CARDIOVASCULAR HEALTH IN ADULTS WITH SPINAL CORD INJURY

CARDIOVASCULAR HEALTH AND PHYSICAL ACTIVITY AMONG INDIVIDUALS WITH SPINAL CORD INJURY

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ABSTRACT

An increased prevalence and earlier onset of cardiovascular disease (CVD) occurs in persons with spinal cord injury (SCI); the higher risk may be explained by novel CVD risk factors of aerobic capacity and peripheral vascular structure and function. Physical inactivity likely contributes to the basis of increased CVD risk after SCI, however evidence on the effectiveness of exercise programs in attenuating CVD risk in SCI is insufficient. The present thesis evaluated novel CVD risk factors in a cohort of individuals with chronic SCI, and examined the effects of a single bout of exercise and exercise training on CVD risk.

The first study demonstrated dramatic decreases in body composition, aerobic capacity, and sublesional endothelial function via flow-mediated dilation (FMD) in adults with chronic SCI vs. able-bodied (AB) controls. The second, third, and fourth studies assessed the role of shear rate (SR) patterns on FMD. Elevated retrograde SR had a detrimental effect on brachial and superficial-femoral-artery (SFA) FMD in both SCI and AB, but elevated anterograde SR had a favorable effect on SFA FMD in AB only. The fifth study demonstrated that sublesional vasculature does not respond to a 4-month combination aerobic and resistance-training program using the recently released physical activity guidelines for adults with SCI (PAG).

The results of this thesis highlight the multilayered regulation of sublesional vasculature, and that it may respond differently to a single bout of exercise and exercise training when compared to an AB population. This information is crucial when designing strategies to combat impaired vascular structure and function after SCI. The results from this thesis also indicate the potential for the PAG to improve aspects of anthropometrics, body composition, and carotid vascular health in adults with SCI. Further investigations are necessary to delineate the effects of SCI itself, and of exercise, on CVD risk in this population.

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LIST OF ABBREVIATIONS

AB	Able-Bodied		
AD	Autonomic Dysreflexia		
AIS	American Spinal Injury Association Impairment Scale		
BA	Brachial Artery		
BP	Blood Pressure		
BWSTT	Bodyweight Supported Treadmill Training		
CFA	Common Femoral Artery		
CSA	Cross Sectional Area		
CVD	Cardiovascular Disease		
DXA	Dual-Energy X-Ray Absorptiometry		
eNOS	Endothelial Nitric Oxide Synthase		
FES	Functional Electrical Stimulation		
FMD	Flow Mediated Dilation		
HDL	High Density Lipoprotein Cholesterol		
HR	Heart Rate		
IL-6	Interleukin-6		
IMT	Intima-Media Thickness		
LDL	Low Density Lipoprotein Cholesterol		
MAP	Mean Arterial Pressure		
NLI	Neurological Level of Injury		
NO	Nitric Oxide		
NTG	Nitroglycerin		
PAG	Physical Activity Guidelines for Adults with Spinal Cord Injury		
PAI-1	Plasminogen Activator Inhibitor		
PARA-SCI	Physical Activity Recall Assessment for People with Spinal Cord		
	Injury		
PP	Pulse Pressure		
PWV	Pulse Wave Velocity		
RCT	Randomized Controlled Trial		
SBP	Systolic Blood Pressure		
SCI	Spinal Cord Injury		
SFA	Superficial Femoral Artery		
SR	Shear Rate		
SR _{AUC}	Shear Rate Area Under the Curve		
TC	Total Cholesterol		
TG	Triglycerides		
TNF-alpha	Tumor Necrosis Factor-Alpha		
VAT	Visceral Adipose Tissue		
VO ₂ peak	Peak Oxygen Consumption		
VO ₂ max	Maximal Oxygen Consumption		
WC	Waist Circumference		
YPI	Years Post Injury		

DECLARATION OF ACADEMIC ACHIEVEMENT

This thesis, presented in sandwich format, is based on the following four original manuscripts.

CHAPTER 2:

Totosy de Zepetnek J.O., Luijben T.A., Doublet I., MacDonald M.J. Cardiovascular health after spinal cord injury: a comprehensive look at cardiovascular disease risk factors. In Preparation, Expected Submission: *Archives of Physical Medicine and Rehabilitation*, 2014.

Contributions

JOT and MJM were involved in study conception and design. MJM obtained funding, and assisted with interpretation of the data and manuscript editing. JOT authored the ethics application at McMaster University, was the lead investigator responsible for data collection, analysis, and interpretation, and was the primary author of the manuscript. TAL and ID contributed to data collection, analysis, and manuscript editing.

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CHAPTER 1

PREAMBLE and REVIEW of LITERATURE

PREAMBLE

Decreased activity levels promote atherosclerosis and subsequent cardiovascular disease (CVD) (1), and conversely exercise is thought to improve CVD risk and vascular structure and function (2, 3). Individuals who have sustained a spinal cord injury (SCI) are at an increased risk of CVD likely due to both physiological adaptations to the injury itself as well as lifestyle factors. Persons with SCI experience autonomic nervous system impairments and perturbed cardiovascular homeostasis, as well as substantially decreased physical activity levels and body composition changes (4, 5). Immobility leads to remodeling of peripheral arteries as the cardiovascular system adapts to revised metabolic demands, and disrupted autonomic pathways lead to blood pressure instability (i.e. orthostatic hypotension, autonomic dysreflexia [AD]) (5). Physical and physiological barriers to exercise also contribute to declines in physical activity, aerobic fitness, and body composition following SCI.

CVD can be predicted by traditional risk factors, however over the past decade these factors have failed to predict cardiovascular events in almost half of cases in able-bodied (AB) persons, and do not fully explain CVD risk after SCI (6, 7). It has been suggested that the CVD risk prediction gap can be explained by novel risk factors including aerobic capacity and peripheral vascular structure (arterial stiffness) and function (endothelial function) (3). Vascular function changes are thought to precede structural changes (8). Shear rate (SR) magnitude and pattern against endothelial cells provide a potent stimulus for endothelial function regulation. It has been documented that elevations in retrograde SR are detrimental to endothelial function, while increased anterograde SR are

considered atheroprotective (9). For example, repeated increases in mean and anterograde SR from exercise have been reported to be beneficial for peripheral arteries (10). Increased mean SR has been reported in the femoral artery of individuals with chronic SCI when compared AB (11), but comprehensive examinations of SR patterns on endothelial function have not been investigated previously in the SCI population. A closer look at potential mechanisms of altered vascular health post-injury may advance our understanding of the effects of extreme inactivity and loss of sympathetic innervation on peripheral vascular function.

Exercise training has shown to be an effective intervention by which to improve CVD risk in multiple clinical populations. The research assessing physical activity interventions in SCI reveal incomplete and conflicting results. Evidence-based physical activity guidelines for adults with SCI (PAG) that take into account the specific needs and capabilities of individuals with SCI were released in 2011 (12). A recent randomized controlled trial showed improved fitness outcomes (aerobic fitness and muscle strength) after following the PAG for 4-months (13). However, it is unknown whether the PAG can improve CVD risk.

Determining the pathophysiological basis of cardiovascular alterations associated with neurological impairment, and how implementing exercise influences cardiovascular health, will facilitate the development of effective strategies for treating or preventing CVD after SCI. The overall purpose of this review of literature will be to describe CVD risk factors in the SCI population, and discuss the role of exercise interventions in the treatment of CVD after SCI. A brief overview on spinal cord anatomy, pathophysiology of SCI, and classification of SCI will be provided. Details on traditional and novel CVD risk factors, and the effects of exercise training on these risk factors after SCI will be discussed.

REVIEW OF LITERATURE

Approximately 44,000 individuals in Canada live with a spinal cord injury (SCI), and this number is almost doubled to 85,000 when non-traumatic SCI are included; the incidence is estimated to be 4,300 per year (14). The most common cause of traumatic SCI in Canada are motor vehicle accidents (35%), falls (31%), other vehicle accidents (12%), and sports/recreation accidents (9%) (15). Life expectancy has increased over the past decade owing to improvements in medical treatment within the first year post-injury (16). Advancing age among persons living with this devastating and debilitating injury has a profound impact on the prevalence of secondary complications and chronic disease, particularly cardiovascular disease (CVD).

1.1. Anatomy of the Spinal Cord

The spinal cord is comprised of white and grey matter responsible for transmission of sensory information to the brain and regulation of motor and autonomic functions. White matter is located in the anterior, lateral, and posterior columns of the spinal cord and is functionally arranged in ascending and descending tracts for the communication of sensory and motor information via upper motor neurons. Ascending tracts to the brain include dorsal for touch and proprioception, and anterolateral for coarse touch, pain, and temperature. Descending tracts from the brain include lateral for distal muscles, and medial for postural support. Grey matter is located in the interior of the spinal cord and is responsible for directing the sensory information from the extremities through dorsal roots to the white matter ascending tracts, and for directing the motor information from

the white matter descending tracts through ventral roots to the extremities, via lower motor neurons.

Thirty-one pairs of spinal nerves emerge from the spinal cord. Each spinal nerve has a dorsal and a ventral root that join together to form a mixed spinal nerve as it exits the spinal cord. Each segment is responsible for transmitting sensory (dermatome) and motor (myotome) function to and from a specific part of the body at that level of the spinal cord. Motor neurons can be either somatic or autonomic. The function of the somatic nervous system is for voluntary movement. The function of the autonomic nervous system is to maintain the internal environment of the body, regulating blood pressure, heart rate, respiration, digestion, glandular secretion, reproduction, and body temperature via synergy between the sympathetic and parasympathetic divisions (17).

Three layers of covering (meninges) protect the spinal cord including dura, arachnoid, and pia mater. The vertebral column then surrounds the spinal cord and meninges; the vertebral foramen holds the spinal cord, and the mixed spinal nerves exit through the intervertebral canal.

1.2. Pathophysiology of Spinal Cord Injury

Due to the anatomy of the white and grey matter, damage to the white mater often results in interruption of both ascending and descending tracts at all levels below the site of injury, whereas damage to grey matter results in damage to that segment only. In other words, often an upper motor neuron injury (i.e. loss of voluntary movement, spasticity, increased deep reflexes, loss of superficial reflexes) will occur at levels below the lesion, while a lower motor neuron injury (i.e. loss of muscle tone, atrophy of muscles, loss of all reflex and voluntary movement) will occur at the level of injury. The acute phase responses to trauma occur immediately and can progress for up to 3 weeks post-injury. Mechanical trauma such as traction or compression disrupts axons and neuronal membranes causing them to release toxic chemicals that damage neighboring neurons (18). In addition, damage to the blood vessels causes hemorrhage. The result is a swollen spinal cord that may occupy the entire spinal canal, causing ischemia. The inflammatory response to the injury occurs to combat bacterial infection, however further cellular damage ensues in parallel. Macrophages are believed to mediate the degradation of myelin (19); demyelination in turn impairs conduction in ascending and descending axons (20). Evidence exists for some re-myelination during the late phase (a few weeks to months post-injury), and recovery of some function (21).

1.3. Classification of Spinal Cord Injury

A standard neurological examination endorsed by the International Standards for Neurological Classification of Spinal Cord injury has become the most accurate way to assess a person who has sustained an SCI. The standards do not provide information regarding autonomic function and cannot predict individual functional outcomes or neurological recovery, but are able to document neurological parameters at the time of the exam (22). The exam assesses both sensory and motor components to determine sensory, motor, and neurologic levels, sensory and motor index scores, determination of completeness of injury, and classification of the impairment.

Using the international standards assessment, an SCI is then typically defined by the level and severity of injury, as well as years post-injury. The neurological level of lesion

(NLI) refers to the highest spinal segment affected, and typically is termed tetraplegia or paraplegia. Tetraplegia is impairment or loss of motor and/or sensory function in the cervical segments of the spinal cord, and results in impairments of function in the arms as well as possibly the trunk, legs, and pelvic organs (20). Paraplegia is impairment of motor and/or sensory function in the thoracic, lumbar, or sacral segments of the spinal cord, and results in impairments of the trunk, legs, and/or pelvic organs (20). The severity of SCI is defined by the American Spinal Injury Association Impairment Scale (AIS), used to describe completeness of injury on a scoring system from A-E. Individuals with AIS A and B injuries (motor complete) with no voluntary movement of the legs are wheelchair bound about their home and community; individuals with AIS C injuries (incomplete) may achieve ambulation in their home, but may use a wheelchair for mobility in the community; and most individuals with AIS D injuries (incomplete) are able to achieve ambulation in their home and community. AIS E refers to normal motor and sensory function (23) (Table 1).

Letter	Complete/Incomplete	Definition
А	Complete	No motor or sensory function is preserved in the
		sacral regions S4-S5
В	Incomplete	Sensory but not motor function is preserved below
		the neurological level and includes the sacral
		segments S4-S5.
С	Incomplete	Motor function is preserved below the neurological
		level, and more than half of key muscles below the
		neurological level have a muscle grade less than 3.
D	Incomplete	Motor function is preserved below the neurological
		level, and at least half of key muscles below the
		neurological level have a muscle grade of 3 or more.
Е	Normal	Motor and sensory function are normal.

Table 1: American Spinal Injury Association Impairment Scale

An acute SCI is typically defined as up to one year post-injury (YPI), and is considered to be the phase when the most drastic and rapid physiological changes take place. Chronic SCI is typically defined as anything ≥ 1 YPI (20).

Diverse sensory, motor, and autonomic impairments result from various levels and severities of injury. Autonomic impairments are more common and more severe at lesions above thoracic segment 6 (T6), including the inability to regulate blood pressure (BP) effectively, impaired thermoregulation, inability to sweat below the level of the lesion, and chronic pain. Autonomic instability can lead to cardiovascular disturbances of arrhythmias, ischemic heart disease, autonomic dysreflexia (AD), peripheral arterial disease, deep vein thrombosis, hypertension, orthostatic hypotension, and a blunted cardiovascular response to exercise (24, 25). Due to a combination of advancing age among persons living with SCI, autonomic instability, and a sedentary lifestyle, CVD has emerged as the leading cause of mortality in chronic SCI (4).

1.4. Cardiovascular Disease

Individuals with SCI have a higher incidence of hyperlipidemia, obesity, diabetes, coronary artery disease, and ischemic stroke when compared to their able-bodied (AB) counterparts (26-28). CVD risk is typically determined based on the following traditional criterion as described by the American Heart Association: abdominal obesity via waist circumference (WC), elevated triglycerides (TG), low high-density lipoprotein cholesterol (HDL), high BP, and elevated fasting glucose (29). A person with at least 3 of these 5 risk factors is defined as having the metabolic syndrome, and CVD is the primary clinical outcome of metabolic syndrome. However, models based on traditional risk

factor assessment fail to predict CVD in ~50% of cases in AB persons (6, 7), and do not fully explain the increased CVD risk in the SCI population (30). Previous studies have reported similar prevalence of metabolic syndrome in SCI and AB, but different risk factor patterns (31, 32). For example, increased BP is thought to be the most important risk factor for premature CVD in the AB population (33), however individuals with high thoracic/cervical SCI exhibit low BP and a greater prevalence of CVD. In addition to traditional risk factors, behavioral influences such as smoking, diet, and a sedentary lifestyle have been suggested to contribute to the elevated CVD risk in individuals with chronic SCI (4). Blood biomarkers may also provide insight into CVD risk; insulin resistance, a pro-inflammatory state, a pro-thrombotic state, and altered serum adipokine secretion are likely mechanisms that link traditional risk factors to CVD (34, 35). Inflammatory cytokines, thrombotic agents, and adipokines are all secreted from adipocytes, particularly in visceral fat (36-38), and adipose tissue accumulation has been implicated as the primary driver of the metabolic syndrome (39-42).

Novel risk factors include physical inactivity, aerobic fitness, and peripheral vascular structure and function. Physical inactivity has been reported as an indirect measure of CVD risk (43), and can lead to decreased aerobic fitness, decreased conduit artery blood flow and diameter, increased vascular resistance, and endothelial dysfunction (44, 45). These novel risk factors may account, in part, for the remaining 50% of CVD risk. The remainder of this literature review will assess how chronic paralysis and the associated autonomic disturbances and impaired energy metabolism affects the internal environment, aerobic fitness, and peripheral vasculature.

1.5. Body Composition and Biomarkers

SCI has a profound impact on energy metabolism both through the autonomic and somatic nervous system, augmenting CVD risk factors. Because the impact of the sympathetic nervous system is often blunted, individuals with SCI have a parasympathetic-dominant nervous system, resulting in neurogenic hypotension and a diminished cardiovascular response to exercise (46, 47). One outcome of a blunted sympathetic nervous system is reduced metabolic demand at rest and during activities (47, 48). Atrophy of affected myotomes results in sarcopenia, resulting in a loss of both the quality and quantity of muscle tissue. It has been reported that the cross-sectional area (CSA) of lower extremity musculature is 45-80% lower in SCI vs. age- and weight-matched AB controls (49). Given that the predominant peripheral action of insulin and 85% of total glucose uptake occurs in skeletal muscle (50, 51), decreased quantity and quality of skeletal muscle mass following SCI has been suggested to be the largest contributor to peripheral insulin resistance in this population.

It has been suggested that obesity is present in more than two thirds of those with SCI (52, 53), and fat mass is 8-18% higher among those with SCI when compared to AB controls (54). A reduction in energy expenditure contributes considerably to the observed increase in adipose tissue post-SCI (55-57). In addition to general accumulation of adipose tissue, the altered distribution of excess adipose tissue to the visceral region is atherogenic and is an independent predictor of CVD (58, 59). Individuals with SCI often have a characteristic distribution of adipose tissue, including increased visceral adipose tissue (VAT) (52, 60, 61). Excess adipose tissue also contributes to insulin resistance

through a number of different mechanisms including enhanced gluconeogenesis and hepatic glucose output contributing to hyperglycemia (62-64). Due to the combination of a loss of lean and gain of adipose tissue mass, total body mass may remain stable, highlighting the importance of comprehensive body composition assessment rather than simply mass based assessments after SCI.

Formerly thought of as benign stored energy, adipose tissue is now known to secrete a number of pro-inflammatory cytokines, pro-thrombotic agents, and adipokines that mediate the metabolic syndrome (36-38, 62). It has been demonstrated that inflammatory cytokines are secreted in large amounts from VAT (37). Interleukin-6 (IL-6) is released from adipocytes and exerts its effects on the adrenal cortex, stimulating the release of corticosteroids that further exacerbate hyperglycemia associated with obesity and the metabolic syndrome (65). Evidence suggests that circulating IL-6 levels at rest can be used as a prognostic marker for outcome of CVD (66). Tumor necrosis factor-alpha (TNF-alpha) is also secreted from adipocytes and contributes directly and indirectly to vascular endothelial cell injury (67). Elevated serum concentrations of IL-6 and TNFalpha have been reported after SCI when compared to AB controls (68-70).

Adipocytes have been strongly associated with plasminogen activator inhibitor (PAI-1) (38), a pro-thrombotic agent that increases the risk of blood clotting and thromboembolism, and is itself associated with increased risk for CVD (71). To date, only two studies have described PAI-1 levels in SCI and reported a correlation with VAT (61, 72). However it is unclear whether PAI-1 contributes to additional CV risk in SCI.

Leptin and adiponectin are two adipokines directly secreted from adipocytes; both might play an important pathogenic role in CVD (73). Leptin acts to suppress appetite and stimulate basal metabolism, partly through the sympathetic nervous system (74). However, as adipose tissue accumulates it suppresses the effects of leptin, creating leptinresistance (75). Elevated leptin levels have been reported after SCI (76-78), and leptin has been shown to be an independent determinant of abdominal obesity after SCI (61). Adiponectin has cardio-protective effects such as increasing glucose disposal and energy oxidation. Serum levels of adiponectin decrease with increasing adiposity (79), and adiponectin deficiency has been identified as an independent risk factor for CVD (80). Limited research has assessed adiponectin levels after SCI; two studies reported no difference between SCI vs. AB, and trending towards higher values in SCI (60, 76). These findings are unexpected; possible rationale is persons with SCI producing biologically inactive forms of adiponectin (81, 82). The analysis methodology measures various forms of adiponectin including both biologically active and inactive forms, possibly impacting the results (76). Alternatively, low resting metabolism after SCI may protect against hypoadiponectinemia (83).

In summary, individuals with SCI experience drastic body composition changes that may result in an altered internal metabolic regulatory environment and potentially the pathophysiology of CVD risk. Further research needs to be conducted to determine the contribution of these biomarker risk factors to CVD in SCI.

1.6. Physical Inactivity and Aerobic Capacity

Physical inactivity has been described as the largest contributor to low aerobic fitness

and CVD in AB persons (1). It has been reported that there is a 40 to 50% lower risk of CVD among AB individuals who participate in regular physical activity when compared to their sedentary counterparts (2). A growing body of research suggests that physical activity can play an important role in reducing chronic disease after SCI (84). Measures of physical activity are generally via self-report survey and the majority of available selfreport physical activity measures have been developed for use in the general population, typically focusing on activities requiring ambulation (i.e. walking, jogging, biking, etc.). Due to limited ambulation and different energy expenditure patterns after SCI (i.e. brushing teeth may be of moderate intensity), SCI-specific physical activity measures are likely more appropriate for use in SCI related research. The Physical Activity Recall Assessment for People with Spinal Cord Injury (PARA-SCI) has been developed for use among people with SCI, and includes a means of measuring the type, frequency, duration, and intensity of physical activity performed by individuals with SCI whose primary mode of mobility is a wheelchair (85). The PARA-SCI is a valid and reliable measure for assessing physical activity levels among individuals with SCI (85, 86), and has been shown to correlate well with estimates of physical activity energy expenditure when compared to the reference standard doubly labeled water approach (87). Aerobic capacity can also be measured as an index of physical inactivity, and itself has been reported as an indirect measure of CVD risk (43). Low aerobic fitness has been associated with the inability to perform activities of daily living as well as with increased frequency of urinary tract infections in individuals with SCI (88).

Aerobic capacity can be assessed using sub-maximal or maximal exercise tests. Sub-

maximal exercise testing can be used to predict maximal capacity, making use of the linear heart rate (HR)-power output relationship. However there are no validated submaximal arm ergometer exercise tests to predict maximal capacity in the SCI population, primarily due to altered autonomic function affecting the HR-power output relationship. Maximal aerobic capacity tests are conducted via measuring maximal oxygen consumption (VO₂max). Physiological factors that are commonly used to identify VO_2 max are: (1) the "plateau phenomenon", the point at which oxygen consumption stops increasing with increasing workloads; (2) respiratory exchange ratio of ≥ 1.10 ; and/or (3) a lactate level >8-9mM (89). VO₂max is, in reality, rarely reached unless an individual is highly trained and willing to tolerate the pain of maximal exercise. At the cessation of exercise three things happen simultaneously: pain, fatigue, and substrate depletion. Among untrained or unmotivated persons, subjective pain or fatigue might very well be the limiters to an exercise test, rather than objective physiological factors. A more appropriate term for the assessments of peak aerobic capacity in the general population might therefore be VO₂peak. VO₂peak can be represented as an absolute (L/min) or relative (mL/kg/min) value; the latter allows comparison of aerobic capacity levels between individuals of different sizes.

To determine as close to a true VO₂peak as possible, the test chosen must be of appropriate duration (8-12 minutes), utilize at least \sim 50% of the muscle mass, be independent of skill, and be performed in the upright position (90). Incremental tests to exhaustion on a bike or treadmill are the most common modalities of VO₂peak testing. Since individuals with SCI are not able to conduct lower body exercise, VO₂peak testing

is most commonly done using arm ergometry. A recent study comparing VO₂peak using functional electrical stimulation (FES) on the lower extremities combined with arm ergometry versus arm ergometry alone reported significantly higher VO₂peak values with the addition of FES (91). Further, studies in AB persons have reported arm VO₂peak values that are 60-80% of leg VO₂peak values (92), with the differences between tests attributed to the smaller muscle mass utilized in arm exercise. Other factors to take into consideration when assessing VO₂peak in individuals with SCI are physiological limitations from the injury including impairments in skeletal muscle, respiratory, cardiac, and autonomic function. These factors suggest that arm ergometry VO₂peak testing should be interpreted with caution when comparing to the AB population, particularly if the AB group has completed a leg cycle or treadmill VO₂peak test. Not surprisingly, VO₂peak has been reported to be lower in SCI vs. AB, as well as in tetraplegia vs. paraplegia (93).

In summary, including regular physical activity into the lifestyle of individuals with SCI may reverse the cardiovascular and metabolic effects of injury related deconditioning and precipitate desirable multisystem adaptations. Even independent of fitness level, however, SCI disrupts supraspinal control of sympathetic circuits that innervate sublesional blood vessels, rendering sublesional arteries susceptible to adverse structure and function changes. Therefore, individuals with SCI are at high risk of experiencing the negative effects of physical inactivity in addition to detrimental sublesional vascular modifications.

1.7. Peripheral Vasculature

With each heart beat blood moves from large elastic central arteries to muscular peripheral arteries and arterioles to capillaries. Peripheral arteries and arterioles are made up of three anatomical layers: the tunica intima, media, and adventitia. The tunica adventitia is the outermost layer that surrounds the artery, predominantly made up of collagen fibers with some elastic fibers. The tunica media is the middle layer, composed of smooth muscle cells, collagen, and elastic fibers. It is separated from the adventitia by external elastic lamina and from the intima by internal elastic media. The role of the tunica media involves contraction or relaxation of the smooth muscle cells to cause vasoconstriction or vasodilation, respectively. The proportional amount of smooth muscle cells and connective tissue in the media determines the function of the vessel. For example, large conduit arteries (i.e. aorta) have a thicker media made up of more smooth muscle cells and elastin, allowing for passive expansion with each heartbeat. Arterioles are composed of just a few layers of smooth muscle cells, resulting in more resistance to blood flow. The tunica intima is the innermost layer of the artery, and consists of a single cell layer of endothelial cells and a basement membrane. The luminal surfaces of endothelial cells are in direct contact with blood as it flows through the artery, and respond to a variety of mechanical and chemical signals from the blood to regulate vasomotor tone.

Degenerative stiffness and calcification of the arterial wall occurs in the medial layer and is considered arteriosclerosis, while lipid oxidation and plaque formation in the intimal layer is considered atherosclerosis (94). Stiffer arteries propagate faster pulse

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waves that amplify systolic blood pressure (SBP), over time leading to damaged endothelial cells, further stiffening, and arterial remodeling. The main determinants of arterial stiffness include arterial wall composition, smooth muscle tone, and mean arterial pressure (MAP). Arterial stiffness occurs with aging, and is accelerated in several disease states such as obesity, hypertension, diabetes, and CVD (95). With age, large elastic central arteries progressively stiffen while peripheral muscular arteries change to a lesser degree (96). Arterial stiffness is typically measured noninvasively in humans via a number of surrogates such as local artery compliance and distensiblity, or regional pulse wave velocity (PWV); these measures will be described below.

It has been suggested that endothelial dysfunction of the intimal layer precedes increased arterial stiffness (8, 97, 98). Endothelial dysfunction can occur due to decreases in nitric oxide (NO), increases in oxidative stress, and/or decreases in antioxidant capacity. The result of decreased NO is increased vascular tone of small arteries, that in turn leads to structural and functional changes of larger arteries upstream. NO is one of the primary vasoactive substances that is released from endothelial cells for the regulation of smooth muscle tone. Several stimuli induce NO release, including changes in shear stress against the endothelial cell wall. Endothelial function is assessed via measuring endothelial-dependent vasodilation (flow-mediated dilation, FMD), and can be interpreted in comparison to measures of endothelial-independent vasodilation.

Changes in shear stress from decreases in activity or other modifications affect both the structure and function of arteries, in particular arterial stiffness and endothelial function in AB persons (3, 10, 99-106). Negative changes in arteries exacerbate the atherosclerotic process, and are an independent risk factor for CVD (106). Studies looking at peripheral vascular adaptations are limited in the SCI population.

1.7.1. Arterial Stiffness

1.7.1.1. Intima Media Thickness

Intima-media thickness (IMT), measured using brightness-mode ultrasound from the adventitial-media to the intima-lumen interface, is used as a surrogate for arterial wall thickness. Two limitations include that the technique: (1) is unable to differentiate between media remodeling and intima thickening, and (2) widely differs between laboratories making study comparisons difficult (107).

Increased IMT of the carotid artery is associated with CVD and reflects generalized atherosclerosis (108-110). In individuals with SCI, increased (111, 112) or no difference (113) in carotid IMT has been observed, however physical activity seems to be associated with improved carotid IMT (112, 114). One study assessed upper and lower extremity IMT in SCI vs. AB and reported no differences in carotid or brachial (BA) IMT between groups, but an increased superficial femoral artery (SFA) IMT when normalized to lumen diameter in the SCI group (113). These results suggest increased lower extremity atherosclerotic burden in SCI. To the author's knowledge, no studies have stratified based on lesion level to determine its effect on arterial IMT.

