legs throughout training. At the beginning of training, initial treadmill speed will be set at 0.5 miles/hour. The participant will walk at 0.5 miles/hour for three to four minutes to warm up. Subsequently, the treadmill speed will be gradually increased by 0.2 miles/hour every two minutes until participant's heart rate reaches 40-50% of his/her heart rate reserve (maximal heart rate – rest heart rate) as recommended by American College of Sports Medicine guidelines.[65] Maximal heart rate will be determined using age-predicted maximal heart rate (220 – participant's age).[182] The participant will be allowed to take three to five minutes rest after 10 minutes of training. Participants can request rest at any time when needed. In the last three minutes, participants will walk at low speed (0.5 miles/hour) for cooling down. To ensure the safety of training, heart rate and blood pressure will be monitored throughout training. Participant's heart rate should not exceed 85% of age-predicted maximal heart rate, and participant's blood pressure should be maintained below 220/110 mm Hg.

Figure 3: Body weight supported treadmill

E.3. Study plan and Timeline:

After a participant signed a consent form and agreed to participate in the study, pre-training measurements will be taken. Then, the participant will receive walk-training three times per week for eight weeks. After completing intervention, participants will be given one day as a break to wash out immediate effects of the intervention, and then the participant will be scheduled for collecting post-training measurements within 72 hours (*Figure 4*).



Figures 4: Study plans

The timeline of this project will be one year (*Table 1*). First, we will begin recruiting and enrolling participants after the study is approved by Institutional Review Board (Human Subjects Committee) at the KUMC. Our goal is to enroll at least three participants a month in the study. We expect that we will meet our enrollment goal within 7 months of the study timeline. At 9 months, we expect that we will finish with providing training to participants. Second, we will analyze the blood sample and clinical assessments at 10 and 11 months. Finally, we will conduct statistical analysis at 12 months.

Table 1: Study timeline

Project activities	One-year timeline											
	1	2	3	4	5	6	7	8	9	10	11	12
Enrollment	■	■	■	■	■	■	■					
Intervention	■	■	■	■	■	■	■	■	■			
Outcome measures analysis										■	■	
statistical analysis												■

F. Risk/benefit assessment:

1. Physical risk:
   During laboratory testing, participants might feel minor pain at the needle site, when blood sample is drawn from his/her body. Pain sensation will go away after blood drawing. Participants might feel muscles fatigue and sore, sweating, and physical discomfort during or after exercise. Also, there are possibility that participants may experience abnormal blood pressure response, fainting, irregular, fast, or slow heart rate during walking exercise, and in rare case, heart attack, stroke, or death. Throughout exercise session, we will carefully monitor participants to reduce the risk associated with walking exercise. We will immediately stop exercise if we observed any sign of risks on participants. Our research teams are trained to do CPR when it is need. Walk-training will be performed at moderate-intensity which has minimal risk and is convenient for individuals with SCI. Participants will get medical clearness from their physicians in order to participate in the study.
2. Psychological risk: N/A
3. Social risk: N/A
4. Economic risk: N/A
5. Potential benefit of participating in the study:
   Participants may or may not benefit from participating in this study. Our research team hope that information gathered in this study will contribute to scientific knowledge of the benefit of walking exercise on cardiovascular health in individuals with SCI

G. Location where study will be performed:

The entire study will take place in Neuromuscular Research Laboratory at KUMC. The blood analysis will be conducted at the Diabetes Research Laboratory at KUMC. The participants' data and file will be kept in locked cabinets at the Neuromuscular Research Laboratory located in the fifth floor of Robinson at KUMC

H. Collaboration (with another institution, if applicable): N/A

I. Single IRB Review for a Multi-site study (if applicable): N/A

J. Community-Based Participatory Research (if applicable): N/A

K. Personnel who will conduct the study, including:

1. Indicate, by title, who will be present during study procedure(s):

   Wen Liu, PhD, faculty member; Jason Frederick, MD; Ramzi Alajam, PhD student; Abdulfattah Alqahtani, PhD student; Sakher Obaidat; PhD students; Sanghee Moon; PhD students; Viswa Gangeddula, PhD students; Caio Vinicius Messias Sarmento, PhD student; Brooke Seever, DPT student; and Kirsten Krull, DPT student.