1.7.1.2. Local Stiffness

A major role of arteries is to dampen pressure oscillations and maintain steady flow to the peripheral tissues and organs. Healthy large elastic arteries (i.e. the aorta) will momentarily store \sim 50% of stroke volume before the blood is transferred to the periphery through elastic recoil, ensuring continuous flow at the level of the tissues. The ability of arteries to accommodate this instantaneous increase in volume is termed compliance, the inverse of stiffness. Arterial compliance is a marker of arterial elasticity and is a principle determinant of blood pressure. Arterial compliance is influenced by the interaction of arterial mechanical properties and vessel geometry, and is modulated by endothelial function, blood vessel wall structure, and muscle tone. Compliance describes the absolute change in volume due to a change in transmural pressure, while distensibility takes into account initial dimensions of an artery, allowing for comparisons between different arteries in the body or between subject populations that may have different arterial diameters (115). If an arterial segment is tethered and flows are pulsatile (both of which occur in the intact circulation), compliance of any artery can be estimated as a change in radius, diameter, flow, or CSA for a given change in pulse pressure (PP: difference between diastolic and systolic pressure). Arterial compliance and distensibility are often measured noninvasively using a combination of applanation tonometry and brightnessmode ultrasound imaging, and calculated as shown in equation 1 and 2 below:

- (1) Compliance $(\text{mm}^2/\text{mmHg}) = \Delta \text{CSA} / \text{PP} = (\pi (d\text{max}/2)^2 \pi (d\text{min}/2)^2) / \text{PP}$
- (2) Distensibility (mmHg⁻¹) = Δ CSA / PP * CSAmin

where d_{max} is the maximum lumen diameter, d_{min} is the minimum lumen diameter, and PP is artery pulse pressure. Decreased arterial compliance means a reduction in arterial elasticity in the absence of compensatory dilation, and has been shown to occur with age and disease (95, 116). Decreased compliance in the aorta in response to age and disease are responsible in large part for increases in aortic PP, indicating stiffer and more atherosclerotic vessels. The relationship between local artery stiffness and CVD risk is unclear in the AB population, with some studies assessing carotid artery compliance reporting no association with mortality (117), while others have shown a correlation between carotid artery compliance and cardiovascular risk (118).

Comparable carotid artery diameters and blood flow have been reported when comparing SCI and AB (119, 120). A few studies have documented decreased (120, 121) or no difference (114) in carotid artery compliance after SCI. Differences in physical activity levels rather than physiological changes from the injury are likely responsible for the contradictory findings between studies. Future work is required to delineate the association between local arterial stiffness and CVD risk, particularly in the SCI population.

1.7.1.3. Regional Stiffness

Arterial stiffness can also be measured regionally. Aortic pulse wave velocity (PWV), commonly termed as central PWV (cPWV), is a regional index of arterial stiffness, measuring the speed of the arterial pulse wave between the carotid and femoral arteries (122). cPWV is the most widely accepted noninvasive technique for indirectly measuring arterial stiffness, and is a significant independent risk factor for the development of CVD in the AB population (103-105). Peripheral PWV in the arm is assessed between the carotid and radial arteries (aPWV), while leg PWV is commonly assessed between the femoral and posterior tibial arteries (IPWV). Although cPWV is considered to be the best indicator of atherosclerotic burden (123), aPWV has also been shown to correlate with

coronary artery plaque volume (124). PWV is commonly assessed using applanation tonometry, however other methods of assessment including photoplethysmography (PPG) (125) or Doppler (126) have shown high repeatability in the SCI population. PWV is calculated as shown in equation 3 below:

(3) Pulse Wave Velocity (m/s) = Distance (m) / Pulse Transit Time (s)

Only two studies have examined PWV in SCI and age- and sex-matched AB persons; both reported higher cPWV after SCI (125, 127) with no differences between groups for aPWV (127) or IPWV (125, 127). These findings are reasonable, as cPWV has been shown to be more sensitive than peripheral PWV to changes associated with coronary artery disease, cerebrovascular, or peripheral arterial disease (128). Future studies should investigate the effects of lesion level on regional arterial stiffness, as well as its relationship to cardiovascular events in SCI.

Studies conducted on measures of local and regional stiffness have reported correlations between carotid artery distensibility and cPWV in healthy populations (129), but evidence of these relationships among individuals with CVD risk factors is unclear. Atherosclerosis is being increasingly recognized as a systemic disease rather than a focal disease, thus several indices of vascular structure including artery IMT, local stiffness, and regional stiffness should be taken into account when determining atherosclerotic burden. As mentioned above, arterial functional changes likely precede structural changes (8), and so a combination of assessments of peripheral vascular structure and function should be considered when evaluating peripheral vascular health.

1.7.2. Endothelial Function

The endothelium is a heterogeneous, disseminated organ that lines the intimal surface of blood vessels of the entire circulatory system. Endothelial cells are highly structured and can acutely alter functionality to provide changes in permeability. The functions of the endothelium include retaining blood within the circulation, allowing rapid nutrient movement between blood and tissue, releasing clotting factors, participating in the inflammatory defense against pathogens, and initiating new blood vessel formation (130). In addition, the endothelium is a dynamic tissue that regulates vascular tone and homeostasis, acting as an autacoid generator for the release of primarily NO but also prostacyclins and endothelium-derived hyperpolarization factors. NO is the most potent vasodilator released from endothelial cells, and is often used as an index of endothelial function (131). Although several chemical stimuli cause the production of NO (acetylcholine, thrombin, bradykinin, adenosine triphosphase), shear stress of blood flow moving against the endothelial cells is the most important stimulus for its production under normal conditions (130). Increased shear stress sensed by integrins that secure the endothelium to the basement membrane (130) causes potassium channels to open and hyperpolarize the endothelial cells, which subsequently increases the driving force for calcium entry. The calcium-calmodulin complex then stimulates endothelial nitric oxide synthase (eNOS) activity that cleaves NO from the amino acid L-arginine. NO diffuses from the endothelium into the smooth muscles cells of the tunica media to cause relaxation and subsequent vasodilation. Therefore, increased shear stress during exercise for example leads to a greater production of NO that, in turn, causes greater vasodilation.
Both the magnitude and pattern of shear stress acting on the vessel wall modulate endothelial structure and function.

1.7.2.1. Shear Stress Patterns and Endothelial Function

Elevations in anterograde shear rate (SR; a surrogate for shear stress in the absence of blood viscosity) commonly observed with exercise are considered atheroprotective, while low flow, retrograde, or oscillatory SR (i.e. hypertension, diabetes, obesity, age) are considered atherogenic (9). SR patterns differ throughout the vascular tree such that arteries farther from the heart (i.e. SFA) typically experience lower mean and greater retrograde SR (132, 133), with implications regarding higher incidence of atherosclerotic lesions. Recent in vivo work in humans has suggested a detrimental effect of acute increases in retrograde SR on endothelial function (134-136). These investigations have led to the advancement of the theory that chronic increases in conduit artery retrograde SR, associated with conditions such as hypertension, diabetes, obesity, and with age, may have negative impacts on endothelial function and vascular health. Numerous studies have assessed the role of increased mean and anterograde SR in response to exercise on endothelial function responses, reporting increases (137-142), decreases (137, 139, 143-145) or no change (138, 143-146) in BA FMD following lower body aerobic or resistance exercise. Several factors may contribute to the variable BA FMD responses including intensity of exercise, subject characteristics (age, sex, body composition, training status), timing of post-FMD measures, diurnal variation, sympathetic changes associated with a single exercise bout, or technical differences in data acquisition and analysis.

It is clear that acute alterations in SR magnitude and direction have considerable influences on endothelial health, however the effects of a single exercise bout are unclear. In addition, all the studies conducted so far assessing SR patterns on endothelial function have been done in healthy AB persons. Little is known regarding SR patterns and endothelial function responses in conditions of sympathetic disturbances, smaller arterial diameters, and increased mean SR, observed in sublesional arteries in individuals with SCI.

1.7.2.2. Endothelial-Dependent Vasodilation: Flow Mediated Dilation

Noninvasive assessment of endothelial function is conducted via FMD, based on the phenomenon of increased SR causing vasodilation. FMD, in general terms, can describe vasodilation of any artery following increased luminal flow and internal wall SR. However, over the past two decades, FMD has become synonymous with investigating peripheral conduit artery diameter change following a period of distal limb ischemia induced by sphygmometer cuff inflation. FMD of the peripheral arteries can provide a measure of endothelial health that has been shown to correlate with invasive measurements of endothelial function in the coronary arteries (147, 148). Therefore, FMD of peripheral arteries has become a conventional method of endothelial function assessment. FMD is believed to represent an endothelium-dependent and largely NO-mediated vasodilation of BA (149) and SFA (150), if standardized protocols are followed (151). Typically duplex ultrasound is used to assess both blood velocity and arterial diameter, and absolute and relative FMD values are then calculated as shown in equations 4 and 5, respectively:

(4) Absolute FMD (mm) = (Peak Diameter – Baseline Diameter)

Blood velocity measures are an important component of the FMD response, and can be used to quantify the SR response to cuff occlusion. Mean blood velocity can be used to calculate SR and blood flow, as shown in equations 6 and 7 below:

(6) SR (1/sec) = (8 * Blood Velocity) / (Lumen Diameter)

(7) Blood Flow (ml·min⁻¹) = $\pi r^2 *$ Blood Velocity

Since the initiation of widespread use of FMD to evaluate endothelial function, it has been observed that a smaller arterial diameter experiences larger SR and therefore a greater FMD response (152). It has been suggested, therefore, that FMD should be normalized to SR area under the curve (SR_{AUC}) up to max post-occlusion diameter (153), as long as a correlation between SR_{AUC} and FMD% exists (154). Normalized FMD is calculated as the ratio of FMD% to the SR_{AUC}.

The impaired capacity of the endothelium to elicit vasodilation in response to physical or hormonal influences is termed endothelial dysfunction (155). Impaired endothelial function is an early indication of atherosclerosis, and has been associated with increased incidence of cardiac events in the AB population (156-161). Much of the research looking at vascular function adaptations after SCI has focused on those occurring below the level of the lesion. Within 6 weeks following injury, femoral artery diameter adaptation is complete (162), and several studies have reported a 20-30% smaller femoral artery diameter when compared to controls (44, 162, 163). In addition, increased mean SR through the common femoral artery (CFA) (162, 164) and SFA (11,

165) have been observed after SCI. One study evaluating posterior tibial artery FMD% reported lower values in SCI vs. AB (166). However studies assessing CFA or SFA FMD following SCI have reported preserved or enhanced FMD% when compared to AB controls (11, 121, 165). When normalizing FMD% to SR_{AUC}, no differences were found between SCI and AB (11, 165). The finding of preserved FMD in the lower extremities opposes the known positive relationships between FMD and physical activity in the AB population (10, 167-169). It is possible that the preserved FMD response in sublesional vasculature after SCI is due to decreased sympathetic tone (170), or due to the chronically enhanced SR resulting in an upregulation of eNOS (171). Another explanation is that these studies did not account for differences in baseline diameter between groups; perhaps the smaller femoral artery baseline diameter in the SCI group artificially increased the FMD response. Further, no study has validated the use of FMD% as an indicator of endothelial function in deconditioned limbs.

Only one study has assessed BA FMD after SCI, and reported similar BA diameter, SR, and FMD% in SCI vs. AB (11). When normalizing FMD% to SR_{AUC}, the SCI group had significantly lower FMD response compared with controls. Although the SCI group had injuries between T1 and L1, the study did not specify whether manual or electric chairs were used; the reduced BA FMD could simply be due to physical inactivity if electric chairs were used. Further research is required to determine vascular function changes in both the upper and lower extremities that occur following SCI.

1.7.2.3. Endothelial-Independent Vasodilation

When assessing endothelial function, it is important to consider the impact of the magnitude of endothelial-dependent (FMD) in comparison to endothelial-independent vasodilation, since endothelial dysfunction could be due to impairments in the NOpathway or at the smooth muscle cells of the tunica media. Endothelial-independent vasodilation is typically assessed noninvasively through the use of an exogenous vasodilator, such as sodium nitroprusside, glycerol trinitrite, or nitroglycerin (NTG). These exogenous vasodilators are able to bypass the endothelium to act directly on smooth muscle cells, and are thus appropriate for assessing smooth muscle function. Guidelines recommend a 0.4mg sublingual dose of an exogenous donor (i.e. NTG) and ultrasound assessments for up to 10 minutes following administration (172). Similar to FMD calculations, endothelial-independent responses can be presented in absolute and relative terms. The relationship between impaired endothelial-independent vasodilation and CVD is less definitive. Two studies assessing SFA endothelial-independent vasodilation in SCI reported similar relative responses to glycerol trinitrite (165) and NTG (11) in SCI and AB, indicating preserved smooth muscle function. Both studies were conducted in individuals with complete SCI (AIS A-B). To the author's knowledge, no previous studies have assessed endothelial-independent vasodilation among individuals with incomplete injuries, or in the BA in individuals with SCI.

1.8. Summary of Cardiovascular Disease after Spinal Cord Injury

Assessing the available literature on CVD risk and subsequent morbidity and mortality in the SCI population reveals limited and contradictory findings. The unique physiological alterations occurring after SCI including autonomic impairment and reduced energy expenditure result in detrimental body composition changes, reduced aerobic capacity, and altered peripheral vasculature. These unfavorable modifications seem to contribute to an internal atherogenic milieu and impairments in peripheral artery structure and function (Figure 1). However, the research is limited and presents contradictory findings. To the author's knowledge, no previous study has conducted a comprehensive assessment of CVD risk in the SCI population including traditional and novel CVD risk factors. Due to the increased prevalence of CVD in the SCI population, it is essential to understand the pathophysiologic mechanisms that contribute to CVD among individuals with SCI.



Figure 1. Overview of potential cardiovascular complications after spinal cord injury.

Implementing regular physical activity into one's lifestyle successfully protects an individual from CVD and CVD complications, and is a common remedy for CVD across multiple patient groups. Research investigating the effects of physical activity on improving traditional or novel CVD risk in the SCI population is inadequate and inconsistent. Further, individuals with SCI have far less choice of physical activity and lower capacity for physical activity than their AB counterparts. Uncovering an effective and feasible means of physical activity for the SCI population is therefore a priority for patients and healthcare providers.

1.9. Exercise Training in Spinal Cord Injury

A review from 2008 highlighted the fact that in AB individuals, while exercise results in significant decreases in cardiac risk, 40% of the risk reduction cannot be explained by improvements in traditional risk factors (3). Increasing evidence suggests that exercise training exerts direct changes on blood vessels, which result in measurable changes in the structure and function of those blood vessels (3). For example, chronic repeated increases in SR in response to regular exercise have been shown to enhance endothelial function (10). It has also been suggested that because different forms of exercise cause different patterns and magnitudes of stress on blood vessels in addition to surrogate measures of cardiovascular health such as the traditional CVD risk factors (e.g., lipid levels, BP, WC) and blood biomarkers (insulin resistance, inflammatory markers, thrombotic agents, adipokines) (3).

1.9.1. Exercise on Traditional Cardiovascular Risk Factors

Systematic reviews in both 2008 (173) and 2009 (174) concluded that there is insufficient evidence to determine whether exercise improves carbohydrate or lipid metabolism in adults with SCI. Two cross-sectional studies conducted among athletes with SCI reported that regular physical activity can maintain insulin sensitivity after SCI (175), and that regular physical activity is effective in reducing WC in individuals with SCI (176). However, these studies did not implement any specific physical activity intervention to look at the effects of physical activity on CVD risk. Previous studies using arm crank, wheelchair ergometry, or swimming interventions have reported improved post-training blood lipid profiles, with the magnitude of improvement inversely proportional to the level of injury (93, 177-183). Other studies have shown no change or decreases in total cholesterol (TC), low-density lipoprotein cholesterol (LDL), TC/HDL, TG, and elevated or reduced HDL levels following exercise interventions in individuals with SCI (174). The quality of the study design in the majority of these studies has been questioned in a recent systematic review looking at the effectiveness of physical interventions for people with SCI (184).

A systematic review in 2011 assessed the effects of various exercise interventions on body composition after SCI (185). They reported on training protocols including functional electrical stimulation (FES), body weight supported treadmill training (BWSTT), aerobic and resistance exercise, and vibration exercise with training frequencies ranging from 2-7 times per week for 8-52 weeks in duration. Studies reporting on body mass changes were assigned a Pedro score of 3 or 4 (case-control, prepost, or case series studies); none reported significant changes in body mass. Studies reporting on lean mass were assigned a Pedro score of 3 or 4, with one study achieving level 2 (prospective controlled trial). The lower-quality studies (Pedro level 3-4) reported increases in lean mass following BWSTT, FES, and vibration training: these studies used a variety of body composition assessment tools including dual-energy x-ray absorptiometry (DXA), computed tomography, bioelectrical impedance, magnetic resonance imaging, and skin folds (185). The level 2 study reported significant increases in leg muscle mass using magnetic resonance imaging among 15 males with complete tetraplegia following 26-weeks of FES treadmill training twice weekly for 20 minutes per session (186). Most studies reporting on fat mass changes reported no change following training, with the exception of two lower-quality studies showing decreases in fat mass following FES cycling (185), and one more recent publication reporting decreased fat mass following 6 weeks of FES rowing (187). A recently published randomized controlled trial (RCT) among 34 individuals with chronic incomplete SCI investigated the effects of FES (n=17) or aerobic and resistance training (n=17) thrice weekly for 16 weeks on whole body fat mass, whole body lean mass, and leg lean mass using DXA, as well as lower leg muscle CSA using peripheral quantitative computed tomography. They reported increases in leg muscle CSA in the FES group, but no changes in any other body composition outcomes for either exercise protocol (188). It is clear from these studies (most of which are low in quality) that contradictory evidence exists regarding the effects of exercise training on body composition in SCI.

The number of studies assessing biomarkers after an exercise intervention is limited. Six months of circuit training (a combination of aerobic and resistance training) three times per week did not affect postprandial inflammatory markers (IL-6 and CRP) among persons with chronic paraplegia (189). A recent study measured leptin, adiponectin, TNF-alpha, IL-6, and PAI-1 following a thrice-weekly 12-week arm cranking exercise program in males with chronic paraplegia, and reported reduced leptin, TNF-alpha, and IL-6 in the exercising group, but no change in adiponectin or PAI-1 (72).

1.9.2. Exercise on Peripheral Vasculature

Limited research has looked at implementing exercise interventions to help improve arterial stiffness and endothelial function in the SCI population. Previous cross-sectional studies have reported a smaller carotid (112, 190), BA (190) and SFA (190) IMT in active vs. sedentary persons with SCI, suggesting that physical activity improves arterial structure post-injury. Other studies have reported improved local stiffness of the femoral artery but no change at the carotid artery following 4 weeks of FES training (121) or 16 weeks of BWSTT (191), however both interventions were focused on lower extremity exercise. One case study report showed improved regional stiffness (arm and leg PWV) following 6 weeks of wheelchair ergometry in an individual with acute (5 months postinjury) paraplegia (192). These studies assessing local and regional stiffness following exercise in SCI are limited, and most do not incorporate exercise programs that can be conducted from home or even at a standard fitness center.

Regarding lower extremity vascular function, studies have shown normalized femoral artery FMD following 2 weeks (193) and 4 weeks (121) of FES training, and one study

showed increased posterior tibial artery FMD following 18 weeks (194) of neuromuscular electrical stimulation training in persons with SCI. Sample size ranged from 5 to 9 participants in these pre-post study designs, and all were focused on lower extremity exercise. To the author's knowledge, no studies have investigated BA FMD or endothelial-independent vasodilation following an exercise intervention in persons with SCI.

According to the literature, it appears as though there is weak evidence for exercise improving traditional risk factors in the SCI population. Further, limited research has been conducted assessing the effects of exercise on peripheral vascular structure and function, and it appears as though no studies have been conducted assessing peripheral vascular structure and function following an aerobic or combination aerobic and resistance training program in SCI (Figure 2).



Figure 2. Effects of physical activity interventions on cardiovascular health in individuals with spinal cord injury.

1.9.3. Exercise Training Programs for Individuals with Spinal Cord Injury

Several barriers to exercise exist that contribute to SCI being the most sedentary population (195). Barriers include secondary complications (i.e. pressure sores, osteoporosis, osteoarthritis, joint degradation, overuse injuries, tendonitis, contractures, urinary tract infections, renal dysfunction), physiological limitations (i.e. blunted cardiovascular response to exercise, reduced energy metabolism, muscle atrophy, respiratory disorders), psychological barriers, and limited resources or inadequate architecture (196). Due to all of these obstacles, AB physical activity guidelines may not be appropriate for individuals with SCI.

1.9.3.1. The Physical Activity Guidelines for Adults with Spinal Cord Injury

The Canadian physical activity guidelines for adults with SCI (PAG) were released in 2011 in order to provide evidence-based guidelines specific to the needs and capabilities of the SCI population (12). The guidelines recommend that for important fitness benefits, persons with SCI should engage twice weekly in at least 20 minutes of moderate-vigorous intensity aerobic exercise, and 3 sets of 10 repetitions of resistance training using every major muscle group. A recent RCT reported that these guidelines do, in fact, improve fitness outcomes of aerobic capacity and muscle strength (13). However, it is unknown whether these guidelines are sufficient to improve indices of CVD risk.

1.10. The Knowledge Gap

A review of the literature on CVD risk factors in the SCI population is limited and inconsistent. Small samples sizes, heterogeneous subject characteristics, and variations in measurement techniques and outcome measures across studies in SCI make it difficult to compare or generalize findings. Further, due to the potential unique balance of factors contributing to the etiology of increased CVD after SCI, traditional CVD risk factors used in the AB population may not be appropriate. The effect of SCI on traditional and novel CVD risk factors is unclear.

Endothelial dysfunction is believed to be an early indicator of atherosclerosis and subsequent CVD, preceding arterial stiffness and other traditional and nontraditional CVD risk factors (8). SR is the most potent stimulus for endothelial release of NO, and the pattern of SR at rest and in response to exercise has important implications for endothelial function. Anterograde SR is thought to be atheroprotective, while retrograde SR is thought to be atherogenic (9). Almost doubled mean SR through sublesional arteries occurs after SCI (11, 165), but the patterns of SR through arteries above and below the lesion are uncertain. Some investigation into the effects of manipulating SR patterns on FMD has been done in AB using a cuff-inflation model or with a single exercise bout, but the research is limited and it has not been done in a condition of sympathetic disruption and extreme physical inactivity such as SCI.

It has been suggested that exercise training results in significant decreases in chronic risk, and physical activity is a common remedy for CVD across multiple patient groups. Individuals with SCI have multiple physical and physiological limitations to exercise that necessitate SCI-specific physical activity prescription. The majority of the studies assessing exercise on CVD risk reduction in SCI utilize BWSTT and FES training, both specialized exercise modalities that are costly and require trained personnel to conduct.

Evidence-based guidelines specific to the needs and capabilities of the SCI population (PAG) were released in 2011 (12), and have recently been shown to improve fitness outcomes of aerobic capacity and muscle strength (13). Approximately 40% of CVD risk reduction observed with exercise might be attributed to vascular adaptations (3), however it is unknown how the recently released PAG might impact peripheral vascular health in a cohort of individuals with SCI.

1.11. Study Objectives and Hypotheses

The global research objective of this dissertation was to explore cardiovascular health and physical activity in adults with SCI. The hypothesis was that physical inactivity and peripheral vascular structure and function might provide additional information about CVD risk in chronic SCI that is not reflected in traditional risk factors.

In Chapter Two, traditional (lipid levels, BP, WC) and novel (physical inactivity, aerobic fitness, peripheral vasculature) CVD risk factors were explored in a cohort of adults with SCI and a comparison subgroup of AB controls using valid, reliable, and interpretable measures. Care was taken to ensure the validity and reproducibility of novel measures. The study hypothesis was that traditional CVD risk would be similar between SCI and AB, but when assessing novel risk factors the cohort of individuals with SCI would have increased CVD risk.

In Chapters Three, Four and Five, potential mechanisms for altered peripheral vascular function were explored. Chapter Three examined the feasibility of implementing a cuff-inflation model for altering SR in the SFA of young healthy men, and established an appropriate cuff-inflation pressure for manipulation of SFA SR patterns on SFA FMD.

It was hypothesized that cuff pressures of 75 and 100mmHg would induce elevations in retrograde SR while retaining a parabolic flow profile, and impair SFA FMD in young healthy males in a dose dependent manner.

The objectives of Chapter Four were twofold. First, resting SR patterns and FMD in both atherosclerotic-resistant (BA) and -prone (SFA) vessels in individuals with paraplegia and age- sex-, and WC-matched AB controls were determined. The hypothesis was that SR patterns and FMD at rest would be similar between groups through the BA, but that mean and anterograde SR and FMD in the SFA would be higher at rest in paraplegia vs. AB. Second, BA and SFA SR patterns were manipulated using the cuffinflation intervention established in Chapter Three. It was hypothesized that in both groups, cuff-inflation in the arm and leg would induce increases in retrograde SR and acutely attenuate FMD in the conduit artery supplying the cuffed limb.

The objective of Chapter Five was to manipulate the anterograde SR component though the SFA using a single bout of arm-crank ergometry exercise in paraplegia vs. AB. It was hypothesized that a single bout of moderate intensity arm-crank exercise would induce augmented anterograde SFA SR and acutely improve SFA FMD in both groups.

In Chapter Six, an RCT was conducted to assess the effectiveness of implementing 4months of the recently published evidence-based PAG on improving traditional and novel CVD risk factors in a cohort of individuals with chronic SCI.

The findings from these studies will increase our understanding of contributions to CVD risk and how physical activity influences CVD risk after SCI.

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CHAPTER 2

Cardiovascular health after spinal cord injury: a comprehensive look at cardiovascular disease risk factors

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Abstract

Individuals with spinal cord injury (SCI) have an increased prevalence of cardiovascular disease (CVD) compared to their able-bodied (AB) counterparts. Models based on traditional risk factor assessment fail to predict CVD in 50% of cases, and do not fully explain the increased CVD risk after SCI. A reason for this gap in CVD prediction may be due to the exclusion of novel risk factors, such as aerobic capacity and/or peripheral vascular structure and function. The present study assessed traditional (metabolic syndrome) and novel CVD risk factors in individuals with chronic SCI. Thirty-four individuals (n=32 M, n=2 F) with SCI (NLI C1-T11, AIS A-D, YPI 12.7±9.9 years), and 8 AB men of similar age, body-mass-index, and waist-circumference participated. Eighteen and 13% of the SCI and AB cohorts met the criteria for metabolic syndrome. The SCI group had significantly less whole body lean mass (kg) and leg lean mass (kg), more whole body fat (%) and visceral fat (%), and lower peak aerobic capacity (VO₂peak) when compared to the AB group. No differences were found between groups for indices of local (carotid compliance) or regional (central, arm, leg pulse wave velocity [PWV]) artery stiffness. No differences were found in brachial artery (BA) flowmediated-dilation (FMD), but the SCI group had lower superficial femoral artery (SFA) FMD% vs. AB after adjusting for baseline diameter. These results indicate that traditional CVD risk factors likely do not explain the increased CVD prevalence in SCI. Body composition, aerobic fitness, and peripheral vascular measures should be included to generate a more comprehensive risk factor assessment. Mechanisms underlying vascular dysfunction after SCI have not been adequately investigated; future studies with larger representative groups are necessary to determine the relationships between novel risk factors and atherosclerosis in individuals with SCI and the effects of physical activity interventions on vascular structure and function.

Alphabetized Key Words: aerobic capacity, body composition, cardiovascular disease, metabolic syndrome, spinal cord injury, vascular function, vascular structure

Introduction:

The incidence of spinal cord injury (SCI) in Canada is estimated to be 4,300 per year, and the prevalence is approximately 85,556 (51% traumatic and 49% non-traumatic) (1). Historically pulmonary and renal conditions have been the leading causes of death in the SCI population, however in recent years cardiovascular disease (CVD) has emerged as the leading cause of mortality in chronic SCI (2). Individuals with SCI have a higher incidence of hyperlipidemia, obesity, diabetes, coronary artery disease, and ischemic stroke when compared to their able-bodied (AB) counterparts (3, 4). The primary etiology of elevated CVD risk in chronic SCI is considered to be autonomic imbalance that perturbs cardiovascular homeostasis via blood pressure abnormalities (orthostatic hypotension, autonomic dysreflexia, AD), rhythm disturbances (bradyarrhythmias, reduced heart rate variability), and blunted cardiovascular responses to exercise (5). Behavioral influences such as smoking, diet, and a sedentary lifestyle also contribute to the elevated CVD risk (2). Typically CVD risk is determined based on the following traditional criterion as described by the American Heart Association: abdominal obesity measured via waist circumference (WC), elevated triglycerides (TG), low high-density lipoprotein cholesterol (HDL), high blood pressure (BP), and elevated fasting glucose (6). The term metabolic syndrome is used as a diagnosis for a person with at least 3 of these 5 risk factors, and CVD is the primary clinical outcome of metabolic syndrome.

Over the past decade it has been shown that models based on traditional risk factor assessment fail to predict CVD in ~50% of cases among AB persons (7), and do not fully explain the increased CVD risk in the SCI population (8). For example, although low

resting arterial BP is usually considered cardio-protective, individuals with high thoracic/cervical injures that exhibit low BP contradictorily have a greater prevalence of CVD. One reason for this gap in CVD prediction may be the exclusion of novel risk factors such as a more comprehensive body composition assessment, physical inactivity, aerobic capacity, and/or vascular structure and function.

Following SCI dramatic body composition changes occur resulting in a loss of metabolically active skeletal muscle and an increase in adipose tissue. The distribution of adipose tissue is also altered after SCI with increases in the viscera region; increased visceral adipose tissue (VAT) is atherogenic and is an independent predictor of CVD (9). These detrimental body composition changes have a sizeable impact on energy metabolism at rest as well as energy expenditure during activity (10). In addition, excess adipose tissue has been associated with increased artery stiffness (11). Due to the combination of a loss of lean and gain of adipose tissue mass, total body mass may remain stable, highlighting the importance of comprehensive body composition assessment rather than simply body mass index (BMI) or mass based assessments after SCI.

Both physical inactivity and aerobic capacity have been reported as indirect measures of CVD risk (12), and low aerobic fitness has been associated the inability to perform activities of daily living as well as with increased frequency of urinary tract infections in individuals with SCI (13). Physical inactivity can lead to decreased conduit artery blood flow and diameter, increased vascular resistance, and endothelial dysfunction (14). Previous studies have shown that leisure time physical activity (LTPA) is associated with a 30-50% reduction in CVD (15).

Arterial plaque accumulation and subsequent rupture is the cause of ~70% of fatal acute myocardial infarction and/or sudden coronary death (7). Cardiovascular events may be better predicted by factors that render individuals susceptible to plaque formation, such as vascular structure and function (16). Previous research examining the cardiovascular system in chronic SCI has focused on central cardiovascular adaptations, including cardiac atrophy (17) and reduced plasma volume (18). However SCI disrupts supraspinal control of sympathetic circuits that innervate sublesional blood vessels, rendering sublesional arterial vasculature susceptible to adverse structure and function changes. Vascular deconditioning after SCI occurs below the level of lesion as a result of physical inactivity and a disruption of autonomic control. Artery intima media thickness (IMT), local and regional stiffness (19), and endothelial function (20) are important predictors of atherosclerosis and subsequent CVD.

Findings of reduced carotid and femoral (21) or no change in radial (22) arterial compliance (inverse of stiffness) have been reported after SCI when compared to an AB control group, and elevated (23) or no change (24) in carotid IMT has been reported after SCI. Two studies reported increased central arterial stiffness with no differences in peripheral stiffness after SCI (22, 25). The majority of studies assessing endothelial function after SCI have examined responses in the lower extremities and report preserved or enhanced endothelial function via flow-mediated dilation (FMD) when compared to AB controls (21, 26-28). One study assessing brachial artery (BA) FMD reported reduced

values in SCI vs. AB (26). The findings of preserved FMD in the lower extremities and a reduction in FMD above the lesion level is contradictory to the literature reporting positive relationships between FMD and physical activity in the AB population (29). The contribution of peripheral vascular dysfunction to CVD risk in the SCI population has not been adequately addressed, as arterial adaptations following SCI are understudied and unclear.

The purpose of the present study was to report a comprehensive cardiovascular health profile of traditional CVD risk factors, body composition, aerobic capacity, and peripheral vascular structure and function in the upper and lower extremities in a cohort of individuals with chronic SCI and a comparison subgroup of AB controls.

Methods:

Participants

Thirty-four individuals with SCI (NLI C1-T11, AIS A-D, YPI 13±10 years) were recruited from Southern Ontario to participate. Eight AB persons were matched to 8 of the participants with paraplegia for age, sex, race, BMI, and WC (Table 1); the matched pairs were used for a side-study. All SCI and AB participants were Caucasian. The study procedures were approved by the Hamilton Health Sciences Research Ethics committee in Hamilton, Ontario, Canada, adhered to the Declaration of Helsinki, and all of the participants gave previous written informed consent.

Experimental Design

Each participant came to the laboratory on two occasions. The first visit consisted of a body composition scan and a maximal exercise test, while the second visit consisted of resting cardiovascular measures. Participants abstained from caffeine and alcohol for \geq 12 hours and abstained from physical activity for a minimum of 24 hours prior to both testing sessions. For the cardiovascular measures participants arrived in a fasted state (\geq 12 hours), and assessment was conducted in a quiet, temperature controlled room (22-24°C). Participants lay supine for 10-15 minutes prior to any data collection to ensure stability of resting measures. Heart rate (HR; Model ML123, ADInstruments Inc.; Colorado Springs, USA) and blood pressure (BP; FMS MIDI, Finapres Medical Systems, Amsterdam, The Netherlands) were monitored continuously throughout all cardiovascular testing procedures.

Experimental Procedures

Body Composition

Anthropometrics

Basic anthropometric measures of mass (kg), length (m), BMI (kg/m²), and WC (cm) were collected. Mass was measured using a floor scale (Detecto BRW-1000 Digital Bariatric Wheelchair Scale, DETECTO, Webb City, MO, USA). Wheelchair mass was measured and subtracted from total mass to determine participants' body mass to the nearest 0.1 kg. Length measurements were made using a flexible non-elastic Gulick II tape measure (Country Technology Inc., Gay Mills, WI) to the nearest 0.1 cm; all
measurements were taken on the right side of the body from the heel to the crown of the head while the participant lay supine. The participant's feet were stretched into dorsiflexion where possible. Length measures were taken in segments if participants had contractures that prevented the straightening of their legs. BMI was determined by dividing the participants body mass (kg) by their length (m) squared. The measurement for WC was taken in the supine position after normal expiration immediately below the lowest rib with the same tape measure as used for participant length (Gulick II) (30). For each WC measurement, the tape measure was placed directly on the skin with the participants' arms by their sides. Each measurement was taken to the nearest 0.1 cm.

Dual Energy X-Ray Absorptiometry

Dual energy x-ray absorptiometry (DXA) is a "gold standard" measure of body composition and an effective method to characterize body composition in people with SCI (31). Whole body fat (kg, %), whole body lean (kg), VAT (kg, %), arms fat and lean (kg), and legs fat and lean (kg) were determined using a Hologic QDR-4500A (Hologic Inc., Waltham, MA, USA) following the manufacturers' guidelines for scan acquisition and body composition analysis. A skilled DXA technician conducted all scans and analyses. VAT was analyzed from the whole body scan by demarcating the region of interest from lumbar vertebra 1 through 4 (32). Quality control tests were performed using a phantom and measurements were maintained within the manufacturer's standards of <1%. Least significant change (LSC) values were calculated of 0.703 kg, 1.587 kg, and 0.275 kg, for whole body fat, whole body lean, and VAT, respectively. LSC refers to the least amount of change between 2 measurements over time that must be exceeded

before a change can be considered true (with 95% confidence). Low LSC values imply high reproducibility with regards to the technician's ability to position patients and analyze scans, and the DXA machine's ability to acquire consistent scans.

Blood Biomarkers

Blood glucose control (HbA1c) and lipids (TG, total cholesterol [TC], low density lipoprotein cholesterol [LDL], HDL, TC/HDL) were measured from a blood sample taken after a 12 hour fast, and sent to a blood clinic for analysis. HbA1c is reported in mmol/mol, and lipids are reported in mmol/L. Healthy HbA1c values range from 20-41 mmol/mol; desirable lipid levels: TG <1.7 mmol/L, TC <5.1 mmol/L, HDL >1.5 mmol/L, TC/HDL <4.5 (men) and <4.0 (women), and LDL <2.6 mmol/L.

Aerobic Capacity and Physical Activity

Peak Oxygen Uptake

To measure peak oxygen uptake (VO₂peak), all participants performed a symptomlimited graded arm cycle ergometer test (Lode Angio BV, The Netherlands). The AB participants also performed a symptom-limited leg cycle ergometer test (Excalibur Sport V2.0; Lode BV, The Netherlands). Cardiac stability and HR were monitored throughout both tests using a 1-lead electrocardiograph (ECG, PowerLab 15T, ADInstruments) and a Polar HR monitor (Polar T31, Polar Electro, Quebec, Canada). Prior to the arm ergometry VO₂peak test, participants were asked to empty their bladder to minimize episodes of AD. Prior to initiation of exercise all participants rested for 2 min during which baseline measurements of HR, BP, expired gas, and ventilatory parameters were assessed using a metabolic cart (Moxus Metabolic System, AEI Technologies Inc., Pittsburgh, PA).

The midpoint of the arm ergometer was set at shoulder level and the distance was set to allow a slight flexion in the elbow when the arm was extended. Participants with insufficient handgrip had their hands secured to the arm handles with tensor bandages. The incremental arm ergometer test began with no resistance at a cadence of 60-80rpm. After a 1-minute warm-up, the resistance increased every minute by 5W for participants with tetraplegia and 10W for participants with paraplegia or AB. The incremental leg cycle ergometer test consisted of a two-minute warm-up at 50W and thereafter increased 1W every 2 seconds. Participants continued arm or leg cycling until volitional fatigue or if they were unable to maintain a cadence of 30rpm. Expired gas and ventilatory parameters were acquired throughout the protocols, and central as well as peripheral RPE using the Borg's RPE scale (scale 1-10) was assessed every minute. BP was assessed immediately following and throughout recovery to ensure that it returned to baseline values following the exercise test. VO₂peak was determined to be the highest 30-second average oxygen consumption.

Physical Activity Recall Assessment for Individuals with Spinal Cord Injury

Current physical activity levels were evaluated among the participants with SCI from 3-day recall using the Physical Activity Recall Assessment for People with Spinal Cord Injury (PARA-SCI) administered by a trained interviewer. The PARA-SCI is a valid and reliable measure for assessing physical activity among individuals with SCI (33), and has been shown to correlate well with estimates of physical activity energy expenditure when compared to the reference standard doubly labeled water approach (34).

Peripheral Vascular Structure and Function

Local Arterial Stiffness and Intima Media Thickness

Participants were instrumented with an oscillometric automated BP measurement device (FMS MIDI, Finapres Medical Systems, Amsterdam, The Netherlands), and instructed to lie supine and motionless for 10 minutes. The Finapres system uses finger arterial volume via photoplethysmography (PPG) to reconstruct BA beat-to-beat values. To obtain carotid waveforms a hand-held tonometer (SPT-301 Millar Instruments, Houston, TX) was positioned over the right carotid artery at the point of greatest pulsation, and continuous pressure waveforms representative of carotid arterial BP were recorded. Since the hand-held arterial tonometer is sensitive to manual hold-down pressure, the values must be adjusted based on several assumptions: 1) diastolic blood pressure (DBP) and mean arterial pressure (MAP) are systemically similar when an individual is in the supine position, and 2) systolic blood pressure (SBP) is amplified through the arterial tree (35). To adjust the carotid artery BP values, the oscillometric automated BP measurement device was used to provide beat-to-beat measurements of BA SBP, DBP, pulse pressure (PP), and calculated MAP. The mean and minimum values from the carotid waveforms were then equated to the MAP and DBP of the BA, respectively, and the maximum carotid waveform value was used as an extrapolation point from the calibrated MAP and DBP of the BA. PP is indicative of the cushioning capacity of the arterial system to minimize pulsatility (larger values indicate greater

systemic stiffness), and is an independent predictor of adverse cardiovascular outcomes (16).

Carotid images were obtained using brightness mode ultrasound (Vivid Q, GE Medical Systems, Horten, Norway). A 13MHz linear array probe was positioned longitudinal to the left common carotid artery and an image ~2cm proximal to the bifurcation of the common carotid artery was obtained. Participants were also instrumented with an ECG (Medical Systems Corp, Miami, FL, USA) for simultaneous recordings of R-R intervals to permit determination of cardiac cycle phase (i.e. systolic vs. diastolic) during off-line analyses.

Ten sequential pressure waveforms from both the BA and right common carotid artery were collected using a commercially available data acquisition system (PowerLab, ADInstruments, Colorado Springs, USA) to calculate PP. Simultaneously, ultrasound images from the left common carotid artery were collected at a frame rate of 40 frames/second for ten consecutive complete heart cycles. Images were converted to digital imaging and communication in medicine (DICOM) files, and analyzed using custom-designed semi-automated edge-detection software (Artery Measurement System Image and Data Analysis, Tomas Gustavsson; Sweden). Minimum diameter values (enddiastole) from all 10-heart cycles were averaged for one minimum value; the same was done for maximum diameter (end-systole), located using the ECG tracing. Crosssectional carotid arterial compliance and distensibility were calculated as shown in equations 1 and 2, respectively:

(1) Compliance (mm²/mmHg) = Δ CSA / PP

(2) Distensibility (mmHg⁻¹) = Δ CSA / PP * CSAmin

From the same common carotid artery images, IMT was measured in a 10mm segment of the far wall using automated edge tracking software (EchoPac PC, Version 110; GE Medical Systems, Horten, Norway). Using the ECG recordings, end-diastolic frames of 10 consecutive heart cycles were located and average IMT was automatically calculated after selecting a region of interest. Wall to lumen ratio was calculated using IMT and diastolic lumen diameter measures.

SFA and BA IMT were assessed using the same software program. For the SFA, a 10mm segment 3-5cm distal to the common femoral bifurcation was collected, and for the BA a 10mm segment 3-5cm above the antecubital fossa was collected.

Regional Arterial Stiffness

Pulse wave velocity (PWV) is the most widely accepted noninvasive technique for indirectly measuring arterial stiffness (36), and has been shown to be a reliable measure in persons with SCI (37). PWV is calculated using the time delay between pulse pressure waveforms at two different sites a known distance apart as shown in equation 3 below:

(3) PWV (m/s) = Distance (m) / Pulse Transit Time (s)

Blood vessel waveforms were acquired via PPG sensors placed on the skin at the site of interest (IR Plethysmograph; Model MLT1020PPG; ADInstruments, Colorado Springs, USA). PPG has been validated as means of assessing PWV (38). Signals were band-pass filtered to remove frequencies below 5Hz and above 30Hz to improve detection of the onset of the waveform using LabChart software (LabChart 7; ADInstruments Inc., Colorado Springs, USA). The filter portrayed the onset of the waveform as a minimum value that corresponded with end diastole. The time delay between the filter minimum values was considered the pulse transit time. Central distance was determined as ((femoral-umbilicus + umbilicus-sternal notch) – (sternal notch-carotid)), arm distance was determined as ((radial-sternal notch) – (sternal notch-carotid)), and leg distance was determined as (femoral-posterior tibial).

Endothelium-Dependent Vasodilation

FMD is believed to represent an endothelium-dependent and largely NO-mediated vasodilation of the BA (39) and SFA (40). Duplex ultrasound (Vivid Q, GE Medical Systems, Horten, Norway) was used to examine BA and SFA FMD. Simultaneous brightness mode images (13 MHz) and pulse-waved Doppler blood velocity measurements (4 MHz) of the BA 3-5 cm proximal to the antecubial fossa, and SFA 3-5 cm distal to the common femoral bifurcation, were obtained for 30-seconds at baseline. Following baseline measurements a pneumatic cuff was positioned on the forearm or thigh and inflated to an occlusion pressure of 200mmHg (at least 50 mmHg above systolic BP) for 5 minutes using a rapid cuff inflator (E20 Rapid Cuff Inflator, AG 101 Cuff Inflator Air Source, Hokanson; Washington, USA). Upon cuff deflation, simultaneous images of the arteries and blood velocity were collected in duplex mode for 3-minutes (BA) and 5-minutes (SFA).

Images were obtained at a frame rate of 7.7 frames/s, and were ECG-gated. Diameter analyses were conduced off-line using two software programs. The first program was

used to select end diastolic frames from pre- and post-occlusion images (Sante DICOM Editor, Version 3.1.20, Santesoft; Greece); the second program was semi-automated edge-detection software that identified the near and far wall to include the media, intima, and lumen (Artery Measurement System II Image and Data Analysis, Tomas Gustavsson; Sweden). Absolute and relative FMD values were then calculated as shown in equations 4 and 5, respectively:

(4) Absolute FMD (mm) = (Peak Diameter – Baseline Diameter)

(5) Relative FMD (%) = ((Absolute FMD) / (Baseline Diameter))*100.

Blood velocity data was collected using a sample volume (gate width) encompassing the entire lumen of the artery, and outsourced to a spectral analyzer (Neurovision 500M TCD, Multigon Instruments; Yonkers, USA). The data was then passed through an analog to digital data acquisition system and fast Fourier transformation was used to determine intensity weighted mean red blood cell velocity for each beat (PowerLab 16/35 with LabChart 7 Pro, ADInstruments Inc.; Colorado Springs, USA). SR was calculated as shown in equations 6 below:

(6) SR (1/sec) = (8 * Blood Velocity) / (End Diastolic Lumen Diameter)

Test-retest reliability has been calculated previously for SFA FMD% for both SCI and AB in our lab; the intraclass correlation coefficient and coefficient of variation was 0.90 and 9% for SCI SFA FMD and 0.95 and 3% for AB SFA FMD, respectively.

Endothelium-Independent Vasodilation

It is becoming increasingly important to assess endothelial function in a comprehensive manner. Vascular tone is determined by both endothelial cell production of vasoactive substances and smooth muscle responsiveness. Thus, an abnormal vascular response to an endothelial stimulus (i.e. FMD) may be secondary to either an endothelial cell defect or to changes in smooth muscle function. As such, it is necessary to test both endothelium-dependent and -independent vasodilation in order to conclude that an impaired response is indeed related to abnormal endothelial function.

Endothelium-independent vasodilation of the BA and SFA was assessed via sublingual administration of nitroglycerine (NTG) at least 15min following FMD assessment. BA diameter was measured at 2, 4, 6, 8 and 10min post-NTG, while SFA diameter was measured at 3, 5, 7, 9, and 11min post-NTG using duplex ultrasound. A time-window of ~8 minutes has been shown to be sufficient to capture the true peak diameter response in conduit arteries following administration of a nitrate-based agent (glyceryl trinitrate) (41). Diameter analyses were conducted using the same protocol as for FMD, and similar to FMD calculations, endothelial-independent responses can be presented in absolute and relative terms.

Statistics

Statistical analyses were performed using SPSS 20.0 software. Outcome measures are presented as mean±SD; all data was normally distributed and homogenous. Frequencies were used as descriptors for categorical values when assessing traditional cardiovascular

risk factors (e.g. WC, BP, HbA1c, HDL, TG). Independent t-tests were used to assess any differences between groups (SCI vs. AB or tetra vs. para).

If baseline diameter differences were found between groups for FMD or NTG assessments, covariate-adjusted means for diameter change using a univariate analysis with group as the fixed factor was used. Briefly, we logarithmically transformed pre- and peak-diameters and calculated the change in diameter on the logged scale. This value was entered as the dependent variable with log baseline as the covariate. Covariate adjusted means for diameter change during the FMD or NTG assessments were obtained, back-transformed, and then converted to a 'corrected' adjusted percentage change by subtracting 1 from the back-transformed value and multiplying it by 100. Data are presented as mean±SD, with p<0.05 considered statistically significant.

Results:

A complete data set was collected on n=25 SCI, and n=8 AB. Missing data points vary per outcome due to participant compliance to data collection (i.e. attending one testing session and subsequently becoming unreachable; n=1), AD or hypotension during lower extremity vascular function measures (n=6), or body mass exceeding the limits for DXA scan (n=2). Demographic and injury characteristics of the participants are presented in Table 1. No differences were found in demographic information between SCI vs. AB, or between tetraplegic (tetra) vs. paraplegic (para).

Traditional Cardiovascular Risk Factors

All participants were assessed for metabolic syndrome (defined by the presence of at least three of the five traditional risk factors) (Figure 1). HbA1c was used in place of fasting glucose measures as a means of assessing long-term glycemic control (2-3 months) (42); the American Diabetes Association has recommended that a threshold for defining diabetes and metabolic syndrome be HbA1c >47.5 mmol/mol (43). Eighteen percent (6/34) of the participants with SCI and 13% (1/8) of the AB participants met the criteria for metabolic syndrome (Table 2 and Figure 1). Significant differences in BP were found between SCI vs. AB, and in TG between tetra vs. para. No differences were found between groups for other risk factors or any other lipid measures (TC, LDL, TC/HDL) (Table 2).

Body Composition

All anthropometric measures were similar between groups (Table 2). Whole body lean mass (kg), whole body fat mass (%), visceral fat (%), and legs lean mass (kg) were different between SCI vs. AB, while arms lean mass (kg) were different between tetra vs. para (Figure 2).

Aerobic Capacity and Physical Activity

 VO_2 peak measures are shown in Figure 3. Peak relative VO_2 , PO, and HR were lower in SCI vs. AB as well as in tetra vs. para. Respiratory exchange ratio (RER) was not different between groups, and reached 1.08 in the tetra group, and >1.1 for all other groups. The leg cycling VO₂peak conducted among the AB participants was used as a reference.

Overall, LTPA participation rates were good, with 73.3% of the sample engaging in LTPA at any intensity level (i.e. total LTPA). Participation rates for mild, moderate, and heavy LPTA were 60%, 57%, and 33%, respectively. Individuals with tetraplegia engaged in more 'heavy' LTPA when compared to those with paraplegia (p=0.04) (Table 3).

Peripheral Vascular Structure and Function

No differences were found between groups for indices of local (carotid compliance) or regional (central, arm, leg PWV) artery stiffness. No differences were found between groups in the carotid or BA IMT, but the AB group had a higher SFA IMT when compared to the SCI group (Table 4).

We did not calculate normalized FMD values in the present study due to a lack of correlation between SR area under the curve up to max dilation (SR_{AUC}) and FMD%. Individuals with para were found to have a larger BA diameter and lower BA SR when compared to those with tetra. After adjusting for baseline BA diameter, no differences were found between para vs. tetra for BA FMD% or NTG%. AB individuals were found to have a larger SFA diameter and lower SFA artery SR when compared the SCI group. After adjusting for baseline SFA diameter, the SCI group had lower SFA FMD% and no difference in SFA NTG% when compared to AB (Table 5).

Discussion:

The present study sought to provide a comprehensive overview of both traditional and novel CVD risk factors in a cohort of individuals with SCI and a comparison AB group. The major and novel findings of the present study were: 1) similar prevalence of traditional CVD risk factors in the SCI and AB cohorts; 2) decrements in body composition and aerobic fitness in the SCI cohort; 3) reduced SFA FMD% in the SCI cohort. Our findings assessing traditional CVD risk factors support the need to investigate more novel risk factors to help explain the increased CVD prevalence in persons with SCI (44). Underlying risk factors of poor body composition, reduced aerobic capacity, and detrimental peripheral vascular structure and function likely contribute to CVD risk.

Traditional Cardiovascular Risk Factors

The present study found a relatively low prevalence of traditional CVD risk factors in both SCI and AB of 18% and 13%, respectively. These findings agree with a previous study reporting a metabolic syndrome prevalence of 22% among individuals with SCI (n=93) (44). The patterns of risk factors tended to differ between groups however, as SCI tended to have high WC and TG in combination with low HDL when compared to the AB group, consistent with previous literature (45). BP was lower in SCI vs. AB; these results are not surprising considering individuals with high thoracic/cervical SCI typically have low BP (46). In addition, due to a sedentary lifestyle and reduced metabolic rate, individuals with SCI generally have poor lipid profiles; previous studies have reported a higher prevalence of carbohydrate and lipid disorders in SCI vs. AB (45). Contrary to these previous reports we did not observe any significant differences in blood lipid levels between groups. The occurrence of metabolic syndrome, dyslipidemia, and obesity has been shown to be dependent on demographics of the population such as age or race (47). In the present study our SCI and AB groups were similar in terms of age and race and our AB sample size was much smaller compared to the SCI group.

Within the SCI group when stratified by lesion level, differences were found in BP and TG. Lower BP among individuals with high thoracic/cervical injuries is characteristic, whereas lower TG levels in the tetra group was not anticipated. Previous studies have reported higher CVD risk among individuals with higher injury levels (48). Interestingly the individuals with tetraplegia in our SCI cohort participated in significantly more 'heavy' LTPA when compared to the paraplegic group (Table 3), possibly contributing to the lower TG levels. No other differences between lesion levels were observed, consistent with previous literature (49).

Body Composition

The AB comparison group had similar BMI to both to the paraplegic group and the whole SCI cohort, but the more comprehensive body composition assessment [DXA] revealed group differences in % body fat (p<0.001). Twenty-four percent of the present SCI cohort was obese using the AB BMI cut-off of >30 kg/m², compared to 69% classified as obese using the % body fat threshold of >25% for men and >33% for women (50). The discontinuity of obesity definitions in the SCI group raises the persistent concern that AB BMI classifications are not appropriate for the SCI population. The

previously suggested SCI-specific obesity cut-off of >22 kg/m² (51) identifies 74% of the present cohort as being obese, seemingly more appropriate. In the AB cohort one individual was classified as obese using either the BMI or % body fat definitions, suggesting BMI may be an appropriate indicator of obesity in the general AB population.

When correlating WC with VAT in both SCI and AB (p<0.001), we verify previous findings that WC is an acceptable surrogate for assessing VAT (30). A recent study showed that WC was correlated with the traditional CVD risk factors, and suggested an optimal cutoff for identifying adverse CVD risk in persons with SCI of \geq 94cm (52). Twenty-eight percent of the present SCI cohort was at risk of CVD using the AB WC cut-offs of \geq 102 cm for men and \geq 88 cm for women. Using the SCI-specific WC cut-off of \geq 94 cm, 44% of the present SCI cohort was at risk of CVD. It appears that the SCI-specific WC cut-off is more sensitive for persons with SCI than the AB guidelines, however future work should validate the use of SCI-specific WC as an indicator of CVD risk. Overall these findings suggest that measuring % body fat is ideal for determining obesity risk; however anthropometric data of SCI-specific BMI and WC may be adequate to detect obesity when % body fat is unavailable or unfeasible.

DXA scans revealed increased whole body % fat and % VAT, and decreased whole body lean and leg lean mass in the SCI cohort, agreeing with previous studies assessing body composition changes post-injury (53, 54). Increased arm lean tissue mass was also observed in the para vs. tetra group, supporting previous findings (53). These drastic changes in body fat and lean mass are expected, primarily due to autonomic dysfunction and a reduction in energy expenditure.

Aerobic Capacity and Physical Activity

The significantly diminished aerobic capacity in the SCI vs. AB cohort was anticipated; evident contributors are secondary complications, physiological limitations, psychological barriers, and limited resources or inadequate architecture (55). Aerobic capacity was also different in the tetra vs. para groups. The VO₂peak values reported in the present study are similar to others previously reported (56). Since RER reached ~>1.1 for all participants, we are confident that the physiological peak was reached during the exercise test. A VO₂peak of <15 mL/kg/min coincides with more dependency during daily life activities in SCI (13); 35% of the total present cohort, and 69% of those with tetra scored <15 ml/kg/mL on the VO₂peak assessment. Using normative values that were developed from the 20th percentiles of VO₂peak values among persons with SCI (57), participants in the present study were classified as 5 poor, 9 fair, 8 average, 5 good, and 4 excellent. These findings suggest that aerobic capacity is a useful additional tool when assessing CVD risk, and should be conducted in combination with the traditional risk factors and body composition assessment.

Individuals with SCI are considered to be one of the most sedentary populations (58). Findings from the PARA-SCI questionnaire show 73.3% of individuals with SCI participated in some LTPA at any intensity level; 50% engaged in moderate (22±27 min/day), and only 33% engaged in heavy (6±10 min/day) LTPA. The average min/day of LTPA is slightly lower in the present cohort compared to previous reports (59), possibly due to including more weekend days in the analyses, when participants are more likely to be sedentary. Interestingly, the tetra group engaged in more 'heavy' LTPA when

compared to the para group (p=0.04). When looking closer at the demographics of the two groups, it was revealed that 5 of the participants with tetra are competitive athletes, whereas only one of the participants with para is a competitive athlete. Including a measure of physical activity when evaluating CVD risk is important, as daily LTPA is associated with lower levels of CVD risk in individuals living with SCI (59). Future studies should assess whether this relationship explains a lower incidence of chronic disease.

Peripheral Vascular Structure and Function

Recent expert consensus guidelines state that central PWV (cPWV) of ≥ 10 m/s puts an individual at increased risk for morbidity and mortality (60). Twenty-six (9/34) and 13% (1/8) of the present SCI and AB cohorts had cPWV measures ≥ 10 m/s, respectively. Although previous studies have reported higher cPWV in SCI vs. AB (22, 25), no differences were found between SCI and AB for cPWV in the present study. Determinants of arterial stiffness include arterial wall composition, smooth muscle tone, and mean arterial pressure. The absence of increased cPWV in our SCI vs. AB group could be due to smooth muscle denervation; perhaps increased structural arterial stiffness is counterbalanced by decreased functional arterial smooth muscle activation. Our findings of similar peripheral PWV (arm and leg) between SCI and AB agrees with previous literature (22, 25). No differences were found between tetra and para for any of central, arm, or leg PWV.