2. Primary responsibility for the following activities, for example:
   a. Determining eligibility: Ramzi Alajam, PhD student; Abdulfattah Alqahtani, PhD student; Brooke Seever, DPT student; and Kirsten Krull, DPT student
   b. Obtaining informed consent: Ramzi Alajam, PhD student; Abdulfattah Alqahtani, PhD student; and Sakher Obaidat; PhD student

c. Providing on-going information to the study sponsor and the IRB: Ramzi Alajam, PhD student; and Abdulfattah Alqahtani, PhD student

d. Maintaining participant's research records: Ramzi Alajam, PhD student; Abdulfattah Alqahtani, PhD student; Sakher Obaidat; PhD student; Sanghee Moon; PhD students; Viswa Gangeddula, PhD student; and Caio Vinicius Messias Sarmento, PhD student.

e. Completing physical examination: Ramzi Alajam, PhD student; Abdulfattah Alqahtani, PhD student; and Sakher Obaidat; PhD student

f. Taking vital signs, height, weight: Ramzi Alajam, PhD student; Abdulfattah Alqahtani, PhD student; and Sakher Obaidat; PhD student

g. Drawing / collecting laboratory specimens: A registered nurse or certified technician in drawing blood

h. Performing / conducting tests, procedures, interventions, questionnaires: Ramzi Alajam, PhD student; Abdulfattah Alqahtani, PhD student; Sakher Obaidat; PhD student; Sanghee Moon; PhD student; Viswa Gangeddula, PhD student; and Caio Vinicius Messias Sarmento, PhD student

i. Completing study data forms: Ramzi Alajam, PhD student; Abdulfattah Alqahtani, PhD student; Sakher Obaidat; PhD student; Brooke Seever, DPT student; and Kirsten Krull, DPT student

j. Managing study database: Ramzi Alajam, PhD student; Abdulfattah Alqahtani, PhD student; Sakher Obaidat; PhD student; Sanghee Moon; PhD student; and Viswa Gangeddula, PhD student.

L. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

1. Elements of the plan include:

a. Persons/groups who will review the data (study team; independent safety monitor, data monitoring committee or formal DSMB):

Dr. Wen Liu and members of our research team will review the data

b. Data/events that will be reviewed

The data of adverse events and other safety related issues will be reviewed.

c. Frequency of review:

The data and safety review will be performed before and after the intervention, and the data will be viewed bi-weekly during intervention

d. Types of analyses to be performed

Any possible pattern of mistakes/errors will be carefully analyzed, and a plan of action will be made in order to prevent any future potential adverse events

e. Safety-related triggers that would cause the PI to stop or alter the study

The enrollment will be stopped immediately, and the study procedures will be reassessed if more than 20% of the participants experience any adverse effects.

1. Describe how adverse events and unanticipated problems will be ascertained and handled. Explain exactly which type of problems will be considered serious and reported to the IRB. The reporting timeframe should also be detailed.

Any adverse events will be immediately reported to the PI and, if appropriate, to KUMC IRB per KUMC IRB policies. The PI will be notified immediately of chest pain, irregular pulse, fainting, shortness of breath, light headed/dizzy, excessive sweating, slurred speech, blurred vision, leg swelling, falls, or any other adverse events.

3. **Explain exactly what will happen if a patient experiences an adverse event or other problem**

Any participant who experiences chest pain, irregular pulse, fainting, shortness of breath, light headed/dizzy, excessive sweating, slurred speech, blurred vision, leg swelling, and falls will be placed on hold and the PI and Dr. Jason Frederick will make an appropriate decision about the participant's situation.

III. Subject Participation

A. Recruitment:

Study subjects will be recruited from 1) Dr. Jason Frederick's rehabilitation clinic at the KU hospital 2) patients who are enrolled in Frontiers/Pioneers Research Participant Registries using the Healthcare Enterprise Repository for Ontological Narration (HERON) database at KUMC, 3) out-patients rehabilitation center in Kansas City area, and 4) flyers and advertisements. Potential study candidates can either provide their contact information to us or contact our team members by phone or email if they are interested in our study.

B. Screening Interview/questionnaire:

To determine the eligibility of participant, a phone screening will be conducted for each individual who is interested in participating in the study

C. Informed consent process and timing of obtaining of consent

A member of our research team will provide a participant with details and comprehensive information about the entire study and help the individual to make independent and thoughtful decision by providing sufficient time and answering questions. The process of informed consent will be taken place in a private room in our laboratory prior to any research related activity and if a participant agrees, he/she will sign a consent form approved by the IRB at University of Kansas Medical Center (KUMC). Each participant will be given a copy of the signed document. A participant should be able to understand the consent form and sign it; otherwise, they will be excluded. The originally signed consent form will be kept with the participant's confidential file in a locked file cabinet in the research laboratory.

D. Alternatives to Participation: N/A

E. Costs to Subjects: There are no costs to participants for being in the study.

F. How new information will be conveyed to the study subject and how it will be documented:

The participants will be told about anything new that might change their decision to be in this study. New information will be conveyed to participants through phone, email, or meeting. Our team will prepare a new consent form and thus, participants should be asked to sign a new consent form if this occurs.

G. Payment, including a prorated plan for payment:

Participants will not receive any payment in the study.