As expected, carotid stiffness, carotid or BA IMT, and carotid or BA lumen diameter measurements were not different between SCI and AB, and the values are similar to those reported in the literature (24, 61). No differences were found between SCI vs. AB for BA FMD%, agreeing with the only other study assessing upper body FMD in SCI vs. AB (26). Within the SCI cohort, stratifying for lesion level revealed a trend towards those with high lesions to have higher BA FMD% values (p=0.09). When adjusting BA FMD% for differences in baseline diameters, the trend of higher BA FMD% in the tetra group disappeared, indicating that individuals with SCI have preserved upper body vascular structure and function when compared to AB controls.

SFA IMT was smaller in SCI vs. AB, but when normalized to lumen diameter the difference disappeared (Table 4). Perhaps the smaller IMT in the SCI SFA is a result of a smaller diameter in accordance with Laplace's Law that states the vessel diameter is related to vessel wall thickness to compensate for the change in wall tension. Although not significant, the SFA IMT normalized to diameter was larger in the SCI group, suggesting a greater thickening of the arterial intima-media. Increased SFA IMT normalized to diameter in SCI vs. AB has been previously reported (24). Femoral artery diameter adaptation is complete within 6 weeks following injury (62), and previous studies have reported a 20-30% smaller femoral artery diameter when compared to controls (14). A smaller diameter suggests that a greater proportion of the lumen is occupied by the IMT following reductions in internal diameter. No differences were observed in the present study when stratifying based on lesion level. Due to the noninvasive nature of our data collection techniques, it is not possible to differentiate between tunica media remodeling and tunica intima thickening when assessing IMT.

The smaller diameter is likely the cause of increased mean SR through the femoral artery after SCI (26, 27). Results from the present study show similar findings to the literature of smaller SFA diameter and higher SFA SR in the SCI group. Contrary to previous studies, no differences were detected between groups for SFA FMD%. The majority of previous work assessing lower extremity FMD% show preserved or enhanced values in SCI vs. AB (21, 26-28); only one study has reported reduced FMD% after SCI in the smaller posterior tibial artery (63). Studies have suggested the chronic elevations in SR resulting in a heightened sensitivity to NO / up-regulation of endothelial nitric oxide synthase (eNOS) may provide an explanation for preserved or enhanced lower extremity FMD responses (28). Indeed, the present study observed a greater response to NTG (an NO donor) in the SCI group, supporting these speculations. However, after adjusting for baseline SFA diameter the differences in SFA NTG% between groups disappeared, agreeing with previous literature (26, 27). Further, after adjusting for baseline SFA diameter the SCI group had lower SFA FMD% when compared to AB. To the author's knowledge, this is the first report of reduced SFA FMD% when comparing SCI vs. AB, likely attributed to adjusting for baseline diameter, as well as differences in experimental protocol (i.e. occlusion time, baseline conditions, etc.). These results suggest that unadjusted SFA FMD% should be interpreted with caution. Although these findings provide important insight into sublesional vascular health, it is crucial that future studies validate the use of FMD% as an indicator of endothelial function in deconditioned limbs. SCI disrupts supraspinal control of the sympathetic circuits that innervate peripheral blood vessels, so it is possible that altered sympathetic innervation contributes to

sublesional FMD% after SCI. Future studies with larger representative groups of individuals with SCI are necessary to determine the relationship between peripheral arterial vasculature and atherosclerosis.

Limitations

We did not exclude participants with SCI with previous CVD or current cardiovascular risk factors; seventeen individuals (50%) were previous or current smokers, one had sustained a previous heart attack, and two had sustained a previous stroke. We did not include a measure of physical activity for the AB comparison group; comparing physical activity between the SCI and AB groups may have provided additional insight into peripheral vascular differences. In addition, our AB comparison group was small, making it difficult to detect small differences between groups (i.e. increased risk of type II error).

Summary

We found a similar prevalence rate for traditional CVD risk factors in the SCI and AB groups, suggesting novel risks may contribute to the increased CVD risk in individuals with SCI. Detrimental body composition changes, reduced aerobic capacity, limited daily LTPA, decreased sublesional conduit artery diameter, increased SR, and decreased FMD% observed in the SCI group could potentially help explain higher CVD risk and be used to more comprehensively assess risk in the future. Mechanisms underlying vascular dysfunction after SCI have not been adequately investigated; future studies should assess the relationships between vascular structure and function indices

and CVD prevalence in a larger cohort of individuals with SCI. It is likely that exercise improves vascular function and future studies should investigate the effect of physical activity interventions on vascular structure and function.

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Parameter	SCI	AB	Tetra	Para
Sex (M/F)	32/2	8	20/2	12
Age, years	40±11	43±7	39±11	42±11
AIS	17 A-B		9 A-B	8 A-B
AIS	17 C-D		13 C-D	4 C-D
NLI	C1-T11		C1-C6	T4-T11
YPI	13±10		14±10	11±10
Smoker (Y/N)	6/28	0/8	2/20	4/8

Values are mean±SD. SCI = spinal cord injury; AB = able-bodied; BMI = body mass index; AIS = American Spinal Injury Association Impairment Scale; NLI = neurological level of injury; YPI = years post injury. No significance found between SCI vs. AB or between tetra vs. para.

Table 1: Participant characteristics.

Parameter	SCI (n=34)	AB (n=8)	Tetra (n=22)	Para (n=12)
Mass, kg	82.1±16.6	86.2±13.6	84.3±16.9	78.0±15.9
Height, m	1.8±0.1	1.8 ± 0.1	1.8 ± 0.1	1.7±0.1
BMI, kg/m^2	26.6±4.7	25.8±3.8	27.1±5.1	25.6±4.0
WC, cm	93±14	92±9	95±14	90±13
HR, bpm	72±10	64±14	71±10	73±11
SBP, mmHg	112±21	129±17*	102±18	129±13†
DBP, mmHg	68±13	80±9*	62±10	79±11†
MAP, mmHg	83±15	96±11*	75±12	95±11†
HbA1c, mmol/mol	35.4±8.3	36.5±2.4	34.4±7.9	37.1±9.0
HDL, mmol/L	1.09±0.31	1.22 ± 0.17	1.07 ± 0.34	1.13±0.27
TG, mmol/L	1.25 ± 0.62	1.00 ± 0.33	1.00 ± 0.46	1.68±0.64†
TC, mmol/L	4.51±1.08	4.76 ± 0.78	4.15±0.96	5.14±1.00
LDL, mmol/L	2.85 ± 0.86	3.08 ± 0.56	2.62 ± 0.78	3.26 ± 0.87
TC/HDL	4.29±1.05	3.90±0.49	4.05 ± 0.98	4.73±1.06

Values are mean \pm SD. SCI n=33 for blood analyses. SCI = spinal cord injury; AB = able-bodied; WC = waist circumference; VAT = visceral adipose tissue; SBP = seated systolic blood pressure; DBP = seated diastolic blood pressure; MAP = seated mean arterial pressure; HbA1c = glycated haemoglobin; HDL = high density lipoprotein; TG = triglycerides. *p-value <0.05 vs. SCI or vs. tetraplegic; †p-value <0.01 vs. SCI or vs. tetraplegic.

Table 2: Traditional cardiovascular disease risk factors (i.e. metabolic syndrome).



Figure 1: Traditional cardiovascular disease risk factors (i.e. metabolic syndrome).



Figure 2: Body composition from dual energy x-ray absorptiometry (DXA). SCI n=29; AB n=8; tetra n=17; para n=12.



Figure 3: Peak aerobic capacity (VO₂peak) from a peak arm and leg cycle ergometer test. SCI n=31; AB n=8; tetra n=19; para n=12.

Parameter	SCI (n=30)	Tetra (n=18)	Para (n=12)
LTPA			
Total	42.8±9.5	45.5±10.0	38.6±14.4
Mild	15.7±24.6	12.0±17.1	21.3±33.1
Moderate	21.4±26.9	25.1±30.4	16.0±20.7
Heavy	5.6±9.5	8.4±11.0	1.3±4.3*

Values are minutes per day of physical activity as mean \pm SD. LTPA = leisure time physical activity. *p-value <0.05 vs. tetraplegic; †p-value <0.01 vs. tetraplegic.

Table 3: Leisure time physical activity among participants with spinal cord injury.

Parameter	SCI (n=34)	AB (n=8)	Tetra (n=22)	Para (n=12)
Local Stiffness (Carotid)				
Pulse Pressure, mmHg	44±14	39±6	42±16	48±9
Compliance, mm ² /mmHg	0.126 ± 0.060	0.150 ± 0.038	0.134 ± 0.070	0.111 ± 0.035
Distensibility, mmHg ⁻¹	0.0043 ± 0.0025	0.0048 ± 0.0012	0.0048 ± 0.0029	0.0035 ± 0.0011
Lumen Diameter, mm	5.74 ± 0.82	5.81 ± 0.47	5.59±0.56	$6.02 \pm .13$
Regional Stiffness				
cPWV, m/s	9.0 ± 4.7	7.4±1.8	8.6±4.6	9.8±5.1
aPWV, m/s	9.1 ± 3.0	7.9±2.2	9.3 ± 3.0	8.8±3.1
IPWV, m/s	8.1±1.8	8.0±1.3	7.9±1.9	8.4±1.7
Intima-Media Thickness				
Carotid IMT, mm	0.55 ± 0.11	0.55 ± 0.10	0.54 ± 0.08	0.58 ± 0.16
Carotid Wall/Lumen, %	9.7±2.4	9.6±2.4	9.7±0.2	9.6±1.7
Brachial IMT, mm	0.34 ± 0.07	$0.29 \pm .02$	0.33 ± 0.07	0.36 ± 0.08
Brachial Wall/Lumen, %	8.5±2.4	8.0±1.3	8.9 ± 2.8	8.0 ± 2.0
SFA IMT, mm	0.40 ± 0.08	$0.46\pm0.06*$	0.40 ± 0.09	0.39 ± 0.06
SFA Wall/Lumen, %	8.4±1.9	7.8±3.8	8.3±2.3	8.4±1.3
Values are mean±SD. SCI =	spinal cord injury; /	AB = able-bodied;]	IMT = intima medi	a thickness; LD =
lumen diameter; $cPWV = cen$	tral pulse wave velo	city (carotid-femor:	al); $aPWV = arm pu$	ulse wave velocity
(carotid-radial); $IPWV = leg f$	oulse wave velocity	(femoral-dorsalis pe	dis). *p-value <0.0	5 vs. SCI.

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Parameter	SCI	AB	Tetra	Para
Brachial Artery				
FMD				
Sample size, <i>n</i>	34	8	22	12
BL LD, mm	4.30±0.60	3.99±0.39	4.13±0.54	4.62±0.60*
Time to Max, s	60±28	58±15	61±21	57±38
FMD, %	7.37±2.94	8.36±5.25	8.00 ± 3.00	6.21±2.62
'Corrected FMD', %			7.68 ± 1.70	6.72±2.27
Baseline SR, 1/s	126±61	102 ± 46	148±59	86±42†
Peak SR, 1/s	813±272	934±229	833±268	777±288
SR_{AUC} , 10^3	18.9±18.3	22.9±14.0	17.8±15.6	20.9±23.1
NTG				
Sample size, <i>n</i>	34	8	22	12
BL LD, mm	4.31±0.60	4.13±0.49	4.09±0.51	4.72±0.55†
Time to Max, s	409±127	465±42	398±121	430±140
NTG, %	18.57±5.59	21.97±3.97	19.13±5.80	17.54±5.26
'Corrected NTG', %			18.18±2.84	19.01±4.27
SFA				
FMD				
Sample size, <i>n</i>	32	8	20	12
BL LD, mm	5.46 ± 0.71	7.29±0.90†	5.45 ± 0.76	5.48 ± 0.64
Time to Max, s	66±38	72±27	60±21	77±56
FMD, %	6.65 ± 2.68	7.22±1.67	6.78 ± 2.58	6.44±2.95
'Corrected FMD', %	6.18±1.13	8.87±2.84*		
Baseline SR, 1/s	105±71	52±14*	96±59	122±90
Peak SR, 1/s	687±322	694±205	674±297	707±373
SR_{AUC} , 10^3	19.7±19.4	20.9±16.6	16.8 ± 14.4	24.5±25.7
NTG				
Sample size, <i>n</i>	28	8	16	12
BL LD, mm	5.51±0.82	7.64±1.04†	5.49 ± 0.95	5.55 ± 0.62
Time to Max, s	529±103	525±150	510±112	555±89
NTG, %	14.90 ± 5.16	9.22±3.25†	14.68 ± 5.08	15.20±5.47
'Corrected NTG' %	13 66+2 27	13.09 ± 5.14		

Values are mean±SD. SCI = spinal cord injury; AB = able-bodied; FMD = flow mediated dilation; BL LD = baseline lumen diameter; SR = shear rate; SR_{AUC} = shear rate area under the curve up to max dilation; NTG = nitroglycerin; SFA = superficial femoral artery. *p-value <0.05 vs. SCI or vs. tetraplegic; †p-value <0.01 vs. SCI or vs. tetraplegic.

Table 5: Vascular function.

CHAPTER 3

Superficial femoral artery endothelial responses to a short-term altered shear rate intervention in healthy men

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Abstract

In animal and in-vitro models, increased oscillatory shear stress characterized by increased retrograde shear-rate (SR) is associated with acutely decreased endothelial cell function. While previous research suggests a possible detrimental role of elevated retrograde SR on endothelial-function in the brachial artery in humans, little research has been conducted examining arteries in the leg. Examinations of altered shear pattern in the superficial femoral artery (SFA) are important, as this vessel is both prone to atherosclerosis and leg exercise is a common form of activity in humans. Seven healthy men participated; bilateral endothelial-function was assessed via flow-mediated-dilation (FMD) before and after 30-minute unilateral inflations of a thigh blood pressure cuff to either 75mmHg or 100mmHg on two separate visits. Inflation of the cuff induced increases in maximum anterograde (p<0.05), maximum retrograde (p<0.01), and oscillatory shear index (OSI) (p<0.001) in the cuffed leg at both inflation pressures. At 100mmHg the increases in SR were larger in the retrograde than the anterograde direction evidenced by a decrease in mean SR (p<0.01). There was an acute decrease in relative FMD in the cuffed leg alone following inflation to both pressures. These results indicate that in the SFA, altered SR profiles incorporating increased retrograde and OSI influence the attenuation in FMD after a 30-minute unilateral thigh-cuff inflation intervention. Novel information highlighting the importance of OSI calculations and assessments of flow profiles add to current body of knowledge regarding the influence of changes in SR patterns on FMD. Findings from the current study may provide additional insight when designing strategies to combat impaired vascular function in the lower extremity where blood vessels are more prone to atherosclerosis in comparison to the upper extremity.

Alphabetized Key Words: endothelial function, flow mediated dilation, oscillatory shear rate, shear rate pattern, superficial femoral artery

Introduction:

Endothelial dysfunction is widely accepted as a precursor to the development of atherosclerosis, may contribute to later stage cardiovascular disease (CVD) (1), and is predictive of CV events in healthy individuals as well as in those with existing CVD (2). Wall shear stress is an important determinant of endothelial cell function (3). *In vitro* studies indicate that high shear stress induces atheroprotective endothelial gene expression (e.g. increased production of endothelial nitric oxide synthase), while low shear stress stimulates an atherogenic phenotype (e.g. increased production of endothelin 1, thrombomodulin, vascular cell adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1]) (4, 5). The pattern of shear stress also contributes to the regulation of vascular structure and function. In cultured cells and animal models, laminar unidirectional shear stress maintains normal endothelial structure and function, while stasis, turbulent flow, or oscillatory shear stress induces an atherogenic state and is associated with inflammation, atherosclerotic lesions, lower numbers of stress fibers, and high endothelial permeability (6).

Shear stress can be approximated non-invasively *in vivo* by shear rate (SR = blood velocity/lumen diameter). One assumption for SR calculations is that blood velocity moves in a parabolic manner. It has been demonstrated in animals that anterograde blood velocity moves in a parabolic-like shape (7, 8), however one study conducted in dogs reported a non-parabolic profile of retrograde blood velocity (7). A recent study in humans examined the shape of anterograde and retrograde blood velocity profiles through the femoral artery using the relationship between mean (MBV) and peak (PBV)

blood velocity across the vessel lumen; they defined a parabolic velocity when mean velocity is half the peak velocity (MBV/PBV = 0.5) (9). They found mean, anterograde, and retrograde profiles were parabolic in shape at rest (i.e. MBV/PBV ratio not different from 0.5). These findings suggest calculations of mean, anterograde, and retrograde SR are acceptable approximations when evaluating endothelial function via flow-mediated dilation (FMD), and when examining resting SR patterns.

The influence of SR patterns on FMD has not been extensively investigated in humans in vivo, and most investigations have focused on the brachial artery. Three human studies in this area suggest increasing anterograde SR acutely enhances brachial artery FMD and can counteract the potential negative influence of elevated retrograde SR, while increased retrograde SR alone acutely impairs brachial artery FMD (10-12). In contrast to the upper extremity, the vasculature of the lower extremity is highly susceptible to atherosclerotic lesion formation (13, 14). It has been reported that resting SR in the superficial femoral artery (SFA) are lower than those in the brachial artery (15), and that the normal pattern of SR in the SFA includes a larger retrograde component in comparison to the brachial artery (16). This chronically lower wall shear stress, along with higher turbulence through the SFA due to anatomical structure, has been suggested to contribute to higher incidence of atherosclerosis in comparison to the upper extremity blood vessels (15, 17). Further, vascular disease risk is often detectable in the blood vessels of the legs rather than the arms (i.e. intermittent claudication, peripheral arterial disease) (18). One recent study assessing SFA FMD response to induced retrograde SR reported decreased FMD following a 60mmHg thigh cuff inflation intervention; however
the researchers were unable to create an exclusively retrograde shear stimulus as had been done previously in the brachial artery with similar interventions (19). The purpose of the present study was to extend this work by examining the acute effects of higher cuff inflation pressures (75 and 100mmHg) designed to induce greater increases in retrograde SR and thereby maximize the impact on SFA endothelial function as measured by FMD. We hypothesized that cuff pressures of 75 and 100mmHg would induce elevations in retrograde SR that would retain a parabolic flow profile and would impair SFA FMD in young healthy males in a dose dependent manner.

Methods:

Participants

Nine healthy recreationally active men (age: 26.6 ± 5.9 years; waist circumference: 82.8 ± 5.8 cm; body mass index: 23.8 ± 2.2 kg/m²) were recruited from McMaster University in Hamilton, ON, Canada. None of the participants had been diagnosed with CVD or any risk factors leading to the development of CVD such as hypercholesterolemia, hyperlipidemia, or hypertension, and none were on any medications that are known to affect the cardiovascular system. The study procedures were approved by the Hamilton Health Sciences Research Ethics committee in Hamilton, Ontario, Canada, adhered to the Declaration of Helsinki, and all of the participants gave previous written informed consent.

Experimental Design

Each participant came to the laboratory on two occasions for approximately two hours in a fasted state (\geq 8 hours), abstained from caffeine and alcohol for \geq 12 hours, and abstained from physical activity for a minimum of 24 hours. Both visits were done at the same time of day to eliminate diurnal variation. Assessment was conducted in a quiet, temperature controlled room (22-24°C). SFA FMD was assessed in both legs (cuffed [altered flow intervention] and non-cuffed [control]) before and immediately after a 30-minute unilateral thigh cuff-inflation intervention designed to alter SR.

Experimental Procedures

Flow Mediated Dilation and Shear Rate

FMD is an established and reliable method of assessing endothelial-dependent vascular function and is considered to be primarily nitric oxide mediated in the more commonly assessed brachial artery (20, 21). There is recent evidence that SFA FMD is also primarily nitric oxide mediated (2, 22). Prior to the baseline FMD assessments, participants rested in the supine position for at least 15-minutes to ensure stability in resting supine leg blood flow, blood pressure (BP), and heart rate (HR) measures. HR was monitored continuously throughout all testing procedures using a single lead electrocardiogram (ECG; Model ML123, ADInstruments Inc.; Colorado Springs, USA), and supine BP was monitored in triplicate at baseline and immediately following cuff release using an automated blood pressure device (Dinamap, GE Healthcare; Horten, Norway).

At baseline a three-heart cycle brightness mode image and 30-seconds of MBV using pulsed-wave Doppler were recorded of the SFA approximately 3-5cm distal to the common femoral artery bifurcation using high-resolution ultrasound (System FiVe, GE Medical Systems; Horten, Norway). A large cuff (CC17 Hokanson; Washington, USA) was placed around the upper thigh approximately 10cm distal to the greater trochanter and instantaneously inflated to 200mmHg for 5-minutes (E20 Rapid Cuff Inflator, AG 101 Cuff Inflator Air Source, Hokanson; Washington, USA). Upon cuff deflation reactive hyperemic MBV through the SFA was assessed for the first 30-seconds and subsequently three-heart cycle SFA diameter images were digitally stored at standardized time points for 5-minutes following cuff release (45s, 60s, 75s, 90s, 120s, 180s, 240s, 300s) (2).

Images were ECG-gated, collected at a frame rate of 15 frames/s, and were stored for off-line analyses (23). End diastolic frames were selected from each three-heart cycle image to create a 3-frame stacked digital imaging and communications in medicine file (Sante DICOM Editor, Version 3.1.20, Santesoft; Greece). Stacked SFA end diastolic diameters were analyzed from the near wall to the far wall to include the intima, media, and lumen using custom-designed semi-automated edge-detection software (Artery Measurement System Image and Data Analysis, Tomas Gustavsson; Sweden). Relative FMD was calculated as shown in equation 1 below:

(1) Relative FMD (%) = ((Peak – Baseline Diameter) / (Baseline Diameter))*100.

Sample volume (gate width) encompassed the entire lumen (from intima-to-intima) for MBV measurements using pulsed-wave Doppler. Raw blood velocity profiles were outsourced to a spectral analyzer (Neurovision 500M TCD, Multigon Instruments;

Yonkers, USA). Intensity weighted MBV was determined using fast Fourier transformation and acquired with an analog to digital data acquisition system for offline beat-to-beat analyses (PowerLab 16/35 with LabChart 7 Pro, ADInstruments Inc.; Colorado Springs, USA). Velocity measures were collected at an insonation angle of 68 degrees for all participants. MBV SR was calculated by dividing the blood velocity values by the end diastolic arterial diameters as shown in equations 2 below:

(2) SR (1/sec) = (8 * Mean Blood Velocity) / (End Diastolic Lumen Diameter)

Reactive hyperemic SR from 0-30s (SR_{AUC}0-30s) after cuff release has been shown to significantly correlate with relative FMD in young adults similar to the correlation between FMD and the commonly assessed area under the SR curve until the time to peak diameter (24). We therefore used the SR_{AUC}0-30s to represent the SR stimuli after cuff release as the full SR response until peak dilation was not technically possible to obtain with our equipment.

Oscillatory shear index (OSI) was used as an indicator of the magnitude of shear oscillation or shear reversal. For purely oscillatory flow, the OSI attains a maximum value of 0.5. Consistently high values of OSI have been associated with endothelial dysfunction (25). OSI was calculated as shown in equation 3 below:

(3) OSI = (|retrograde SR|) / (|retrograde SR| + |anterograde SR|)

Altered Shear Intervention

The same large leg cuff used for the assessment of FMD% was used to induce altered SR patterns in the dominant leg. Leg dominance was determined to be the same side as

hand dominance. Following baseline diameter and blood velocity measures, the cuff was inflated to 75mmHg on the first visit and 100mmHg on the second visit for 30-minutes. The cuff inflation pressures were chosen based on a previous study reporting impaired brachial artery FMD% following 30-minutes of forearm cuff pressure at 75mmHg (10). Owing to the larger SFA diameter and in keeping with the aim of creating an exclusive retrograde stimulus, we further selected the 100mmHg inflation pressure. SFA diameters as well as blood velocity measures were obtained every 5-minutes throughout the 30-minute intervention and stored for off-line analyses (Figure 1). Mean, anterograde, and retrograde SR were determined over a 30-second period every 5-minutes throughout the intervention.

Blood Velocity Shape

At baseline and at each data collection time point throughout the 30-minute cuffinflation intervention, intensity-weighted MBV and PBV were outsourced separately to the same spectral analyzer as described above (Neurovision 500M TCD, Multigon Instruments; Yonkers, USA; PowerLab 16/35 with LabChart 7 Pro, ADInstruments Inc.; Colorado Springs, USA).

Values for MBV, PBV, and MBV/PBV ratio were determined for mean, anterograde, and retrograde phases of the cardiac cycle during rest. Excess retrograde blood velocity is purposefully created during the cuff inflation intervention, resulting in altered ratios of MBV to PBV that influence the entire cardiac cycle calculations. We therefore calculated MBV, PBV, and MBV/PBV for the anterograde and retrograde components only during the cuff-inflation intervention. The anterograde phase included all anterograde blood velocity and the retrograde phase included all retrograde blood velocity within an entire cardiac cycle. When MBV/PBV=0.5 parabolic velocity was present, MBV/PBV=1.0 represented a plug-like profile, and MBV/PBV≈0 represented a sharpened parabolic profile (9).

Statistics

Statistical analyses were performed using SPSS 17.0 software. Paired t-tests were used to assess the baseline characteristics between the two testing days. Repeated measures analysis of variance was used to determine the effects of time (pre vs. postintervention) and leg (cuffed vs. non-cuffed) on FMD and SR. Post-hoc t-tests were performed when a significant main or interaction effect was found. Paired t-tests were used to determine the differences in SR and SR patterns at baseline and at 30-minutes of the intervention.

Single sample t-tests were used to determine if MBV/PBV ratios were different from 0.5 at rest and during increased retrograde SR, and paired t-tests were used to determine if MBV/PBV ratios were different between baseline and increased retrograde SR conditions.

Results:

Due to discomfort, one participant could not complete the 75mmHg and another could not complete the 100mmHg testing session; therefore n=8 participants completed both testing days, and n=7 were included in the final analysis. Pre-intervention BP, HR, SR, and FMD were not different across the two testing days (Tables 1 and 2). There were significant increases in peak retrograde (p<0.01, p<0.01) and anterograde (p<0.01,

p=0.05) SR in the cuffed leg but not the control leg during the 75 and 100mmHg interventions, respectively (Figures 2 and 3). There was no dose response change in SR parameters to the increasing inflation pressure; however during the 100mmHg cuff pressure intervention the larger increase in retrograde SR vs. anterograde SR resulted in a decrease in mean SR (p<0.01). There was a significant increase in OSI in the cuffed leg only during cuff inflation intervention to both 75 and 100mmHg (p<0.001; Table 2).

A significant interaction (time x leg) indicated a decrease in FMD% in the cuffed leg only after both the 75 and 100mmHg interventions (p<0.05, Figures 2 and 3). Baseline SFA reproducibility of FMD% assessment between testing days agrees with previous literature; the ICC and CV was 0.57 and 25%, respectively.

At baseline the mean ratio of MBV/PBV was greater than 0.5 indicating a blunted parabolic shape; the retrograde ratio was less than 0.5 indicating a sharpened parabolic shape; the anterograde ratio was not different from 0.5 indicating a parabolic shape. At 30-minutes of the cuff inflation intervention the retrograde ratio was less than 0.5 and the anterograde ratio was greater than 0.5. Both anterograde and retrograde ratios increased from baseline to 30-minutes of the cuff intervention (Table 3).

Discussion:

Our results provide novel information about changes in OSI as well as the shape of the flow profile while supporting the concept that a brief period of enhanced oscillatory SR acutely attenuates FMD% in the legs of humans. Our results also challenge previous conclusions that these FMD% changes can be exclusively attributed to retrograde SR changes induced by cuff inflation interventions. Consistent with previous reports examining both the upper and lower limbs (10, 19), our unilateral leg cuff procedures induced altered SFA SR patterns that resulted in acute decreases in FMD% in the cuffed leg only. Despite using higher cuff pressures than previous work, we were not able to induce an exclusive retrograde SR alteration. In the cuffed leg we observed changes in both anterograde and retrograde SR at both cuff inflation pressures, however we observed larger increases in retrograde SR at the 100mmHg cuff pressure, evidenced by a decrease in mean SR (Figure 3). No alterations in flow patterns were observed in the non-cuffed leg.

Decreases in mean and increases in oscillatory SR have been shown to be detrimental to endothelial cells (26, 27), and are observed in arteries where the propensity for atherosclerosis is higher (1, 15, 28). Although OSI cannot differentiate between uniaxial and multidirectional flows, high OSI has been is thought to trigger atherosclerosis (29). A recent review assessing the relationship between various methodologies of wall shear stressors and atherosclerosis reported that while a few previous studies have shown no relationship between wall shear stress and disease, the majority of evidence points to the involvement of OSI in atherosclerotic progression (30). Our findings of an increase in OSI following the cuff-inflation interventions and subsequent decrease in FMD% may support the proposed influence of OSI on endothelial cells.

Our intention was to induce a retrograde stimulus without affecting anterograde SR through the SFA at rest, thereby replicating the stimulus applied to the brachial artery in a previous study (10). As both retrograde and anterograde SFA SR were augmented in response to our 30-minute thigh cuff inflations, we cannot comment on the effects of

isolated increases in retrograde SR on endothelial health. Since anterograde SR is considered to be atheroprotective (6), it is likely the acute increases in anterograde SR observed with the present protocol provided some cardio-protective effect to the SFA. It is possible that a more pronounced decrease in SFA endothelial function could occur following an exclusive retrograde stimulus. In contrast to previous work in the upper extremity (10), it seems it is not possible to induce an exclusive retrograde SR stimulus without impacting anterograde SR through the SFA using the present protocol. However, the mixed SR pattern changes induced with the thigh cuff may be more reflective of the complex SR changes commonly observed with enhanced vascular tone.

The same group who previously presented an isolated retrograde stimulus in the brachial artery recently examined the effects of an altered SR profile on both brachial and SFA FMD but were not able to replicate their previous observations of an exclusive increase in retrograde SR (19). In this recent study, cuff inflation to 60mmHg resulted in mixed SR effects with increases in both anterograde and retrograde SR through both the SFA and the brachial artery (19). In agreement with their previous work, they reported a correlation between change in FMD% and change in retrograde SR, and a decrease in FMD% in both arteries. They combined these findings with their initial study results and suggested that a threshold for external pressure exists between 30 and 50mmHg; however it is difficult to conclude that this threshold is the same for the SFA as the initial study included the brachial artery only. In our current study we found a greater relative retrograde SR response to 100mmHg vs. 75mmHg evidenced by a decrease in mean SR at 100mmHg. Future studies should explore a variety of cuff pressures and intervention

lengths to ascertain whether creating an exclusive retrograde stimulus is possible through the SFA. Future studies should also calculate OSI when assessing altered SR patterns as it may be that simply evaluating the change in retrograde SR is not sufficient to assess the complex relationship between SR and endothelial function.

When calculating peripheral artery SR in humans it is assumed that the blood velocity profile is parabolic in shape; a recent study in humans by Ade et al. (2012) reported parabolic profiles for mean, anterograde and retrograde phases at rest, but during an increased downstream resistance intervention (cold pressor test), the mean and anterograde components exhibited a plug-like profile (MBV/PBV >0.5) (9). In the present study we observed a sharpened retrograde parabolic profile (ratio <0.5), a pluglike mean profile (ratio >0.5), and a parabolic anterograde profile (ratio =0.5) at rest. During the cuff inflation intervention, we observed an increase in both anterograde and retrograde ratios; the retrograde profile remained a sharpened parabolic shape, while anterograde became a plug-like profile. The disparity between study results may be due to a number of factors: a) our intervention was local (on the thigh), whereas the previous study used a cold pressor test on the hand to induce sympathetic activation; b) our intervention assessed blood velocity profiles in the SFA, whereas the previous study assessed the common femoral artery, likely introducing variant responses due to the proximity of our measurements to the common femoral artery bifurcation; and c) our analysis included anterograde and retrograde blood velocity throughout the entire cardiac cycle, whereas the previous study only considered the first two phases of the cardiac cycle (see Figure 1). Caution should be employed when interpreting the results from Ade et al. due primarily to the last rationale. Our work extends the knowledge in the field regarding true blood velocity shape across the entire cardiac cycle; however our findings of non-parabolic profiles suggests some error may be introduced into SR calculations. Future work should examine each phase of the cardiac cycle separately for blood velocity shape.

Limitations of our study include sample size and method of data acquisition. Although we saw significant results with the current sample size, it is possible the results would be more pronounced with a larger sample size. Our method of data acquisition was such that we were not able to simultaneously collect FMD measures on the cuffed and non-cuffed leg. Post-FMD measurement on the non-cuffed control leg was conducted after data collection on the cuffed leg (approximately 15-minutes following the cuff inflation intervention); theoretically FMD in this contra-lateral leg could have also been depressed immediately following the 30-minute intervention and then returned to baseline after 15-minutes. However, our SR data in the control leg were obtained during the cuff intervention and showed no changes in local flow environments as a result of the contralateral cuff inflation. In addition, it has been shown that alterations in FMD are persistent for up to 2-hours post-exercise (31). Several other studies looking at endothelial health changes following various acute exercise interventions found alterations were still evident 30-minutes to 1-hour post-intervention (32, 33); it is therefore unlikely that FMD in the control leg would have been attenuated and then returned to baseline within 15-minutes.

This study directly advances the current knowledge about the influence of modifications in oscillatory SR on arterial structure and function and presents novel data about the regulation of endothelial function in clinically important lower limb arteries. Endothelial dysfunction is common in conditions where there is increased vascular tone, such as age, obesity, and hypertension. Chronic changes in endothelial function may be related to alterations in both SR magnitude and pattern; effective interventions to improve vascular health should take into account these regulatory mechanisms. Future studies should explore the acute effects of inducing an exclusively retrograde shear stimulus on endothelial function in the lower extremity in healthy as well as clinical populations.

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	75mmHg		100mmHg		
Parameter	Before	After	Before	After	p-value
SBP, mmHg	129 ± 21	134±19	127±8	130±10	0.78
DBP, mmHg	75±14	79±16	69±6	73±7	0.31
MAP, mmHg	98±21	102±27	88±6	92±8	0.25
HR, bpm	65±10	67±13	66±11	66±11	0.65

Values are mean \pm SD. Abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate. P value refers to paired t-tests between the pre-intervention values between the two testing days. No differences were found pre- to post-intervention at either cuff pressures.

Table 1: Supine blood pressure and heart rate. Data are for participants before and after both interventions (n=7).



Figure 1: SFA Cuff-Inflation Intervention (Doppler Screen Capture). Screen capture of the Doppler velocity profile before and at 30-minute of the (A) 75mmHg and (B) 100mmHg cuff inflation in the cuffed leg.

		Cuffed		Ι	Non-Cuffed	
Parameter	Before	After	p-value	Before	After	p-value
75mmHg						
Baseline D, mm	6.5 ± 0.4	6.4 ± 0.4	0.506	6.7 ± 0.5	6.7 ± 0.6	0.390
Peak SR, s ⁻¹	810 ± 218	910 ± 158	0.231	793±354	942±176	0.153
SR_{AUC} 0-30, 10 ³	5.32 ± 1.73	6.08 ± 2.31	0.372	5.80 ± 3.41	6.72 ± 2.06	0.219
OSI	0.30 ± 0.06	0.43 ± 0.03	<0.001	0.24 ± 0.07	0.27 ± 0.07	0.148
SFA FMD, mm	0.47 ± 0.19	0.19 ± 0.17	0.018	0.50 ± 0.19	0.48 ± 0.20	0.636
100mmHg						
Baseline D, mm	6.0 ± 0.6	6.1 ± 0.7	0.756	6.0 ± 0.6	6.0 ± 0.6	0.794
Peak SR, s ⁻¹	958±127	866±264	0.320	903±307	1001 ± 372	0.455
SR_{AUC} 0-30, 10 ³	5.57±1.74	5.18 ± 1.20	0.609	5.71 ± 2.30	6.48 ± 3.15	0.437
OSI	0.28 ± 0.05	0.42 ± 0.06	<0.001	0.27 ± 0.07	0.29 ± 0.07	0.450
SFA FMD, mm	0.58 ± 0.23	0.40 ± 0.26	0.012	0.51 ± 0.27	0.51 ± 0.32	0.993
Values are mean±SD.	D = diameter;	SR_{AUC} 0-30 = s	shear rate are	a under curve for	r 30sec; OSI =	oscillatory
shear index; SFA FML) = superficial f	emoral artery flo	ow mediated	dilation. P value	refers to paired	t-tests pre-
vs. post-cuff inflation i	ntervention. No	differences wer	e found betw	een baseline valu	es on the two te	sting days,
or between cuffed vs. n	ion-cuffed leg ba	aseline measures				
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characteristics	d leg (n=7).
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Table 2: Su	Data is of pa



Pre-Intervention
During/Post-Intervention





Figure 3: SFA 100mmHg Cuff-Inflation Intervention. On the left (A): mean, anterograde (+ve), and retrograde (-ve) SR patterns pre- and during the intervention in the cuffed leg. On the right (B): FMD% before and after the intervention in the cuffed and non-cuffed leg; mean and individual data are presented (n=7). Error bars represent SD.

Parameter	Before	p-value	At 30min	p-value	p-value
		vs. 0.5		vs. 0.5	pre vs. post
Cardiac Cycle					
$MBV (cm s^{-1})$	6.4±2.6				
PBV (cm s^{-1})	10.1±4.6				
MBV/PBV	0.65 ± 0.14	< 0.001			
Anterograde					
MBV (cm s ⁻¹)	11.0 ± 4.5		20.0±9.3		< 0.001
PBV (cm s^{-1})	22.2±8.9		35.6±15.1		0.001
MBV/PBV	0.49 ± 0.05	0.600	$0.55 \pm .05$	0.003	0.006
Retrograde					
$MBV (cm s^{-1})$	-4.5±2.4		-15.1±7.3		< 0.001
PBV (cm s^{-1})	-12.1±5.7		-34.7±14.7		< 0.001
MBV/PBV	0.37±0.05*	0.001	$0.42 \pm 0.07 *$	< 0.001	0.034

Values are mean±SD. Abbreviations: MBV = average mean blood velocity; PBV = average peak blood velocity; "P value vs. 0.5" refers to single sample t-tests between the MBV/PBV ratio and 0.5 (the blood velocity profile is a perfect parabola). "P value pre vs. post" refers to paired t-tests between preintervention (before) and at 30-min of intervention (at 30min). *p<0.001, significantly different from anterograde MBV/PBV ratio.

Table 3: Superficial femoral artery hemodynamics. Data is responses to 30min cuff-inflation at 75mmHg and 100mHg (n=16).

CHAPTER 4

Upper and lower limb conduit artery endothelial responses to a shortterm altered shear rate intervention in spinal cord injured and ablebodied men

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Abstract

Individuals with spinal cord injury (SCI) experience a loss of sympathetic innervation and arterial remodeling of the sublesional peripheral vasculature, resulting in altered shear-rate (SR) profiles and endothelial function when compared to able-bodied (AB) persons. In vitro and recent in vivo evidence suggest increased retrograde SR is associated with acutely decreased endothelial cell function. It is unknown how modified SR patterns influence sublesional vasculature. The present study assessed: 1) resting SR patterns and endothelial function via relative flow-mediated dilation (FMD%) in both upper extremity (brachial artery, BA) and lower extremity (superficial femoral artery, SFA) vessels in SCI and AB; and 2) examined the effects of augmented retrograde SR and oscillatory shear index (OSI) on FMD in both BA and SFA. Eight individuals with SCI and eight age, sex, and waist-circumference-matched AB controls participated. BA SR patterns and FMD% were similar between groups, while SFA anterograde SR was higher (p<0.01) and FMD% was lower (p=0.04) in SCI vs. AB. Retrograde SR was augmented through both arteries at rest using a sub-systolic cuff inflation model. Bilateral FMD assessments were conducted before and after 30-minutes of unilateral inflation of a forearm or thigh blood pressure cuff to 75mmHg. Cuff-induced increases in both anterograde (p<0.05) and retrograde (p<0.05) SR resulted in acute decreases in BA and SFA FMD% (p<0.05) in the cuffed limbs in both groups. These results highlight that manipulating retrograde SR can acutely impair FMD% in conditions of both normal and reduced sympathetic innervation. Further, it is likely that decreased sympathetic innervation and arterial remodeling contribute to sublesional vasculature SR patterns and FMD% after SCI. This information is crucial when designing strategies to combat impaired vascular function in healthy and clinical populations.

Alphabetized Key Words: endothelial function, flow mediated dilation, oscillatory shear index, retrograde shear rate, spinal cord injury

Introduction:

The vascular endothelium plays an important role in the regulation of vasomotor tone via the synthesis and release of nitric oxide (NO) (1). Changes in the magnitude and pattern of shear stress acting on the vessel wall are potent stimuli for NO-mediated arterial vasodilation. Alterations in shear stress are thought to modulate endothelial structure and function through mechanotransduction. It is generally accepted that retrograde shear rate (SR; a surrogate for shear stress in the absence of blood viscosity) is atherogenic, as elevations in retrograde and oscillatory shear are associated with inflammation, atherosclerotic lesions, lower numbers of stress fibers, increased leukocyte adhesion proteins, and high endothelial permeability (2). Elevations in anterograde SR are considered to be atheroprotective; increased laminar flow tends to elongate and align the endothelial cells in the direction of the shear stress and result in increased production of endothelial nitric oxide synthase (eNOS) as well as up-regulation of antioxidant, antiinflammatory, anticoagulant, and antiapoptotic genes (2). In other words, increases in oscillatory, turbulent, and retrograde SR result in decreased endothelial cell function, while increased anterograde and mean SR result in improved endothelial cell function (3, 4).

SR patterns differ throughout the vascular tree such that at rest the superficial femoral artery (SFA) typically experiences lower mean (5) and greater retrograde SR than the brachial artery (BA) (6). The resultant chronically lower wall SR, along with higher turbulence through the SFA due to anatomical structure, have been suggested to contribute to the higher incidence of atherosclerosis in the SFA in comparison to blood

vessels in the upper extremity (5, 7). More recently, the acute effects of induced increases in retrograde SR have been investigated in humans in efforts to determine the *in vivo* impact of altered SR patterns and provide an evidence base for interventions focused on improving vascular health. Previous research in young healthy men has shown that brief periods of elevated retrograde SR [induced through a forearm or thigh cuff-inflation model] resulted in acute impairments in endothelial function measured via flow-mediated dilation (FMD) (8-10). These investigations have led to the advancement of the theory that chronic increases in conduit artery retrograde SR, associated with conditions such as hypertension, diabetes, obesity, and with age, may have negative impacts on endothelial function and vascular health.

It is clear that alterations in SR magnitude and direction can have substantial influences on endothelial health, however little is known regarding peripheral vascular responses to SR modifications in conditions of altered sympathetic innervation or drastic arterial remodeling such as after spinal cord injury (SCI). Studies have reported a 50-100% increased resting SFA mean SR (11, 12) and preserved or enhanced SFA FMD (13, 14) in individuals with SCI compared to able-bodied controls (AB). Only one study assessing SR patterns through the common femoral artery (CFA) reported a 30% increase in resting anterograde SR, but no difference in retrograde SR in SCI vs. AB (15). It is unclear whether the unexpected preserved or enhanced SFA and subsequent up-regulation of eNOS (16), or perhaps explained by structural adaptations or neural factors. There is a

lack of information on resting SR patterns and FMD, as well as the influence of altered SR (in particular augmented retrograde SR) on FMD in the SCI population.

The first objective of the present study was to investigate resting SR patterns and FMD in both the BA (atherosclerotic-resistant) and SFA (atherosclerotic-prone) in individuals with SCI and AB controls. The second objective was to manipulate SR patterns, and in particular retrograde SR, in those same arteries in both groups. Our approach was to increase retrograde SR through the BA and SFA at rest using a sub-systolic cuff inflation model, and assess its effects on endothelial function via FMD. We hypothesized that resting SR patterns and FMD would be similar between groups through the BA, but that resting mean and anterograde SR and FMD would be higher through the SFA in SCI vs. AB. We hypothesized that in both groups cuff-inflation of the arm and leg would induce increases in retrograde SR and acutely attenuate FMD in the conduit artery supplying the cuffed limb.

Methods:

Participants

Eight individuals with SCI (level of injury T4-T11, AIS A-C, 11.9±11.4 years postinjury) were recruited from Southern Ontario to participate. Eight AB persons matched for age, sex, height, body mass index (BMI), and waist circumference (WC) were recruited as controls (Table 1). The study procedures were approved by the Hamilton Health Sciences Research Ethics committee in Hamilton, Ontario, Canada, adhered to the Declaration of Helsinki, and all of the participants gave previous written informed consent.

Experimental Design

Each participant came to the laboratory on two occasions for approximately two hours in a fasted state (\geq 8 hours), abstained from caffeine and alcohol for \geq 12 hours, and abstained from physical activity for a minimum of 24 hours. Both visits were conducted at the same time of day to eliminate differences due to diurnal variation, and all assessments were conducted in a quiet, temperature controlled room (22-24°C). Participants lay supine for 10-15 minutes prior to any data collection to ensure stability of resting measures. Heart rate (HR) was monitored continuously throughout all testing procedures using a single lead electrocardiograph (ECG, Model ML123, ADInstruments Inc.; Colorado Springs, USA). Supine blood pressure was monitored continuously throughout the FMD assessments and cuff-inflation interventions using a pneumatic finger cuff (FMS MIDI, Finapres Medical Systems, Amsterdam, The Netherlands).

The first visit included baseline BA vascular assessments followed by 30-minutes of 75mmHg cuff inflation on the dominant forearm to assess the effects of augmented retrograde SR through the BA. The second visit included baseline SFA vascular assessments followed by 30-minutes of 75mmHg cuff inflation on the dominant thigh (determined to be the same side as the dominant forearm) to assess the effects of augmented retrograde SR through the SFA. BA and SFA FMD were assessed in both limbs (cuffed [altered flow intervention] and non-cuffed [control]) before and

immediately after the 30-minute, unilateral 75mmHg cuff-inflation intervention (Figure 1).

Experimental Procedures

Baseline Endothelial Function and Shear Rate

To examine BA and SFA vasculature, duplex ultrasound (Vivid Q, GE Medical Systems, Horten, Norway) was used to obtain simultaneous brightness-mode images of the arteries (13 MHz) and pulsed-wave blood velocity measurements (4 MHz). Preocclusion data of the arteries were collected for 30-seconds. BA images were acquired 3-5 cm proximal to the antecubial fossa, and SFA images were acquired 3-5 cm distal to the common femoral bifurcation. A 5-minute period of ischemia was initiated by inflating a pneumatic cuff positioned on the forearm or distal thigh to an occlusion pressure of 200mmHg (at least 50mmHg above systolic blood pressure) using a rapid cuff inflator (E20 Rapid Cuff Inflator, AG 101 Cuff Inflator Air Source, Hokanson; Washington, USA). Upon cuff deflation, post-occlusion data were collected continuously for 3minutes (BA) and 5-minutes (SFA).

Brightness-mode images were obtained at a frame rate of 7.7 frames/s. End diastolic frames from pre- and post-occlusion images were selected and saved to digital imaging and communications in medicine file format (Sante DICOM Editor, Version 3.1.20, Santesoft; Greece). End diastolic lumen diameter analyses involved using custom-designed semi-automated edge-detection software (Artery Measurement System Image and Data Analysis, Tomas Gustavsson; Sweden) to identify the near and fall wall to

include the intima, media, and lumen. Relative FMD values were then calculated as shown in equation 1 below:

(1) Relative FMD (%) = ((Peak - Baseline Diameter) / (Baseline Diameter))*100.

Test-retest reliability has been calculated previously for SFA FMD% for both SCI and AB in our lab; the intraclass correlation coefficient and coefficient of variation was 0.90 and 9% for SCI SFA FMD% and 0.95 and 3% for AB SFA FMD%, respectively.

Pulsed-wave velocity measures were collected to encompass the entire lumen of the artery of interest (from intima-to-intima) so that measurements of blood velocity represent a mean of the entire cross-sectional area of the vessel. Raw blood velocity profiles were outsourced to a spectral analyzer (Neurovision 500M TCD, Multigon Instruments; Yonkers, USA), and intensity weighted mean red blood cell velocity was determined using fast Fourier transformation and acquired with an analog to digital data acquisition system for offline beat-to-beat analysis (PowerLab 16/35 with LabChart 7 Pro, ADInstruments Inc.; Colorado Springs, USA). To obtain baseline SR patterns, mean, anterograde, and retrograde components during pre-occlusion data collection were analyzed using PowerLab. SR was calculated as shown in equation 2 below:

(2) SR $(1 \cdot \sec^{-1}) = (8 * blood velocity) / (end diastolic lumen diameter)$

Oscillatory shear index (OSI) represents a measure of the SR acting on the luminal surface due to either cross-flow or reverse flow velocity components occurring during pulsatile flow. OSI was calculated (6) as shown below:

(3) OSI = (|retrograde SR|) / (|retrograde SR| + |anterograde SR|)

Cuff Inflation Interventions

The same forearm and thigh cuff used for the assessment of FMD% were used to induce altered SR patterns in the dominant limb. Following bilateral baseline FMD% and blood velocity measures, the cuff was inflated to 75mmHg around the dominant forearm on visit 1, and around the dominant thigh on visit 2. The cuff inflation pressure was chosen based on a combination of previous data from our lab (manuscript under review (17)), as well as a previous study reporting impaired BA FMD% following 30-minutes of forearm cuff pressure at 75mmHg (8). BA and SFA diameters as well as blood velocity measures (mean, anterograde, retrograde) were obtained every five minutes during the 30-minute intervention and stored for off-line analyses. Immediately following the 30-minute cuff inflation intervention, bilateral FMD% was re-assessed (Figure 1).

Statistics

Statistical analyses were performed using SPSS 20.0 software. Baseline characteristics (demographics and vasculature) are presented as mean \pm SD; independent t-tests were used to assess any differences between groups (SCI vs. AB). A 2-way repeated measures analysis of variance was used to determine the effects of time (pre vs. post-intervention) and limb (cuffed vs. non-cuffed) on FMD%. Post-hoc t-tests were performed when a significant main or interaction effect was found. Paired t-tests were used to determine the differences in SR components (mean, anterograde, retrograde) at baseline at and end-intervention. Pearson correlations were used to examine the relationships between change in FMD% and change in SR parameters. Statistical significance was determined at p<0.05.

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If FMD% values do not scale properly for differences in baseline diameter, it is unclear whether the group differences represent true differences in endothelial function or simply differences in baseline artery diameter. Evidence of improper scaling can be seen if: a) baseline differences between groups exist, or b) the upper confidence interval of the slope between logarithmically transformed pre- and peak-diameters is lower than 1 (18). If logarithmic transformation was found to be appropriate in the present study, covariate-adjusted means for diameter change using a univariate analysis with group as the fixed factor was used. Briefly, we logarithmically transformed pre- and peak-diameters and calculated the change in diameter on the logged scale. This value was entered as the dependent variable with log baseline as the covariate. Covariate adjusted means for diameter change during the FMD% assessments were obtained, back-transformed, and then converted to a 'corrected' adjusted percentage change by subtracting 1 from the back-transformed value and multiplying it by 100 (18). Data are presented as mean±SD, with p<0.05 considered statistically significant.

Results:

All participants completed the entire protocol, save one person with SCI who did not complete the non-cuffed FMD% assessment portion of the leg cuff inflation intervention due to a history of deep vein thrombosis in that leg. No differences were found in age, anthropometrics, or blood pressure between SCI and AB controls (Table 1).

Heart Rate and Blood Pressure

HR did not change during the cuff-inflation interventions in either group. During the BA cuff-inflation intervention, systolic blood pressure (SBP) (127 ± 13 to 138 ± 13 mmHg, p<0.01), diastolic blood pressure (DBP) (73 ± 10 to 77 ± 8 mmHg, p<0.01), and mean arterial pressure (MAP) (91 ± 9 to 97 ± 7 mmHg, p<0.01) increased in SCI but not in AB. During the SFA cuff-inflation intervention, DBP (68 ± 8 to 73 ± 10 mmHg, p<0.01) and MAP (88 ± 10 to 92 ± 10 mmHg, p<0.05) increased in AB but not in SCI.

Baseline Endothelial Function and Shear Rate

BA and SFA FMD% values were not different between SCI and AB, however baseline BA diameter was larger and baseline SFA diameter was smaller in SCI vs. AB. After adjusting for baseline BA and SFA diameters, the SCI group had no difference in BA FMD% (p=0.77) and lower SFA FMD% (p=0.04) when compared to AB (Figure 2). BA SR patterns were not different between SCI and AB. Mean (p<0.01) and anterograde (p<0.01) SFA SR were higher with similar retrograde (p=0.92) SFA SR in individuals with SCI compared to AB (Table 2 and Figure 2).

Cuff Inflation Interventions

Doppler blood velocity profiles at baseline and at 30-minutes of the cuff-inflation intervention in SCI and AB are shown in Figures 3 (BA) and 4 (SFA). There were increases in retrograde SR (p=0.01, p<0.01), anterograde SR (p=0.01, p=0.01), and OSI (p<0.001, p<0.001) in the cuffed limb but not the control limb in SCI and AB, respectively (Table 3, and Figures 5 and 6). A significant interaction (time x limb)

indicated a decrease in FMD% in the cuffed limbs only (BA p=0.03, p<0.01 and SFA p<0.01, p=0.01) in SCI and AB, respectively (Figures 5 and 6). A baseline diameter difference was observed in the cuffed leg after the SFA cuff inflation intervention in the AB group (Table 3). However the upper confidence interval of the slope between logarithmically transformed pre- and peak-diameters was lower than 1, therefore it was deemed that the FMD% values were scaling appropriately (18). There were no correlations between change in FMD% and change in any of the SR parameters (mean, anterograde, retrograde, OSI).

Discussion:

The major findings in the present study were: 1) similarities in baseline BA, but differences in baseline SFA vascular characteristics between SCI and AB, and 2) a detrimental effect of augmented retrograde SR on BA and SFA FMD% in a model of altered sympathetic innervation and arterial remodeling (SCI) was well as in a control group (AB). These novel findings advance the knowledge of endothelial function regulation, and highlight the requirement to consider SR magnitude and pattern, as well as neural influences, in attempts to discern the effects of SCI on sublesional vasculature.

Baseline Shear Rate and Endothelial Function

Typical resting peripheral arterial blood flow patterns are comprised of anterograde blood flow during systole due to pressure generated from left ventricular contractions, and retrograde blood flow during early diastole due to downstream peripheral resistance. Arteries farther from the heart experience greater amounts of retrograde blood flow and can experience a third phase of late diastole anterograde flow generated from elastic stored energy of the conduit vessels from the second phase. We observed these patterns using ultrasound Doppler in the BA (Figure 3) and SFA (Figure 4) in both SCI and AB. Aging and physical function impairments are typically associated with greater retrograde SR and OSI in peripheral conduit arteries (19, 20), likely due to an increase in downstream resistance and possibly due to a decrease in basal NO bioavailability (21). Increased sympathetic activity has also been associated with increased retrograde SR and OSI (22), likely due to vasoconstriction of resistance vessels downstream.

Due to our groups being age-matched and the upper limbs being unaffected in the SCI group, it is logical that our baseline BA SR patterns were similar between groups. Interestingly, baseline BA diameter was larger in the SCI group vs. AB, likely due to the requirement of constant arm use for wheelchair propulsion, activities of daily living, and leisure time physical activity as all participants with SCI were in manual wheelchairs. A recent study reported similar findings of a larger BA diameter in SCI vs. AB (23).

In the present study we observed enhanced baseline SFA mean and anterograde SR with comparable retrograde SR in SCI vs. AB (Figure 2). We also observed smaller SFA diameter in the SCI group, likely due to disuse and inward remodeling (24). Several previous studies reported similar findings of smaller diameters and increased mean SR through the CFA (11, 12) and SFA (13, 14) after SCI. A few studies assessing CFA or SFA SR patterns in SCI reported increased anterograde SR (15, 23), but differences in mean and retrograde SR have been inconclusive when comparing to AB controls. After SCI, in addition to disuse there are neurological changes including loss of sympathetic

innervation to sublesional vasculature. It is possible that decreased sympathetic tone, increased arterial stiffness, and arterial remodeling all contribute to the unique SFA blood velocity and SR patterns after SCI (25, 26).

Resting BA FMD% values were similar between groups, confirming previous literature (13). Resting SFA FMD% values were lower after SCI after adjusting for baseline diameter (p=0.04) (Figure 2). These lower extremity FMD% findings agree with only one previous study evaluating a smaller lower extremity artery (posterior tibial artery) (27). The majority of the current literature has reported preserved or even enhanced SFA FMD% after SCI (13, 14, 25). However, these studies have utilized either a different occlusion time frame (10min) (13, 25), conducted the FMD under different baseline conditions (FMD assessment during saline solution infusion via CFA cannula) (14), and did not control for baseline diameter differences. One of the studies reporting enhanced SFA FMD% after SCI found no difference between groups after normalizing FMD values to SR_{AUC} (13). We did not calculate normalized FMD values in the present study due to a lack of correlation between SR_{AUC} and FMD%. Further, five of the eight participants with SCI were either previous or current smokers while none of the AB participants were smokers; most evidence suggests that smoking decreases FMD% (28). The unique SFA SR pattern and lower SFA FMD% after SCI suggest that atheroprotective anterograde SR may not be the key determinant of FMD% response, and that perhaps altered retrograde SR may be a stronger determinant of endothelial (dys)function.