H. Payment for a research-related injury:

According to KUMC institutional disclaimer statement "If you think you have been harmed as a result of participating in research at the KUMC, you should contact the Director, Human Research Protection Program, Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160. Under certain conditions, Kansas state law or the Kansas Tort Claims Act may allow for payment to persons who are injured in research at KUMC."

IV. Data Collection and Protection

A. Data Management and Security:

Each participant will have a unique numerical code that will be used to identify participant's documents and data. This code will be placed on the consent form and correspond with the participant's data sheets. The consent form will have both participant's name and the code. The consent form and other documents will be kept on locked cabinets in Neuromuscular Research laboratory at KUMC. REDcap website will be used to create and manage data collection forms. Participants' data will be stored on a secure KUMC network drive which will be only accessible to the investigators and the members of research team.

B. Sample / Specimen Collection:

Blood drawing will take place in a clinical laboratory at Neurology Clinic in the KU hospital or at Neuromuscular research laboratory in KUMC. Blood sample will be drawn from each participant at two-time points in baseline and after completing the study. About 20 ml of blood will be collected into a tube containing heparin. The blood sample will be centrifuged at 1,800 g for 10 min at room in order to separate plasma from cells. Subsequently, blood sample will be stored at $-80\ C^{0}$ until analysis at KUMC Diabetes Research Laboratory. Each blood sample tube be labeled by a code which does not has identifiable participant information.

C. Tissue Banking Considerations: N/A

D. Procedures to protect subject confidentiality:

Each participant will have a unique numerical code that will be used to identify participant's documents and data. This code will be placed on the consent form and correspond with the participant's data sheets. The consent form will have both participant's name and the code. The consent form and participants' documents will be kept on locked cabinets in Neuromuscular Research laboratory at KUMC. Also, participants' data will be stored on a secure KUMC network drive which will be only accessible to the investigator (PI) and the members of research team.

E. Quality Assurance / Monitoring:

The data will be reviewed periodically by PI to make sure the data collected are accurate, consistent, complete and reliable.

V. Data Analysis and Reporting

A. Statistical and Data Analysis:

Statistical analysis will be conducted using SPSS statistical software. Descriptive statistics of frequency, mean, and standard deviation will be generated on the data collected during the study. Assumption of normal and variance of data will be assessed using Shapiro-Wilk test and Brown-Forsythe Levene test, respectively. For *primary hypothesis*, first, we will conduct the simple linear regression to determine whether there is a linear relationship between each factor and the outcome measure, which heart rate adaptation. If it is true that more than one factor is correlated

with outcome measure. As an Exploratory manner, we will conduct multiple linear regression to identify contribution of these factors to outcome measure and determine the factor that is strongly contributed to outcome measure based on contribution other factors. The level of significance for multiple regression will be set at 0.1 due to small sample of this pilot study. For *secondary hypothesis and exploratory aims*, the paired sample t-test will be utilized to assess difference in outcome measures pre- and post-training. The level of significance for paired-sample test will be set at 0.05.

B. Outcome:

Aim#1 expected results: For recruitment rate, we expect that one of every four candidate participants who are screened eligibility will enroll in the study (ratio of recruitment rate 4:1) as described in a past study in individuals with SCI.[183] For perception, we expect that participants will be satisfied with walk-training program in regard to time duration of walk-training and use of the assistive training device and time needs to step the assistive training device, and safety and comfortability of training. For compliance, we expect that participants will complete more than 80% of total training sessions. For retention, we expect that drop out during the study will be less than 20%. In regard to walking performance, we expected increase time of walk-training, treadmill walking speed, the number of stepping per session throughout training period.

Aim# 2 expected results: We expect that decreases in resting and exercise heart rate after 8 weeks of walking will show significant correlation with increased leg muscles activity, which will be stronger than its correlations with changes in cardiac autonomic function, leg muscle spasticity, or vital lung capacity

Aim#3 expected results: As compared to baseline measures, we expect that 8 weeks of walk-training program would decrease the levels of TC, HbA1c, CRP, and IL-6 and increase the level of HDL-C.

Exploratory aims expected results: We expected that 8 weeks of walk-training would 1) increase muscle strength of the lower extremities, 2) decrease muscle spasticity of the lower extremities and the level of depression, anxiety, and stress and 3) improve functional independence measure and quality of life in individuals with chronic SCI.

The Criteria for success or failure of the study will be depended on participants' compliance, the rat of drop out, and adverse events. For example, if compliance of participants is more than 80% and the rate of drop out is low, these will be considered success, vice versa. The study will be ended if most participants experience many adverse events due to the study.

C. Study results to participants:

We will not give any individual results to the participants since our study is not for diagnosis purpose. Participants will be notified when all results of the study are published.

D. Publication Plan:

Manuscripts and final reports will be published within one year after completing the study. The results will be published without any identifiable data for our participant.

VI. Bibliography / References / Literature Cited

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