Cuff Inflation Interventions

Our findings extend the knowledge in the field of retrograde SR and endothelial function *in vivo* by reporting that alterations in SR patterns including augmented retrograde and OSI results in a decrease in FMD% in middle-aged men with and without SCI in both the BA (atherosclerosis-resistant) and SFA (atherosclerotic-prone). Several pathways have been proposed linking elevated retrograde SR and OSI to reduced endothelial function, including down-regulated eNOS (29), enhanced endothelin-1, up-regulated adhesion molecules (VCAM-1 (30), ICAM-1 (31)), and increased reactive oxygen species (ROS) production in cultured cells (29).

It is interesting to note that both groups experienced similar increases in retrograde (8-fold increase) and anterograde (1.8-fold increase) SR in response to the BA cuffinflation intervention, but the SCI group experienced a larger retrograde SR (5.5- vs. 4fold increase) and slightly smaller anterograde SR (1.4- vs. 2-fold increase) response to the SFA cuff-inflation intervention when compared to AB. Upon visual inspection of the Doppler profile during the SFA cuff-inflation intervention it is clear that the SCI group had a greater retrograde response during diastole when compared to the AB group (Figure 4). Further, a trend towards a decreased mean SR during the SFA cuff-inflation intervention was observed in SCI (p=0.08) but not in AB (p=0.60), indicating a greater retrograde SR response in SCI (Figure 6). It is possible that altered sympathetic innervation and/or stiffer arterial walls after SCI contributed to the larger sublesional retrograde SR response. The present study interventions were unable to create an exclusive retrograde stimulus through either limb in either group, and no correlations were found between change in SR parameters (anterograde, retrograde, OSI) and change in FMD%. Although the mixed SR pattern changes observed provides a more accurate representation of the complex SR changes commonly observed with enhanced vascular tone, future studies should endeavor to create a model of exclusive retrograde SR to more clearly determine the influence of changing SR patterns on endothelial function. Evaluating a variety of cuff pressures and intervention lengths, as well as elevating the leg during cuff-inflation, may be considerations for future studies. Further, determining the level of autonomic impairment in SCI will help to elucidate the effects of altered sympathetic innervation on both resting sublesional vasculature and in conditions of altered SR.

Summary

The present study observed similar baseline SR patterns and FMD% through the BA in SCI and AB. When investigating the SFA, we found enhanced mean and anterograde SR but lower FMD% in SCI vs. AB. These findings suggest that the atheroprotective properties of anterograde SR may not be the primary determinant for endothelial function in sublesional vasculature. When investigating the effects of augmented retrograde SR in both atherosclerotic-resistant (BA) and –prone (SFA) arteries, we observed a decrease in FMD% in both arteries in both SCI and AB. These findings demonstrate the potential detrimental effects of short-term augmented retrograde SR on endothelial function among individuals with altered sympathetic innervation (SCI) and AB controls. Since both endothelial dysfunction and enhanced retrograde SR have been associated with

conditions of increased vascular tone (i.e. stiffer arteries), the present results may shed some light on future interventions taking into account these regulatory mechanisms.

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Parameter	SCI	AB	p-value
Age, years	43±7	43±7	0.598
Height, m	1.8±0.1	1.8±0.1	0.540
Mass, kg	78.8±18.2	86.2±13.6	0.369
BMI, kg/m^2	25.1±4.2	25.8±3.8	0.764
WC, cm	89.2±14.5	91.9±9.5	0.671
HR, bpm	66±6	64±14	0.674
Supine SBP, mmHg	127±13	127±13	0.962
Supine DBP, mmHg	73±10	71±8	0.672
Supine MAP, mmHg	91±9	89±9	0.809

Values are mean \pm SD. SCI = spinal cord injury; AB = able-bodied; BMI = body mass index; WC = waist circumference; HR = heart rate; SPB = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure. P-value refers to independent t-tests between groups (SCI vs. AB)

Table 1: Participant characteristics.

Visit 1



Figure 1: Schematic of experimental design. Visit 1: 30min 75mmHg cuff inflation intervention at rest designed to augment retrograde shear rate through the brachial artery (BA). **Visit 2:** 30min 75mmHg cuff inflation intervention at rest designed to augment retrograde shear rate through the superficial femoral artery (SFA).

	B	srachial Artery	7	Superf	icial Femoral _/	Artery
Parameter	SCI	AB	p-value	SCI	AB	p-value
Mean BV, cm/s	3.8 ± 1.0	3.6±1.2	0.747	9.6±5.3	4.2 ± 1.0	0.013
Retrograde BV, cm/s	4.1 ± 1.2	4.4 ± 1.3	0.669	11.3 ± 4.9	6.4 ± 1.3	0.016
Anterograde BV, cm/s	-0.5 ± 0.1	-0.6 ± 0.7	0.693	-1.6 ± 0.7	-2.2±0.9	0.214
OSI	0.12 ± 0.03	0.10 ± 0.09	0.683	0.15 ± 0.08	0.25 ± 0.05	0.008
D, mm	4.80 ± 0.57	3.99 ± 0.39	0.005	5.62 ± 0.58	7.45±0.90	<0.001
FMD, mm	0.27 ± 0.10	0.33 ± 0.20	0.451	0.34 ± 0.08	0.54 ± 0.10	<0.001
Values are mean±SD. SCI	= spinal cord in	ijury; $AB = able$ -	-bodied; BV =	= blood velocity;	OSI = oscillator	y shear
index; D = baseline end dia tests between groups (SCI v	astolic lumen dia vs. AB).	umeter; FMD = fl	low mediated	dilation. P-value	refers to indeper	ndent t-
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3rachial artery and superficial femoral artery	acteristics in SCI and AB.
aseline Vascular Characteristics. l	city and flow-mediated dilation char-
Table 2: B	plood veloc



Figure 2: Baseline Vascular Characteristics. Brachial artery (BA) and superficial femoral artery (SFA) shear rate and flow-mediated dilation (FMD) characteristics in spinal cord injury (SCI) and able-bodied (AB). Corrected FMD% refer to covariate-adjustments for baseline diameter differences. Error bars represent SD.

	Sp	inal Cord Inj	iury		Able-Bodied	
			Brach	ial Artery		
	$AUC_{SR}, 10^3$	OSI	D, mm	AUC_{SR} , 10 ³	ISO	D, mm
Cuffed						
Before	17.5 ± 18.2	0.12 ± 0.03	4.80 ± 0.57	22.0 ± 11.0	0.10 ± 0.09	3.99 ± 0.39
After	24.8 ± 27.8	0.32 ± 0.08	4.85 ± 0.52	18.2 ± 6.0	0.35 ± 0.12	4.06 ± 0.55
p-value	0.357	<0.001	0.543	0.334	<0.001	0.587
Non-Cuffed						
Before	8.8 ± 4.9	0.10 ± 0.03	4.58 ± 0.56	15.9 ± 5.3	0.08 ± 0.06	4.05 ± 0.52
After	7.4±5.0	0.10 ± 0.03	4.71 ± 0.51	15.2 ± 7.9	0.08 ± 0.06	4.11 ± 0.43
p-value	0.471	0.635	0.112	0.846	0.916	0.489
			Superficial]	Femoral Artery		
Cuffed						
Before	25.0 ± 30.0	0.17 ± 0.08	5.65 ± 0.49	19.3 ± 14.8	0.24 ± 0.07	7.29 ± 0.90
After	25.1 ± 17.0	0.46 ± 0.08	5.61 ± 0.53	14.1 ± 8.0	0.42 ± 0.07	7.52±0.97
p-value	0.992	<0.001	0.420	0.417	<0.001	0.002
Non-Cuffed						
Before	23.9 ± 13.4	0.18 ± 0.08	5.56±0.58	25.6 ± 15.6	0.22 ± 0.05	7.38 ± 0.90
After	21.9 ± 11.8	0.17 ± 0.06	5.66±0.59	23.4 ± 12.3	0.24 ± 0.07	7.44±0.96
p-value	0.729	0.555	0.571	0.761	0.151	0.278
Values are m	ean±SD. AUC _{SI}	x = shear rate a	rea under curve	up to maximum	artery dilation c	luring pre- (before)
and post- (afi	ter) intervention	FMD; $OSI = 0$	oscillatory shear	index during bas	eline (before) a	ind at 30min of the
intervention (cuff-inflation	(after); D = base intervention in b	eline end diasto	dic lumen diam	eter. P-values refe imb	er to paired t-tee	sts before and after
Tahle 3. R	trachial and	Sunerficial	Femoral Art	erv Cuff Infla	tion Interve	ntions Brachial
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and superficial femoral artery characteristics of participants before and after cuff intervention in the cuffed and non-cuffed forearm and thigh in SCI and AB.



Figure 3: Brachial Artery Cuff Inflation Intervention (Doppler screen capture). Brachial artery blood velocity profiles at baseline and at 30-minutes of the forearm cuffinflation model for an individual with SCI and an AB control.



Figure 4: Superficial Femoral Artery Cuff Inflation Intervention (Doppler screen capture). Superficial femoral artery blood velocity profiles at baseline and at 30-minutes of the thigh cuff-inflation model for an individual with SCI and an AB control.



Figure 5: Brachial Artery Cuff Inflation Intervention. On the left: mean, anterograde (+ve), and retrograde (-ve) shear rate patterns pre- and during the intervention in the cuffed leg. On the right: relative flow-mediated dilation (FMD%) before and after the intervention in the cuffed and non-cuffed leg; mean and individual data are presented. Data are for SCI (A and B) and AB controls (C and D). Error bars represent SD.



Figure 6: Superficial Femoral Artery Cuff Inflation Intervention. On the left: mean, anterograde (+ve), and retrograde (-ve) SR patterns pre- and during the intervention in the cuffed leg. On the right: relative flow-mediate dilation (FMD%) before and after the intervention in the cuffed and non-cuffed leg; mean and individual data are presented. Data are for SCI (A and B) and AB controls (C and D). Error bars represent SD.

CHAPTER 5

Lower limb conduit artery endothelial responses to acute upper limb exercise in spinal cord injured and able-bodied men

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Abstract

It is well known that regular exercise improves cardiovascular health. Recent work has revealed that a single bout of exercise results in vascular adaptations in the non-active regions, such as improved upper extremity endothelial function via flow-mediated dilation (FMD) following leg cycling. The vascular improvements are likely mediated by increased anterograde shear-rate (SR) during exercise. It is unknown whether upper body exercise produces the same increases in SR through the lower extremities, and if these SR modifications influence FMD. Individuals with spinal cord injury (SCI) experience sublesional vascular deconditioning; the SCI population could benefit from these possible upper body exercise-induced increases in lower body SR and FMD improvements. The present study utilized a single bout of arm ergometry to determine the effects of exerciseinduced SR changes through the superficial femoral artery (SFA) on FMD. Eight individuals with SCI and eight age, sex, and waist-circumference-matched able-bodied (AB) controls participated. Nine minutes of incremental arm exercise increased anterograde (p=0.02 and p<0.01) and retrograde (p<0.01 and p<0.01) SFA SR in both SCI and AB, respectively. However, the SR alterations resulted in an increased FMD in the AB group only (SCI p=0.74; AB p<0.01). These results highlight that while SR patterns are one factor linked to changes in endothelial function, other regulatory factors (i.e. metabolic or neural) must be considered in the assessment of interventions designed to improve endothelial function after SCI. This information is crucial when designing strategies to combat impaired vascular function in healthy and clinical populations.

Alphabetized Key Words: endothelial function, exercise, flow mediate dilation, spinal cord injury, shear rate

Introduction:

It is clear from *in vitro*, and more recently human studies, that alterations in the magnitude and pattern of shear stress acting on the endothelial cell wall can have substantial influences on endothelial health. Elevations in anterograde shear rate (SR; a surrogate for shear stress in the absence of blood viscosity) commonly observed with exercise are associated with increased cardiac output and muscle blood flow, and have been described as atheroprotective (1, 2). Anterograde laminar flow tends to elongate and align the endothelial cells in the direction of the SR and result in increased production of endothelial nitric oxide synthase (eNOS) as well as up-regulation of antioxidant, anti-inflammatory, anticoagulant, and antiapoptotic genes (3). It has been shown *in vitro* that low flow, oscillatory, or turbulent SR results in decreased endothelial cell function (1, 2). Further, retrograde SR is considered to be atherogenic as it has been associated with inflammation, atherosclerotic lesions, lower numbers of stress fibers, increased leukocyte adhesion proteins, and high endothelial permeability (3).

Regular exercise has been shown to enhance endothelial function via flow-mediated dilation (FMD) of large peripheral conduit arteries in humans (4); these enhancements may be partially mediated by the repeated increases in blood flow and SR during exercise (5). Exercise not only leads to improvements in vascular structure and function in active regions (6), but also results in vascular adaptations in non-active regions such as improved upper extremity FMD following leg cycling (7). Interestingly, recent work has reported larger releases in nitric oxide (NO) in response to oscillatory vs. pure anterograde SR during exercise, supporting the concept that SR patterns may have

important implications for endothelial health (8). Several studies have investigated the effects of a single bout of lower body exercise on brachial artery FMD. Conflicting results from these studies include increases (9, 10), decreases (11, 12) or no change (13, 14) in brachial artery FMD following lower body aerobic or resistance exercise. Several factors may contribute to the variable brachial artery FMD responses observed, including intensity of exercise, subject characteristics (e.g. age, sex, body composition), timing of post-FMD measures, diurnal variation, sympathetic changes associated with a single bout of exercise, or technical differences in data acquisition and analysis. Few of these studies have examined the components of the SR pattern (mean, anterograde, retrograde) during exercise and important information is likely missed when SR patterns induced with exercise are not considered or when only mean SR is reported.

Individuals with spinal cord injury (SCI) experience arterial remodeling and augmented SR through sublesional vasculature. Previous reports have shown a 50-100% increased resting superficial femoral artery (SFA) SR in individuals with SCI compared to able-bodied controls (AB) (15-17), but a preserved or decreased SFA FMD (17-19). Sublesional FMD responses after SCI could be attributed to the lack of sublesional vasculature sympathetic innervation and/or arterial remodeling (20). It is unknown whether upper extremity exercise could induce increases in SR through the non-active lower extremities leading to improvements in lower extremity FMD. Individuals with SCI who are wheelchair bound could benefit from these upper body exercise-induced increases in lower body SR and potential FMD improvements.

The objective of the present study was to investigate exercise-induced SR pattern changes in the SFA during supine arm cranking among individuals with SCI and AB controls. SFA FMD was assessed before and after arm cranking exercise. We hypothesized that in both groups arm-crank exercise would augment SFA anterograde and retrograde SR and acutely improve SFA FMD.

Methods:

Participants

Eight individuals with SCI (level of injury T4-T11, AIS A-C, 11.9±11.4 years postinjury) were recruited from Southern Ontario to participate. Eight AB persons matched for age, sex, height, body mass index (BMI), and waist circumference (WC) were recruited as controls (Table 1). The study procedures were approved by the Hamilton Health Sciences Research Ethics committee in Hamilton, Ontario, Canada, adhered to the Declaration of Helsinki, and all of the participants gave previous written informed consent.

Experimental Design

Each participant came to the laboratory on two occasions for approximately one and a half hours. The first visit consisted of the peak aerobic capacity test (VO₂peak) and body composition assessment. The second visit consisted of the vascular assessments and supine exercise. For the vascular assessments, participants arrived in a fasted state (\geq 8 hours), abstained from caffeine and alcohol for \geq 12 hours, and abstained from physical activity for a minimum of 24 hours. Participants lay supine for 10-15 minutes in a quiet,

temperature controlled room (22-24°C) prior to any data collection to ensure stability of resting measures. Heart rate (HR) was monitored continuously throughout all testing procedures using a single lead electrocardiograph (ECG, Model ML123, ADInstruments Inc.; Colorado Springs, USA). Supine blood pressure was measured discretely in triplicate at baseline and immediately post-exercise using an automated blood pressure device (Dinamap, GE Healthcare; Horten, Norway).

SFA FMD was assessed in the dominant leg (determined to be the same side as the dominant arm) before and after 9 minutes of continuous incremental supine arm cranking exercise (increasing in intensity every 3 minutes) (Figure 1).

Experimental Procedures

Peak Oxygen Uptake

Peak oxygen uptake (VO₂peak) was assessed via a symptom-limited graded arm cycle ergometer test (Lode Angio BV, The Netherlands). Participants with insufficient handgrip had their hands secured to the arm handles with tensor bandages. Cardiac stability and HR were monitored throughout the test using a 1-lead ECG (PowerLab 15T, ADInstruments) and a Polar HR monitor (Polar T31, Polar Electro, Quebec, Canada). The test began with no resistance at a cadence of 60-80rpm; after a 1-minute warm-up the resistance increased every minute by 10W. Participants continued arm cycling until volitional fatigue or if they were unable to maintain a cadence of 30rpm. Expired gas and ventilatory parameters were acquired throughout the protocol. BP was assessed immediately following and throughout recovery to ensure that it returned to baseline

values following the exercise test. VO₂peak was determined to be the highest 30-second average oxygen consumption.

Body Composition

Dual energy x-ray absorptiometry (DXA; Hologic Inc., Waltham, MA, USA) was utilized to assess for whole body fat (%). DXA is a "gold standard" measure of body composition and an effective method to characterize body composition in people with SCI (21).

Endothelial Function

To examine SFA FMD, duplex ultrasound (Vivid Q, GE Medical Systems, Horten, Norway) was used to obtain a simultaneous brightness-mode image of the SFA (13 MHz) and pulsed-wave blood velocity measurements (4 MHz). SFA images were acquired 3-5 cm distal to the common femoral bifurcation. Pre-occlusion data was collected for 30-seconds followed by a 5-minute period of ischemia via inflating a pneumatic cuff positioned on the distal thigh to an occlusion pressure of 200mmHg (at least 50mmHg above systolic blood pressure) using a rapid cuff inflator (E20 Rapid Cuff Inflator, AG 101 Cuff Inflator Air Source, Hokanson; Washington, USA). Upon cuff deflation, post-occlusion data was collected continuously for 5-minutes.

Images were ECG-gated and obtained at a frame rate of 7.7 frames/s. Off-line analyses involved selecting end diastolic frames from pre-occlusion and post-occlusion images and saving them to digital imaging and communications in medicine file format (Sante DICOM Editor, Version 3.1.20, Santesoft; Greece). End diastolic diameters were analyzed from the near wall to the far wall to include the intima, media, and lumen using

custom-designed semi-automated edge-detection software (Artery Measurement System Image and Data Analysis, Tomas Gustavsson; Sweden). Relative FMD was then calculated as shown in equation 1 below:

(1) Relative FMD (%) = ((Peak - Baseline Diameter) / (Baseline Diameter))*100.

Test-retest reliability has been calculated previously for SFA FMD% for both SCI and AB in our lab; the intraclass correlation coefficient and coefficient of variation was 0.90 and 9% for SCI SFA FMD% and 0.95 and 3% for AB SFA FMD%, respectively.

Sample volume (gate width) for the pulsed-wave velocity measures encompassed the entire SFA lumen (from intima-to-intima) so that measurements of blood velocity represent a mean of the entire cross-sectional area of the SFA. Raw blood velocity profiles were outsourced to a spectral analyzer (Neurovision 500M TCD, Multigon Instruments; Yonkers, USA). Intensity weighted mean red blood cell velocity was fast Fourier transformed and acquired with an analog to digital data acquisition system for offline beat-to-beat analysis (PowerLab 16/35 with LabChart 7 Pro, ADInstruments Inc.; Colorado Springs, USA). Mean, anterograde, and retrograde components of the pre-occlusion data were analyzed using PowerLab. SR was calculated as shown in equiation 2 below:

(2) SR $(1 \cdot \sec^{-1}) = (8 * blood velocity) / (end diastolic lumen diameter)$

Arm Crank Exercise Intervention

Following baseline FMD% and blood velocity measures in the dominant SFA, each participant performed 9-minutes of continuous supine arm-exercise (3x3-min of

increasing intensity: 40, 50, 60W) using a Monark arm ergometer (Monark Rehab Trainer 881e, Monark Exercise AB, Varberg, Sweden). Participants were instructed to maintain a comfortable cadence of 60rpm. During the last 30-seconds of each exercise intensity, SFA diameters and blood velocity measures (mean, anterograde, retrograde) were obtained. Velcro straps were secured around the participant's hips and thighs to minimize lower body movement and to facilitate data collection. Following the 9-minute exercise intervention, dominant leg FMD% was re-assessed (Figure 1).

Statistics

Statistical analyses were performed using SPSS 20.0 software. Baseline characteristics (demographics and vasculature) are presented as mean±SD; independent t-tests were used to assess any differences between groups (SCI vs. AB). A factorial-repeated measures analysis of variance was used with the factor being group (SCI vs. AB) and the repeated measures being time (pre vs. post-intervention) to determine the effects of arm exercise on SFA FMD%. A post-hoc t-test with Bonferonni correction was performed when a significant interaction effect was found. Paired t-tests were used to determine the differences in SR components (mean, anterograde, retrograde) at baseline at and end-intervention. Statistical significance was determined at p<0.05.

If baseline diameter differences were found between groups for FMD% assessments, covariate-adjusted means for diameter change using a univariate analysis was used. Briefly, we logarithmically transformed pre- and peak-diameters and calculated the change in diameter on the logged scale. This value was entered as the dependent variable with log baseline as the covariate. Covariate adjusted means for diameter change during

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the FMD assessments were obtained, back-transformed, and then converted to a 'corrected' adjusted percentage change by subtracting 1 from the back-transformed value and multiplying it by 100 (22). Data are presented as mean \pm SD, with p<0.05 considered statistically significant.

Results:

All participants completed the entire protocol; no differences were found in age, anthropometrics, or blood pressure between SCI and AB controls. Aerobic capacity (VO_2peak) was lower in SCI (p<0.01) (Table 1).

HR was significantly elevated in both groups following the exercise bout (SCI 62±12 to 125±17 bpm p<0.001; AB 56±11 to 113±12 bpm, p<0.001). After the exercise intervention DBP decreased in the SCI group (79±7 to 69±3 mmHg, p<0.01). In the AB group SBP increased (124±13 to 137±16 mmHg, p<0.01) and DBP decreased (77±13- to 70±12mmHg, p<0.05).

Doppler blood velocity profiles for SFA at baseline and at 9 minutes of arm exercise in SCI and AB are shown in Figure 2. During arm exercise, there were increases in SFA peak anterograde (p=0.02) and retrograde (p<0.01) SR in the SCI group, but no change in FMD% post-exercise (p=0.74). There were increases in all SFA SR components (mean p=0.03, anterograde p<0.01, retrograde p<0.01) during arm exercise in the AB group, and an increase in FMD% post-exercise (p<0.01) (Figure 3). A difference in baseline diameter pre- to post-exercise in the AB group was observed (Table 2); after adjusting for baseline diameter the significant improvement in FMD% remained (p=0.03) (Figure 3).

Discussion:

The major and novel finding in the present study was different acute SFA FMD% responses to arm exercise-induced SFA SR patterns between SCI and AB. These findings advance the knowledge of the multilayered regulation of endothelial function, and highlight the requirement to consider many factors including SR magnitude and pattern as well as metabolic and neural influences in attempts to discern the optimal intervention for the treatment and prevention of vascular dysfunction after SCI.

Previous work has shown that exercise-induced increases in oscillatory SR in the nonworking limb results in a shear-mediated release of NO in AB individuals (8). A review of the literature reveals conflicting results regarding changes in brachial artery FMD% following a single bout of lower body exercise, likely due to study design differences. The majority of these studies did not assess SR patterns in response to the single exercise bout, likely missing important information. One study assessed common femoral artery (CFA) SR patterns following seated arm exercise in both SCI and AB and found increases in mean and anterograde CFA SR in both groups but a larger CFA retrograde increase in the SCI vs. AB group (23). No previous research has been conducted assessing the impact of SFA SR patterns on FMD% following arm exercise in either SCI or AB.

In agreement with previous work we observed increases in anterograde and retrograde SR through the non-working limb (SFA) in both SCI and AB following a single exercise bout (9-minutes of arm ergometry). We found improved SFA FMD% following the augmented SR patterns in response to arm exercise in the AB group only.

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There are several possible reasons for the unchanged FMD% in the SCI group after arm exercise: 1) the existence of a critical threshold of SR change required to induce changes in FMD%; 2) elevated systemic oxidative stress; and/or 3) lack of sympathetic nervous system activity.

Previous work in humans has suggested the existence of a critical threshold of exercise intensity required for endothelial adaptations, likely related to increases in BP and SR (24). Although it is largely unknown the specific exercise intensity threshold needed for FMD% changes, it has been proposed that FMD% increases after moderate intensity exercise (25), and decreases after high intensity exercise (14, 26). Both the SCI and AB groups exercised at the same power output resulting in the groups exercising at 60% and 46% of their peak power output, respectively. These intensity levels are both considered moderate, so the inconsistent FMD% response is more likely to be attributed to BP and SR changes with exercise. The AB group experienced increases in SBP and HR while the SCI group experienced increases in HR only. It has been demonstrated that systemic NO-dependent exercise hyperemia depends on increases in BP and HR, and cannot be achieved with increases in HR alone (27). With regards to SR, it has been shown that elevating brachial artery retrograde SR component during lower body exercise above normal physiological levels attenuated brachial artery FMD% (25). In the present study the SCI group experienced more atherogenic retrograde SR (400% vs. 265% increase) and less atheroprotective anterograde SR (55% vs. 135% increase) compared to AB. Perhaps the lower relative anterograde increase from baseline values resulted in less alteration in SR on the endothelial cells. Alternatively, the SCI group had

significantly higher baseline anterograde SR (p<0.01); it is possible that when exercising at a moderate intensity the SCI group achieved an absolute anterograde SR level close to what is expected of high intensity exercise in AB. The consequence of these different SR pattern responses between groups could have contributed to an unchanged SFA FMD% in the SCI cohort.

The pathophysiology of SCI is characterized by increased oxidative stress secondary to the primary injury (28). In addition, reactive oxygen species (ROS) production is greater in obese compared to lean adults (29), and in the present study the SCI cohort was obese (body fat >25%) while the AB cohort was not (body fat 21%) (Table 1). ROS production can scavenge NO and thereby impair NO-dependent vasodilation (i.e. FMD%) (30). Exercise is also known to increase ROS (31) and can reduce NO bioavailability (32); elevations in ROS have been suggested as a potential mechanism for the observed reduction in FMD% immediately following high intensity exercise (26). It is probable that individuals with SCI have higher levels of basal oxidative stress, and may respond to exercise by producing a greater quantity of ROS when compared to AB controls. Increased oxidative stress may have contributed to the unchanged SFA FMD% in the SCI group in the present study.

The final factor to take into consideration when examining the lack of FMD% response to exercise in the SCI group is sympathetic activity. Since sympathetic nerve activity decreases for several hours following an exercise bout (33), it is possible that improvements in FMD% following a single bout of moderate intensity exercise can be partially explained by attenuation of sympathetic flow in addition to increased NO

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bioavailability in AB persons. It is likely that sympathetic innervation to the peripheral vasculature was altered in all participants with SCI in the present study; evidence of altered sympathetic activity in the SCI group is seen when examining SFA diameter responses to the exercise intervention. In the AB group, SFA diameter decreased during the exercise intervention (Table 2) providing an indication of supra-spinal sympathetic regulation. No changes in SFA diameter were seen in the SCI group. In a state of altered sympathetic modulation such as SCI, it is conceivable that the lack of change in sympathetic tone post-exercise could contribute to the absence of FMD% change.

Limitations

Several limitations need to be addressed. We did not exclude participants with SCI with previous cardiovascular disease or current cardiovascular risk factors; five individuals with SCI were previous or current smokers, and one had sustained a previous stroke. We did not include EMG to assess the quantity of muscular activity occurring in the non-working lower limbs, however we did attempt to stabilize the lower extremities to reduce movement. Another limitation is that all participants in both the SCI and AB groups exercised at the same intensities, resulting in slightly different relative intensities. A final limitation in the present study was the lack of any direct examination of sympathetic nervous system activity. Knowledge of sympathetic innervation at rest and during the exercise intervention could provide insight on the distinct responses between SCI and AB.

Summary and Future Directions

The SCI and AB cohorts responded differently to arm exercise-induced SFA SR alterations: the SCI group experienced no change in SFA FMD% while the AB group had a significant improvement in SFA FMD%. Upon closer inspection of SR patterns between groups, it was found that the SCI group had significantly higher resting anterograde SR (p<0.01), and in response to exercise the SCI group experienced smaller relative anterograde (atheroprotective) and a greater relative retrograde (atherogenic) SR increases when compared to AB. Perhaps baseline and exercise SR patterns influence endothelial cell responses. It is likely that altered metabolic (elevated ROS) and neural (lack of sublesional vasculature sympathetic innervation) factors contribute to the way in which endothelial cells respond to altered SR environments.

It is possible that arm-only exercise is not enough to influence peripheral vessels that lack sympathetic innervation; perhaps lower extremity exercise (i.e. functional electrical stimulation, body weight supported treadmill training, NuStep) could evoke upper extremity vascular function improvements. Future studies should explore the short and long-term effects of different modes, intensities, and durations of exercise on endothelial health in both healthy and clinical populations, as well as resolve the influence of metabolic and neural factors on endothelial function responses.

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No conflict of interest in accordance with journal policy.

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Parameter	SCI	AB	p-value
Age, years	43±7	43±7	0.598
Height, m	1.8±0.1	1.8±0.1	0.540
Mass, kg	78.8±18.2	86.2±13.6	0.369
BMI, kg/m^2	25.1±4.2	25.8±3.8	0.764
WC, cm	89.2±14.5	91.9±9.5	0.671
Body Fat, %	25.1±6.4	20.7±5.9	0.236
HR, bpm	66±6	64±14	0.674
Supine SBP, mmHg	127±13	127±13	0.962
Supine DBP, mmHg	73±10	71±8	0.672
Supine MAP, mmHg	91±9	89±9	0.809
VO ₂ peak, mL/kg/min	22.3±4.1	31.1±5.5	0.009
Peak PO, W	103.6±17.8	129.4±29.3	0.085

Values are mean \pm SD. SCI = spinal cord injury; AB = able-bodied; BMI = body mass index; WC = waist circumference; SPB = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; VO_{2peak} = peak oxygen uptake; Peak PO = peak power output. P-value refers to independent t-tests between groups (SCI vs. AB)

Table 1: Participant characteristics.



Figure 1: Schematic of experimental design. 9-minute incremental supine arm exercise intervention designed to augment oscillatory SR through the SFA.

Parameter	Before	After	p-value
Spinal Cord Injury			
EDLD, mm	5.62±0.58	5.51±0.54	0.693
Peak SR, s ⁻¹	705±325	681±401	0.718
AUC_{SR} , 10^3	23.4±15.7	23.5±21.7	0.988
Able-Bodied			
EDLD, mm	7.45±0.90	7.12±0.93	< 0.001
Peak SR, s ⁻¹	633±123	636±143	0.943
AUC_{SR} , 10^3	22.8±15.8	29.4±16.5	0.213

Values are mean \pm SD. AUC_{SR} = shear rate area under curve up to maximum artery dilation during pre-(before) and post- (after) intervention flow mediated dilation; EDLD = baseline end diastolic lumen diameter. P-values refer to paired t-tests before and after exercise intervention.

Table 2: Arm Crank Exercise Intervention. SFA FMD characteristics of participants

 before and after exercise intervention. Data are for both SCI and AB controls.



Figure 2: Arm Crank Exercise Intervention (Doppler Screen Capture). SFA blood velocity profiles at baseline and at 9-min of the exercise model for an individual with SCI and an AB control.



Figure 3: Arm Crank Exercise Intervention. On the top: mean, anterograde (+ve), and retrograde (-ve) shear rate patterns pre- and at 9 minutes of exercise for SCI and AB. On the bottom: relative flow-mediated dilation (FMD%) before and after the exercise intervention in SCI and AB; mean and individual data are presented. Corrected FMD% refers to covariate-adjustments for baseline diameter differences. Error bars represent SD.

CHAPTER 6

The physical activity guidelines for adults with spinal cord injury do not improve vascular health: a randomized controlled trial

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Abstract

Objective: To evaluate the effects of implementing 4-months of the physical activity guidelines for adults (PAG) with spinal cord injury (SCI) on traditional cardiovascular disease (CVD) risk factors and vascular health outcomes.

Design: Parallel-group randomized controlled trial.

Methods: Twenty-three individuals with chronic (≥ 1 year post-injury) SCI (NLI C3-T11, AIS A-C, YPI 12.0±9.9 years, age 41.4±11.6 years) were randomized into PAG training (n=12) or active control (CON; n=11) groups. PAG training was 2x/wk and involved ≥ 20 min of moderate-vigorous aerobic exercise (RPE 3-6 on 10-point scale) and 3x10reps of upper-body strengthening exercises (50-70% 1RM). The CON group maintained existing physical activity levels with no guidance on training intensity. Outcome measures were obtained at baseline and after 4-months of training. Fasted blood samples were analyzed for lipids, insulin, adipokines, inflammatory, and thrombotic markers. Body composition was assessed via anthropometrics and with dual-energy x-ray absorptiometry. The vascular health indicators included arterial stiffness via local carotid distensibility and regional pulse wave velocity, and endothelial function via brachial artery (BA) and superficial femoral artery (SFA) flow-mediated dilation (FMD) and response to nitroglycerin.

Results: Twenty-one individuals completed the 4-month intervention (PAG=12; CON=9). No adverse events related to study participation occurred. No baseline differences between groups were found for any outcome measure. Significant group-by-time interactions were observed for whole body mass (p=0.03), whole body fat (p=0.04), visceral adipose tissue (p=0.04), and carotid artery distensibility (p=0.05), suggesting maintained body composition and carotid health in the PAG group concurrent with declines in the CON group. No changes were found for regional arterial stiffness or BA and SFA FMD with training.

Conclusions: Four months of PAG in adults with SCI is insufficient to improve many aspects of cardiovascular health. However, there were some indications of positive body composition and carotid distensibility changes with 4-months of PAG. The PAG should continue to be promoted as a means to increase physical fitness and maintain body composition in individuals with SCI, but may need to be modified for the realization of other health outcomes. Increases in the duration and/or intensity of the aerobic exercise component of the existing PAG may be needed to improve vascular health outcomes in adults with SCI.

Alphabetized Keywords: body composition, cardiovascular disease, exercise, spinal cord injury, vascular function, vascular structure
Introduction:

It has been reported that there is a 40 to 50% less risk of cardiovascular disease (CVD) among individuals who participate in regular physical activity when compared to their sedentary counterparts (1). Due to the multifactorial effects of exercise on cardiovascular health, training studies should monitor both traditional and novel CVD risk factors for comprehensive assessments of the impact of exercise interventions. The American Heart Association has defined traditional risks as abdominal obesity, elevated triglycerides (TG), low high-density lipoprotein cholesterol (HDL), high blood pressure (BP), and elevated fasting glucose (2). A person with \geq 3/5 of the risk factors is labeled as having the metabolic syndrome, and CVD is the primary clinical outcome of metabolic syndrome.

Blood biomarkers may also provide insight into CVD risk; insulin resistance, a proinflammatory state (i.e. interleukin-6 [IL-6], tumor necrosis factor alpha [TNF-alpha]), a pro-thrombotic state (i.e. plasminogen activator inhibitor-1 [PAI-1]), and altered serum adipokine secretion (i.e. leptin, adiponectin) are likely mechanisms that link traditional risk factors to CVD (3, 4). IL-6 stimulates the release of corticosteroids that exacerbate hyperglycermia associated with obesity and the metabolic syndrome; elevated levels of IL-6 have been reported in patients with CVD (5). TNF-alpha acts locally to promote insulin resistance, contributes directly and indirectly to vascular endothelial cell injury, and is positively associated with atherogenic dyslipidemia (6). PAI-1 increases the risk of blood clotting and thromboembolism, and is present in increased levels in obesity and metabolic syndrome (7). Leptin regulates body adiposity and acts to suppress appetite and stimulate basal metabolism, partly through the sympathetic nervous system; leptin levels increase with obesity and are considered an independent CVD risk factor (8). Adiponectin protects against CVD through its vasodilator, anti-apoptotic, antiinflammatory and anti-oxidative activities in both cardiac and vascular cells; adiponectin deficiency has been identified as an independent risk factor for CVD (9). All these biomarkers are secreted from adipose tissue, and in particular visceral adipose tissue (VAT) (10, 11). Some evidence exists regarding the effects of exercise on these blood biomarkers in able-bodied persons (12).

Novel risk factors include peripheral vascular structure and function (13). Arterial stiffness refers to changes in the mechanical properties within an artery or a segment of the arterial tree; stiffer arteries propagate faster pulse waves that amplify systolic blood pressure, over time leading to damaged endothelial cells, further stiffening, and arterial remodeling. Arterial stiffness is a strong independent risk factor for CVD (14). Endothelial function typically refers to the regulation of arterial vascular tone via the synthesis and release of nitric oxide (NO) (15). Impaired endothelial function is an early indication of atherosclerosis, and has been associated with increased incidence of cardiac events (16). Growing evidence in able-bodied literature suggests that exercise training exerts direct changes on blood vessels, which results in measureable changes in the structure and function of those blood vessels (13).

Individuals with spinal cord injury (SCI) are at an increased risk for CVD when compared to their able-bodied counterparts (17). Due to altered etiology of cardiovascular risk, altered autonomic regulation, and a blunted cardiovascular response to exercise post-injury, able-bodied physical activity guidelines may not be appropriate for individuals with SCI. The Canadian physical activity guidelines for adults with SCI (PAG) were released in 2011 in order to provide evidence-based guidelines specific to the needs and capabilities of the SCI population (18). The guidelines recommend that for important fitness benefits, persons with SCI should engage twice weekly in at least 20 minutes of moderate-vigorous intensity aerobic exercise, and 3 sets of 10 repetitions of resistance training using every major muscle group.

Including regular physical activity into the lifestyle of individuals with SCI may reverse the cardiovascular and metabolic comorbidities associated with injury and precipitate desirable multisystem adaptations (19). However, limited literature exists regarding effective exercise interventions for slowing the progression of multiple risk factors for CVD in persons with SCI. Strong evidence exists regarding the effects of exercise on fitness outcomes after SCI (20), and a recent randomized controlled trial (RCT) run in parallel with the present study reported that the evidence-based PAG do improve fitness outcomes of VO₂peak and muscle strength (21). Therefore, the purpose of the present study was to determine the effects of implementing the PAG on vascularhealth outcomes.

Methods:

Participants

This prospective RCT was part of a larger study examining additional outcomes (21). Participants with chronic SCI (≥ 1 year post-injury) aged 18-65 years who were community wheelchair users were recruited from southern Ontario to participate. Exclusion criteria included any progressive loss of neurologic function within the previous six months. Data collection and implementation of the intervention took place at McMaster University in Hamilton, Ontario, Canada. The study procedures were approved by the Hamilton Health Sciences Research Ethics committee in Hamilton, Ontario, Canada, adhered to the Declaration of Helsinki, and all of the participants gave written informed consent.

Randomization

Following baseline testing, participants were randomly assigned into PAG training or active control (CON) groups (GraphPad Software, Inc., La Jolla, California, United States). The active control group was selected due to ethical considerations regarding withholding exercise from a clinical population (22).

Intervention

Participants randomized to the PAG group attended supervised training sessions twice weekly for 4-months (32 sessions in total) for approximately 60 minutes per session. Training was progressive and involved at least 20 minutes of moderate-vigorous aerobic exercise (RPE 3-6 on 10-point scale) and 3x10reps (50-70% 1RM) of resistance exercises for major upper-body muscle groups. The aerobic exercise equipment available for use throughout the study included arm ergometer (Monark Rehab Trainer 881e, Monark Exercise AB, Varberg, Sweden), vitaglide (Vitaglide, RMT Fitness, Miami, Florida, United States), or NuStep (NuStep T5XR Recumbent Cross Trainer, NuStep, Inc., Ann Arbor, Michigan, United States). The resistance exercise equipment available included a multi-station accessible weight stack (Equilizer Exercise Machines, Red Deer, AB, Canada), wall pulleys (Endorphin Pulleys, Patterson Medical Canada, Mississauga, ON, Canada), and free weights. Participants randomized to the CON group maintained existing physical activity levels with no guidance on training intensity.

Adherence to the training program was calculated based on the percentage of a maximum of 32 sessions. If participants in the PAG group missed any sessions, up to four additional weeks of training were permitted to complete the additional sessions.

Assessments

In addition to participant demographic and injury-related characteristics, the presence or past history of chronic conditions (i.e. high blood pressure, diabetes, heart disease, arthritis, etc.) and current medications (name, amount, frequency, duration) were collected via self-report. Participants were asked to empty their bladder before examination and refrained from alcohol and caffeine for ≥ 12 hours and exercise for a minimum of 24 hours prior to both testing days. Medications and vitamins were kept constant throughout the study, except for nitroglycerin (NTG), which was withheld on testing days. Cardiovascular assessments were conducted in a quiet, temperature controlled room (22-24°C).

Blood Biomarkers

Blood glucose control (HbA1c), lipids (triglycerides, TC, LDL-c, HDL-c, TC/HDLc), fasting insulin, adipokines (leptin and adiponectin), pro-inflammatory markers (IL-6 and TNF-alpha), and pro-thrombotic markers (PAI-1) were measured from a blood sample taken after a 12 hour fast. HbA1c and lipids were sent to a blood clinic for analysis. Serum insulin, leptin, IL-6 and TNF-alpha were analyzed in-house on a 4-plex luminex plate and run in duplicate; they were measured in pg/mL and a five parameter curve fitting analysis was used. Serum adiponectin and PAI-1 were analyzed in-house on a 2-plex luminex plate and run in duplicate; they were measured in pg/mL and a best fitting analysis was used.

Body Composition

Basic anthropometric measures of mass (kg), length (m), body mass index (BMI, kg/m²), and waist circumference (WC, cm) were collected as previously reported (23). Whole body fat (kg, %), whole body lean (kg), and visceral adipose tissue (VAT) (kg, %) were determined using dual-energy x-ray absorptiometry (Hologic QDR-4500A, Hologic Inc., Waltham, MA, USA) as previously reported (23). Least significant change (LSC) values among a cohort of n=34 adults with SCI were calculated of 0.703 kg, 1.587 kg, and 0.275 kg, for whole body fat, whole body lean, and VAT, respectively.

Peripheral Vascular Structure and Function

Following the blood draw participants lay supine for 10-15 minutes to ensure stability of resting measures. Heart rate (HR; Model ML123, ADInstruments Inc.; Colorado Springs, USA) and BP (FMS MIDI, Finapres Medical Systems, Amsterdam, The Netherlands) were monitored continuously throughout all cardiovascular testing procedures. Carotid pulse pressure (PP, mmHg), distensibility (mmHg⁻¹), intima media thickness (IMT, mm), lumen diameter, and wall to lumen ratio (WLR) were determined using a combination of applanation tonometry (SPT-301 Millar Instruments, Houston, TX) and brightness mode ultrasound (Vivid Q, GE Medical Systems, Horten, Norway). Central and peripheral (arm, leg) pulse wave velocity (PWV, m/s) was determined as a surrogate for arterial stiffness using photoplethysmography (PPG; IR Plethysmograph; Model MLT1020PPG; ADInstruments, Colorado Springs, USA). Brachial (BA) and superficial femoral artery (SFA) endothelial dependent (flow-mediated dilation, FMD) and independent (NTG) vasodilation was determined using duplex ultrasound (Vivid Q, GE Medical Systems, Horten, Norway) for simultaneous collection of arterial diameter and blood velocity. All peripheral vascular structure and function measures were collected and analyzed as previously described (23). Test-retest reliability has been calculated for SFA FMD% for SCI in our lab; the intraclass correlation coefficient and coefficient of variation was 0.90 and 9%, respectively.

Statistics

Baseline characteristics and outcome measures are presented as mean±SD. The Shapiro-Wilk test was used to verify normal data distributions. Data that did not represent a normal distribution was transformed using Tukeys Ladder of Transformations.

Independent t-tests were performed to determine group differences in baseline characteristics. Repeated-measures analyses of variance (ANOVA) were used to determine time and group x time interaction effects. Effect sizes were calculated (Cohen's d) from absolute change scores and defined as small 0.2, medium 0.5 and large 0.8 (24). Statistical analyses were performed using SPSS 20.0 software with a level of

significance of p<0.05.

Results:

Figure 1 describes the participant flow through the study. Recruitment and training took place from April 2012 to September 2013. Twenty-three participants were randomized (NLI C3-T11, AIS A-C, YPI 12.0 \pm 10.0 years, age 40.5 \pm 11.6 years) into PAG (n=12) and CON (n=11), and there were no differences between groups at baseline (Table 1). Adherence was 98 \pm 3% and 44 \pm 34% in the PAG and CON group, respectively.

Twenty-one participants completed the intervention, and a complete data set was collected on seventeen participants, n=9 in PAG and n=8 in CON. Two participants dropped out of the study, one due to dissatisfaction of group randomization and one due to an adverse event unrelated to the study. Three participants (n=2 PAG, n=1 CON) were unable to complete the NTG protocol due to low BP, and one participant had a body mass exceeding the limits for DXA scan (n=1 PAG).

Traditional CVD Risk Factors and Blood Biomarkers

Results regarding traditional risk factors are presented in Table 2. There were no differences between groups at baseline. There was a group x time interaction for WC (p=0.03) and BMI (p=0.02), indicating maintained body composition in the PAG group and detrimental changes in the CON group following training. Analysis of change scores revealed high effect sizes for WC (d=1.02) and BMI (d=1.14). Glycated hemoglobin (HbA1c), a reliable measure of long-term glycermic exposure, was used in place of fasting glucose measures. Blood biomarkers of fasting insulin, adipokines, inflammatory

markers, and thrombotic markers are presented in Table 3; no differences were found between groups at baseline or following training for any of the biomarkers.

Body Composition

There were no significant differences between groups at baseline for any of the body composition variables. There was a group x time interaction for whole body mass (kg) (p=0.03), whole body fat (kg) (p=0.04), and VAT (kg) (p=0.04), reflective of maintained body composition in the PAG group of -0.77 ± 1.61 kg, -0.88 ± 1.84 kg, and -0.15 ± 0.35 kg, and poorer body composition in the CON group of 0.73 ± 1.13 kg, 0.73 ± 1.33 kg, 0.17 ± 0.26 kg, respectively (Figure 2). Change scores demonstrated large effect sizes for whole body mass (d=1.07), whole body fat (d=1.00), and VAT (d=1.02). There was a trend towards an interaction for leg fat (kg) (group x time interaction p=0.056) indicating maintained leg fat in the PAG group concurrent with increases in leg fat in the CON group. No changes were observed in whole body lean (kg) or segmental lean mass (arm or leg, kg).

Peripheral Vascular Structure and Function

No differences in peripheral vascular structure and function were found between groups at baseline (Table 4). After transforming non-normal data, a group x time interaction was found for carotid distensibility (mmHg⁻¹) (p=0.05), indicating maintained carotid distensibility in the PAG group concurrent with a decline in the CON group following the training intervention (Figure 3); a large effect size was calculated (d=0.9). No interactions were found for other measures of carotid artery structure (PP, IMT,

WLR), indices of regional artery stiffness (central, arm, leg PWV), or vascular function (BA and SFA endothelial dependent [FMD] or independent [NTG]) (Table 4, Figures 4 and 5).

Discussion:

The present study demonstrated that when implemented in a supervised training program, the PAG influences some aspects of body composition and carotid vascular health in a cohort of adults with SCI, but was not of sufficient stimulus to improve traditional CVD risk factors or sublesional vasculature.

Traditional CVD Risk Factors and Body Composition

Neither the PAG nor the CON group experienced a change in metabolic syndrome prevalence (at least 3/5 of BP, WC, TG, HDL, and HbA1c) following the 4-month intervention. In support of these findings, previous reports have concluded that there is insufficient evidence to determine whether exercise improves aspects of traditional CVD risk in adults with SCI (25, 26).

Maintained anthropometrics and body composition in the PAG group concurrent with detrimental changes in the CON group (Table 2 and Figure 2) could be of clinical interest, potentially indicating that: 1) performing regular exercise following the PAG at a specified intensity for 4-months results in body composition maintenance, and/or 2) exercising without specific intensity guidelines defined in the PAG or without regular exercise results in negative body composition changes in just 4 months. Our data support the need for larger longitudinal trials to confirm if individuals with chronic SCI indeed

experience a gradual increase in whole body fat mass and VAT, and if following the PAG might prevent this.

Maintenance of whole body mass and anthropometrics (WC, BMI) following training were observed in the present study (Table 2 and Figure 2). Previous literature has reported either increases, no change, or decreases in body mass and/or anthropometrics following training protocols including aerobic and resistance training, FES-assisted exercise, and BWSTT, but all were of low quality (Pedro level 4-5) (20, 27).

It was unexpected in the current study to find no change in whole body or arm lean mass following exercise training for 4 months, especially considering the observed increase in arm muscle strength (21). It is unclear from previous studies whether sufficient evidence exists regarding the effects of exercise on lean tissue mass. A recently published RCT among 34 individuals with chronic incomplete SCI investigated the effects of FES-assisted walking or control (aerobic and resistance training) thrice weekly for 16 weeks on body composition (28). Following the intervention, increases in leg muscle cross sectional area (CSA) in the FES group were observed, concurrent with decreases in muscle CSA in the CON group (28). A 2011 systematic review assessed the effects of various exercise interventions on body composition among persons with chronic SCI and reported increases in lean mass following BWSTT, FES, and vibration training, however the majority of these studies were of low quality (Pedro level 3-4) (20). Several other studies reported no difference in lean mass post training (20). It may be that the specific resistance training protocol used in the current study (3 sets of 8-10 reps), as well as SCI related muscle denervation, limited the potential to stimulate muscle hypertrophy. Alternatively, it is possible that lean mass improved in certain upper extremity muscle groups; DXA is not sensitive enough to target trained muscles specifically.

To the author's knowledge, the present study is the first to report significant group x time interactions for whole body fat and VAT following aerobic and resistance training (Figure 2). The maintenance / improvements in VAT should be interpreted with caution, as the decrease in the PAG group of -0.15±0.35 kg is not larger than the calculated LSC of 0.28 kg. Previous investigations have shown either no change in fat mass (20) or decreases in fat mass following FES training (20, 27), and one recent cross-sectional study reported an association between exercise and lower total fat mass and regional fat mass (29). Decreased body fat and VAT in the PAG group is of particular interest, as these changes are typically associated with enhanced lipid metabolism (30). However, we did not observe improvements in fasting lipids following training. The lack of change in lipid profiles could be attributed to the ceiling effect: 74% and 91% of the present cohort had TG and HbA1c levels in the normal range, respectively, and consequently limited room for improvement. Alternatively, the absence of lipid changes following training may be due to the use of fasted samples. Postprandial TG levels have been associated with CV events while fasted TG levels showed little independent relationship among 26,509 able-bodied women (31). Among individuals with paraplegia, amplified postprandial lipids have been reported among those with normal fasted TG (32), and higher VAT has been related to delayed TG clearance following a fat load (33). Measuring fasted lipid levels instead of postprandial lipid levels may miss important information regarding potential improvements in lipid metabolism. Further, we were not able to control for lifestyle factors and did not measure or control diet among our participants, factors that are important determinants of body composition and lipid metabolism.

Blood Biomarkers

No group or time differences in any of fasting insulin, adipokines, inflammatory markers, or thrombotic markers were found in the present study (Table 3). The number of previous studies assessing these biomarkers after an exercise intervention is limited. In agreement with the current study, six months of circuit training 3x/wk (a combination of aerobic and resistance training) did not affect postprandial inflammatory markers (IL-6 and CRP) among persons with chronic paraplegia (34). Contrary to the current study, a 12-week arm cranking exercise program performed thrice-weekly in males with chronic paraplegia reported reduced leptin, TNF-alpha, and IL-6 in the exercising group, but no change in adiponectin or PAI-1 (35). Possible rationale for the inconsistent findings could be the inclusion of individuals with high paraplegia and tetraplegia in the current study (17 of the 23 participants had an injury level >T6). It has been postulated that the sympathetic nervous system plays a role in adipokine and cytokine response to exercise (36, 37), and so including persons with high thoracic/cervical injuries with altered sympathetic regulation could contribute to the overall lack of change in inflammatory and adipokine markers post-exercise in the current study.

Peripheral Vascular Structure and Function

Our results suggest maintained / improved local stiffness (carotid distensibility) in the PAG group concurrent with declines in the CON group (Figure 3). No other changes in carotid artery structure (PP, IMT, WLR), regional stiffness (PWV), or endothelial function (BA or SFA FMD or NTG) were observed.

Previous cross-sectional studies have reported a smaller carotid IMT and WLR in active vs. sedentary persons with SCI (both paraplegic and tetraplegic) (38), and similar carotid IMT and compliance between SCI ('sedentary') and able-bodied ('active') (39). Both of these studies support the suggestion that physical activity improves carotid artery structure post-injury. It is possible that carotid artery distensibility is the only vascular structure measure that changed following the training intervention in the present study because it is a homogenous artery assessment; in other words the measure is not based on a regional or whole body circulation model. Carotid artery distensibility is likely improved following PAG due to increased arterial pressure and HR that cause the vessels to deform and subsequently alter the connective tissue cross-linking (40).

It has been suggested that regular exercise results in a systemic improvement (BA and SFA) in IMT in individuals with SCI (41). Improved femoral artery compliance following 4-weeks of FES training (42) or 16 weeks of BWSTT (43) have been reported, but in contrast to the current study, both previous interventions were focused on lower extremity exercise. The present study did not assess distensibility or IMT of the BA or SFA; perhaps local stiffness improvements occurred in these arteries in the present study that were not recognized due to study design limitations.

No changes were observed in regional vascular stiffness assessed via central, arm, and leg PWV in the present study (Figure 4). The lack of change in regional vascular stiffness could be due to a number of potential mechanisms. First, the exercise intervention included a combination of aerobic and resistance training that may have had opposing effects on arterial stiffness. In able-bodied persons aerobic training has been shown to improve arterial stiffness (44) whereas resistance training has been shown to be detrimental (45) or have no effect on arterial stiffness (46), suggesting the mode of exercise could have different effects on arterial indices. Second, a parasympathetic dominant resting state and smooth muscle denervation are characteristic after SCI, likely resulting in low tone of sublesional arteries; it is possible our measurement technique was not sensitive enough to differentiate between structural arterial stiffness and functional arterial smooth muscle activation. However, no changes were observed in smooth muscle function (response to NTG) suggesting no changes in vascular tone, and no changes were observed in resting HR or BP supporting the lack of change in PWV. Third, several participants were smokers, had diabetes, and/or were on anti-hypertensive medication, all factors that influence arterial stiffness (47), making definitive conclusions as to the effects of the exercise training program on arterial stiffness difficult. Only one other case study in an individual with acute paraplegia (time since injury 5 months) assessed the effects of exercise on PWV and reported that 6 weeks of wheelchair ergometry improved arm and leg PWV (48). However the acute injury (i.e. unstable cardiovascular system due to ongoing physiological adaptations as a result of the injury), case study design, and estimated instead of measured distance between arterial segments make comparisons

between studies difficult. The limited amount of literature on vascular stiffness after SCI necessitates further investigation regarding mechanistic and intervention studies to explore potential relationships.

A novel aspect of the present investigation was the addition of upper extremity endothelial-dependent vasodilation (FMD) and upper and lower extremity endothelialindependent vasodilation (NTG) as outcome measures. No FMD or NTG changes were observed in either the BA or SFA (Figure 5). Several studies have included lower extremity FMD as an outcome, and have shown normalized or increased lower extremity FMD following lower body FES or neuromuscular electrical stimulation training programs (42, 49, 50). It is possible that the mode of training is a critical component for arterial adaptations. Perhaps lower body electrical stimulation is required to achieve sublesional vascular adaptations. Alternatively, the combination of aerobic and resistance training may have had opposing effects on arterial function; in able-bodied young men, aerobic training improves FMD (51), but 12 weeks of whole body resistance training did not have an effect on BA FMD (52).

The time course of structural and vascular changes should also be taken into consideration when interpreting the present study FMD results. It has been shown in healthy able-bodied persons that BA and popliteal FMD increase at 2 weeks of aerobic exercise training but return to baseline levels by 8 weeks, accompanied by steadily increasing structural adaptations (53). These results suggest that functional changes precede structural changes; arterial remodeling in response to exercise may help to normalize increased levels of SR resulting in FMD values returning to baseline levels

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after 8 weeks of training (53). Implications for the present study are that initial functional changes may have been missed due to measuring FMD at baseline and at 4 months. However in other clinical populations (coronary artery disease or heart failure), BA FMD improvements have been observed at similar training time points to the current study (54, 55). Future studies should assess the time course of artery function and remodeling in response to exercise in SCI, a population with altered autonomic function.

Summary

Our observations suggest that anthropometrics, body composition, and carotid distensibility in the PAG group were maintained or changing in a positive direction whereas these outcome measures were moving in a negative direction for the CON group. More definitive results are likely limited by the high variability of injury characteristics and our use of an active CON group. Difficulty in finding homogenous samples in the SCI population is common, given the variability with regards to age, level, severity, and duration of injury. We chose to use a recreationally active CON group to be more representative of a community sample; it also allowed us to examine more specifically the impact of PAG prescription. There is a need for larger, well-designed studies to verify the physical activity thresholds and dose response characteristics for improvements in body composition and carotid vascular health in individuals with SCI. Further, future studies with larger samples sizes should stratify based on lesion level, and control for lifestyle factors that may affect vascular health (i.e. smoking, diabetes, diet, use of anti-hypertension medications).

No training related changes were detected in any traditional CVD risk factors, blood biomarkers, or in regional stiffness or vascular function measures, suggesting that the current PAG do not provide a sufficient stimulus to induce these adaptations. Although the individual relative intensity of exercise was monitored to ensure a moderate to heavy load in the PAG group, it is possible that due to arm-only exercise and low fitness levels that the absolute intensity was not sufficient to evoke sublesional arterial adaptations. The guidelines were developed with fitness benefits in mind, and have been shown to improve VO₂peak and muscle strength (21). The guidelines should continue to be promoted as a means to increase physical activity, but may need to be modified to induce vascular health improvements.

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Figure 1: CONSORT flow diagram.

Parameter	PAG (n=12)	CON (n=11)
Sex (M/F)	12	9/2
Age, years	39±11	42±13
Height, m	1.8±0.1	1.7±0.1
Mass, kg	83.4±18.9	78.6±15.7
BMI, kg/m^2	27.3±5.2	25.7±4.9
WC, cm	96.2±14.9	89.6±11.4
Injury		
	3 A-B	5 A-B
AIS	9 C-D	6 C-D
NLI	C3-T10	C1-T11
YPI, years	15±10	9±10
Medications		
Antihypertensives	4 (33)	3 (27)
Lipid-lowering agents	3 (25)	2 (18)
Antbiotics	2 (17)	3 (27)
Spasms	6 (50)	5 (45)
Neuropathic pain	6 (50)	7 (64)
Depression / Anxiety	3 (25)	2 (18)
Constipation	5 (42)	3 (27)
Neurogenic bladder	4 (33)	2 (18)
Neurogenic bowel	0 (0)	1 (9)
Asthma	1 (8)	1 (9)
Bisphosphonates	2 (17)	0 (0)
Smoking Status (Y/N)	2/11	3/11

Values are mean±SD or n (%). PAG = physical activity guidelines (intervention group); CON = controls; BMI = body mass index; WC = waist circumference; AIS = American Spinal Injury Association Impairment Scale; NLI = neurological level of injury; YPI = years post injury. No significance found in any baseline measures between PAG vs. CON.

Table 1: Baseline participant characteristics.

	PAG (n=12)		CON	(n=9)
Parameter	Baseline	Post	Baseline	Post
SBP, mmHg	116±18	116±15	118±18	116±17
^a DBP, mmHg	68±9	67±9	74±13	72±11
MAP, mmHg	84±12	84±11	89±14	86±13
HR, bpm	75±13	71±13	75±10	74±10
^b HbA1c, mmol/mol	35.7±11.6	36.6±11.2	34.9±4.8	34.7±3.9
^b HDL, mmol/L	1.01±0.2	1.01±0.3	1.13±0.2	1.17±0.3
TG, mmol/L	1.3±0.6	1.4±0.6	1.1±0.7	1.0 ± 0.7
TC, mmol/L	4.5±0.9	4.3±1.0	4.1±0.9	4.1±0.9
LDL, mmol/L	2.9±0.9	2.7±0.7	2.5±0.7	2.4±0.6
TC/HDL	4.6±0.9	4.4±0.8	3.8±1.1	3.6±1.0
WC, cm	96.2±14.9	95.2±15.0	89.6±11.7	93.1±10.4*
BMI, kg/m^2	27.3±5.2	27.0±5.0	25.7±4.9	26.6±4.7*

Values are mean±SD. PAG = physical activity guidelines group; CON = control group; SBP = seated systolic blood pressure; DBP = seated diastolic blood pressure; MAP = seated mean arterial pressure; HR = heart rate; HbA1c = glycated haemoglobin; HDL = high density lipoprotein; TG = triglycerides; TC = total cholesterol; LDL = low density lipoprotein; TC/HDL = total cholesterol to high density lipoprotein ratio; WC = waist circumference; BMI = body mass index. ^{a,b}Indicates non-normal data that was log (^a) or inverse (^b) transformed prior to calculating ANOVA or effect size. Non-transformed values are reported. *Represents group x time ANOVA interaction p<0.05.

Table 2:	Traditional	cardiovascular	[.] disease risl	x factors	(i.e. meta	abolic sy	yndrome).	
					`		/ /	

	PAG	(n=12)	CON (n=9)		
Parameter	Baseline	Post	st Baseline Po		
^b Insulin, pg/mL	227.9±171.1	283.2±180.5	396.2±452.4	455.8±449.9	
^c APN, μg/mL	76.7±64.0	90.1±42.1	82.02±38.28	117.69±56.82	
^a Leptin, ng/mL	10.12±13.25	11.12±15.97	10.2 ± 12.8	14.3±14.9	
^a TNFα, pg/mL	4.7±1.8	4.4±2.0	4.1±2.2	4.0±1.8	
^a IL-6, pg/mL	2.5±2.2	1.5 ± 1.0	3.7±2.1	5.5±7.1	
PAI-1, ng/mL	30.4±17.7	42.0±15.5	31.1±22.7	42.6±19.5	

Values are mean±SD. PAG = physical activity guidelines group; CON = control group; APN = adiponectin; TNF α = tumor necrosis factor alpha; IL-6 = interleukin-6; PAI-1 = plasminogen activator inhibitor-1. ^{a,b,c}Indicates non-normal data that was log (^a) inverse (^b) or square root (^c) transformed prior to calculating ANOVA or effect size. Non-transformed values are reported.

Table 3: Blood biomarkers.



Figure 2: Body composition. Mass, fat, and lean refer to whole body measures; VAT = visceral adipose tissue. Error bars represent SD. *Represents group x time ANOVA interaction p<0.05. Mass (kg) p=0.031; fat (kg) p=0.042; lean (kg) p=0.891; VAT (kg) p=0.038.

			PAG	CON		1	
Parameter	Baseline		Post	Baseline		Post	
Carotid Artery							
Local Stiffness							
Sample size (<i>n</i>)		12			9		
^a PP, mmHg	41±10		44±11	38±15		45±21	
IMT, mm	0.52 ± 0.14		0.49 ± 0.10	0.49 ± 0.08		0.49 ± 0.09	
^a Mean LD, mm	5.87±0.79		5.86 ± 0.80	5.78 ± 0.46		5.88±0.58	
Wall:Lumen, %	9.1±2.7		8.5±1.7	8.5±1.0	8.3±1.0		
Brachial Artery							
FMD							
Sample size (n)		12			9		
BL LD, mm	4.30±0.74		4.28±0.74	4.38±0.54		4.38±0.52	
Time to Max, s	53±24		52±16	51±17		52±12	
^a FMD, mm	0.34 ± 0.14		0.39 ± 0.13	0.28 ± 0.10		0.29 ± 0.08	
^a Peak SR, 1/s	775±377		845±298	798±187		833±242	
${}^{\mathrm{a}}\mathrm{SR}_{\mathrm{AUC}},10^{3}$	14.1±13.4		14.3±7.0	18.4 ± 11.0		16.0 ± 5.0	
NTG							
Sample size (<i>n</i>)		10			8		
^a BL LD, mm	4.32±0.68		4.30 ± 0.74	4.40 ± 0.52		4.55±0.45	
^a Time to Max, s	370±120		468±132	427±136		525±89	
NTG, mm	0.81 ± 0.16		0.84 ± 0.24	0.95 ± 0.21		0.97±0.26	
FMD:NTG, %	43±14		47±14	31±13		32±10	
Superficial Femoral	Artery						
FMD							
Sample size (<i>n</i>)		12			9		
^b BL LD, mm	5.51 ± 1.05		5.53 ± 1.04	5.64 ± 0.48		5.44±0.74	
^a Time to Max, s	57±18		59±22	80±52		63±27	
FMD, mm	0.39 ± 0.16		0.42 ± 0.22	0.31 ± 0.12	0.27 ± 0.11		
Peak SR, 1/s	680±409		633±231	604±213	657±178		
SR_{AUC} , 10^3	14.6±10.2		13.4±9.4	19.3±16.1		18.3±16.2	
NTG							
Sample size (<i>n</i>)		10			8		
^b BL LD, mm	5.64±1.16		5.55 ± 1.04	5.67±0.59		5.38±0.73	
^a Time to Max, s	551±113		432±132	578±96		555±135	
NTG, mm	0.80 ± 0.34		0.74 ± 0.30	0.67±0.14		0.71±0.14	
FMD:NTG, %	44±17		51±31	44±16		36±12	

Values are mean±SD. PAG = physical activity guidelines group; CON = control group; PP = pulse pressure; IMT = intima media thickness; BL LD = baseline lumen diameter; FMD = flow mediated dilation; SR = shear rate; SR_{AUC} = shear rate area under the curve up to max dilation; NTG = nitroglycerine. ^{a,b,c}Indicates non-normal data that was log (^a) or inverse (^b) transformed prior to calculating ANOVA or effect size. Non-transformed values are reported.

Table 4: Vascular structure and function.



Figure 3: Local stiffness (carotid artery distensibility, $mmHg^{-1}$). Error bars represent SD. *Represents group x time ANOVA interaction p<0.05.



Figure 4: Regional Stiffness (Pulse Wave Velocity, m/s). Error bars represent SD. p-values represent group x time ANOVA interaction. PAG n=12; CON n=9.





Figure 5: Vascular function. Data are for: (A) endothelial-dependent vasodilation via flow mediated dilation (FMD), PAG n=12, CON n=9, and (B) endothelial-independent vasodilation via nitroglycerine (NTG), PAG=10, CON=8. Error bars represent SE. p-values represent group x time ANOVA interaction.

CHAPTER 7

DISCUSSION

DISCUSSION

7.1. Discussion Overview

The main objective of this dissertation was to evaluate cardiovascular disease (CVD) risk and the role of exercise in ameliorating CVD risk in adults with spinal cord injury (SCI). It has been established that individuals with SCI experience an earlier onset of CVD and/or an increased prevalence of CVD when compared to their able-bodied (AB) counterparts (1), likely due to physical inactivity, altered cardiovascular regulation, and reduced energy metabolism (2). Due to injury-related changes in metabolic and neural function, other non-traditional novel risk factors (i.e. physical inactivity, peripheral vascular health) may assist in the accurate assessment CVD risk in the SCI population.

Literature has established the importance of implementing regular physical activity into one's lifestyle to protect from CVD and CVD complications for AB individuals (3). The same benefits are becoming evident in the SCI population as a growing body of research reveals the effectiveness and benefits of exercise programs in this vulnerable population (4). However, the evidence is insufficient and/or scarce regarding the effects of exercise training interventions on traditional and novel CVD risk factors after SCI. Further, AB physical activity guidelines are not appropriate for the SCI population due to altered metabolic demands and a blunted cardiovascular response to exercise post-injury. Canadian physical activity guidelines for adults with SCI (PAG) take into account the specific needs and capabilities of the SCI population (5), and have been shown to improve fitness outcomes of VO₂peak and muscle strength (6), but have not been evaluated for their effectiveness related to improvements in CVD risk. Taken together, the series of studies in the present dissertation present novel CVD risk factors that can be assessed in the SCI population to provide a more comprehensive cardiovascular health profile, and may help to elucidate the benefits of the incorporation of regular exercise into the daily lives of individuals with chronic SCI. This dissertation takes steps to fill some of the substantial gaps in knowledge surrounding the impacts of physical activity on cardiovascular health after SCI.

7.2. Summary of Main Findings

The purpose of the first study (Chapter 2) was to describe traditional and novel CVD risk factors among adults with chronic SCI. This was an important first step in the characterization of risk factors that render individuals with SCI more susceptible to CVD. It appears as though both injury-related physiological limitations (impairments in skeletal muscle, respiratory, cardiac, and autonomic function) as well as modifiable lifestyle factors (i.e. physical inactivity, obesity) affect CVD risk after SCI. This study confirmed that traditional CVD risk factors (blood pressure [BP], waist circumference [WC], lipid profile) are not sensitive enough to differentiate CVD risk between SCI and AB (7), yet revealed that body composition, physical activity, aerobic capacity, and indices of peripheral vascular health were negatively impacted after SCI. Results from this first study showed that a comprehensive body composition assessment including whole body and segmental fat and lean mass is important for evaluating obesity and CVD risk after SCI. Simply assessing mass based changes in individuals with chronic SCI failed to represent lean tissue losses, fat tissue gains, and relative fat tissue redistribution. In the absence of available equipment for comprehensive body composition assessment, in the

very least, SCI-specific anthropometric thresholds are required for evaluating CVD risk (i.e. WC \geq 94cm, body mass index [BMI] \geq 22 kg/m2) (8, 9). Findings from this first study also indicate that aerobic capacity should be assessed to provide information regarding fitness levels for CVD risk reduction, as well as insight regarding the ability to perform activities of daily living (10). Lastly, this was the first study to report decreased superficial femoral artery (SFA) endothelial function via relative flow-mediated dilation (FMD%) after adjusting for baseline diameter in comparison to an AB cohort, suggesting previous reports of enhanced SFA FMD% in SCI (11-14) were due, in part, to differences in baseline diameter between groups. Although these findings provide important insight into sublesional vascular health, it is crucial that future studies validate the use of FMD% as an indicator of endothelial function in deconditioned limbs. SCI disrupts supraspinal control of the sympathetic circuits that innervate peripheral blood vessels, so it is possible that altered sympathetic innervation contributes to sublesional FMD% after SCI. Further, future work should discern the relationship between FMD% and CVD risk in SCI. The overall contribution to the literature of this first study is that incorporating novel risk factors into standardized risk factor assessment could potentially help explain the higher CVD risk after SCI.

Findings of decreased sublesional FMD% in the first study lead to questions regarding potential mechanisms for altered endothelial function after SCI. Endothelial dysfunction is the earliest detectable manifestation of atherosclerosis and subsequent CVD (15); it is therefore an important component of vascular health to identify and understand post-injury. Shear rate (SR) magnitude and direction exerted by the blood

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against the endothelial cells is the most potent stimulus for modulating endothelial structure and function (16). It has been shown that anterograde SR is atheroprotective while retrograde and oscillatory SR are atherogenic (17). Research assessing SR through peripheral arteries is scarce in the SCI population, and no studies have investigated the effects of altered SR on endothelial function after SCI. The objectives of the second, third, and fourth studies (Chapters 3-5) were to investigate the effects of altered SR on endothelial function after SCI. The objectives of altered SR on endothelial function after 3) was a feasibility study of an intervention designed to augment retrograde SR in young healthy AB men. The third study (Chapter 4) implemented the intervention to augment retrograde SR through peripheral arteries in both adults with SCI and age- and sex-matched AB controls. The fourth study (Chapter 5) utilized a single exercise bout to augment anterograde SR through peripheral arteries in adults with SCI and age- and sex-matched AB controls.

Previous *in vivo* work has suggested a detrimental effect of increased retrograde SR created using a cuff-inflation model on brachial artery (BA) FMD% in young healthy men (18-20). The purpose of the second study (Chapter 3) was to expand on those findings and determine the feasibility of using the same cuff-inflation intervention in the lower extremities among young healthy men. Two cuff inflation pressures (75mmHg and 100mmHg) were utilized; it was determined that the 75mmHg pressure enhanced retrograde and oscillatory SR enough to acutely attenuate SFA FMD% in young AB men. The purpose of the third study (Chapter 4) was two-fold: 1) identify resting SR patterns and FMD% through the upper extremity (BA) and sublesional lower extremity (SFA) vessels in SCI and age and sex-matched AB controls, and then 2) manipulate SR patterns

using the cuff inflation pressure established from the previous study to augment retrograde SR through the BA and SFA. BA SR patterns and FMD% were similar between groups, while SFA anterograde SR was higher (p<0.01) and FMD% was lower (p=0.04) in SCI vs. AB. These findings suggested that the elevated atheroprotective anterograde SR at rest may not be the key determinant of SFA FMD% after SCI, and that perhaps altered retrograde SR may be a stronger determinant of endothelial function. Retrograde SR was augmented through both arteries at rest using the sub-systolic cuff inflation model, resulting in attenuated FMD%. Interestingly, the SCI group experienced a larger retrograde SR component during the SFA cuff-inflation intervention compared to AB, perhaps indicating that structural (stiffer arterial walls) or neural (reduced sympathetic contribute innervation) factors to SR pattern responses in deconditioned/paralyzed limbs. Findings from these two studies demonstrate the potential detrimental effects of acute elevations in retrograde SR among individuals with extensive arterial remodeling and reduced sympathetic innervation (SCI) and AB controls.

Numerous studies conducted among AB persons reveal that a single exercise bout can result in beneficial vascular adaptations in both active and non-active regions, such as improved BA FMD% following leg cycling (21, 22). The vascular improvements are likely mediated by repeated increases in anterograde SR experienced during exercise. It is unknown whether upper body exercise produces comparable increases in SR through the lower extremities and if these SR modifications influence FMD%, particularly in sublesional vasculature. The purpose of the fourth study (Chapter 5) was to determine the effects of a single arm-cranking exercise bout on SFA SR patterns and FMD%. Nine
minutes of arm-cranking exercise augmented SFA anterograde and retrograde SR in both SCI and age- and sex-matched AB controls, but improved SFA FMD% in the AB group only. Interestingly, the SCI group once again experienced a larger atherogenic retrograde SR component compared to AB. These findings highlight the importance of considering other endothelial regulatory factors (i.e. oxidative stress, sympathetic innervation) when interpreting the effects of a single bout of arm exercise on sublesional vasculature. It is also possible that a single bout of moderate intensity arm exercise did not provide the appropriate stimulus to generate acute sublesional vascular responses in SCI.

These series of studies were the first to assess the effects of SR patterns on FMD% in the SCI population. In sum, the results provide support for continued investigation of endothelial function responses to changes in SR patterns, and may shed light into potential vascular adaptations to exercise training.

The fifth study (Chapter 6) of the present dissertation assessed the effects of exercise training on traditional and novel CVD risk factors. Knowledge gained from the first study informed selection of outcome measures: comprehensive body composition assessment, aerobic capacity, and peripheral vascular health including arterial stiffness and endothelial function. Based on the findings from the first study regarding significantly elevated body fat and VAT in SCI, analyses of inflammation, thrombosis, and adipokine secretion were included, biomarkers known to be released from excess adipose tissue and in particular VAT (23-25). Four months of implementing the PAG that take into account the specific needs and capabilities of the SCI population (5) influenced aerobic capacity (6), body composition, and carotid vascular health. No changes in traditional CVD risk

factors, blood biomarkers, or sublesional vascular health were observed. The lack of change in traditional CVD risk factors was not surprising since these measures did not seem sensitive enough to differentiate CVD risk between SCI and AB in the crosssectional cohort (Chapter 2). The observation of no change in any of the adipose-derived biomarkers with exercise training may be due to inclusion of participants with high thoracic/cervical injuries in which autonomic regulation is affected; it has been postulated that the sympathetic nervous system plays a role in adipokine and cytokine response to exercise (26, 27). The lack of change in sublesional vascular health may have been due to a number of factors: 1) perhaps, in agreement with findings from Chapter 5 showing no improvement in sublesional FMD% following a single arm-cranking exercise bout, armonly exercise training does not provide a sufficient SR stimulus to influence sublesional vascular health; 2) a combination of aerobic and resistance exercise may have had conflicting effects on peripheral vascular health; 3) several participants were smokers, had diabetes, and/or were on hypertension medication, factors that may affect vascular health; and/or 4) perhaps the overall absolute training stimulus from the PAG and low initial individual fitness levels were simply not sufficient to evoke arterial adaptations. This is one of very few randomized controlled exercise training studies in the SCI population, and the first randomized controlled trial to evaluate the effectiveness of the PAG on cardiovascular health. The results from this study revealed the potential of the PAG to improve aerobic capacity (6), body composition, and carotid vascular health compared to an active control group. In addition, the study findings advance the

important question of how SCI influences exercise-induced adaptations to peripheral vasculature.

7.3. Interpretation of Overall Findings

Reflecting on overall findings from the five studies incorporated in the present dissertation, it is clear that a more comprehensive assessment of CVD risk is necessary to delineate the effects of injury on the increased prevalence of CVD in this population. In particular, the effects of the SCI itself, as well as the effects of both a single bout of exercise and exercise training on sublesional vasculature deserve further investigation. Results from the cross-sectional study (Chapter 2) showed a decreased SFA FMD% in SCI vs. AB after controlling for baseline diameter, suggesting sublesional endothelial dysfunction after SCI. SR magnitude and direction are the most potent stimuli for endothelial adaptations; results from the third study (Chapter 4) revealed elevated anterograde SFA SR in SCI vs. AB at rest. Since anterograde SR is generally considered atheroprotective, other factors such as arterial remodeling or sympathetic innervation must be taken into consideration when interpreting sublesional vascular health assessments after SCI. Exercise is known to protect AB individuals from CVD and CVD complications, however the effects of a single bout of exercise or exercise training on sublesional vascular health after SCI is unknown. Results from the fourth study (Chapter 5) assessing the effects of a single arm exercise bout on sublesional vascular function showed that augmented anterograde and retrograde SFA SR improved SFA FMD% in AB but not in SCI. These findings support the conclusions made in the previous study that factors other than SR patterns may contribute to sublesional endothelial function

regulation after SCI (i.e. metabolic, neural). Results from the fifth study (Chapter 6) showed no influence of 4-months of upper body exercise training on sublesional vascular health.

Considering results from Chapter 5 (single exercise bout) and Chapter 6 (exercise training) together, it is clear that non-active vasculature responds differently to exercise stimuli in individuals with SCI compared to AB (21, 22, 28), perhaps due to disturbed autonomic regulation. Although the findings of no change in FMD% following exercise training is also contradictory to previous studies in the SCI population, all the previous studies utilized lower body functional electrical stimulation (FES) (12, 29, 30). The present study was the first study to assess a combination of aerobic and resistance exercise training on vascular adaptations.

An alternative explanation to the lack of change in sublesional vasculature in response to a single bout of exercise or exercise training is that arm-only exercise does not provide a sufficient stimulus to influence the peripheral vessels that lack sympathetic innervation. Perhaps lower extremity exercise (i.e. FES, body weight supported treadmill training [BWSTT], NuStep) could evoke upper extremity vascular function improvements. It is also possible that the arm-only exercise employed in the present dissertation did not provide a sufficient SR or metabolic stimulus to evoke sublesional adaptations in individuals with SCI. Although the individual relative intensity of the single exercise bout was similar between SCI and AB groups in study four (Chapter 5), and the relative intensity of the exercise training sessions was monitored to ensure a moderate to heavy load in study five (Chapter 6), it is possible that due to arm-only

exercise and low initial fitness levels that the absolute intensity was not enough to evoke sublesional arterial adaptations. Perhaps more frequent, higher intensity, or longer duration of training stimuli would result in beneficial vascular adaptations.

7.4. Difficulties Experienced Throughout Data Collection

Research conducted in the SCI population is not without its difficulties. Recruitment is a persistent and frequent concern, particularly when longitudinal interventions are initiated. Heterogeneous participant groups with regards to age, sex, or severity and level of injury are a common occurrence in SCI literature. In addition, the majority of individuals with SCI are on several medications to control secondary complications such as cardiovascular abnormalities (anti-hypertensive or hypotensive medications), lipid disorders, depression/anxiety, infection (pressure sores, ingrown toenails, urinary tract infections, etc.), osteoporosis, osteoarthritis, pain, spasticity, contractures, constipation, neurogenic bladder and bowel, etc. Variability across SCI participants in addition to medications that may influence cardiovascular function makes it difficult to draw definitive conclusions regarding cardiovascular health after SCI from cross-sectional cohorts. Ideally recruitment would take into consideration potential confounders or effect modifiers, but the difficulty in recruiting large numbers of individuals with SCI make it near impossible. Further, when conducting an exercise intervention many barriers including personal health issues, medical appointments, transportation, and other commitments contribute to study adherence. Although in in present training study (Chapter 6) a high level of adherence of 98±3% was observed, the majority of the

participants required additional time, past the 4-months, to complete all training sessions due to the barriers listed above.

Difficulties that arose over the course of data collection with respect to assessing cardiovascular health in the SCI population were mostly attributed to altered cardiovascular regulation. Several participants experienced autonomic dysreflexic (AD) attacks during leg FMD assessment, likely due to the noxious stimulus of the cuff pressure or uncomfortable positioning. In some instances the test was terminated, while in others the issue was circumvented by providing additional cushioning or adjusting positioning of the cuff. During the nitroglycerine (NTG) assessment, several participants experienced a drop in BP that required termination of the assessment, and continuous monitoring until BP reached baseline levels. Circumstances of spasticity or history of deep vein thrombosis resulted in challenging data collection during pulse wave velocity (PWV) and FMD assessment. Other data collection difficulties that arose included participants with diabetes unable to fast for a full 12 hours prior to blood draw, the potential of long supine cardiovascular testing sessions (3+ hours) resulting in pressure sores, spasticity or contractures during positioning for dual energy x-ray absorptiometry (DXA) scans, and spasticity or joint pain affecting peak aerobic capacity testing. All these obstacles to data collection are common to the SCI population and should be anticipated to avoid any adverse events.

7.5. Limitations and Future Directions

Several limitations from the present series of studies need to be addressed. In the cross-sectional study (Chapter 2) the AB comparison group was small, likely resulting in

underpowered statistics (type II error). Although the SCI cohort was fairly large for a clinical population (n=34), future studies with a greater sample size should be conducted assessing the same outcome measures to enable controlling for covariates such as smoking, diabetes, anti-hypertensive medications, etc. There was no measure of physical activity for the AB group, which may have provided additional insight into peripheral vascular differences in SCI vs. AB. Future studies should also consider the use of accelerometers to better characterize daily physical activity. Although a comprehensive measure of body composition was included, assessing adipose-derived biomarkers (i.e. inflammatory markers, thrombotic agents, adipokines) may better characterize the effects of altered body composition on an atherogenic internal environment in individuals with SCI in a cross-sectional study design. The finding of decreased SFA FMD% after adjusting for baseline diameter in SCI needs to be confirmed; future studies should incorporate controlling for baseline diameter when assessing endothelial function between populations as well as between arterial sites. The next steps in CVD risk assessment in the SCI population is to validate the use of FMD% as an indicator of endothelial function in deconditioned limbs, and to determine the relationship between vascular structure and function indices and CVD prevalence.

A limitation from the two studies assessing augmented retrograde SR and FMD% (Chapters 3 and 4) include the inability to create an exclusive retrograde SR stimulus with the cuff inflation model. Further, no relationships were observed between any of the SR patterns and FMD%, making it difficult to comment on the influence of isolated SR

parameters on endothelial health. However it does provide a more accurate representation of *in vivo* SR observed with enhanced vascular tone.

In Chapter 5, no changes in FMD% were observed following a single bout of arm exercise in individuals with SCI; future studies should explore the effects of different modes (i.e. FES, BWSTT, NuStep), intensities, and durations of exercise on endothelial health in both healthy and clinical populations. Considering potential confounding factors such as oxidative stress and sympathetic modulation may help delineate the effects of different exercise stimuli on endothelial function responses.

Limitations in the exercise training study (Chapter 6) may have affected the observed results. It has been shown that postprandial lipid assessment is more representative of lipid metabolism than fasting assessments; amplified postprandial lipids have been reported among individuals with SCI with normal fasted triglycerides (31). Therefore, measuring fasted lipids instead of postprandial lipids may have missed important information regarding potential improvements in lipid metabolism following 4-months of the PAG. Further, lifestyle factors or diet were not controlled, factors that certainly could have affected CVD risk factor outcomes. The small heterogeneous sample size did not allow for stratification based on injury level; the majority of participants had high thoracic or cervical injuries and therefore altered cardiovascular homeostasis. Future studies should recruit a larger sample size to enable consideration of confounding factors including smoking, diabetes, medications that affect vascular health, and sympathetic nervous system modulation. The PAG should continue to be promoted as a means to increase physical activity and fitness (6), but future studies should evaluate the effects of

a more frequent, higher intensity, or longer duration training stimulus on vascular adaptations in SCI.

7.6. Conclusions

Overall, the results from the present dissertation highlight the importance of evaluating a comprehensive CVD risk factor profile among individuals with SCI to begin to delineate what factors contribute to the elevated CVD prevalence in this population. The current dissertation has presented insight into the effects of SCI on CVD risk, and the effects of exercise on CVD risk reduction in SCI. This dissertation demonstrated that people with SCI have a different CVD risk factor profile when compared to their AB counterparts including detrimental body composition changes, physical inactivity, reduced aerobic capacity, and poor peripheral vascular health. The data here suggest that sublesional vasculature may respond differently to a single bout of exercise and exercise training when compared to AB, likely due to autonomic dysregulation. Further research is necessary to elucidate the effects of metabolic and neural influences on sublesional vascular structure and function in SCI. Finally, the current work has shown that the PAG can be implemented within a community setting, and that it is effective in improving aerobic fitness (6) and some aspects of body composition and vascular health in adults with chronic SCI.

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APPENDIX

Physical Activity Recall Assessment for People with Spinal Cord Injury (PARA-SCI)

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Assessors Initiate. Cardiovascular Fitness among Individuals with Chronic Spinal Cord Injury

PARA-SCI

Participant ID

Page 1 of 3

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PARA-SCI

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viduals with Chronic Spinal Cord Injury	PARA-SCI	/ / Phone Call
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